

**The Synthesis of Imidazo[1,2-*a*]pyrazines as Inhibitors of the VirB11 ATPase
and their Incorporation into Bivalent Compounds**

James Richard Sayer

Department of Chemistry

University College London

Submitted to the Department of Chemistry for the Degree of Doctor of Philosophy

I, James Richard Sayer, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

James Sayer

In Loving Memory of Dad

Graham John Sayer

2nd February 1945 – 21st November 2012

I would like to take this opportunity to thank Prof Alethea Tabor and Prof Gabriel Waksman for the opportunity to work on this project and for their ongoing support and guidance throughout. I would also like to express my gratitude towards Dr Lisa Haigh for running countless mass spectrometry samples, Dr Abil Aliev for his assistance with NMR analysis, Jill Maxwell for elemental analysis and past and present members of the Tabor and Hailes research teams for their friendly help and advice.

My thanks also go to Prof David Selwood, Dr Karin Wallden, Dr Paul Gane, Dr Thomas Pesnot, Dr Fred Campbell and Hans Koss for their contribution to this work.

Last but not least, I would like to thank my wife Danielle for her support, patience and understanding throughout my PhD.

Abstract

Helicobacter pylori are gram negative bacteria that colonise in the human stomach causing illnesses such as peptide ulcers and various cancers. Through targeting a component of their secretion apparatus, it is envisaged that translocation of toxic molecules will be inhibited and therefore virulence would be reduced.

Virtual high throughput screening of the *Helicobacter pylori* VirB11 ATPase, HP0525, identified imidazo[1,2-*a*]pyrazine compounds as potential ATP mimics and ATPase inhibitors.

Synthesis of these target compounds was carried out, with two routes established to deliver 2- and 3- aryl regioisomers. *In vitro* screening identified compound **14** as the lead compound ($IC_{50} = 7 \mu M$) and studies have shown it to be a competitive inhibitor of ATP. Based upon *in silico* interactions of this compound within the ATP binding site, a second generation of compounds were then designed and synthesised.

Whilst these compounds showed no major improvements in potency, two further inhibitors were identified as potential leads. Trends in the structure-activity-relationship of these imidazo[1,2-*a*]pyrazine compounds have also been identified, paving the way for the design of further novel compounds.

In addition, the lead compound **14** was successfully incorporated into peptide-small molecule bivalent compounds. The objective of the peptide moiety was to disrupt hexamer formation of the VirB11 ATPase, whilst the small molecule targets the enzyme active site. Initial studies have shown this class of bivalent compound not to inhibit below 500 μM . Further investigation of these bivalent compounds will be required.

Presented in this thesis are details of the synthesis, biological testing and computational evaluation of imidazo[1,2-*a*]pyrazines and their incorporation into the synthesis of bivalent compounds.

Contents

Abstract.....	v
Contents	vi
List of Abbreviations.....	xi
Chapter 1: Introduction.....	1
1.1 Biological Background.....	1
1.1.1 <i>Helicobacter Pylori</i>	1
1.1.2 Secretion Systems.....	3
1.1.3 Type IV Secretion Systems.....	3
1.1.4 VirB11 ATPase: HP0525.....	8
1.1.4.1 HP0525 Monomer.....	8
1.1.4.2 HP0525 Hexamer.....	8
1.1.4.3 Nucleotide Binding Site.....	10
1.1.4.4 Subunit-Subunit Interface	11
1.1.4.5 HP0525 Mechanism of Action.....	12
1.1.4.6 HP1451: Regulatory Protein of HP0525.....	13
1.1.4.7 Current Inhibitors of HP0525	14
1.2 Imidazo[1,2-<i>a</i>]pyrazines	15
1.2.1 Target Compounds	15
1.2.2 Synthesis, Reactivity and Therapeutic Applications of Imidazo[1,2- <i>a</i>]pyrazines..	16
1.2.2.1 Synthesis of Imidazo[1,2- <i>a</i>]pyrazines.....	18
1.2.2.1.1 Imidazo[1,2- <i>a</i>]pyrazines from Pyrazine Rings	18
1.2.2.1.2 Imidazo[1,2- <i>a</i>]pyrazines from Imidazole Rings.....	26
1.2.2.1.3 Alternative Approaches to the Formation of Imidazole and Pyrazine Rings.....	28
1.2.2.1.4 Multicomponent Reactions.....	29
1.2.2.2 Reactivity of Imidazo[1,2- <i>a</i>]pyrazines	32
1.2.2.2.1 Electrophilic Addition	32
1.2.2.2.2 Nucleophilic substitution.....	35
1.2.2.2.3 Metalation of Imidazo[1,2- <i>a</i>]pyrazines.....	39
1.2.2.2.4 Reduction of Imidazo[1,2- <i>a</i>]pyrazines.....	39
1.2.2.3 Therapeutic Applications of Imidazo[1,2- <i>a</i>]pyrazines	40
1.2.2.3.1 Imidazo[1,2- <i>a</i>]pyrazines as Anticancer Agents	40
1.2.2.3.2 Additional Therapeutic Applications of Imidazo[1,2- <i>a</i>]pyrazines.....	45
1.3 Project Aims & Objectives	48

Chapter 2: Results & Discussion: Target Compounds.....	49
2.1 Initial Synthetic Approach	49
2.2 Synthesis of 3-Aryl Imidazo[1,2-<i>a</i>]pyrazines	51
2.2.1 Overview of the Synthesis	51
2.2.2 Formation of Amino Alcohol Moiety <i>via</i> Epoxide	51
2.2.3 Formation of Amino Alcohol Moiety <i>via</i> Azido Ketone	54
2.2.4 Synthesis of 3-Aryl Imidazo[1,2- <i>a</i>]pyrazine from Amino Alcohol	56
2.2.5 Alternative Approach to 3-Aryl Imidazo[1,2- <i>a</i>]pyrazines	58
2.3 Synthesis of 2-Aryl Imidazo[1,2-<i>a</i>]pyrazines	58
2.3.1 Optimisation of Condensation Reaction	60
2.3.2 Tautomerisation of Imidazo[1,2- <i>a</i>]pyrazines	62
2.4 Buchwald-Hartwig Cross Coupling.....	64
2.4.1 Optimisation of Buchwald-Hartwig Cross Coupling Reaction of Imidazo[1,2- <i>a</i>]pyrazines.....	66
2.4.2 Displacement of Methyl Sulfone Moiety of Imidazo[1,2- <i>a</i>]pyrazines as an Alternative to Buchwald-Hartwig Cross Coupling Reaction.....	71
2.4.3 Exploring the Synthesis of 2-Aryl Imidazo[1,2- <i>a</i>]pyrazin-8-amine	73
2.5 Evaluation of Target Compounds.....	75
2.5.1 Biochemical Assay.....	75
2.5.1.1 Biochemical Assay Data	76
2.5.1.2 Compound 14 is a competitive inhibitor of ATP	79
2.5.2 Molecular Modelling.....	80
2.5.2.1 Results of Molecular Modelling	83
2.6 Concluding Remarks to Chapter	87
Chapter 3: Results & Discussion: 2nd Generation Compounds	89
3.1 Imidazo[1,2-<i>a</i>]pyrazine Analogues	89
3.1.1 Variations at Position 8	90
3.1.2 Variations with the Aromatic Group in Position 2	94
3.2 Maybridge Analogues.....	96
3.2.1 Maybridge Fragment Substructure Search.....	97
3.3 Compound Analysis	101
3.3.1 Imidazo[1,2- <i>a</i>]pyrazines	101
3.3.2 Maybridge Compounds.....	108
3.3.3 BioFocus Compounds	110
3.4 Concluding Remarks to Chapter	114
Chapter 4: Results & Discussion: Peptide-Small Molecule Bivalent Reagents	117

4.1 Introduction to Bivalent Inhibitors	117
4.1.1 Peptide-Small Molecule Bivalent Inhibitors.....	118
4.1.2 Conjugation Techniques and Protein Modification.....	124
4.2 Bivalent Inhibitor Reagents as Potential ATPase Inhibitors.....	128
4.3 Synthesis of PEGylated Imidazo[1,2-<i>a</i>]pyrazines.....	134
4.3.1 Synthesis of the Carbamate Linker for PEG attachment to Imidazo[1,2- <i>a</i>]pyrazine Inhibitors	135
4.3.2 Synthesis of PEGylated Free Hydroxyl Imidazo[1,2- <i>a</i>]pyrazines	139
4.3.2.1 PEGylated-Benzenesulfonamide-bound Imidazo[1,2- <i>a</i>]pyrazines.....	139
4.3.3.2 Attempted Synthesis of PEGylated- <i>N</i> -(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2- <i>a</i>]pyrazines	141
4.3.3 Synthesis of PEGylated Maleimide Linked Imidazo[1,2- <i>a</i>]pyrazines.....	142
4.3.3.1 PEGylated-Benzenesulfonamide-bound Imidazo[1,2- <i>a</i>]pyrazines.....	142
4.3.3.2 Approaches to PEGylated- <i>N</i> -(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2- <i>a</i>]pyrazines	146
4.3.4 Synthesis of PEGylated Allyl and Alkyne Linked Imidazo[1,2- <i>a</i>]pyrazines	149
4.3.4.1 PEGylated-Benzenesulfonamide-bound Imidazo[1,2- <i>a</i>]pyrazines.....	149
4.3.4.2 Approaches to PEGylated- <i>N</i> -(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2- <i>a</i>]pyrazines	150
4.4 Peptide Synthesis.....	152
4.4.1 Synthesis of <i>N</i> -Fmoc protected <i>S</i> -Allyl Cysteine.....	152
4.4.2 Peptide Synthesis.....	155
4.5 Peptide-Imidazo[1,2-<i>a</i>]pyrazine Conjugation to give Bivalent Inhibitor Reagents.....	157
4.5.1 Trial Reactions	158
4.5.2 Solution Based Conjugation of Peptides and PEGylated Imidazo[1,2- <i>a</i>]pyrazines	159
4.5.3 Resin-Bound Conjugation of Peptides and PEGylated Imidazo[1,2- <i>a</i>]pyrazines	160
4.6 Compound Evaluation	164
4.6.1 PEGylated-Imidazo[1,2- <i>a</i>]pyrazines	164
4.6.2 Wild-Type Peptides.....	167
4.6.3 Bivalent Inhibitor Reagents	167
4.7 Concluding Remarks to Chapter	167
Chapter 5: Conclusions.....	169
5.1 Future Directions	170
Chapter 6: Experimental	173
6.1 Compound Synthesis	173
6.1.1 General Considerations.....	173
6.1.2 Synthesis of Target Compounds	175

6.1.2.1 Initial Synthetic Approach	175
6.1.2.2 Synthesis of 3-Aryl Imidazo[1,2-a]pyrazines	177
6.1.2.2.1 Formation of Amino Alcohol Moiety via Epoxide.....	177
6.1.2.2.2 Synthesis of α -bromo aryl ketones.....	178
6.1.2.2.3 Synthesis of α -azido aryl ketone.....	181
6.1.2.2.4 Synthesis of α -azido aryl alcohols.....	184
6.1.2.2.5 Synthesis of α -amino aryl alcohols	189
6.1.2.2.6 Synthesis of α -amino aryl ketones	193
6.1.2.2.7 Synthesis of 2-[(3-chloropyrazin-2-yl)amino]-1-(2-aryl)ethanols.....	194
6.1.2.2.8 Synthesis of 2-[(3-chloropyrazin-2-yl)amino]-1-(2-aryl)ethanones	199
6.1.2.2.9 Synthesis of 3-aryl-8-chloro-imidazo[1,2-a]pyrazine.....	204
6.1.2.2.10 Synthesis of 4-methyl-N-(3-Arylimidazo[1,2-a]pyrazin-8-yl)benzenesulfonamides.....	209
6.1.2.2.11 Synthesis of 4-methyl-N-(4-(3-Aryl-imidazo[1,2-a]pyrazin-8-ylamino)phenyl)benzenesulfonamides.....	214
6.1.2.2.12 Alternative Approach to 3-Aryl Imidazo[1,2-a]pyrazines	216
6.1.2.3 Synthesis of 2-Aryl Imidazo[1,2-a]pyrazines	218
6.1.2.3.1 Synthesis of 2-aryl-8-chloro-imidazo[1,2-a]pyrazines	218
6.1.2.3.2 Synthesis of 4-methyl-N-(2-Arylimidazo[1,2-a]pyrazin-8-yl)benzenesulfonamides.....	225
6.1.2.3.3 Synthesis of Synthesis of 4-methyl-N-(4-(2-Aryl-imidazo[1,2-a]pyrazin-8-ylamino)phenyl)benzenesulfonamides.....	231
6.1.2.3.4 Alternative Compounds used during Buchwald-Hartwig Optimisation	235
6.1.3 Synthesis of 2nd Generation Compounds	241
6.1.3.1 Imidazo[1,2-a]pyrazine Compounds	241
6.1.3.1.1 Variations in Position 8.....	241
6.1.3.1.2 Variations with aromatic Group in Position 2.....	255
6.1.3.2 Maybridge Analogues	260
6.1.4 Synthesis PEGylated Imidazo[1,2-a]pyrazines	269
6.1.4.1 Synthesis of the Carbamate Linker	269
6.1.4.2 Synthesis of PEGylated Free Hydroxyl Imidazo[1,2-a]pyrazines	275
6.1.4.2.1 PEGylated-Benzenesulfonamide-bound Imidazo[1,2-a]pyrazines	275
6.1.4.2.2 PEGylated-N-(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2-a]pyrazines	284
6.1.4.3 Synthesis of PEGylated Maleimide Linked Imidazo[1,2-a]pyrazines.....	288
6.1.4.3.1 PEGylated-Benzenesulfonamide-bound Imidazo[1,2-a]pyrazines	288
6.1.4.3.2 PEGylated-N-(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2-a]pyrazines	303
6.1.4.4 Synthesis of PEGylated Allyl and Alkyne Linked Imidazo[1,2-a]pyrazines	307
6.1.4.4.1 PEGylated-Benzenesulfonamide-bound Imidazo[1,2-a]pyrazines	307
6.1.4.4.2 PEGylated-N-(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2-a]pyrazines	313
6.1.4.5 Alternative Synthesis of PEGylated Imidazo[1,2-a]pyrazines	316

6.1.4.5.1 PEGylated-N-(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2- <i>a</i>]pyrazines	316
6.1.5 Synthesis of S-Allyl Cysteine.....	317
6.1.6 Trial Peptide-Small Molecule Conjugation	325
6.2 Peptide Synthesis and PEGylated Imidazo[1,2-<i>a</i>]pyrazine Conjugation	328
6.2.1 General Methods.....	328
6.2.1.1 HPLC Methods	331
6.2.1.1.1 Preparative HPLC.....	331
6.2.1.1.2 Analytical HPLC	333
6.2.2 Synthesis of Non-conjugated Peptides	334
6.2.3 Solution-Based Conjugation	336
6.2.3.1 Solution-Based R240C-Maleimide Conjugation	336
6.2.3.2 Solution-Based R240SAC-Allyl Cross Metathesis.....	338
6.2.4 Resin-Bound Conjugation.....	339
6.2.4.1 Resin-Based R240C-Maleimide Conjugation.....	339
6.2.4.2 Resin-Based R240SAC-Allyl Cross Metathesis	341
6.2.4.3 Resin-Based R240AzLys-Alkyne Click	341
6.3 Biochemical Assay.....	342
6.4 Molecular Modelling.....	344
References	345
Chapter 7: Appendix.....	367
7.1 Dose Response Curves	367
7.2 Characterisation of Peptides and Peptide Conjugates.....	369

List of Abbreviations

Acm - Acetamidomethyl	BINAP - 2,2'-	(<i>N,N</i> -dimethylamino)-
ADP – Adenosine diphosphate	Bis(diphenylphosphino)- 1,1'-binaphthyl	biphenyl
ADT – AutoDock Tools	Boc – <i>tert</i> -	DEAD – Diethyl
AGT - <i>O</i> ⁶ -alkylguanine- DNA alkyltransferase	Butyloxycabonyl	azodicarboxylate
Aib – α -aminoisobutyric acid	Cag - Cytotoxin-associated genes	Dha - dehydroalanine
Ala – Alanine	CagPAI - Cytotoxin- associated genes	DIPEA – <i>N,N</i> - Diisopropylethylamine
ARC - adenosine-arginine conjugates	Pathogenicity island	DMAP -
Arg – Arginine	cAMP – Cyclic adenosine monophosphate	Dimethylaminopyridine
Asn – Asparagine	C.D. - Circular dichroism	DMF –
Asp – Aspartic acid	CI – Chemical ionisation	Dimethylformamide
ATP – Adenosine triphosphate	CLP - <i>O</i> ⁴ -benzyl-2-chloro- 6-aminopyridine	DMSO -
ATPase – Adenosine triphosphate hydrolase	COSY – Correlation spectroscopy	Dimethylsulfoxide
AzLys – Azido lysine	CTD – Carbon terminal domain	DNA – Deoxyribonucleic acid
BG - <i>O</i> ⁶ -benzylguanine	Cys – Cysteine	dppf - 1,1'-Bis(diphenyl- phosphino)ferrocene
	DavePhos - 2- Dicyclohexylphosphino-2'-	EDT – 1,2-Ethanedithiol
		EGFR – Epidermal growth factor receptor
		EI – Electron ionisation
		ES – Electrospray

Eq - Equivalents	HRMS – High resolution mass spectrometry	Met – Methionine
FBDD – Fragment-based drug discovery	HTS – High throughput screening	MIC – Minimum inhibitory constant
Fmoc - 9-Fluorenylmethyl-oxycarbonyl	IC ₅₀ – Half maximal inhibitory concentration	Mpt – Melting point
GABA – γ -Aminobutyric acid	IM – Inner membrane	MSH - <i>O</i> -mesitylene-sulfonylhydroxylamine
Glu – Glutamic acid	IPA - Isopropanol	NMP – <i>N</i> -methyl-2-pyrrolidone
Gln – Glutamine	IR – Infra-Red	NMT - <i>N</i> -myristoyl transferase
Gly – Glycine	JNK - c-Jun N-terminal kinases	NOESY – Nuclear Overhauser effect spectroscopy
HBTU - <i>O</i> -(Benzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate	LCMS – Liquid Chromatography – Mass Spectrometry	MS – Mass spectroscopy
His – Histidine	Lle – Isoleucine	NBS – <i>N</i> -Bromosuccinimide
HIV - Human immunodeficiency virus	Leu – Leucine	NMR – Nuclear Magnetic Resonance
HMBC - Heteronuclear Multiple Bond Coherence	LRMS - Low resolution mass spectrometry	<i>m/z</i> – Mass-to-charge ratio
HSQC - Heteronuclear Single Quantum Coherence	Lys – Lysine	NMM – <i>N</i> -methyl-morpholine
HPLC – High Performance Liquid Chromatography	MALDI – Matrix-assisted laser desorption/ionisation	NTD – Nitrogen Terminal Domain
<i>H. pylori</i> – <i>Helicobacter pylori</i>	<i>m</i> CPBA – <i>meta</i> -Chloroperbenzoic acid	OM – Outer membrane
	MCR – Multicomponent reaction	Su - Succinimide

Pd(dba) ₂ - bis(dibenzylideneacetone)- palladium(0)	Q-Phos - 1,2,3,4,5- pentaphenyl-1'-(di- <i>tert</i> - butylphosphino)ferrocene	Thr – Threonine
Pd ₂ (dba) ₃ - Tris(dibenzylideneacetone) dipalladium (0)	RT – Room Temperature	TIC – Total ion count
PEG – Poly(ethylene) glycol	SAC – <i>S</i> -allyl cysteine	TIPS - Triisopropylsilane
Pet. ether – Petroleum ether	SAR – Structure activity relationship	TLC – Thin Layer Chromatography
Phe – Phenylalanine	sat. aq. - Saturated aqueous	TMS – Trimethyl silyl
PKA – Protein Kinase A	Ser – Serine	Trp – Tryptophan
PKI – Protein Kinase Inhibitor	SPP – Solid phase peptide synthesis	Trt - Trityl
PP - polyproline	TBAF – Tetra- <i>n</i> -butyl ammonium fluoride	Tyr – Tyrosine
Pro – Proline	TBDMS – <i>tert</i> -butyl dimethyl silyl	Val - Valine
PTM – Posttranslational modification	^t Bu-XPhos - 2-Di- <i>tert</i> - butylphosphino-2',4',6'- triisopropylbiphenyl	UV – Ultra-violet
<i>R_f</i> – Retardation Factor	TES – Triethylsilane	WT – Wild type
RNA – Ribonucleic acid	TFA – Trifluoroacetic acid	
	THF – Tetrahydrofuran	

Chapter 1: Introduction

1.1 Biological Background

1.1.1 Helicobacter Pylori

In 1983, Marshall and Warren described the successful isolation and culture of a spiral bacterial species,¹ which later became known as *Helicobacter pylori* (*H. pylori*).² It is the most common bacterial infection amongst humans and is present in approximately half the world's population.³

Infection is acquired in childhood, and once the stomach is colonized, the same organism can persist for decades, and sometimes a lifetime.³ In northern Europe and the United States of America, *H. pylori* is present in 30-50% of adults who acquired the infection at childhood, and it is almost universal amongst adults in developing countries.³ Males in many populations appear to have 20-30% higher rates of infection than females.³ The mode of transmission is unknown, but is believed to be through fecal-oral or gastric-oral spread or improperly cleaned endoscopic equipment.^{3,5}

An example of gram negative bacterial found in the human stomach, they cause illnesses such as gastric ulcers, gastritis and various cancers including mucosa-associated lymphoid tissue (MALT)-lymphoma and gastric adenocarcinoma.^{4,5} *H. pylori*-positive patients have a 10-20% lifetime risk of developing ulcer disease and a 1-2% risk of developing gastric cancer,² and as such the bacteria have been classified as a category 1 carcinogen.⁶

H. pylori possess 2 to 6 flagella to propel itself through the mucus lining. They adopt an S-shaped or curved rod, are 2.4-4.0 µm long and 0.5-1.0 µm wide and contain a urease enzyme in order to create an alkaline environment meaning they are able to survive in the acidic conditions of the stomach.⁵

H. pylori can be grouped into two classes:^{7,8} the more virulent type I strains which contain the cytotoxin-associated genes pathogenicity island (*cagPAI*), and are referred to as CagA⁺ strains; and type II strains which lack this segment and are therefore less virulent.

The *cag*PAI consists of 31 genes, the majority of which code for the Type IV secretion system (T4SS),^{2,7} which is responsible for penetrating the gastric epithelial cells and facilitating the translocation of toxic bacterial factors into host cells:

- The highly immunogenic protein CagA which is encoded by the *cagA* gene,⁷ present in approximately 50-70% of *H.pylori* strains;²
- Peptidoglycan, which is recognised by the intracellular receptor Nod1,⁹ which in turn promotes inflammation, possibly by induction of proinflammatory cytokines such as IL-8,¹⁰
- The vacuolating cytotoxin (VacA), encoded by the *vacA* gene, and induces massive vacuolisation in epithelial cells *in vitro*¹¹ and plays an important role in the pathogenesis of peptide ulceration and gastric cancer.^{12,13}

Attachment of *H. pylori* induces cell spreading and elongation in the host (“Hummingbird Phenotype”),¹⁴ and is accompanied by cytoskeletal rearrangements as a result of CagA phosphorylation.^{7,15,16} CagA becomes tyrosine phosphorylated, at EPIYA motifs within the C-terminus, by multiple members of the Src family of tyrosine kinases.¹⁵ The resulting CagA^{P-Tyr} inactivates c-Src. This is an example of a classical negative feedback loop and could explain how the level of accumulated CagA^{P-Tyr} within the host is regulated.¹⁵

c-Src inactivity leads to the dephosphorylation of host proteins such as cortactin,¹⁵ which in turn enhances actin cross-linking and induces the characteristic rearrangement of the actin cytoskeleton. Another protein within the host that becomes dephosphorylated is ezrin, which could result in the development and metastasis of *H. pylori* induced gastric cancer.⁷ CagA^{P-Tyr} also specifically binds and activates SHP2, the first phosphatase found to act as a human oncogene.¹⁷ Deregulation of SHP2 by CagA is an important mechanism by which *H. pylori* promotes gastric carcinogenesis.

The treatment of *H. pylori* requires concurrent administration of two or more antimicrobial drugs.¹⁸ None of the drug treatments eradicate the infection in 100% of patients and many have undesired side effects. “Triple therapy” has been considered the standard eradication method and consists of a proton pump inhibitor (PPI), notably Omeprazole, and a choice of two antibiotics, traditionally clarithromycin, tetracycline, amoxicillin or metronidazole.^{19,20} Bismuth compounds can also be used in “quadruple therapy”.^{18,19,20}

1.1.2 Secretion Systems

Gram-negative bacteria possess seven secretion systems to mediate the transport of a variety of substrates across their cell membranes.²¹ They are classified as type I (T1SS) to type VI (T6SS) secretion systems and the chaperone-usher (CU) system. They utilize one of two secretion mechanisms:²¹

- One-step, Sec-independent mechanism: T1SS, T3SS, T4SS, T6SS transport substrates directly from the bacterial cytoplasm to the extracellular medium or into the eukaryotic cell;
- Two-step, Sec-dependent mechanism: T2SS, T5SS and CU systems lack an inner membrane transporter and so substrates are first translocated across the inner membrane *via* the general secretory pathway (GSP) (Sec translocation) or the Tat pathway as used by some T2SS substrates. Once in the periplasm the substrates are targeted to one of the secretion systems that mediate transport across the outer membrane.

Table 1 illustrates different bacterial examples of each of the secretion systems together with a brief description of their structures.

1.1.3 Type IV Secretion Systems

As previously mentioned, *H.pylori* utilises the T4SS to translocate toxic molecules into host epithelial cells. These secretion machineries are also found in gram positive bacteria and can be broadly divided into 3 functional types (Figure 1).^{26,27,28,22}

- Conjugation systems: transfer of plasmid DNA from one cell to the other (cell-to-cell contact dependent), helping microorganisms to adapt. It is a major mechanism in the spread of antibiotic resistance;
- DNA uptake and release systems: translocation of DNA to or from the extracellular milieu;
- Effector translocation systems: play a major role in virulence in establishing pathogen-host interactions and/or transferring toxic effector proteins or protein complexes into eukaryotic target cells.

Secretion System	Brief Description	Bacterial Examples
T1SS ^{21,23}	Three components: an inner membrane ABC protein, an outer membrane protein and a membrane fusion protein	Esherichia coli Erwinia chrysanthemi Pasteurella haemolytica Bordetella pertussis
T2SS ²⁴	Spans both membranes but requires the Sec or Tat pathways for transporting across the inner membrane	Esherichia coli Vibrio cholera Pseudomonas aeruginosa Klebsiella oxytoca Neisseria gonorrhea
T3SS ²⁵	Large supramolecular structures spanning both membranes	Salmonella typhimurium Shigella flexneri Chlamydia psittaci Yersinia pestis
T4SS ^{26,27,28}	Consists of at least 12 proteins and are ubiquitous in bacterial pathogens	Helicobacter pylori Legionella pneumophila Bordetella pertussis Agrobacterium tumefaciens
T5SS ²⁹	Auto-transporters assembled only in the outer membrane	Esherichia coli Brucella abortus Bartonella species Pseudomonas species
T6SS ³⁰	Recently discovered and still largely uncharacterised	Rhizobium leguminosarum Vibrio cholera Pseudomonas aeruginosa
CU system ³¹	Simple system assembled in the outer membrane and is responsible for the assembly and secretion of virulence factors	Esherichia coli Salmonella enteric Yersinia species Klebsiella pneumoniae Pseudomonas aeruginosa

Table 1: Table giving brief descriptions and bacteria examples of each of the seven secretion systems

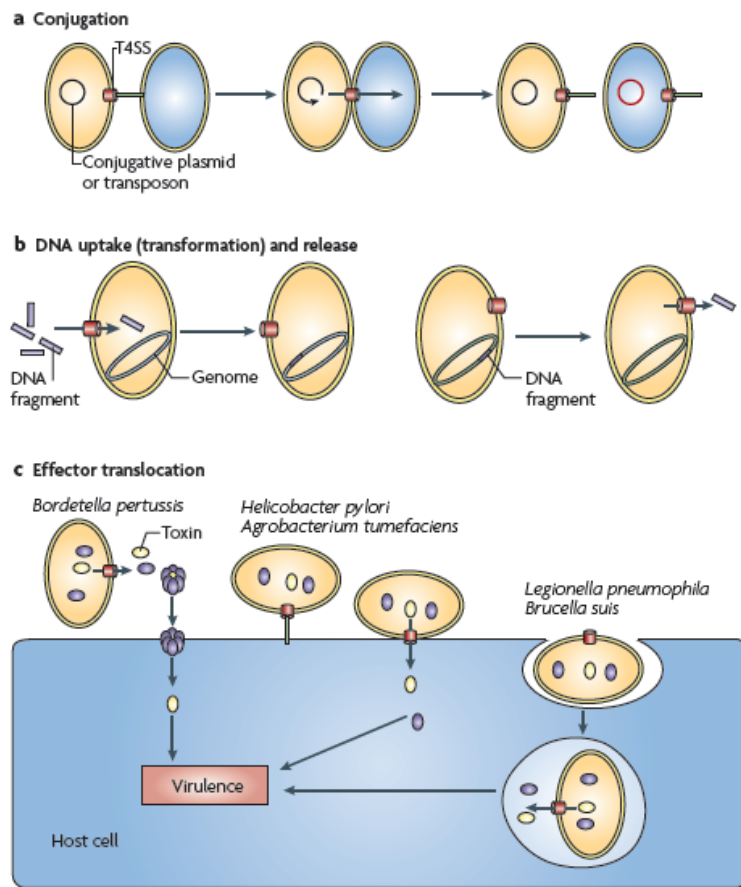


Figure 1: Schematic of the three different groups of T4SS in bacteria.²⁷ Reproduced with permission.

A single bacterial genome can code multiple T4SS, for example, in *H. pylori* the effector protein delivery system (encoded by *cagPAI*) coexists with a DNA release and uptake system (encoded by the *comb* gene cluster).²⁷

All bacterial T4SS are evolutionary related, and although variations exist, many are similar to the *Agrobacterium tumefaciens* (*A. tumefaciens*) VirB-VirD system. This system serves as a prototype of T4SS.^{26,27,22}

The VirB-VirD system comprises 12 proteins (Figure 2, a), VirB1-11 and VirD4. They can be grouped according to their function and/or cellular location:^{26,27,28,22,32}

- Cytoplasmic ATPases: VirB4, VirB11 and VirD4:
- Translocation pore complex proteins: VirB6-10:
- Pilus and other surface structure proteins: VirB2 and VirB5.

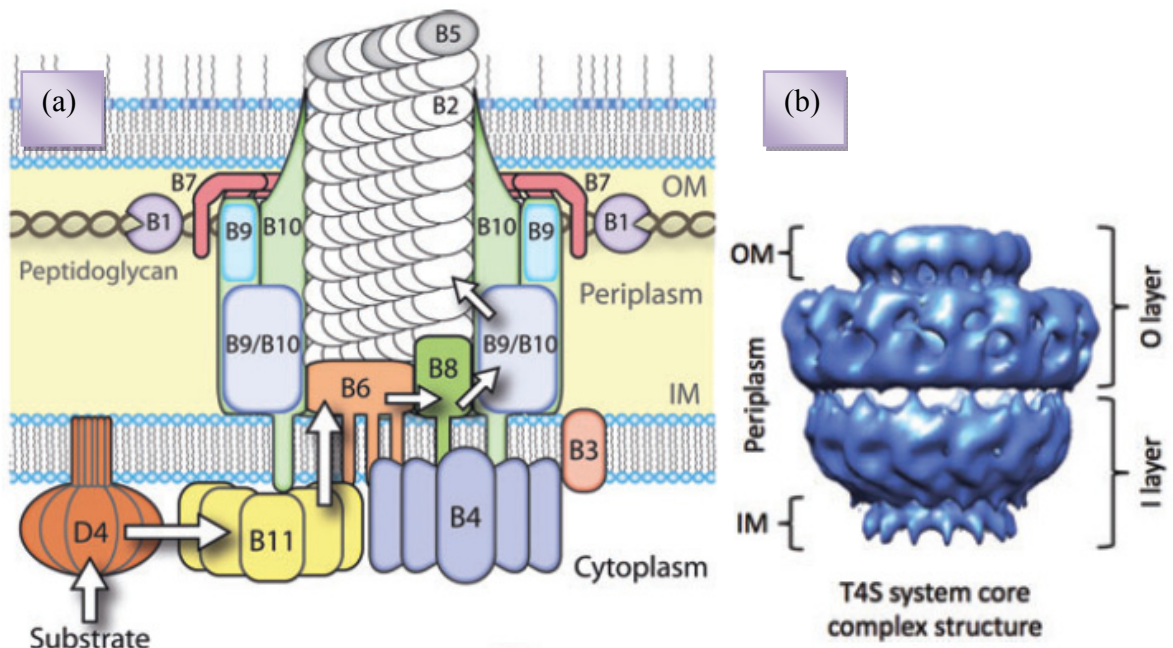


Figure 2: (a) Schematic model of the VirB-VirD system of *A. tumefaciens*, arrows indicate steps in the translocation sequence; (b) T4SS translocation pore complex.²⁶ Reproduced with permission.

The three cytoplasm or inner-membrane (IM) associated ATPases exhibit the highest sequence conservation among VirB-VirD complexes. ATPases are enzymes which catalyse the desphosphorylation reaction of ATP to ADP and a free phosphate (P_i). The reaction releases energy which the enzyme can use to drive other chemical reactions.

In the T4SS, the VirB4 protein is the least characterised and to date there is no crystal structure. It is thought to be a homodimer protein which could represent a building block for a more likely hexameric structure.^{26,27,28} The VirB11 protein belongs to a family of “traffic ATPases” and is also found in T2SS, T3SS and T6SS.^{26,27,28} They are peripheral IM proteins, form hexameric ring structures and they are proposed to act as portals in the transfer of toxic molecules (see Section 1.1.4).^{26,27,28}

The VirD4 protein forms a hexameric globular ring and is a substrate receptor, also known as a coupling protein in DNA transfer systems.^{26,27,28} They interact directly with T4SS substrates and mediate their transfer to the specific subunits of the secretion machinery.

The translocation pore complex (Figure 2, b) consists of 14 copies of the VirB7, VirB9 and VirB10 proteins, forming a cylindrical structure with a diameter and length of 185 Å. It

comprises two layers: I-layer and O-layer, which are double walled, ring-like structures with a hollow chamber.^{26,27,28,33}

The I-layer consists of the NTDs of the VirB9 and VirB10 proteins, which are anchored into the IM, with a 55 Å diameter hole at the base. However, the VirB6 and VirB8 proteins are more likely to form this pore as they come into direct contact with the substrate during secretion.

The O-layer consists of a main body with 10 Å diameter cap on the outermost side of the complex, inserted into the outer-membrane (OM). It comprises the VirB7 protein and the CTDs of the VirB9 and VirB10 proteins.³⁴

There is evidence suggesting that the VirB1 and VirB3 proteins are not part of the secretion apparatus, but contribute to the formation of the secretion machinery.²⁷ The VirB1 is a periplasmic protein consisting of lytic transglycosylases which make holes in the peptidoglycan cell wall to allow assembly of surface structures. The VirB3 is a small protein, and although its location remains unclear, it has been shown to be stabilised by the VirB4 protein.²⁷

The VirB2 and VirB5 proteins form pilus structures extending from the extracellular surface.²⁷ The VirB2 protein forms the major component and is essential for substrate transfer, by stabilising the donor-host contact and forming a channel for passage of substrates. The VirB5 protein is the minor component and is thought to function as an adhesin and is therefore located at the tip of the pilus.

As an example the well characterised F-pilus (encoded by *E. coli* F-plasmid),²⁷ has been shown to contain DNA within the tip, and thus indicating its importance in secretion. It has an outer diameter of 8-10 nm, length of 2-20 µm and channel diameter of 2 nm. CagA has also been found in the tip of the T4SS pili of *H. pylori*.²⁷

1.1.4 VirB11 ATPase: HP0525

The VirB11 ATPase is a vital component of the T4SS and as such there has been much interest in the structure and function of the protein. There have been extensive studies into the crystal structures of the *H.pylori* VirB11 homolog HP0525 bound to both ADP³⁵ and the non-hydrolysable ATP γ S³⁶ as well as the nucleotide-free, *apo*-form.³⁶

1.1.4.1 HP0525 Monomer³⁵

The HP0525 monomer (Figure 3) consists of two domains: the nitrogen terminal domain (NTD) from residues 6 to 136, and the carbon terminal domain (CTD) from residues 137 to 328, linked *via* a short loop between residues 134 and 141. The NTD has six β strands, with the central sheet packed against two α -helices on one side only, whereas the CTD has seven β strands, with the central β sheet flanked by three α -helices on one side, and four on the other.

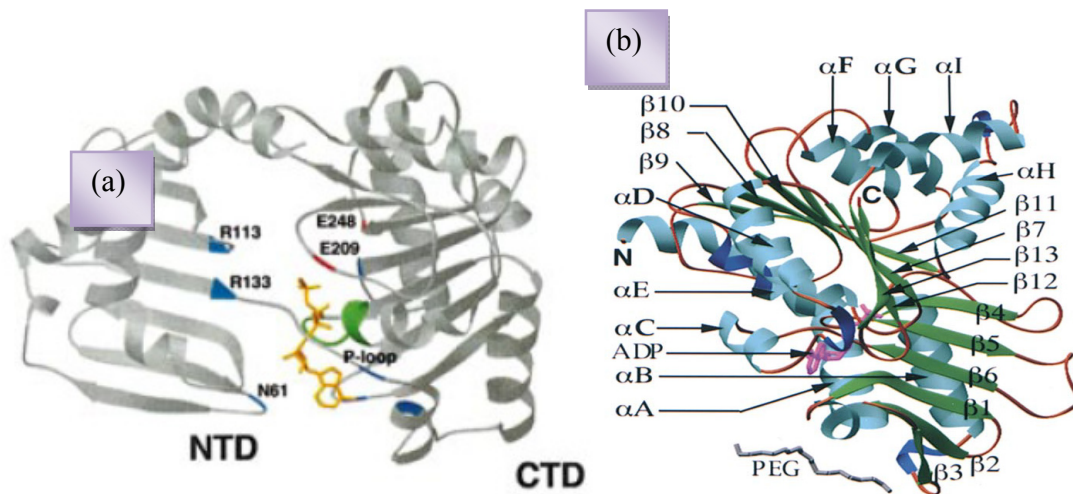


Figure 3: Crystal structures of HP0525 monomer³⁶ indicating the two domains (a) and the various helices and strands (b). Reproduced with permission.

1.1.4.2 HP0525 Hexamer³⁵

HP0525 forms a hexameric ring (Figure 4) where each of the subunit monomers assemble in an intertwined propeller shape with residues in both domains of each subunit participating in subunit-subunit interactions. In fact, each of the NTDs and CTDs form two separate ring

structures with the CTD ring forming a 6-clawed grapple mounted on the hexameric ring formed by the NTD creating a cylindrical chamber, with a volume of approximately 60,000Å³.

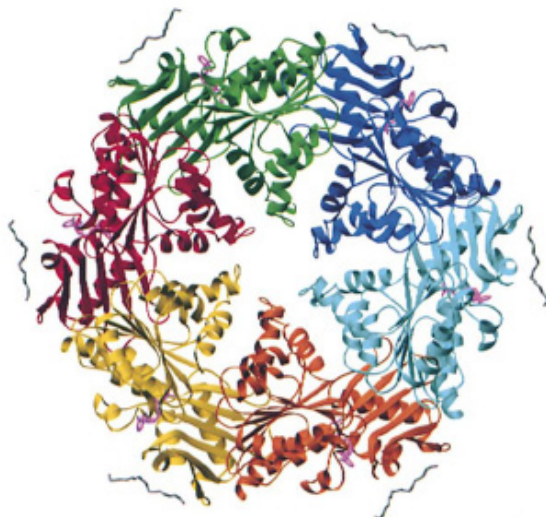


Figure 4: Illustration of the hexameric structure of HP0525.³⁵ Reproduced with permission.

A 9-mer of poly(ethylene)glycol (PEG) is found within the NTD indicating that this domain might be embedded into the IM. This hydrophobic nature continues when examining the outside surface of the remaining NTD and part of the CTD, but the tip of the CTD grapple shows strong negative electrostatic potential in the region facing the bacterial cell cytoplasm.

The structures of ADP- and ATP γ S-HP0525 are virtually identical; however, *apo*-HP0525 exists as an asymmetric hexamer, very different to that of the nucleotide bound forms, where the NTDs exhibit rigid-body rotations (between 2° and 15°) about the linker region and away from the centre of the chamber. This indicates that nucleotide binding and not hydrolysis is responsible for ATP-induced conformational changes.^{35,36}

There are two different types of subunit (A and B) which alternate around the hexameric ring. This is evident from the observation of varying rotations of the NTD, as well as ATP γ S being present in full occupancy in only one of the two molecules in the crystal structure dimer.³⁶

1.1.4.3 Nucleotide Binding Site³⁵

The nucleotide binding site of HP0525 is located between the NTD and CTD (Figure 3 a) with residues from both domains contributing to the binding site. From the NTD, Thr46 and Asn61 make interactions with the ribose and Arg133 and Arg136 make interactions with the phosphates. In ATP γ S-HP0525, the Arg113 undergoes a major conformational change to interact with the γ -phosphate (in ADP-HP0525 the residue is 7.5 Å away from the β -phosphate and therefore makes no interactions).³⁶ Figure 5 illustrates some of the key interactions made between the ATP γ S and the protein.

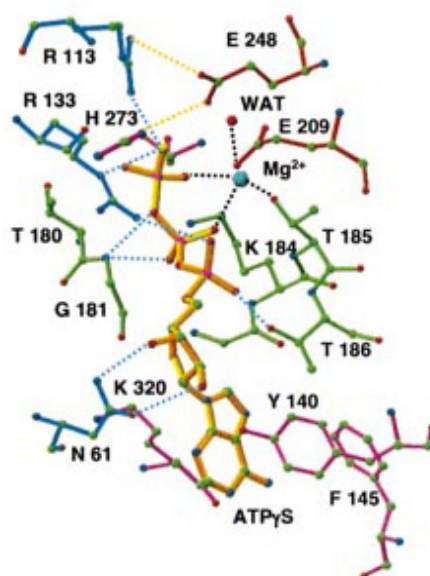


Figure 5: Key residues in HP0525 that interact with ATP γ S.³⁶ Reproduced with permission.

In the CTD, the major interactions with the phosphate are made by the “P-loop” which is located between the β 7 strand and the α E helix. Tyr140, Phe145 and Lys320 also interact with the adenine. In addition, Glu209 makes strong hydrogen-bonding interactions to the H₂O ligated to Mg²⁺ and is proposed to have a stabilising role, with Glu248 acting as a H₂O activator.

1.1.4.4 Subunit-Subunit Interface

In order to form a hexameric structure, each subunit interacts with two flanking subunits giving rise to subunit-subunit interactions with the interface area totalling 2260 Å². There are three major contacts (Figure 6).³⁵

1. NTD-NTD, with an area of 463 Å²;
2. NTD-CTD, with an area of 1372 Å²;
3. CTD-CTD, with an area of 425 Å².

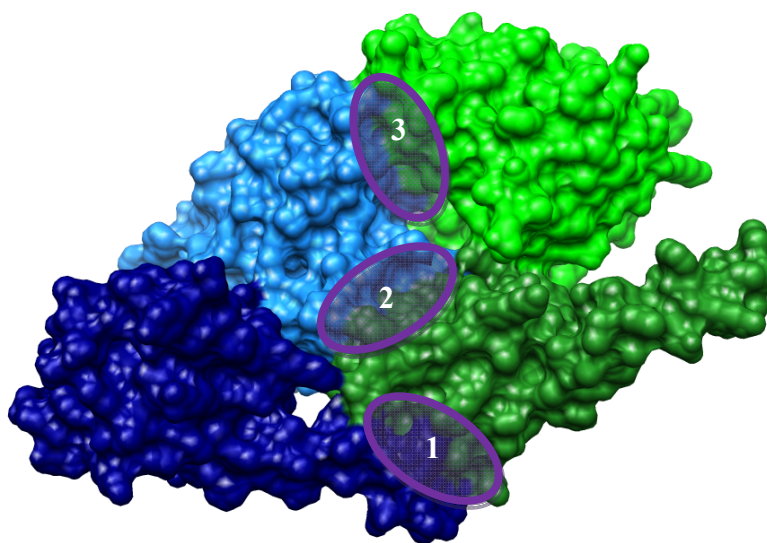


Figure 6: Illustration of the three major contacts in subunit-subunit interface. Blue: Subunit A (Dark blue = NTD, light blue = CTD), Green: Subunit B (Dark green = NTD, light green = CTD); 1: NTD-NTD interactions, 2: NTD-CTD interactions, 3: CTD-CTD interactions. Image generated using Chimera³⁷

The NTD-NTD interface comprises interactions between residues of the αB helix of one subunit and residues of the β5-β6 loop. This includes two salt bridges between Arg18 with Asp125 and Glu126, and Glu9 with Lys74; as well as hydrophobic contacts of Phe13 and Leu14 with Leu78.³⁵

NTD-CTD interactions make up the majority of subunit-subunit interfaces. Most of the solvent accessible surfaces of the β-sheet of the NTD makes contact with residues of the β9 sheet, αF helix, αF-β10 loop and αE-β8 loop of the CTD.³⁵

There is also a cluster of acidic and basic residues within the NTD-CTD interface that plays a pivotal role in maintaining the hexameric structure of HP0525. Glu47 from the NTD makes ionic interactions with Arg238 from the adjacent α F- β 10 loop in the CTD; however Arg113 and Arg133 from the NTD and Arg240 from the α F- β 10 loop in the CTD would clash electrostatically if it were not for the presence of a nucleotide neutralising these like-charges. In the absence of nucleotide these clashes would have a destabilizing effect on the interface, causing the NTD to be released and swivel into an open conformation.³⁵

In further studies, the different arginines in question were mutated to glutamic acid (Arg113Glu, Arg133Glu, Arg240Glu and Arg113Glu/Arg133Glu double mutant) in order to provide favourable interactions between glutamic acid and arginine. In doing so, this locks the hexamer into a closed conformation and as a result, a dramatic decrease in ATPase activity was observed. In addition the fact that the *apo* form exhibits asymmetry and structural variability among the NTD, support the prediction that in the absence of nucleotide there is little to hold the NTD and CTD together.³⁵

Interactions between the claws of the grapple make up the CTD-CTD interface, possibly helping to maintain the grapple in the close conformation.³⁵

1.1.4.5 HP0525 Mechanism of Action

The various observations made from the different crystal structures of HP0525 prompted a mechanism for the mode of action of VirB11 ATPase to be proposed (Figure 7). This consists of the following steps:³⁶

1. Asymmetric hexamer of the nucleotide-free form with the NTD (pale pink) exhibiting mobility and the CTD (pale blue) maintaining the pseudo-scaffold. This flexibility is likely to mean effective binding of target molecules;
2. Binding of three ATP molecules locks three of the subunits into a rigid conformation;
3. Hydrolysis of these three ATPs to ADP together with binding of a further three ATP molecules to the remaining subunits results in a perfectly hexameric rigid form;
4. The structure retains its symmetry and rigidity until all ATPs are hydrolysed, at which point HP0525 reverts to its nucleotide-free asymmetric form.

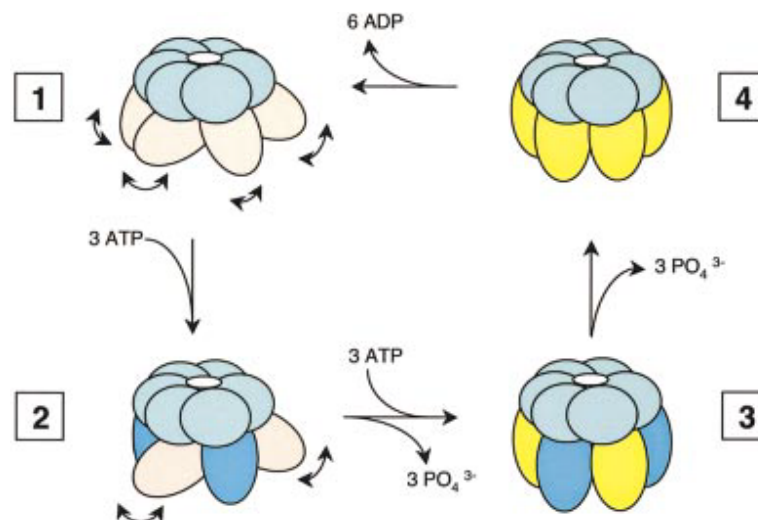


Figure 7: Schematic representation for the mode of action of VirB11 ATPases. NTD (light pink), CTD (light blue). ATP binding (Blue) and ADP (Yellow).³⁶ Reproduced with permission.

In light of the structural evidence and postulated mechanisms highlighted, VirB11 is viewed as a viable target in the search for antimicrobial agents.

1.1.4.6 HP1451: Regulatory Protein of HP0525³⁸

Studies have shown that HP0525 interacts with a protein known as HP1451,³⁸ with the crystal structure suggesting it functions as a regulator of HP0525 activity. Two molecules of HP1451 are positioned opposite each other, interacting with the NTDs of two adjacent monomers of HP0525 and weakly with a third. There are two sites of interaction, which locks the HP0525 NTDs into a closed state. Testing the ATPase activity shows the hydrolysis drops to 30% of the native value indicating that four of the six subunits are being inhibited. It was postulated that HP1451 has the role of modulating HP0525 ATPase activity to regulate Cag-mediated secretion.³⁸

1.1.4.7 Current Inhibitors of HP0525

A previous group has carried out a high throughput screen (HTS) of a diverse chemical library, from Chiron Corporation, of 524,400 compounds to assess ATPase activity of Cag α , a VirB11 homologue of *cag*.³⁹ Using BIOMOL green reagent and measuring absorbance at inhibitor concentrations of 1-5 μ M, 675 compounds were shown to inhibit the enzyme activity by 50% or more. Further studies revealed three compounds with IC₅₀ values of less than 1 μ M (Figure 8).

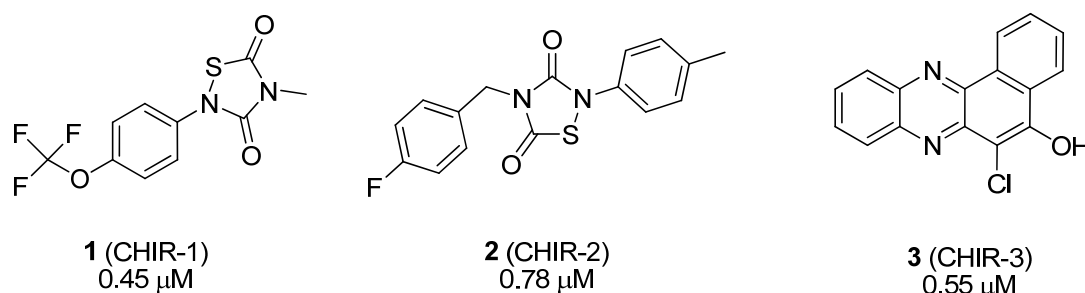


Figure 8: Small molecule inhibitors of *H. pylori* CagA as determined by a HTS of 524,000 Chiron Corporation compounds.³⁹

Chir-1, **1**, the compound with the lowest IC₅₀ was then used in further testing to assess its inhibition properties and the following results were observed:³⁹

- Chir-1 reduced the number of *H. pylori* infected AGS cells showing hummingbird phenotype, with an MIC of 90 μ M (hummingbird phenotype is an indicator of the *H. pylori* T4SS functionality);
- Chir-1 inhibits the secretion of CagA into AGS cells, observed by a 3-4-fold decrease in fluorescence intensity of CagA in AGS cells, and 3-fold increase within the bacterial cell, compared to untreated controls;
- By investigating the amount of phosphorylated CagA (CagA^{P-Tyr}) as a readout for CagA translocation, it was observed that 50 μ M of Chir-1 is sufficient to inhibit translocation of CagA into AGS cells to limits of detection, with a period of pre-incubation (<3 min) required. Experiments with no pre-incubation of *H. pylori* cells and inhibitor show that CagA reaches the AGS cells;

- The amount of IL-8 produced by AGS cells was inhibited in a concentration dependent manner: 50 μ M Chir-1 resulted in a decrease of 19.8% which was further increased to 36.6% with a 15 min incubation period;
- Initial *in vivo* experiments showed that increasing concentrations of Chir-1 resulted in decreased colonisation of *H. pylori* in mice, with a concentration of 50 μ M of Chir-1 reaching a significant level of reduction compared with the control group.

The results of the CagA^{P-Tyr} experiments indicate that Chir-1 interferes with the early steps of the T4SS mechanism, but not with the translocation step, and therefore the inhibitor is only effective for a short time period. The data also confirms HP0525 as a viable target for the inhibition of the *H. pylori* secretion system.

1.2 Imidazo[1,2-*a*]pyrazines

1.2.1 Target Compounds

Using AutoDock 4.0, previous work within the group screened, *in silico*, a drug-like fragment database from the ZINC collection⁴⁰ against the structure of ATP _{γ} S-HP0525 (1NLY – Chain A, ligand removed prior to docking).^a High binding affinity fragments were identified and were used to search full molecules from ZINC using substructure similarity. This created a smaller ligand database, which was then re-screened against 1NLY and a number of hit compounds were generated that were proposed to bind within the nucleotide binding site as putative ATP inhibitors. Within this selection were compounds with an identical heterocyclic core, and therefore these structures were suggested for synthesis (Figure 9).

^a Work Carried out by Dr F. Buelens & Dr T. Boyle – unpublished data, not shown

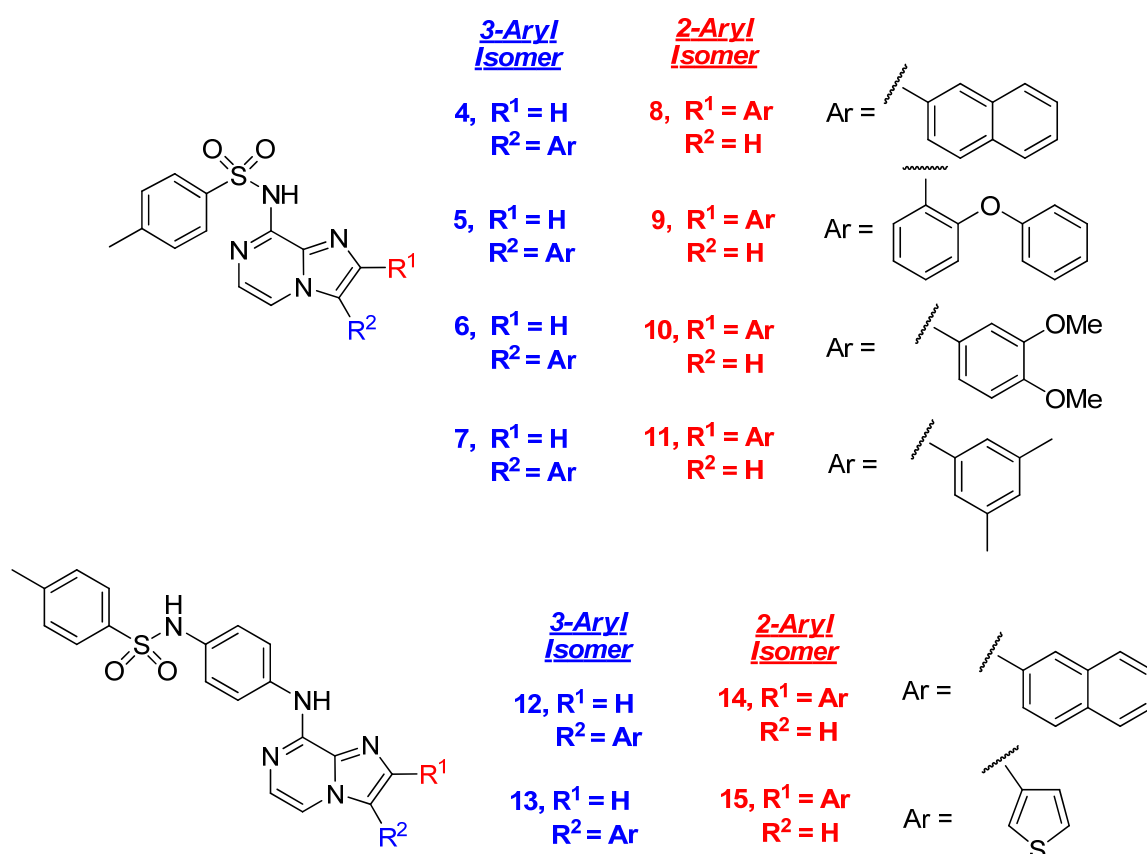


Figure 9: Target compounds identified from previous *in silico* screening carried out within the group

Each of the target compounds have an identical imidazo[1,2-*a*]pyrazine core, with either a 4-toluenesulfonamide or *N*-(4-aminophenyl)-4-methylbenzenesulfonamide moiety in the 8-position and an aromatic group in either the 2- or 3-positions. Synthesis of these compounds (Chapter 2) would give a small library of potential ATPase inhibitors investigating two different regioisomers.

1.2.2 Synthesis, Reactivity and Therapeutic Applications of Imidazo[1,2-*a*]pyrazines

Whilst imidazo[1,2-*a*]pyrazines are a well known class of heteroaromatic compound, their use within the literature remains relatively sparse. To give an indication, running a search using SciFinder and removing duplicates and conference abstracts, 184 hits were returned, consisting 93 patents and 91 journals.⁴¹ Figure 10 shows a graphical representation, illustrating that interest in imidazo[1,2-*a*]pyrazines has increased in recent years.

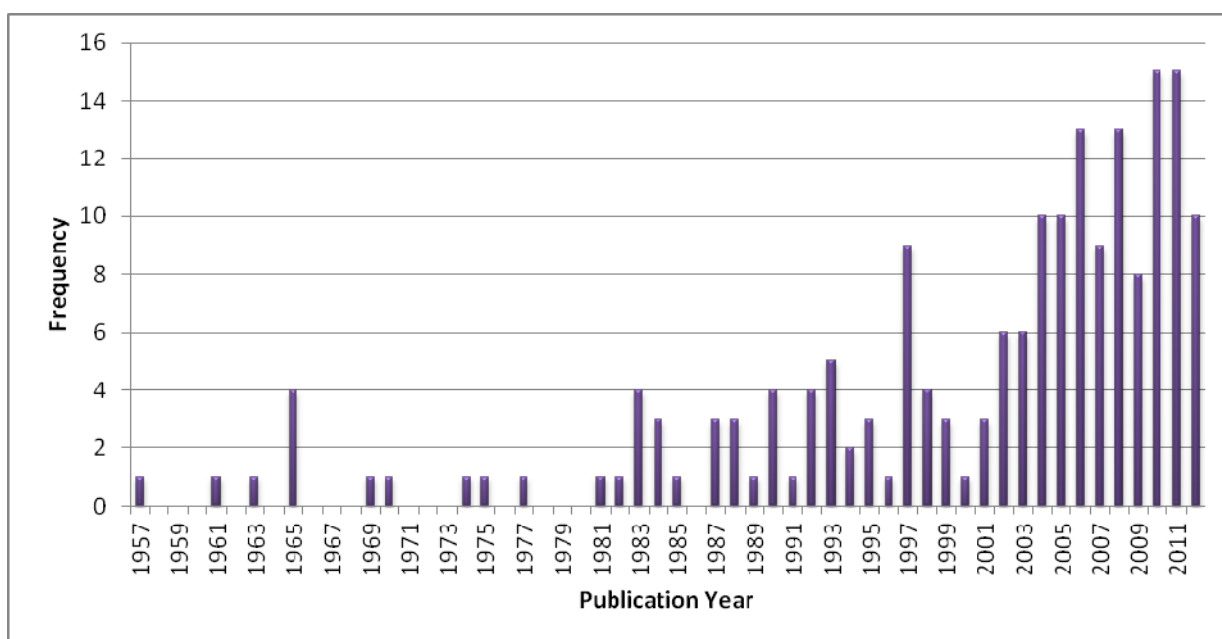


Figure 10: Graphical representation of number of imidazo[1,2-*a*]pyrazine publications (patents and journals) per year using SciFinder⁴¹

The naming and numbering of these bicyclic systems, as established by von Baeyer,⁴² is illustrated in Figure 11.⁴³

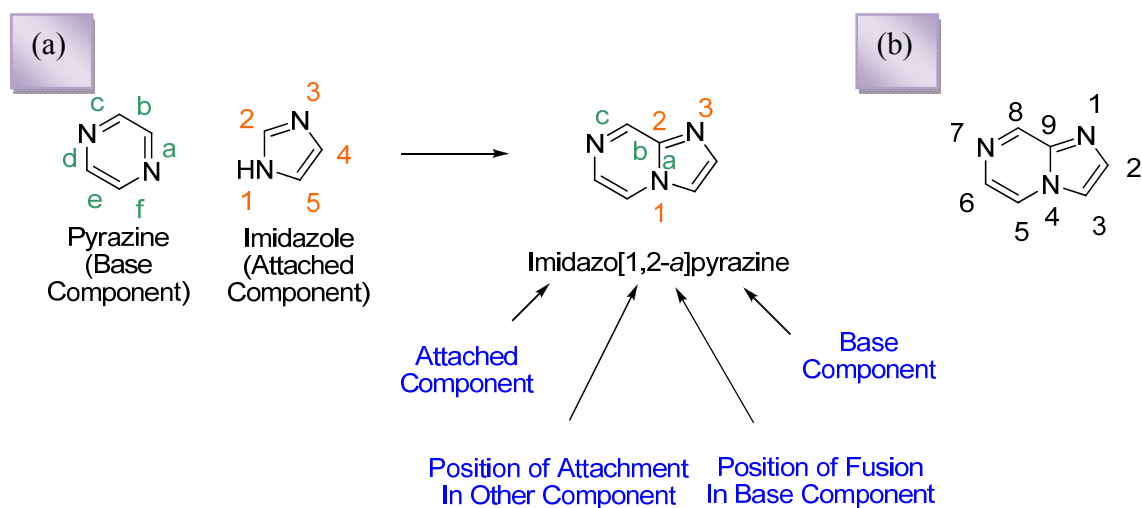


Figure 11: Naming structure of imidazo[1,2-*a*]pyrazines (a) and the proposed numbering of each of the positions (b)^{42,43}

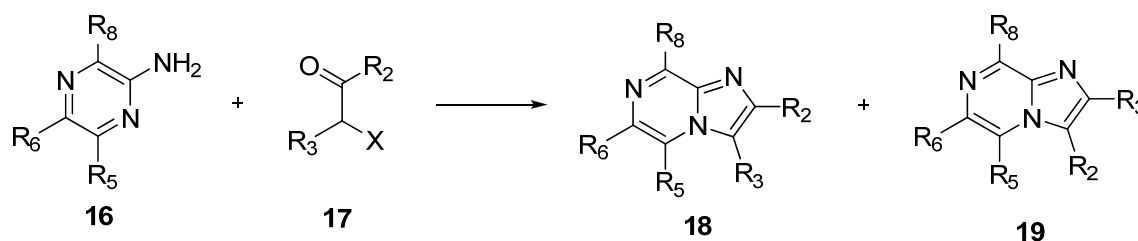
The discovery of natural products containing the imidazo[1,2-*a*]pyrazine core, such as luciferins, has highlighted the potential therapeutic applications of this class of compound.^{44,45} The main motivation for the development of imidazo[1,2-*a*]pyrazines in recent years is that they are good ATP mimics and possess anti-cancer properties (see Section 1.2.2.3.1). This section explores the synthesis and reactivity of imidazo[1,2-*a*]pyrazines and their therapeutic applications.

1.2.2.1 Synthesis of Imidazo[1,2-*a*]pyrazines

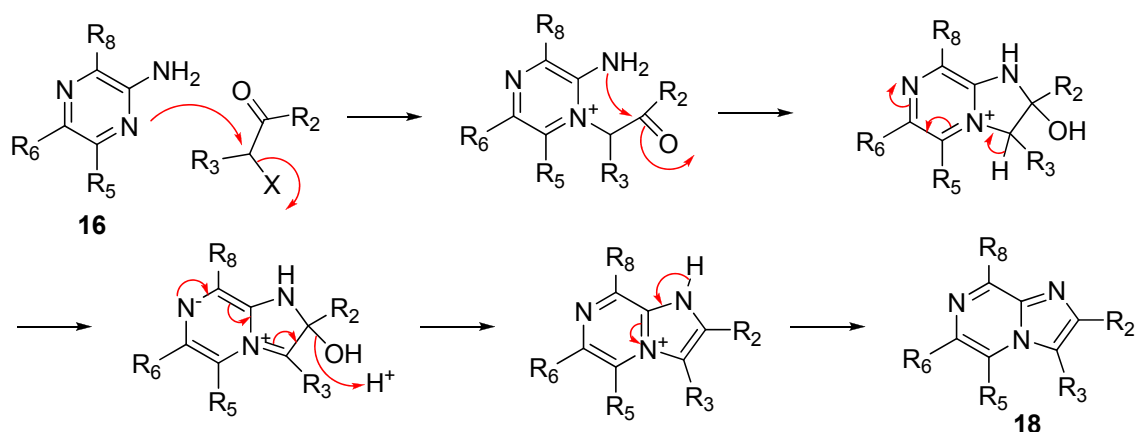
The first instance of imidazo[1,2-*a*]pyrazine synthesis was reported in 1957.^{44,46} Since then, three different approaches to synthesising imidazo[1,2-*a*]pyrazines have been reported: starting from the pyrazine or imidazole ring; simultaneous formation of the two rings; and more recently, multicomponent reactions.

1.2.2.1.1 Imidazo[1,2-*a*]pyrazines from Pyrazine Rings

The traditional method of synthesising imidazo[1,2-*a*]pyrazines involves the condensation between aminopyrazines and α -halocarbonyl compounds (Scheme 1).^{44,47,48} The reaction is initiated by the nucleophilic attack of the aminopyrazine endocyclic nitrogen on the alkyl-halo position, followed by cyclisation between the primary amine functionality and the carbonyl group.⁴⁷ An E1CB mechanism completes the aromatisation (Scheme 2). Kaminski postulated that when unsymmetrical carbonyls are used, different isomers are possible depending on which nitrogen initiates the reaction (Scheme 1).⁴⁸



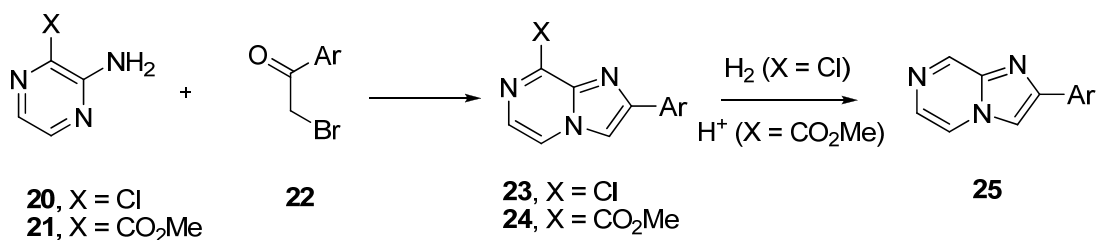
Scheme 1: Synthesis of imidazo[1,2-*a*]pyrazines *via* the condensation of aminopyrazines and α -halocarbonyl compounds



Scheme 2: Mechanism for the condensation reaction between 2-aminopyrazines and α -halocarbonyl compounds

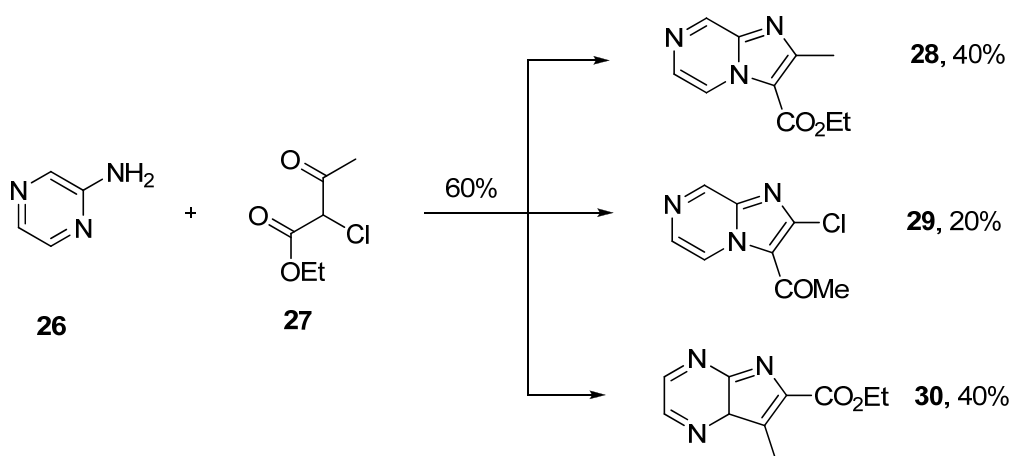
Spitzer *et al*⁴⁹ showed that in the absence of any other functionality in aminopyrazine, the endocyclic nitrogen not adjacent to the primary amine moiety was the most nucleophilic and that alkylation with α -bromoacetophenone did not result in the desired imidazo[1,2-*a*]pyrazine bicyclic system. By introducing a chlorine in the position adjacent to the primary amine moiety (2-amino-3-chloropyrazine, **20**), the nucleophilicity of the N₄ endocyclic nitrogen is reduced, and so the reaction proceeds *via* the N₁ nitrogen, with removal of the chlorine by catalytic hydrogenation (Scheme 3).

Alternatively, the heterocycle can be accessed by using a carboxymethyl derivative in place of the chlorine (**21**), which can then be removed by ester hydrolysis and acid de-carboxylation (Scheme 3).⁴⁹ The group then showed the importance of the nitrogen in position 7 in inotropic activity.⁴⁹



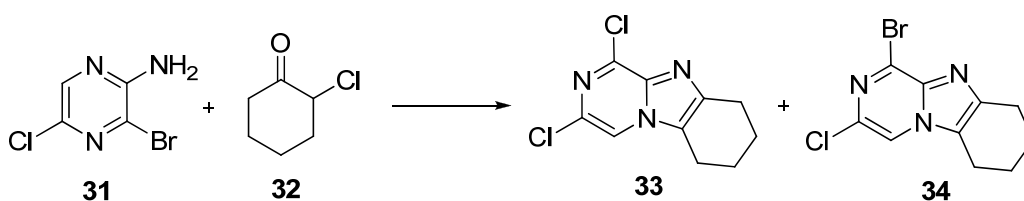
Scheme 3: Synthesis of imidazo[1,2-*a*]pyrazine as illustrated by Spitzer *et al*⁴⁹

Early studies carried out by Sablayrolles *et al*⁴⁷ investigated the reaction between 2-aminopyrazine and ethyl 2-chloroacetoacetate and three competing reactions were observed (Scheme 4). Products **28** and **29** result from the initial standard nucleophilic reaction between the endocyclic nitrogen at the alkyl-chloro position, with product **28** forming *via* cyclisation between the primary amine and the ketone carbonyl. Product **29**, however, forms from cyclisation between the primary amine and the ester carbonyl, followed by rearrangement of the resulting lactam-lactim. Product **30** forms when the primary amine initiates the nucleophilic attack with the cyclisation directed from the *ortho* position of the pyrazine ring.



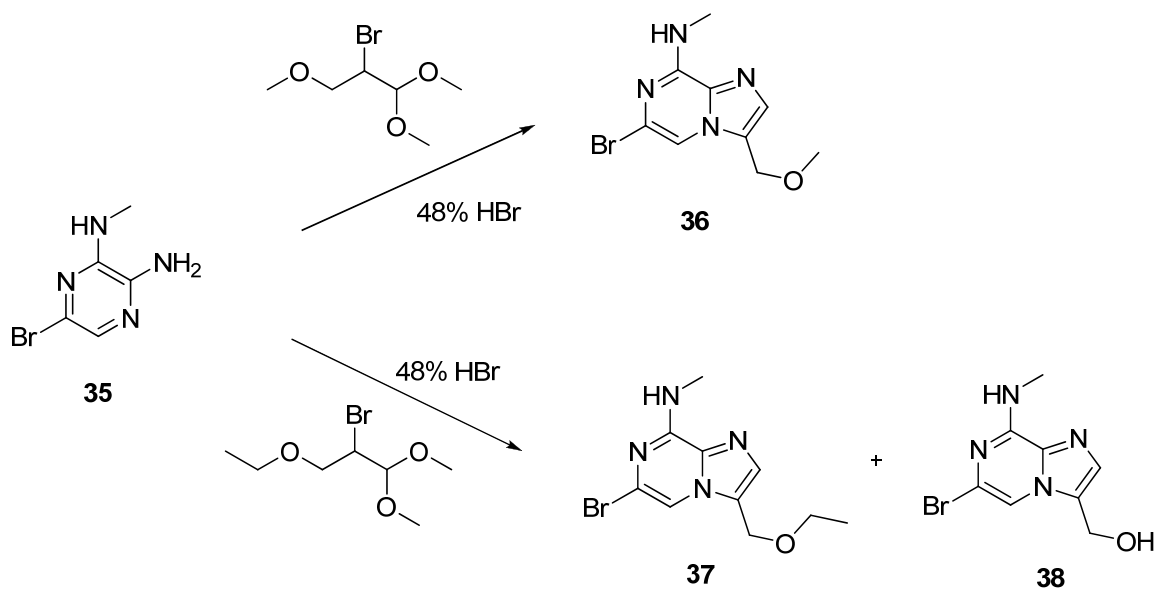
Scheme 4: Competitive reactions between aminopyrazine and ethyl 2-chloroacetoacetate as observed by Sablayrolles⁴⁷

Interestingly, Lumma *et al* reported halogen scrambling products as a result of the condensation between halo-aminopyrazines and halo-ketones as illustrated in Scheme 5.⁵⁰ Many groups have also used 2-amino-3,5-dibromopyrazine (from reacting 2-aminopyrazine and NBS) in the condensation reaction in order to obtain 6,8-dibromoimidazo[1,2-*a*]pyrazine, and thereby introducing functionalities into the ring, which can be further reacted (see Section 1.2.2.2).^{51,52}



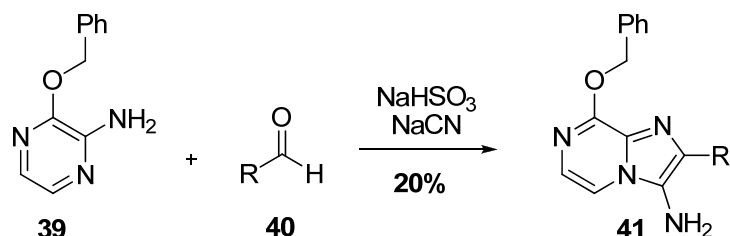
Scheme 5: Condensation between 2-amino-3-bromo-5-chloropyrazine and 2-chlorocyclohexanone illustrating halogen scrambling⁵⁰

In the case of unstable aldehydes, the use of the corresponding acetals has also been reported, whereby acid hydrolysis (heating with HBr, 48% aq.) produces the α -halocarbonyl *in situ*. An early instance of this was carried out by Bonnet *et al*⁵¹ using 2-amino-5-bromo-8-(methylamino)pyrazine, **35**, and two different acetals (Scheme 6). The second acetal used, and more specifically the ether functionality, has a different stability to acidic conditions compared to the first. This gives rise to two different aldehydes and therefore two different condensation products.



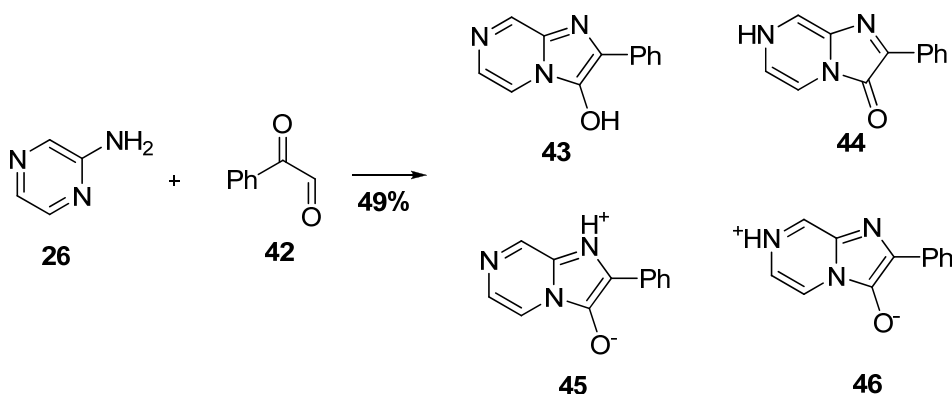
Scheme 6: An example of the use of acetals in the condensation reaction to form substituted imidazo[1,2-*a*]pyrazines⁵¹

Kaminski carried out a condensation reaction between substituted aminopyrazine **39** and the appropriate aldehyde compounds in the presence of sodium bisulfite and sodium cyanide in order to give substituted 3-aminoimidazo[1,2-*a*]pyrazines, **41** (Scheme 7).⁴⁸



Scheme 7: Formation of 3-aminoimidazo[1,2-*a*]pyrazines as demonstrated by Kaminski⁴⁸

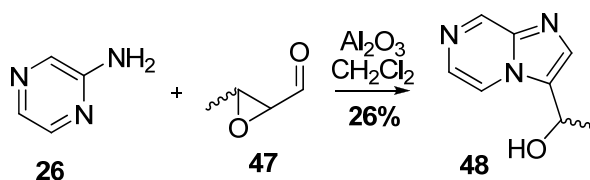
Condensation of 2-aminopyrazine with phenyl glyoxal gives rise to four possible forms of imidazo[1,2-*a*]pyrazine (Scheme 8).⁵³ The dipolar form, **46**, is the most plausible structure owing to the quarternisation at N₇ giving rise to an unusual coupling constant ($J_{6,8}$ 0.5 Hz) observed in the ¹H NMR spectrum.



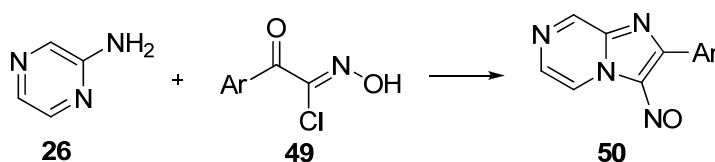
Scheme 8: Reaction of 2-aminopyrazine and phenyl glyoxal⁵³

Some alternative condensation reactions include the reaction of 2-aminopyrazine and 2,3-epoxybutanal in the presence of alumina to synthesise 3-(1-hydroxyethyl)imidazo[1,2-

a]pyrazines (Scheme 9).⁵⁴ In addition, 2-aminopyrazine can be reacted with α -ketohydroximoyl chlorides to form 3-nitrosoimidazo[1,2-*a*]pyrazines (Scheme 10).⁵⁵

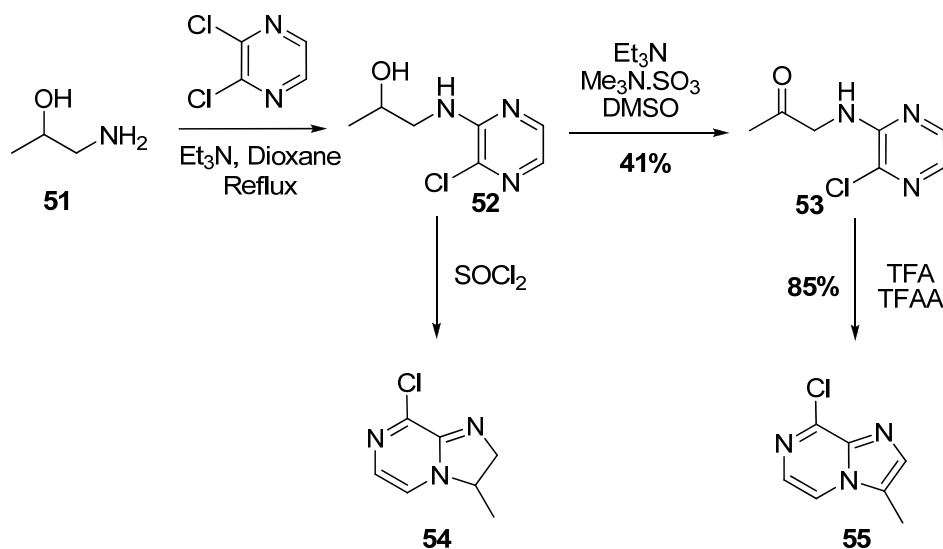


Scheme 9: Synthesis of 3-(1-hydroxyethyl)imidazo[1,2-*a*]pyrazine using 2,3-epoxybutanal⁵⁴



Scheme 10: Use of α -ketohydroximoyl chlorides to form 3-nitrosoimidazo[1,2-*a*]pyrazines⁵⁵

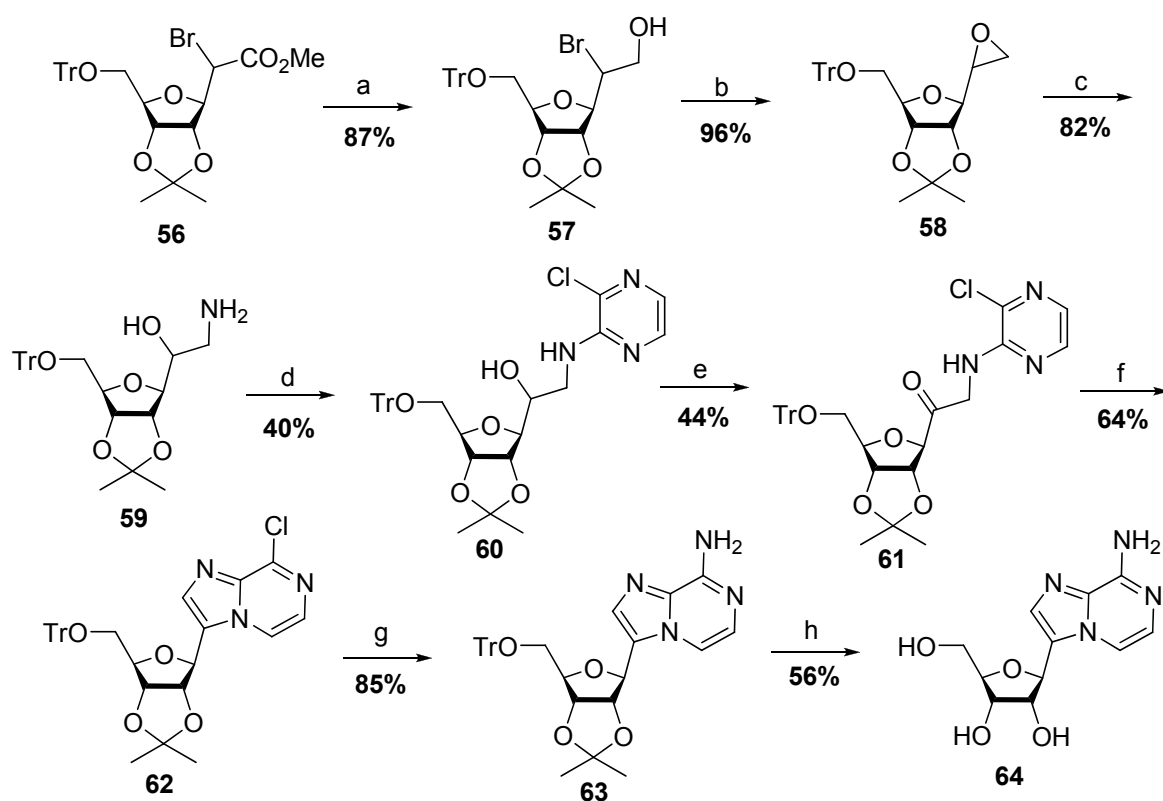
In addition to the traditional condensation reaction, Meurer *et al*⁵⁶ and Lumma *et al*⁵⁰ explored an alternative route to imidazo[1,2-*a*]pyrazines (Scheme 11). This involves the reaction of a vicinal amino alcohol (1-amino-2-propanol) and chloropyrazine followed by oxidation using trimethylamine-sulfur trioxide complex, and dehydration-cyclisation using trifluoroacetic acid and trifluoroacetic anhydride. Also explored was the formation of the di-hydro species *via* reaction of the intermediate amino alcohol with thionyl chloride.



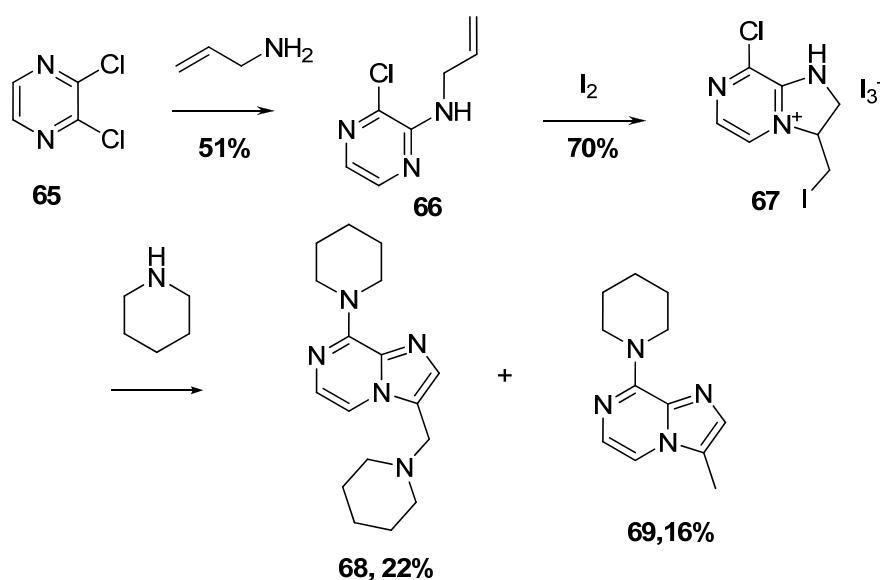
Scheme 11: Regioselective synthesis of imidazo[1,2-*a*]pyrazines and dihydroimidazo[1,2-*a*]pyrazine using vicinal amino alcohols and chloropyrazines as described by Meurer⁵⁶ and Lumma⁵⁰

MacCoss *et al* followed up this work by incorporating ribose moieties into the synthesis to create nucleosides containing 8-aminoimidazo[1,2-*a*]pyrazines (Scheme 12).⁵⁷ Starting with the saccharidic α -bromoester **56** (prepared from D-ribose in four steps), reduction with lithium borohydride was followed by formation of the epoxide. Ring opening of the epoxide afforded the key vicinal amino alcohol. Pyridine was used in the dehydration-cyclisation step due to the acid-labile protecting groups. It is worth noting that the group also attempted this synthesis by subjecting the α -bromoaldehyde of the ribose moiety to condensation with 2-amino-3-chloropyrazine, but no reaction was observed owing to the instability of the aldehyde.

Slepukhin *et al* synthesized 3,8-disubstituted imidazo[1,2-*a*]pyrazines through the cyclisation of 2-allylamino-3-chloropyrazine, **66**, by the action of iodine in diethyl ether (Scheme 13).⁵⁸ The resulting dihydroimidazo[1,2-*a*]pyrazine, **67**, reacts with piperidine, to give piperidine substitution at the 8-position and aromatisation.

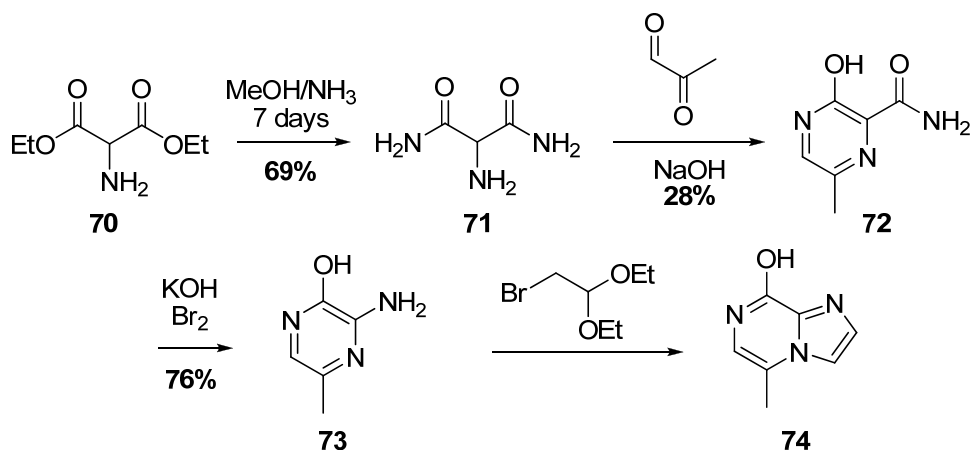


Scheme 12: Synthesis of nucleosides containing 8-aminoimidazo[1,2-*a*]pyrazines.⁵⁷ (a) LiBH₄, THF; (b) NaH, DMF; (c) NH₃, MeOH; (d) Et₃N, Dioxane, 2,3-dichloropyrazine; (e) pyridine/SO₃, DMSO or TFAA, Et₃N, DMSO; (f) TFA, TFAA, pyridine, PhMe; (g) NH₃, IPA; (h) 90% aq. TFA



Scheme 13: Synthesis of imidazo[1,2-*a*]pyrazines as outlined by Slepukhin *et al*⁵⁸

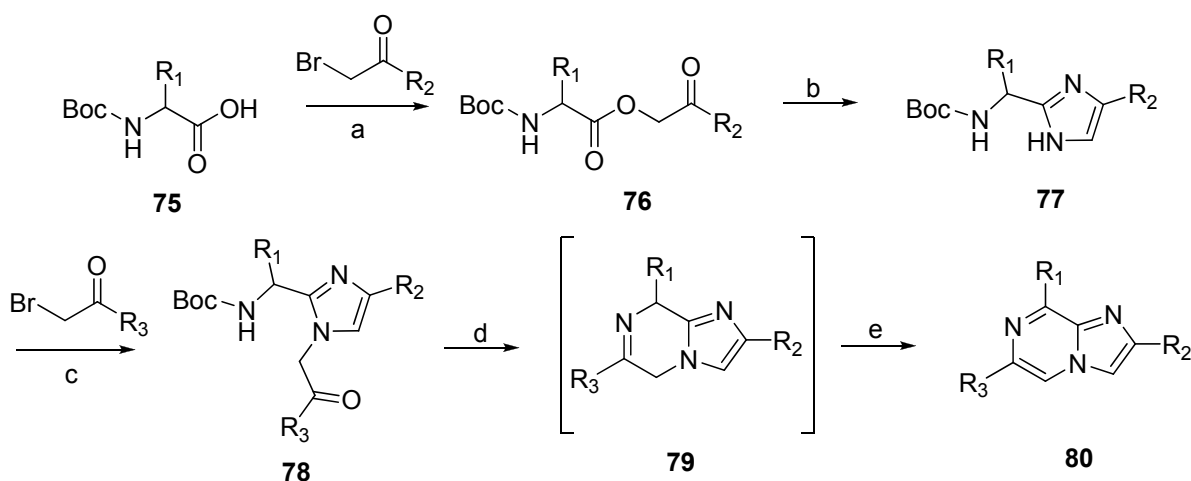
Diethyl aminomalonate (**70**) has been used as the starting material in the synthesis of 5-substituted imidazo[1,2-*a*]pyrazines (Scheme 14).⁵⁹ Reaction with methanolic ammonia gives 2-amino-malonamide, which upon reaction with pyruvic aldehyde in the presence of sodium hydroxide affords the pyrazine-2-carboxaldehyde. Further reaction with potassium hydroxide and bromine, followed by condensation with bromoacetaldehyde diethyl acetal furnishes 5-methyl-imidazo[1,2-*a*]pyrazine-2-ol (**74**).



Scheme 14: Synthesis of 5-methyl-imidazo[1,2-*a*]pyrazine-2-ol⁵⁹

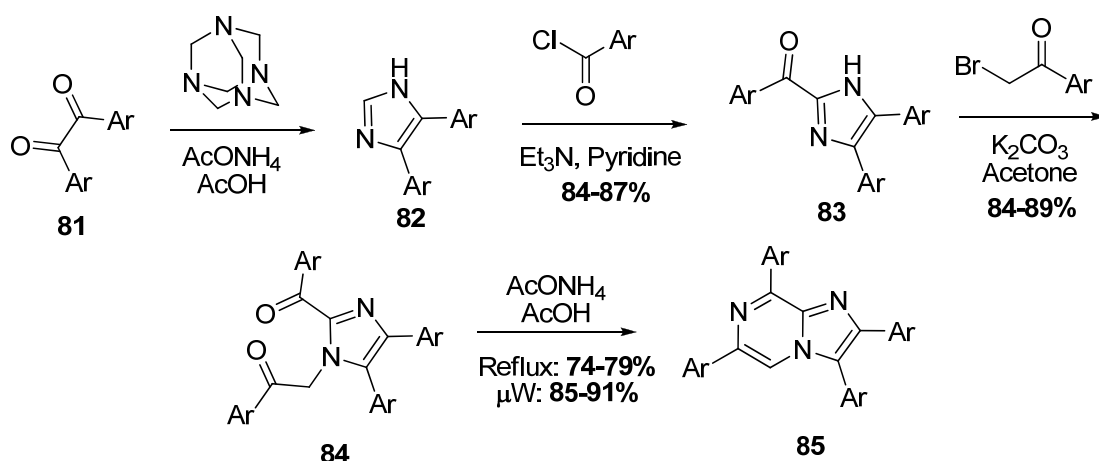
1.2.2.1.2 Imidazo[1,2-*a*]pyrazines from Imidazole Rings

A method by Contour-Galcéra *et al* described the reaction of an imidazole core with an α -bromoketone (Scheme 15).⁶⁰ The imidazoles can be first made by alkylating *N*-protected amino acids with an α -bromoketone, followed by cyclisation of the resulting keto ester in ammonium acetate. *N*-alkylation of the imidazole ring, facilitated by polymer supported *N*-methyl morpholine, was followed by Boc-deprotection and spontaneous cyclisation to yield the dihydroimidazo[1,2-*a*]pyrazine. In all but one of the analogues, this species was unstable and so oxidation to the fully aromatised product was carried out.



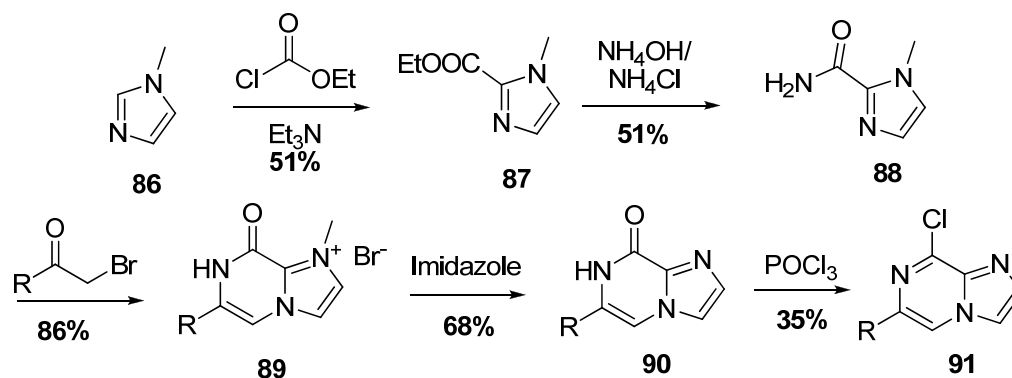
Scheme 15: Synthesis of imidazo[1,2-*a*]pyrazines via alkylation and cyclisation of an imidazole core.⁶⁰
 (a) (i) Cs₂CO₃, EtOH/H₂O; (ii) bromoketone, DMF; (b) NH₄OAc, xylene, 150°C; (c) polymer supported NMM, DMF; (d) 10% TFA/CH₂Cl₂; (e) DMSO or MeOH or Magtrieve, MeOH

A similar approach was adopted by Kayağil and Demirayak⁶¹ in their synthesis of 2,3,6,8-tetraaryl imidazo[1,2-*a*]pyrazines. Using classical reflux conditions or microwave irradiation they reacted substituted diketo imidazoles with ammonium acetate in AcOH to yield the functionalised imidazo[1,2-*a*]pyrazine core (Scheme 16).



Scheme 16: Synthesis of 2,3,6,8-tetraarylimidazo[1,2-*a*]pyrazine⁶¹

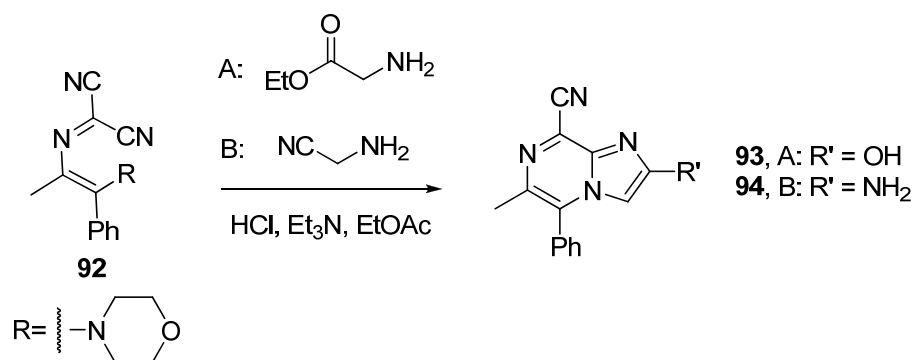
In an alternative approach, the reaction of 1-methylimidazole with ethyl chloroformate, followed by either aqueous ammonia or ammonium hydroxide and ammonium chloride affords the imidazole-2-carboxamide **88** (Scheme 17).^{59,62} Continuing the reaction with an α -bromoketone forms the imidazo[1,2-*a*]pyrazine salt which upon heating with imidazole, forms the imidazo[1,2-*a*]pyrazin-8-one. Reacting with phosphorus oxychloride affords 8-chloroimidazo[1,2-*a*]pyrazine.⁵⁹



Scheme 17: Synthesis of 6-methyl-8-chloroimidazo[1,2-*a*]pyrazine starting from imidazole^{59,62}

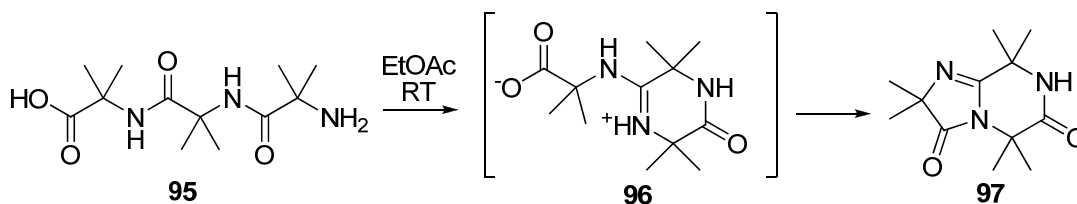
1.2.2.1.3 Alternative Approaches to the Formation of Imidazole and Pyrazine Rings

Treatment of 5-dialkylamino-1,1-dicyano-2-aza-1,3-dienes with α -amino esters gives substituted imidazo[1,2-*a*]pyrazines (Scheme 18).⁶³ If ethylenediamine is used, the resulting heterocycle is the 2,3-dihydroimidazo[1,2-*a*]pyrazine.⁶⁴



Scheme 18: Formation of imidazo[1,2-*a*]pyrazines through the reaction of 5-dialkylamino-1,1-dicyano-2-aza-1,3-dienes with α -aminoesters⁶³

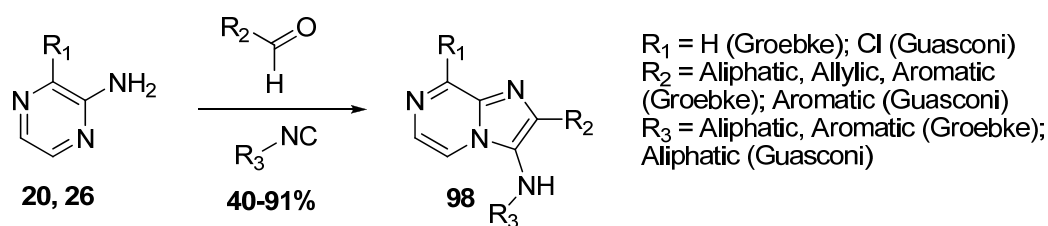
Tripeptides of the sequence Aib-Aib-Aib readily isomerise in ethyl acetate at RT to give monocyclic amidines, which in turn cyclises to form imidazo[1,2-*a*]pyrazine-3,6-diones (Scheme 19).⁶⁵



Scheme 19: Isomerisation of tripeptides to give monocyclic amidine, which cyclises to give imidazo[1,2-*a*]pyrazine derivatives⁶⁵

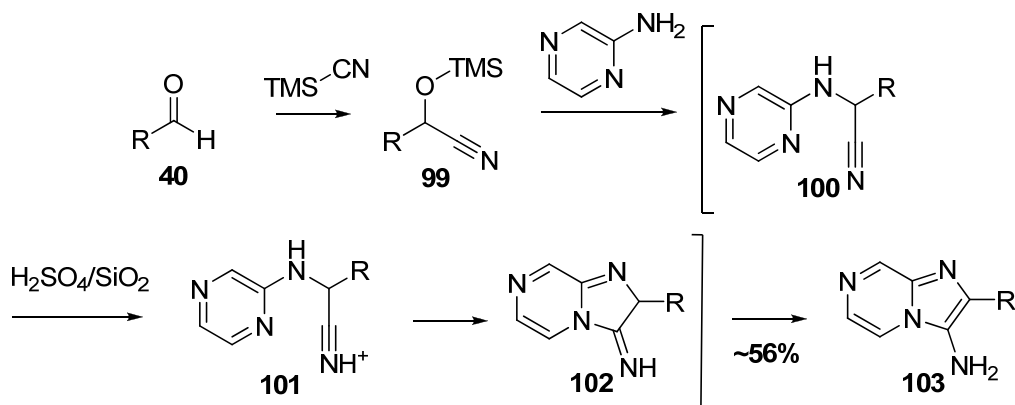
1.2.2.1.4 Multicomponent Reactions

Different multicomponent reactions (MCR) have also been used to deliver various substituted imidazo[1,2-*a*]pyrazines. The first, reported in 1968 by Goto *et al.*,⁶⁶ combined 2-aminopyrazine, formaldehyde and sodium cyanide to produce 3-aminoimidazo[1,2-*a*]pyrazine. Using an isonitrile, aldehyde and aminopyrazine, the three independent groups of Blackburn,⁶⁷ Bienaymé⁶⁸ and Groebke⁶⁹ utilised a Ugi-type MCR in order to synthesise a series of 2-substituted-3-aminoimidazo[1,2-*a*]pyrazines. This was then repeated more recently by Guasconi *et al.*,⁷⁰ with an additional nucleophilic aromatic substitution step, to synthesise 2-substituted-3,8-diaminoimidazo[1,2-*a*]pyrazines, as illustrated in Scheme 20.



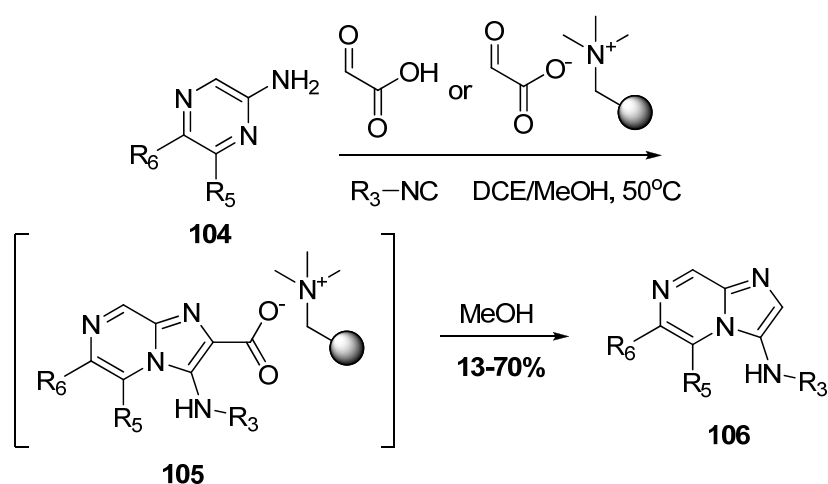
Scheme 20: Ugi-type multicomponent reaction to deliver 2-substituted-3-aminoimidazo[1,2-*a*]pyrazines^{69,70}

TMSCN has been used as an isonitrile replacement in a MCR to synthesise 3-aminoimidazo[1,2-*a*]pyrazines.⁷¹ It was postulated that a cyanohydrin is formed by the reaction of an aldehyde and TMSCN, which is then followed by a Strecker reaction with 2-aminopyrazine to form an aminonitrile intermediate. Catalysed by silica-sulfuric acid, the intermediate undergoes cyclisation, followed by a [1,3] proton-shift to afford the desired bicycle (Scheme 21).



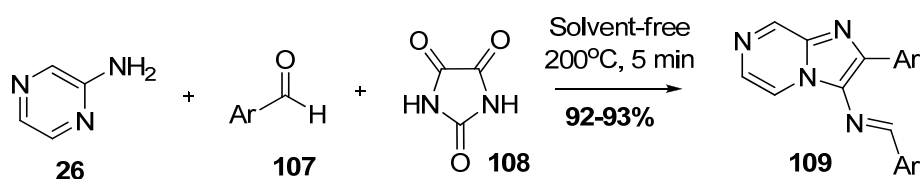
Scheme 21: MCR of 2-aminopyrazine, an aldehyde and TMSCN catalysed by $\text{H}_2\text{SO}_4/\text{SiO}_2$ ⁷¹

Glyoxylic acid, either in solution or immobilised on a macroporous polystyrene carbonate, has been used as a formaldehyde equivalent in the synthesis of 2-unsubstituted-3-aminoimidazo[1,2-*a*]pyrazines (Scheme 22).⁷²



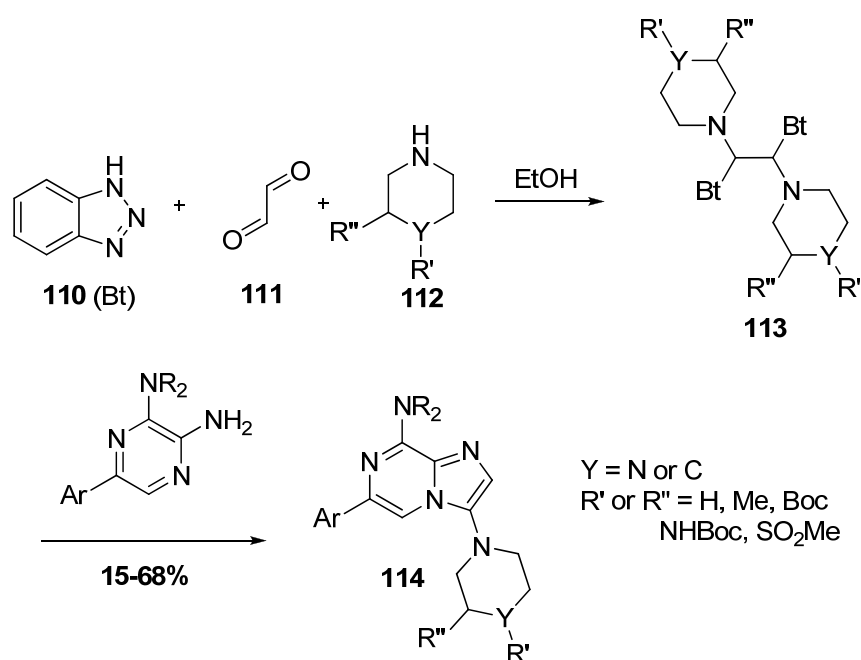
Scheme 22: MCR utilising glyoxylic acid or MP-glyoxylate as formaldehyde equivalents⁷²

Adib *et al*⁷³ have since suggested an alternative MCR by reacting 2-aminopyrazine, benzaldehyde derivatives and imidazoline-2,4,5-trione under solvent-free conditions to yield 3-amino-2-arylimidazo[1,2-*a*]pyrazine imine derivatives in high yields.



Scheme 23: Solvent-free multicomponent reaction to yield -amino-2-arylimidazo[1,2-*a*]pyrazine imine derivatives⁷³

Pastor *et al* has described the first direct synthesis of 3-*N*-dialkylimidazo[1,2-*a*]pyrazines⁷⁴ via a one-pot cyclisation utilising 1,2-bis(benzotriazolyl)-1,2(dialkylamino)-ethanes (Scheme 24). It is an alternative synthesis to that proposed by Lyon *et al*,⁷² and it builds on the work carried out by Katritzky *et al* in the synthesis of imidazopyridines and pyrimidines.⁷⁵



Scheme 24: Direct synthesis of 3-*N*-dialkylimidazo[1,2-*a*]pyrazines using benzotriazole⁷⁴

1.2.2.2 Reactivity of Imidazo[1,2-*a*]pyrazines

Imidazo[1,2-*a*]pyrazines contain both π -excessive imidazole and π -deficient pyrazine rings and as such undergo reactions suited to both types of system. Numerous early studies showed these features, and these will now be discussed.

1.2.2.2.1 Electrophilic Addition

The electron density surface map of neutral imidazo[1,2-*a*]pyrazine (Figure 12 a),⁷⁶ predicts the C₃ position to be the most electrophilic, and this is confirmed by bromination with NBS yielding the 3-bromo species exclusively.^{47,76} However, protonated or hydrogen bonded heterocycles can have vastly different charge distributions relative to their neutral counterparts and as such the electron density surface map of protonated imidazo[1,2-*a*]pyrazine (Figure 12 b) predicts the C₅ position to be the preferential site of electrophilic attack. Synthetic experiments with Br₂ in EtOH confirm this prediction, with the 5-bromo species resulting either through protonation of N₁ of imidazo[1,2-*a*]pyrazine by HBr generated, or by hydrogen bonding to EtOH.

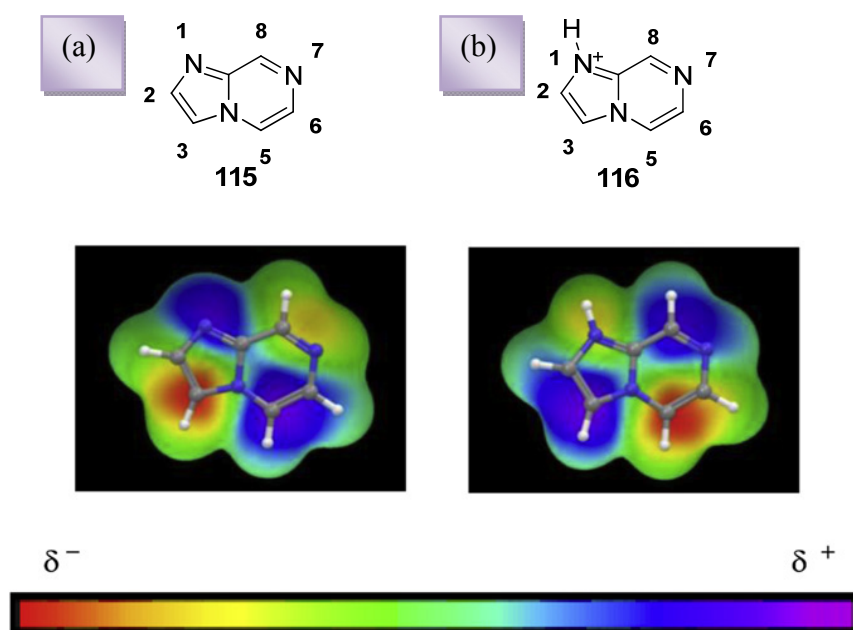
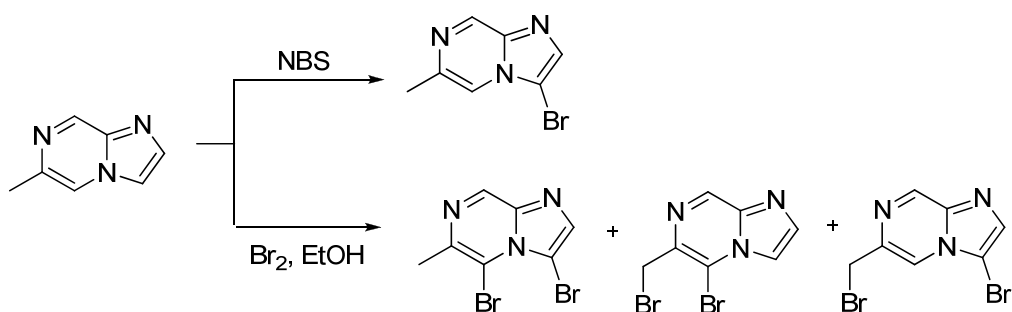


Figure 12: Electron density map for (a) neutral and (b) protonated imidazo[1,2-*a*]pyrazines.⁷⁶
Reproduced with permission.

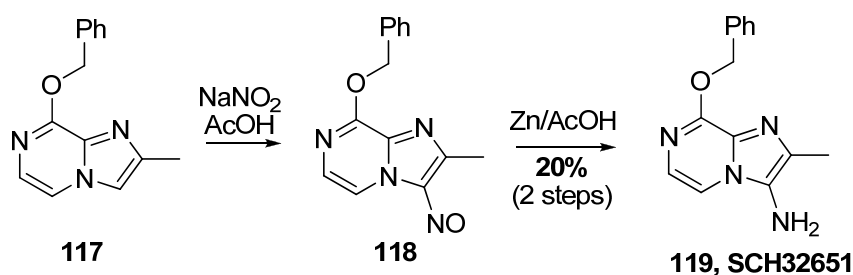
Similar electron density experiments with 6-methylimidazo[1,2-*a*]pyrazine also showed the C₃ position as the preferential site of electrophilic addition (again confirmed by synthetic work with NBS) and the C₅ position preferential for the protonated form. However, when 6-methylimidazo[1,2-*a*]pyrazine was reacted with Br₂ in EtOH, no mono-bromination at the C₅ position was evident. Instead, various di-brominated species were observed: 3,5-dibromo, as well as bromination of the methyl group and either the 3- or 5- positions (Scheme 25). This indicates that steric effects outweigh the electronic effects in this system.⁷⁶



Scheme 25: Bromination of 6-methylimidazo[1,2-*a*]pyrazine using either NBS or Br₂/EtOH

Other groups have also shown electrophilic halogenation using NBS or NCS to initially occur at the C₃ position.⁵² Electron withdrawing halogens in the 8-position facilitates this electrophilic reaction, whereas if electron donating groups are present at C₈, halogenation is now observed at the C₅ position owing to an increased electron density relative to C₃.⁵⁶

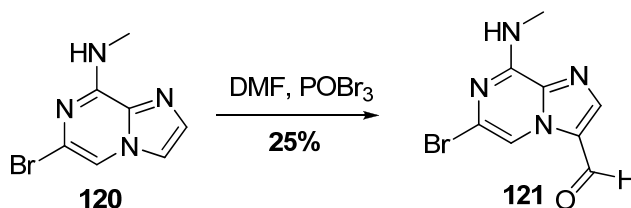
In an alternative to yield 3-aminoimidazo[1,2-*a*]pyrazines, Kaminski *et al*⁴⁸ showed that nitrosation of 2,8-disubstituted imidazo[1,2-*a*]pyrazines with sodium nitrite and AcOH occurs at the 3-position. Reduction with Zn/AcOH can then be used to afford the desired product (Scheme 26). However, in the absence of these two activating groups, the nitrosation reaction fails.⁵¹ The compound shown, SCH32651, has been shown to be a reversible potassium competitive acid blocker (P-CABs) in inhibiting the gastric H⁺/K⁺ ATPase.^{48,77,78}



Scheme 26: Nitrosation of imidazo[1,2-*a*]pyrazine⁴⁸

Vitse *et al* showed that nitration of various 6,8-disubstituted imidazo[1,2-*a*]pyrazines occurs regioselectively at C₃.⁷⁹ It is also possible to directly hydroxymethylate similar systems in the C₃ position using formaldehyde.^{51,80}

The same group then demonstrated Vilsmeier-Haack formylation of 6-bromo-8-(methylamino)imidazo[1,2-*a*]pyrazine using DMF and phosphorous oxybromide (Scheme 27).⁸¹ The use of the classical reagent phosphorous oxychloride was avoided due to the likely elimination/addition of the C₆ bromine.



Scheme 27: Vilsmeier-Haack formylation of 6-bromo-8-(methylamino)imidazo[1,2-*a*]pyrazine⁸¹

Under neutral and acidic conditions, imidazo[1,2-*a*]pyrazines do not undergo hydrogen-deuterium exchange; however, in an alkaline solution (aq. NaOD in DMSO), exchange is observed with H₃ and H₅,⁵² further indicating the electrophilic nature of these positions.

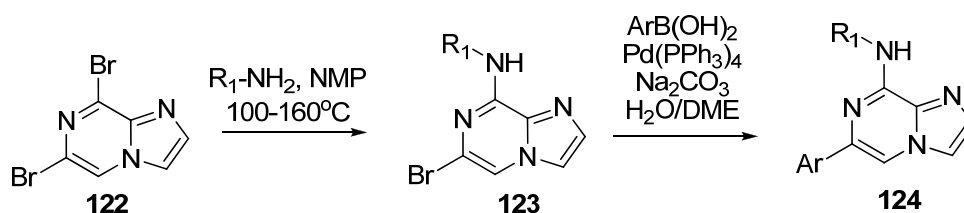
Computational studies⁴⁸ have shown that N₇ is the nitrogen of greatest electron density, but the most stable protonated species results from N₁ protonation, as illustrated by X-ray crystallography.⁴⁸ The pK_a value for the N₁ protonated species of imidazo[1,2-*a*]pyrazine has been reported as 3.59 ± 0.02.⁸² In addition, by reacting imidazo[1,2-*a*]pyrazines with MeI at RT for 5 days, quaternisation of N₁ and N₇ is observed in a 1.6:1 ratio.⁵² NMR studies have been reported which determine the site of protonation and methylation in imidazo[1,2-*a*]pyrazines.^{82,83}

1.2.2.2.2 Nucleophilic substitution

Early studies showed that nucleophilic substitution only occurs at the C₅ and C₈ positions. Bradač *et al* showed mono-displacement of 6,8-dibromoimidazo[1,2-*a*]pyrazine by a methoxy moiety at the 8-position only.⁵² Similar observations were made by Bonnet *et al* by displacement with a variety of alcohols and amines⁵¹ and by Meurer *et al* by displacement with piperazine.⁵⁶ The later also showed that with a piperazine in position 8, displacement of chlorine in positions 3 and 6 with piperazine does not occur, whilst displacement of bromine at C₆ tentatively occurs with methylamine, with unsuccessful isolation of the reaction product. Unsuccessful methoxide displacement was also observed showing the resistance to nucleophilic substitution at C₃, C₅ and C₆ in compounds bearing a piperazine moiety at C₈.⁵⁶

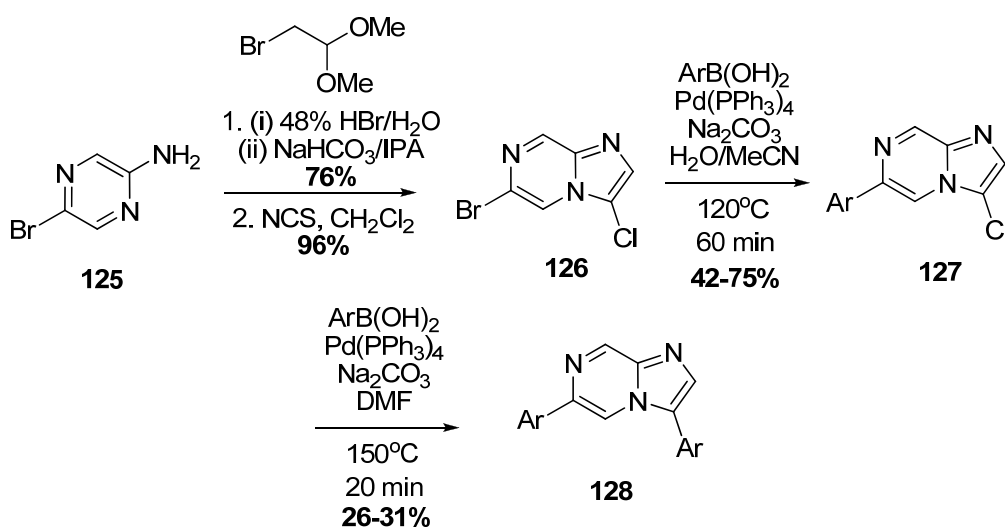
Lumma *et al* showed that the chlorine in the 8-position in 6,8-dichloroimidazo[1,2-*a*]pyrazine can be exchanged for bromine using sodium bromide and HBr in butanone.⁵⁰

More recently, however, a variety of methods have been established to allow substitution at previously thought inaccessible positions. In order to achieve substitution of halogens in the 6-position, palladium coupling reactions have been used. Examples of these include work carried out by Mitchell *et al* in the synthesis of inhibitors of the receptor tyrosine kinase EphB4.⁸⁴ Starting with 6,8-bromoimidazo[1,2-*a*]pyrazine **122**, amine displacement of the 8-bromo proceeds under thermal conditions, but displacement of the 6-bromo requires a Suzuki coupling reaction (Scheme 28).



Scheme 28: Example of the use of Suzuki coupling to displace halogens in the 6-position of imidazo[1,2-*a*]pyrazine⁸⁴

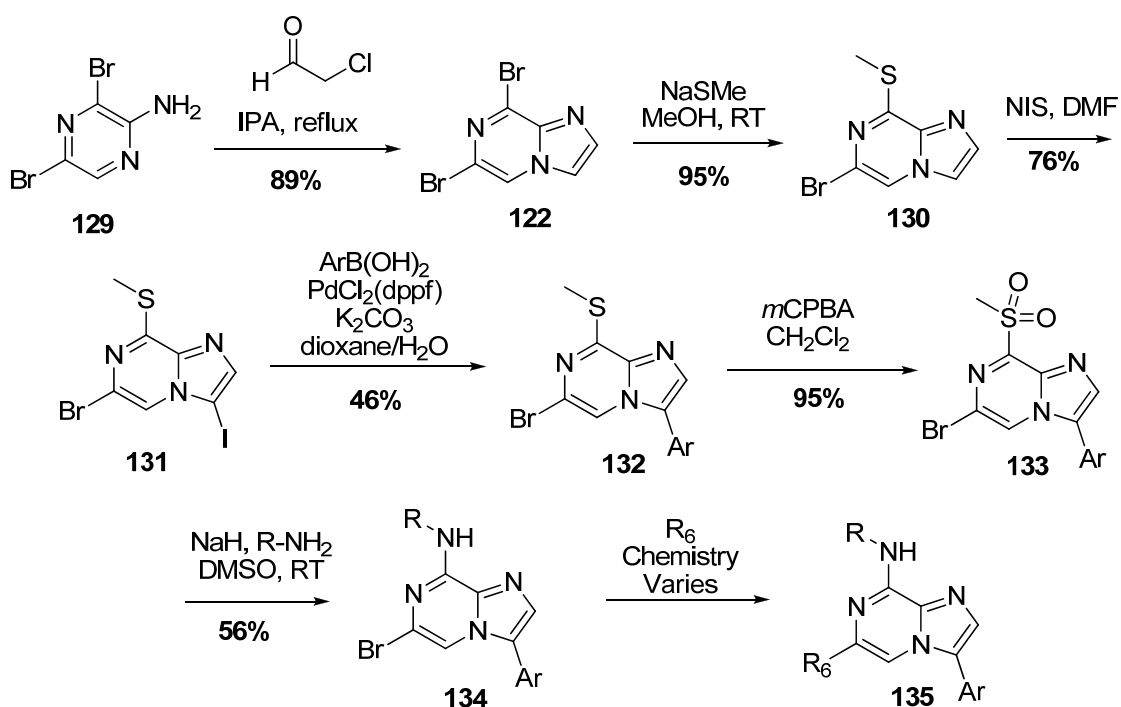
Matthews *et al* identified specific conditions that enabled selective Suzuki coupling in 3-chloro-6-bromoimidazo[1,2-*a*]pyrazine.⁸⁵ Using $Pd(PPh_3)_4$, Na_2CO_3 , MeCN/ H_2O , 120°C, 60 min, microwave, the 6-bromo was selectively displaced; changing the solvent to DMF and heating at 150°C for 20 min under microwave enables the 3-position to now be displaced (Scheme 29).



Scheme 29: Illustration of selective Suzuki coupling conditions⁸⁵

More recently, activation of the 8-position for nucleophilic substitution of less reactive nucleophiles has been reported.^{86,87} Starting with 3-bromo-8-chloroimidazo[1,2-*a*]pyrazine, selective substitution of the 8-chloro group is achieved with sodium thiomethoxide. This moiety can then be oxidised to the methyl sulfone using *meta*-chlorobenzoic acid, which in turn has been shown to be displaced by a range of amine nucleophiles. The 3-position is then free to be functionalised *via* Suzuki coupling chemistry.

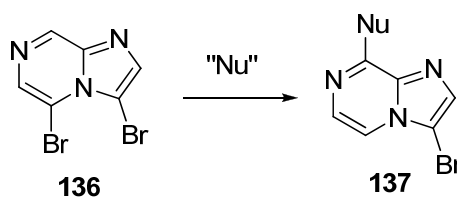
The same group also demonstrated further selective functionalisation, as depicted in Scheme 30. By having three orthogonal leaving groups at the 3-, 6- and 8-positions, varying chemistries can be utilised to install three different moieties around the heterocyclic core.



Scheme 30: Synthesis of 3,6,8-trisubstituted imidazo[1,2-*a*]pyrazines using three orthogonal leaving groups⁸⁶

It has also been shown that displacement of 3-bromoimidazo[1,2-*a*]pyrazines occurs by refluxing copper cyanide in DMF.⁸⁰

Another interesting transformation is the telesubstitution reaction whereby the entering functionality adopts a position more than one atom away from the atom to which the leaving group was attached.⁸⁸ Such reactions with imidazo[1,2-*a*]pyrazines have previously been reported with methoxy groups^{52,89} as well as amino and alkyl amino moieties (Scheme 31).⁵¹ The telesubstitution reaction competes with the addition-elimination reaction at position C₅. It is postulated that the electron withdrawing nature of the nitrogen in position 7 activates C₈ for nucleophilic attack.



Scheme 31: Generic telesubstitution reaction of 3,5-dibromoimidazo[1,2-*a*]pyrazine

To our knowledge there are no literature examples for the synthesis of imidazo[1,2-*a*]pyrazine with a sulfonamide attached directly to the core, such as the target compounds **4-11**.

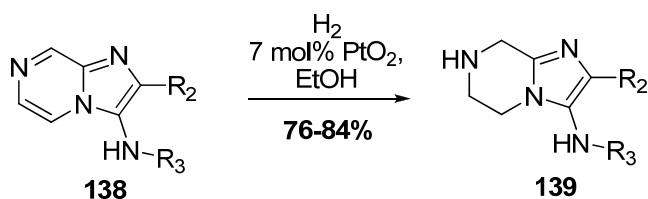
1.2.2.2.3 Metalation of Imidazo[1,2-*a*]pyrazines

Lithiation of position 8 in unsubstituted imidazo[1,2-*a*]pyrazine is observed with both MeLi⁹⁰ and PhLi.⁹¹ In addition, lithiation of imidazo[1,2-*a*]pyrazine, followed by quenching with an electrophile occurs with both *n*-BuLi and the non-nucleophilic lithium tetramethylpiperidide (LTMP) to yield 3- and 5-substituted products.⁹²

Halogen-metal exchange of 3,6-dibromo imidazo[1,2-*a*]pyrazine derivatives occurs with *n*-BuLi at the 3-position with the 6-bromo exhibiting *ortho*-directing effects resulting in 3,5-disubstituted products.⁹² The regioselectivity of the halogen-metal exchange reaction of 3,6-dibromo-8-methoxyimidazo[1,2-*a*]pyrazine also depends on the nature of the quenching electrophile. Even though 3,5-dilithiated species are formed, the more sterically hindered the electrophile is, the more regioselective the reaction is towards C₃.⁹²

1.2.2.2.4 Reduction of Imidazo[1,2-*a*]pyrazines

It has previously been shown that under hydrogen atmosphere with 7 mol% PtO₂ in EtOH, imidazo[1,2-*a*]pyrazine derivatives undergo partial reduction to form tetrahydroimidazo[1,2-*a*]pyrazines, as depicted in Scheme 32.⁹³ An alternative method of achieving reduction involves the use of NaBH₃CN.⁹⁴



Scheme 32: Partial reduction of imidazo[1,2-*a*]pyrazines⁹³

1.2.2.3 Therapeutic Applications of Imidazo[1,2-*a*]pyrazines

Imidazo[1,2-*a*]pyrazines have been reported with a variety of therapeutic uses. These will be discussed in this section, with the inclusion of a few case studies in relation to anticancer treatment.

1.2.2.3.1 Imidazo[1,2-*a*]pyrazines as Anticancer Agents

A series of papers published from the Merck Research Laboratories describe the synthesis of imidazo[1,2-*a*]pyrazines as potent Aurora kinase inhibitors.^{86,87,95,96,97,98} Aurora kinases A-C are cell cycle regulated serine/threonine kinases expressed only during mitosis, and are an important anti-cancer target.⁹⁹ The objective of the group was to identify a potent and selective Aurora A or B inhibitor, showing mechanism-based cell activity (by inhibition of the phosphorylation of Histone H3 (HH3)), and with ideal pharmacokinetic profiles.

Through initial screening of an internal library, an imidazo[1,2-*a*]pyrazine scaffold with a pyrazole ring and isothiazole ring in the 3- and 8-positions respectively was identified with sub-micromolar IC₅₀ values (Figure 13 **140**).⁸⁷ SAR studies showed that the addition of a small hydrophobic moiety (Me, SMe) in the 6-position improves cell penetration and that the removal of the pyrazole methyl improves potency by approximately 100 fold (Figure 13, **141/142**).⁸⁷ The improved potency can be attributed to an important hydrogen-bonding role of the pyrazole as illustrated by the X-ray crystal structure.

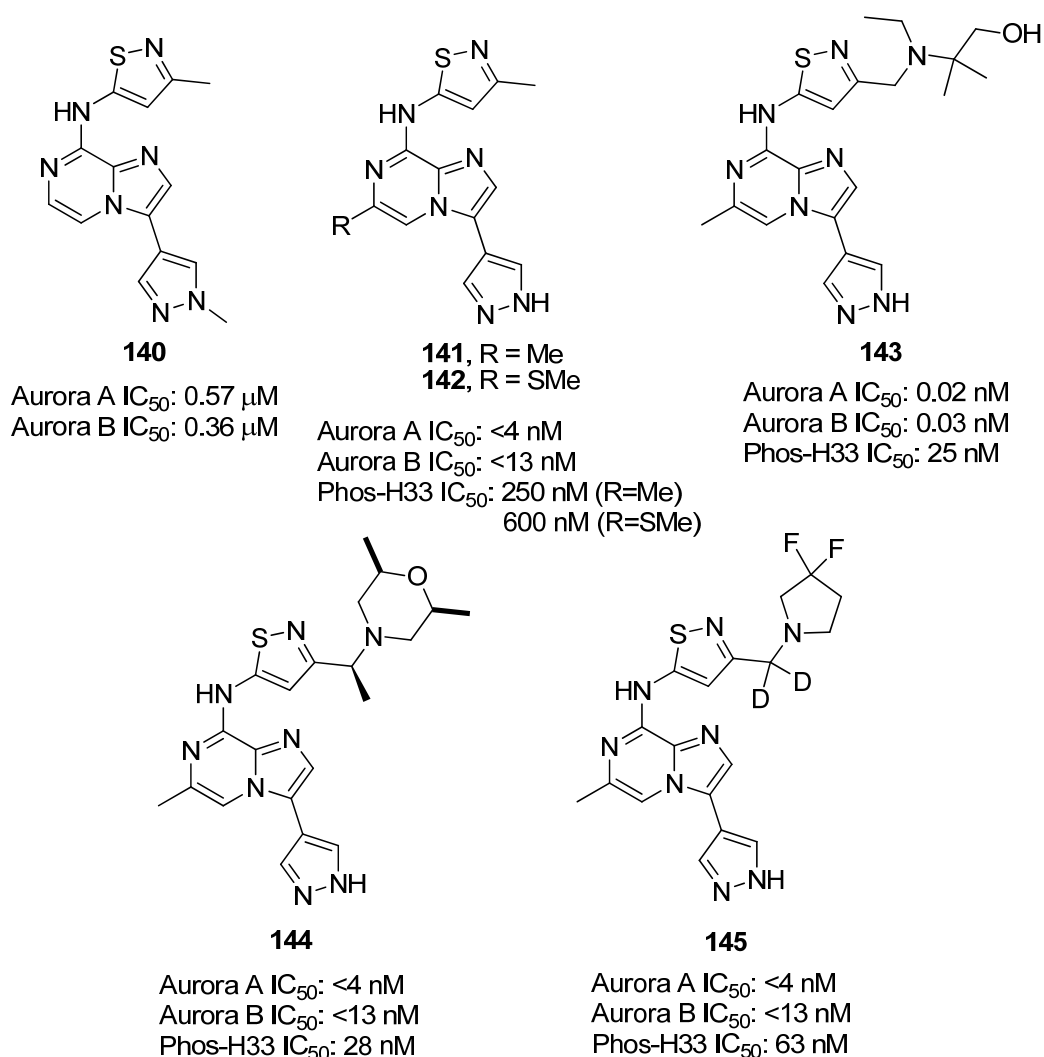


Figure 13: Range of imidazo[1,2-*a*]pyrazine based inhibitors as Aurora kinase inhibitors^{87,95,96,97}

Further studies looked at improving cell potency, pharmacokinetics and aqueous solubility for intravenous formulation, focusing on the solvent accessible isothiazole methyl position. Installation of polar groups (Figure 13, **143**) succeeding in improving potency and solubility.⁹⁵ However it showed multi-kinase inhibition and poor bioavailability and as such compound **144** (Figure 13) was synthesised, showing anti-tumour activity, oral bioavailability and improved off-target kinase activity.⁹⁶

Further studies to improve oral bioavailability of **143** showed that the major site of metabolism occurs at the basic nitrogen, and therefore an approach was sought to block this metabolism and

improve absorption. This was achieved by the incorporation of fluorine and deuterium into the structure (**145**).⁹⁷

Further studies included a bioisoteric approach by replacing the isothiazole with thiophene and thiazoles,⁹⁸ as well as investigating alternative amide moieties on the pyrazole nitrogen.⁸⁶ The synthesis of compounds **141-145** was achieved using the chemistry illustrated in Scheme 30.

Another group looked at imidazo[1,2-*a*]pyrazines as selective Aurora-A kinase inhibitors (Figure 14).¹⁰⁰ HTS of an in-house library yielded two promising hits, one of which is compound **146**. They then initiated a hit-to-lead programme, by identifying the inhibitory effects of C₃, C₆ and C₈ substituents, with the aim of establishing SAR trends. Compounds were synthesised in an analogous manner to those depicted in Scheme 28, with an additional chlorination step of the 6,8-dibromoimidazo[1,2-*a*]pyrazine.

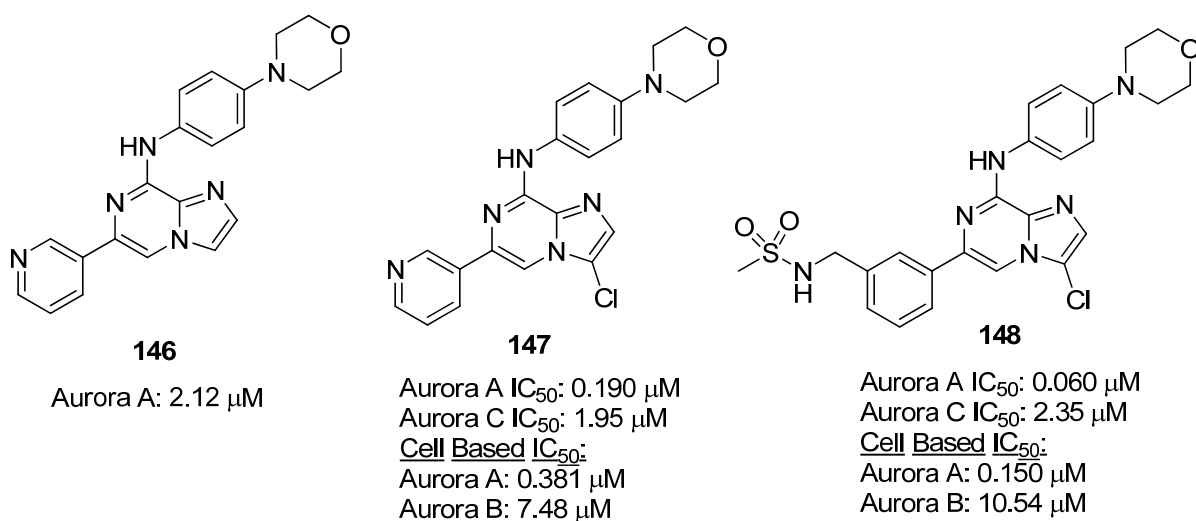


Figure 14: Imidazo[1,2-*a*]pyrazines as selective Aurora-A kinase inhibitors. Initial values refer to inhibition of recombinant human Aurora-A and Aurora-C, and secondary values refer to cell-based *in vitro* assays and are determined using Flashplate and Filterplate assays.¹⁰⁰

Compound **147** proved to be an approximately 10-fold more potent inhibitor, and was found to have a 19-fold selectivity in inhibiting Aurora-A over Aurora-B. In addition, it displayed cellular potency when studying the inhibitory activity of two cancer cell lines. Through co-crystallisation of compound **147** with Aurora-A, the binding mode was elucidated, providing an insight into the interactions of imidazo[1,2-*a*]pyrazines with Aurora kinases. This led to the design of further

analogues, with compound **148** showing improved potency and a 70-fold selectivity in inhibiting Aurora-A over Aurora-B in cell-based assays.¹⁰⁰

The lipid phosphoinositide-3-kinase (PI3K) is a central component in the PI3K/AKT/mTOR signalling pathway, and is an important target for cancer therapeutics. González *et al* have recently published work into the identification of novel PI3K inhibitors, based on the imidazo[1,2-*a*]pyrazine core structure.^{101,102} Initially they adopted a ligand-based rational drug design approach, utilising key morpholinyl and 3-hydroxyphenyl fragments in the 8 and 6 positions respectively and investigating alternative groups in the 3-position (Figure 15).¹⁰¹

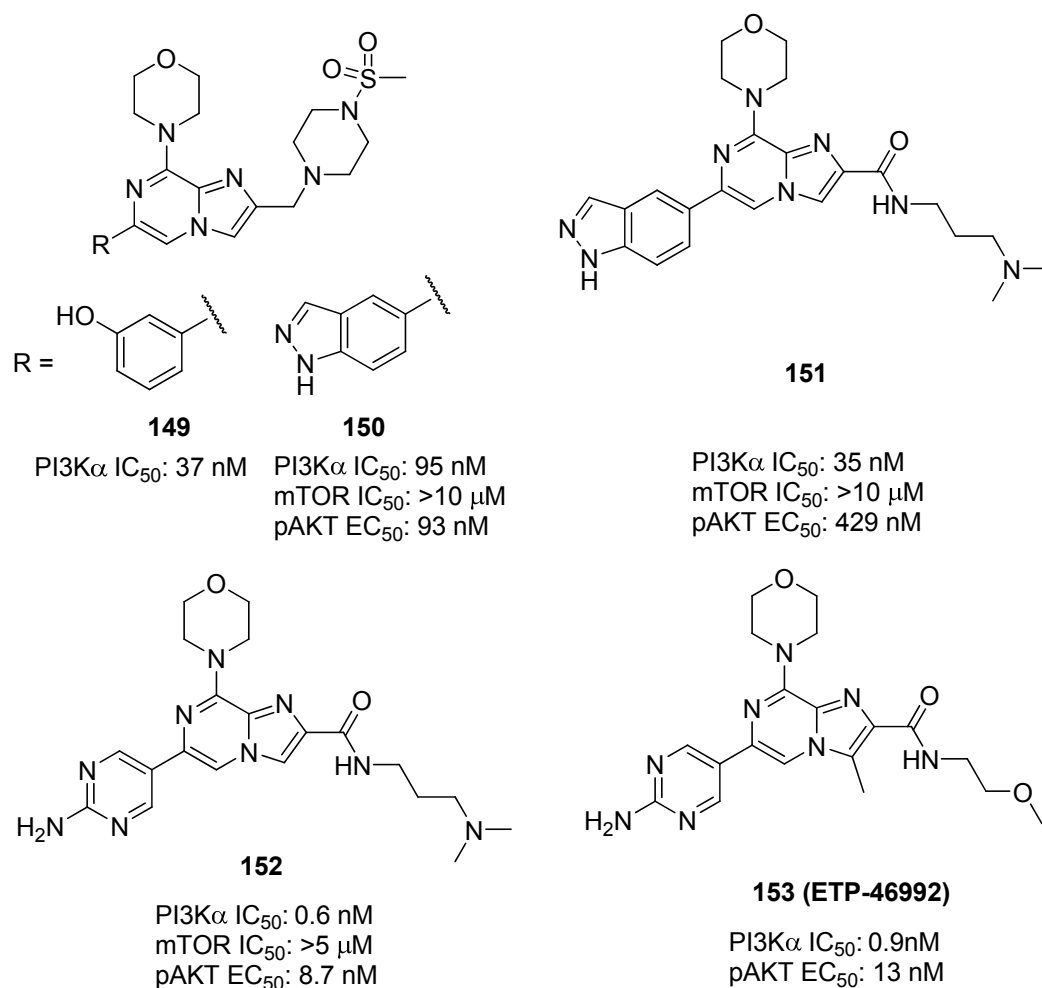


Figure 15: Imidazo[1,2-*a*]pyrazine based PI3K inhibitors. pAKT refers to phosphorylation of AKT^{101,102}

Compound **149** has an IC₅₀ value of 37 nM; however, it has limited *in vivo* potential due to the metabolically unstable 3-hydroxyphenyl group. Through changing this group with the isosteric indazole, the resulting compound **150**, although displaying a slightly higher IC₅₀ was thought of as a viable starting point for different functionalisation of the imidazo[1,2-*a*]pyrazine core.¹⁰¹ The synthesis of **149** and **150** was carried out by substituting a morpholine in the 3-position of 2-amino-3,5-dibromopyrazine, condensation with dichloroacetone, and then nucleophilic displacement of the resulting alkyl chloride. A Suzuki reaction was used to install the R₆ moiety.

The group then prepared various secondary amide derivatives at C₂ with an improved potency of 35 nM observed with compound **151**. In addition this compound displayed good selectivity verses mTOR kinases.¹⁰¹ Taking this hit forward, the indazole was replaced with a 2-aminopyrimidine moiety (**152**), greatly improving the potency to 0.6 nM for a biochemical assay, as well as improving the IC₅₀ in a cell-based assay of the inhibition of AKT phosphorylation from 429 nM to 8.7 nM.¹⁰² Synthesis was carried out by condensing the same morpholino-pyrazine with ethyl bromo pyruvate, Suzuki reaction and then amide bond formation.

Further investigations into positions 2-, 5- and 6- revealed the lead compound ETP-46992 (**153**), with a biochemical and cell-based IC₅₀ of 0.9 nM and 13 nM respectively.¹⁰² The compound shows good pharmacokinetic data, good selectivity, high permeability and targeted inhibition *in vivo*.

The same group from Merck Research Laboratories synthesised imidazo[1,2-*a*]pyrazines, in a similar fashion to before, as Brk/PTK6 inhibitors (Figure 15).¹⁰³ Initial screening identified a compound with an IC₅₀ of 500 nM, with follow up screening identifying compound **154** with an enzymatic activity IC₅₀ of 7 nM, and *p*-SAM68 IC₅₀ of 156 nM (mechanism-based cellular activity measured by inhibition of phosphorylation of SAM68).

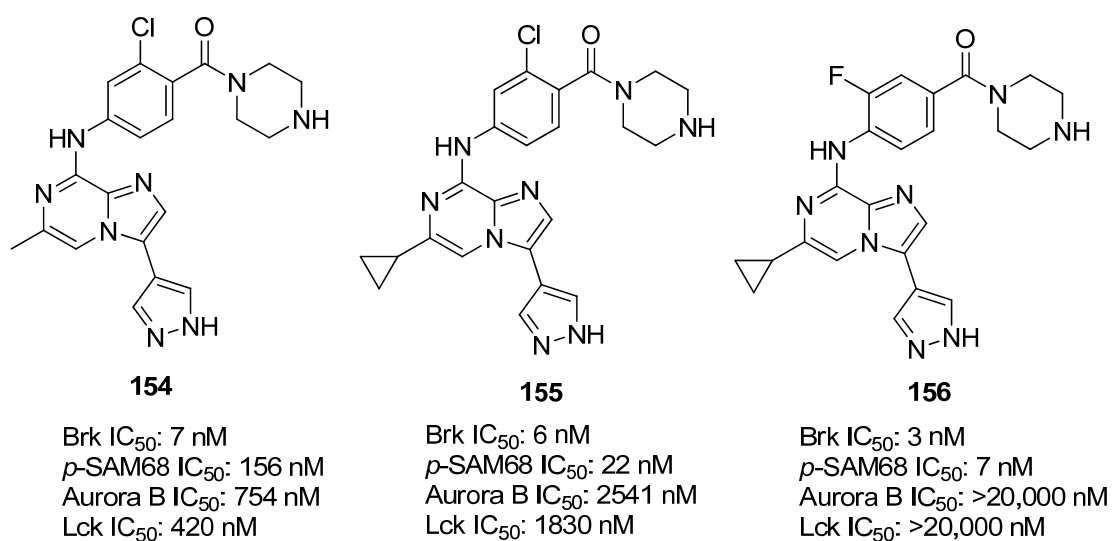


Figure 16: Imidazo[1,2-*a*]pyrazines as inhibitors of Brk/PTK6¹⁰³

The group then looked at different substituents in each of the positions around the ring to improve potency and selectivity over Aurora-B kinase and Lck (an Src family kinase). Methylation of the pyrazole resulted in diminished activity, whilst substituting for the 3-thiophene group gave an IC₅₀ of 51 nM. Changing the methyl at C₆ for either an ethyl or cyclopropyl (**155**) resulted in maintained potency, but improved selectivity.

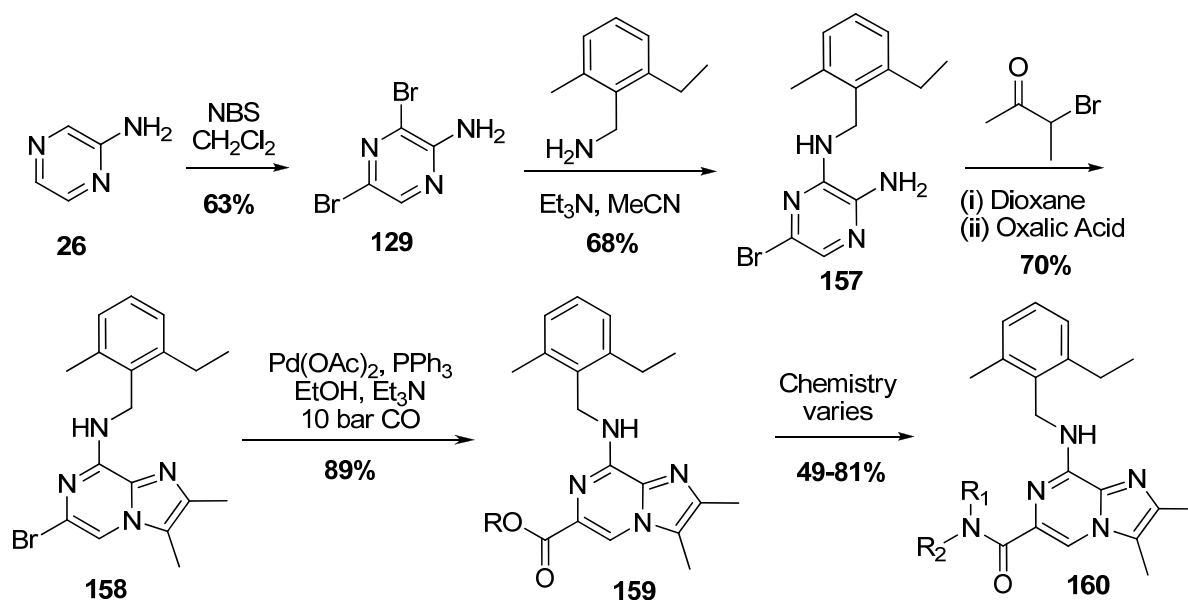
Extensive studies into the amide functionality in the C₈ position resulted in the top compound structurally very similar to the ones preceding it (**156**), with fluorine replacing the chlorine, and moving *ortho* to the amine. Activity is maintained once again, with selectivity improved to greater than 300-fold.

Alternative cancer therapeutic targets for imidazo[1,2-*a*]pyrazines include the serine/threonine kinases of checkpoint kinase 1 (CHK1),⁸⁵ cyclin dependent kinase (CDK),¹⁰⁴ c-Jun N-terminal kinases (JNK)^{105,106} and the receptor tyrosine kinase EphB4.⁸⁴

1.2.2.3.2 Additional Therapeutic Applications of Imidazo[1,2-*a*]pyrazines

As previously mentioned, SCH32651 (Scheme 26) has potential antiulcer properties,^{48,77} and based on this structure, Zimmermann *et al*^{78,107} synthesised a series of potent reversible inhibitors of the gastric H⁺/K⁺ ATPase (Scheme 33). By carrying out an alkoxycarbonylation on the 6-

bromo species, a variety of amides could be accessed. Other derivatives as acid pump antagonists have also been reported.¹⁰⁸



Scheme 33: Synthesis of 6-carboxamide-substituted imidazo[1,2-a]pyrazine synthesised by Zimmermann *et al*⁷⁸

Anti-inflammatory properties of imidazo[1,2-a]pyrazines have been reported, with derivatives acting as CXCR3 antagonists.¹⁰⁹ CXCR3 are chemokine receptors, responsible for trafficking T-cells to inflamed cells, and therefore antagonists can be used in various inflammatory diseases such as multiple sclerosis and rheumatoid arthritis.

Other targets for inflammatory diseases of imidazo[1,2-a]pyrazine inhibitors are: I κ B kinase (IKK), where the inhibitors are reported to additionally prevent and treat immune-related diseases;¹¹⁰ p38 kinases;¹¹¹ Mitogen-Activated Protein Kinase-Activated Protein Kinase 5 (MAPKAP5), also for the treatment of degenerative diseases;¹¹² Monopolar Spindle 1 (Mps-1) kinase, also for the treatment of cancer, arthritis and other hyperproliferative disorders;¹¹³ the ligand-gated ion channel P2X7;¹¹⁴ and the non-receptor spleen tyrosine kinase (Syk), with additional cancer therapeutics.¹¹⁵

Bonnet *et al* synthesised a number of 8-alkoxy- and 8-(alkylamino)imidazo[1,2-*a*]pyrazines which demonstrated high antibronchospastic activities *in vitro* and *in vivo* whilst not exhibiting adverse central nervous system (CNS) stimulant effects observed by Theophylline, an anti-asthma agent.⁵¹ The lead compound from this study, SCA40 (Figure 17) also displayed vasorelaxant properties¹¹⁶ as a phosphodiesterase III (PDE3) inhibitor¹¹⁷ and potassium channel opener.¹¹⁸ Derivates of SCA40 have also been shown to possess similar properties.⁸¹

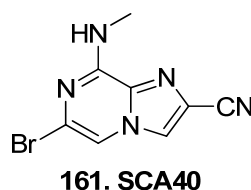


Figure 17: Imidazo[1,2-*a*]pyrazine, SCA40, described by Bonnet *et al*⁵¹

In addition to the PDE3 inhibitor, imidazo[1,2-*a*]pyrazines have been reported as PDE10 inhibitors for the prevention or treatment of neurological, psychiatric and metabolic disorders and diseases.¹¹⁹ Similarly, derivatives have been reported as gamma secretase modulators (GSM), which have potential applications in treating Alzheimer's disease.¹²⁰

Sabluyrolles *et al* synthesised imidazo[1,2-*a*]pyrazine derivatives with uterine-relaxing, antibronchospastic and cardiac-stimulating properties,⁴⁷ whilst Zurbonsen *et al* displayed antiproliferative, differentiating and apoptotic effects of imidazo[1,2-*a*]pyrazine derivatives.¹²¹ Imidazo[1,2-*a*]pyrazines have also been reported to display potent inotropic effects¹²² and to control allergic reactions.¹²³

Lumma *et al* showed imidazo[1,2-*a*]pyrazines to have selective affinity for α -adrenergic receptors, and therefore have potential as non-classical anti-depressants.⁵⁰ Similarly, Meurer *et al* reported imidazo[1,2-*a*]pyrazine derivatives that act as high affinity α_2 -adrenergic antagonists and therefore display potent hypoglycemic activity.⁵⁶ This potential for diabetic therapy was also reported for tetrahydroimidazo[1,2-*a*]pyrazines.¹²⁴

Strongly linked with diabetes is the issue of obesity, and it has been reported that imidazo[1,2-*a*]pyrazine derivatives modulate the activity of melanocortin-4 receptors, which have been implicated in the regulation of body weight.¹²⁵

Finally, the following applications have also been reported for imidazo[1,2-*a*]pyrazines: the prevention and treatment of viral infections, in particular the hepatitis C virus (HCV);⁵⁹ corticotrophin-releasing factor (CRF) antagonists for the treatment of irritable bowel syndrome;¹²⁶ orexin receptor antagonists for the treatment of sleep, mood and anxiety-related disorders;¹²⁷ treatment of diseases related to bone loss, for example osteoporosis;¹²⁸ and treatment and prevention of Duchenne muscular dystrophy.¹²⁹

To our knowledge, there are no applications of imidazo[1,2-*a*]pyrazines as antibacterial agents.

1.3 Project Aims & Objectives

Using the results of the virtual HTS, the initial aims of this project are to synthesise the target imidazo[1,2-*a*]pyrazines, test for inhibition with the VirB11 ATPase HP0525 and to use molecular modelling programs to evaluate potential interactions of these compounds within the enzyme active site.

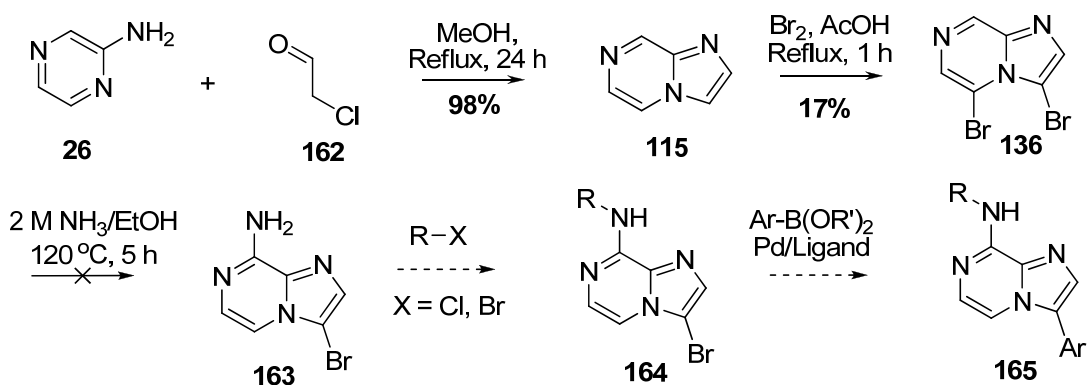
The secondary objective is to incorporate these putative ATP inhibitors into the synthesis of bivalent inhibitor reagents whereby one half of the chimera targets the nucleotide binding site and the other targets the unstable nature of the subunit-subunit interface which is in close proximity to the nucleotide binding site.

By synthesising a peptide based on the α F helix of HP0525 it is postulated that it could substitute the native α F helix and disrupt the opening and closing mechanism of the hexameric portal. This synthetic peptide will form the basis of second part of the bivalent inhibitor.

Chapter 2: Results & Discussion: Target Compounds

2.1 Initial Synthetic Approach

The imidazo[1,2-*a*]pyrazine ring is present in each of the target compounds, therefore, the planned initial synthetic approach was to first synthesise this core heterocycle, followed by functionalisation of the ring (Scheme 34).



Scheme 34: Planned initial synthetic route to access 3-aryl, 8-amino imidazo[1,2-*a*]pyrazines

The classical synthetic route towards imidazo[1,2-*a*]pyrazines involves a condensation between 2-aminopyrazine and chloroacetaldehyde. Previously reported methods using DMF as solvent⁴⁷ proved to be low yielding; however, the use of MeOH improved the yield to 98% (Table 2).

Entry	Solvent	Temp/°C	Time/h	Yield/%
1	DMF	Reflux	16	13
2	MeOH	Reflux	24	98

Table 2: Reaction conditions explored in the condensation reaction to form imidazo[1,2-*a*]pyrazine

Following formation of the core heterocycle **115**, attempts to brominate using Br₂ in AcOH resulted in extremely messy reaction profiles, which proved to be difficult to purify, and therefore low yields were observed. Table 3, entry 7 shows the highest yield obtained was 17%; however, this consisted of two different dibrominated regioisomers (9:1 ratio), which were only separable by reverse phase flash chromatography. After purification on a sample of the mixture, it was difficult to tell by NMR which dibrominated species had formed. COSY showed no coupling between the 3 singlet signals and therefore bromination at the 8-position can be ruled out. As previously discussed (Section 1.2.2.2.1), bromination is likely to occur at the 3,5- positions, with 3-bromo forming primarily; therefore the likely species formed are 3,5-dibromo or 3,6-dibromoimidazo[1,2-*a*]pyrazines. Attempts to obtain a crystal structure, *via* crystallisation using a Craig Tube proved to be unsuccessful with needle like crystals of approximately 0.2 μm observed.

Entry	Solvent	Temp: Br ₂ Addition/°C	Reaction temp/°C	Time/min	Yield/%
1	AcOH	0	0	20	0
2	AcOH	0	0	30	3
3	AcOH	RT	RT	60	Product could not be purified
4	AcOH	0	RT	90	Product could not be purified
5	AcOH	0	0	90	Product could not be purified
6	MeOH	0	RT	30	0
7	AcOH	RT	Reflux	60	17 ^a
8	AcOH	0	RT	60	8 ^b

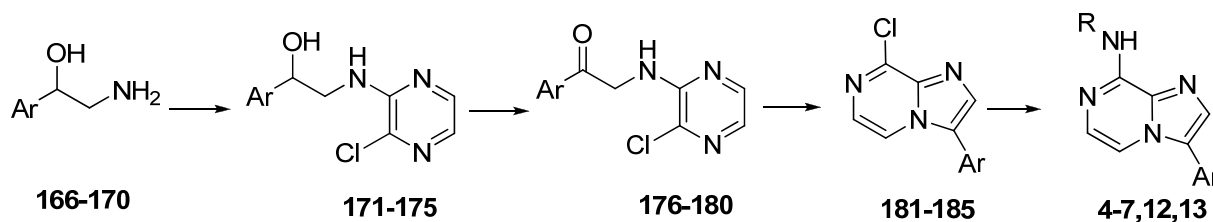
Table 3: Reaction conditions used for the bromination of imidazo[1,2-*a*]pyrazine, **115**. ^a Mixture of di-bromo regioisomers; ^b Mixture of mono-, di-, tri-bromo regioisomers.

The strategy to introduce the amine moiety in the 8-position involves a telesubstitution reaction. Unfortunately, no desired product was observed in the reactions attempted and therefore an alternative route was sought.

2.2 Synthesis of 3-Aryl Imidazo[1,2-*a*]pyrazines

2.2.1 Overview of the Synthesis

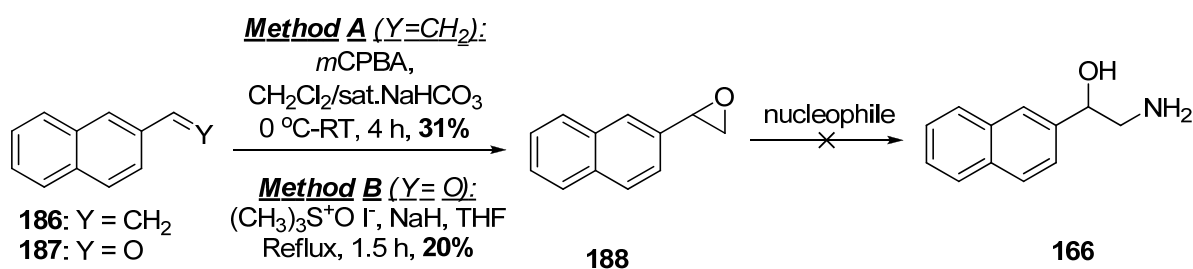
Through adapting the procedures of Meurer *et al*,⁵⁶ Lumma *et al*⁵⁰ and MacCoss *et al*,⁵⁷ and starting from the vicinal amino alcohol, the targeted 3-aryl substituted imidazo[1,2-*a*]pyrazines could be synthesised (Scheme 35). Coupling with 2,3-dichloropyrazine, would be followed by oxidation, dehydration-cyclisation and finally amine coupling. Two strategies were attempted to synthesise the key amino alcohol starting material: regioselective aminolysis of an epoxide; and *via* an azido ketone. These will be discussed in the following sections.



Scheme 35: Overview of the synthetic route towards 3-aryl imidazo[1,2-*a*]pyrazines

2.2.2 Formation of Amino Alcohol Moiety *via* Epoxide

The initial strategy to synthesise the key amino alcohol, was to form an epoxide followed by a regioselective ring opening using ammonia.⁵⁷ The epoxide of naphthalene was successfully synthesised using two methods: either the Prilezhaev reaction of alkenes with *meta*-chloroperbenzoate (mCPBA),^{130,131} or the Corey-Chaykovsky reaction of a sulfur ylide with a carbonyl compound¹³² (Scheme 36).



Scheme 36: Formation of 2-amino-1-(naphthalen-2-yl)ethanol, 166, *via* epoxide 188

Looking at Table 4, it is evident that the formation of the epoxide is low yielding for both methods used. The Prilezhaev reaction goes to near 100% completion in each of the attempts, but after column chromatography, a maximum yield of 31% is isolated suggesting the epoxide formed is unstable on the silica column. The Corey-Chaykovsky epoxidation can be carried out using either trimethyloxosulfonium iodide or trimethylsulfonium iodide where the use of a strong base, such as NaH, produces the nucleophilic sulphur ylide *in situ*. The former reagent was used in this case due to the relative stability of the later.

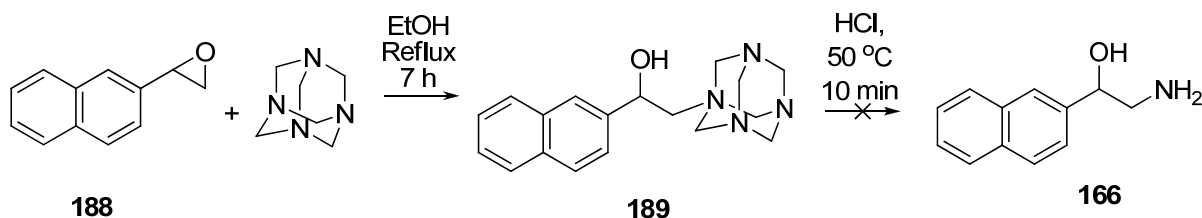
Entry	Method	Solvent	Time/h	Yield/%
1	A	$CH_2Cl_2/NaHCO_3$ (Sat.Aq)	10	0 92 Conversion ^a
2	A	$CH_2Cl_2/NaHCO_3$ (Sat.Aq)	10	14 90 Conversion ^a
3	A	$CH_2Cl_2/NaHCO_3$ (Sat.Aq)	10	31 92 Conversion ^a
4	B	THF	1.5	20 ^b
5	B	THF/DMSO (1:1)	1.5	0
6	B	THF	2	0 ^c
7	B	THF	3	2

Table 4: Variation in reaction conditions for the formation of 188. ^a Determined by ¹H NMR; ^b 2:1 Mixture of SM:Product by ¹H NMR; ^c Diol formation observed. Method A: $mCPBA$, $CH_2Cl_2/NaHCO_3$ (sat. aq.), RT; Method B: $(CH_3)_3S^+O^-I^-$, NaH, THF, Reflux.

It is very important that the solvent used is anhydrous, as any contamination can cause a reduction in the yield and no reaction at all (Entries 5, 6 & 7). The other issue is the difficult separation between the product and the starting aldehyde; numerous attempts to purify the product were necessary leading to degradation of the epoxide.

All subsequent attempts, exploring a number of amine nucleophiles, to ring open the epoxide, **188**, failed. Firstly 2 M ammoniacal ethanol⁵⁷ was used and after 2 h of heating at 100 °C in a sealed tube a green precipitate was isolated. The identity of this species is unknown, but it does not correspond to the desired amino alcohol from MS and NMR observations. There is a possibility that once a molecule of ammonia opens the epoxide, the resulting primary amine will be more nucleophilic. This will act as a competing nucleophile, leading to a complex reaction mixture.¹³³ There is also the known problem^{133,134} that aminolysis of epoxides bonded to an aromatic moiety results in a terminal alcohol, where the initial nucleophilic attack is directed towards the more stable tertiary carbocation. Epoxides bonded to an aliphatic substituent tend to result in the terminal amine being observed.

In light of this, an alternative source of amine nucleophile was investigated. Using a modified Delépine reaction,¹³⁵ reacting hexamethylenetetramine with epoxide **188** and cleaving the resulting tetramine salt with HCl should result in the terminal amine (Scheme 37). When refluxing in CHCl₃ no reaction was observed; however, when switching to EtOH, no signs of epoxide were evident after 7 h, and LCMS showed a minor product mass peak. On working up the reaction it became clear by NMR and LCMS that no desired product was evident and therefore no further work was carried out.



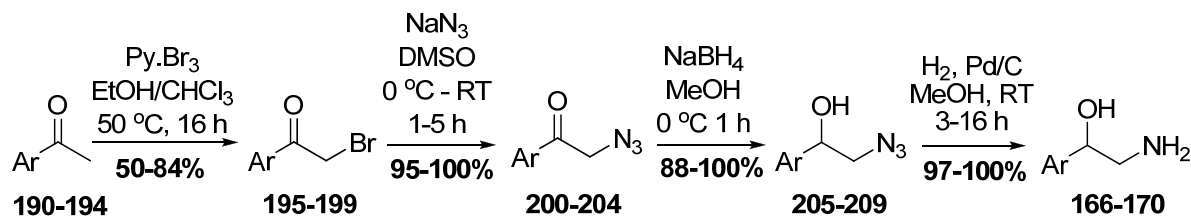
Scheme 37: Modified Delépine reaction between epoxide **188** and hexamethylenetetramine¹³⁵

It has previously been shown that microwaves display regioselective properties in many reactions including the aminolysis of epoxides.^{136,137} Subjecting epoxide **188** and ammonium acetate to microwave irradiation resulted in trace quantities of desired product by NMR and LCMS and no further work was investigated.

Further nucleophiles were explored in the form of sodium azide¹³⁸ and 4-methoxybenzylamine¹³⁹ with the aim of reducing the resulting species to a primary amine using Pd/C and H₂. No product was observed after either attempt. The use of 2-amino-3-chloropyrazine was also explored as a potential short-cut to access the pyrazinyl amino alcohol; however, no desired product was observed.

2.2.3 Formation of Amino Alcohol Moiety *via* Azido Ketone

Due to the lack to success with the epoxide aminolysis, an alternative approach was sought. Scheme 38 outlines the synthesis of the amino alcohol *via* an azido ketone.



Scheme 38: Reaction scheme for the synthesis of key amino alcohol *via* an azido ketone

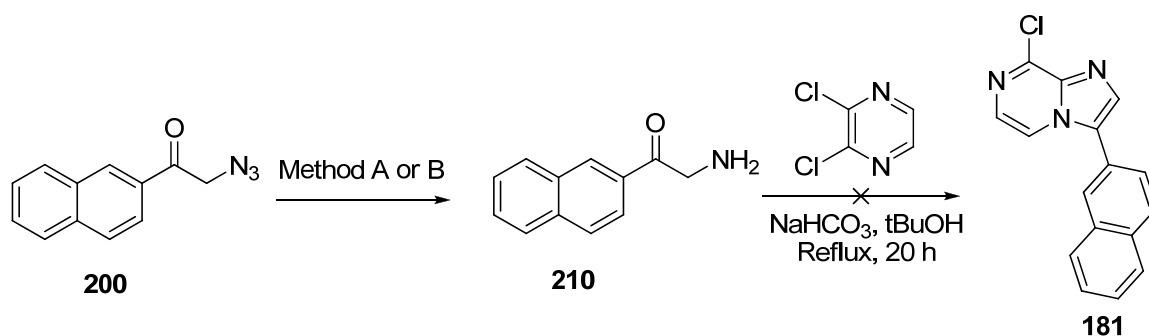
The first stage involves α -bromination of the aryl ketone using pyridinium tribromide.¹⁴⁰ In the majority of the cases, a mixture of mono- and di-bromated species is formed, the separation of which is achievable *via* flash chromatography. Following the reaction by TLC showed that the di-bromo species is formed immediately. Switching to Br₂ in AcOH,¹⁴¹ resulted in an extremely messy reaction profile, with no desired product observed by NMR.

The next three steps were carried out in near quantitative yields, without the use of any purification. Substitution of the α -bromine using sodium azide¹⁴² proceeded in 1-5 h providing at least 1.5-2 equivalents of sodium azide are used. In the cases where 1.2 eq were originally used,

when an extra portion of NaN₃ was added, an immediate change in the colour of the reaction and complete conversion to the desired azido ketone was observed.

Reduction of the ketone to give azido alcohols was successfully carried out using sodium borohydride in MeOH in just 1 h.¹⁴³ The use of sodium borohydride and activated alumina with Et₂O was originally explored,¹⁴⁴ with yields of 50% observed, and therefore these conditions were not employed. Hydrogenation at atmospheric pressure using 10% Pd/C¹⁴⁴ delivered the amino alcohols, with the exception of the thiophene analogue, which required 3 bar of H₂ to proceed. If purification was carried out at this stage, a loss of yield was observed and so it was decided to use the amino alcohols as crude for the next steps.

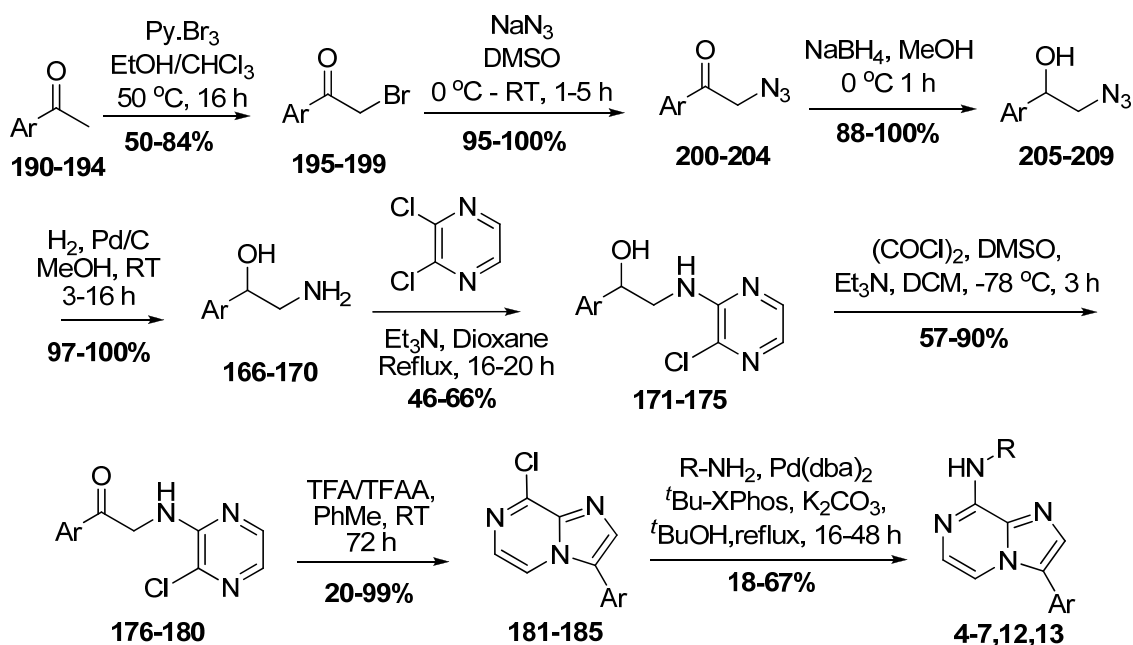
It was originally envisaged that reduction of the azido ketone to an amino ketone could be followed by coupling and cyclisation with 2,3-dichloropyrazine to form 8-chloro-3-arylimidazo[1,2-*a*]pyrazines (Scheme 39). **210** was successfully synthesised *via* hydrogenation of **200** (Method A),¹⁴² but the use of the Staudinger reduction (Method B)¹⁴⁵ proved to be unsuccessful. Stability tests of **210** showed that immediately after purification a single mass, corresponding to the desired product was observed by LCMS; however, after 3 days, a new peak corresponding to a mass of 349 was also apparent. It is not known what this mass relates to, but it is thought that the species could be polymerising. Attempts to react **210** with 2,3-dichloropyrazine to form **181** failed and so no further work was investigated with this approach.



Scheme 39: Potential synthetic route to access 8-chloro-3-(2-naphthyl)imidazo[1,2-*a*]pyrazine, **181**.
Method A: H₂, HCl, 10% Pd/C, MeOH, RT, 48 h: 27%; Method B: PPh₃, THF, RT, 24 h: 0%

2.2.4 Synthesis of 3-Aryl Imidazo[1,2-*a*]pyrazine from Amino Alcohol

With the synthesis of the key amino alcohol complete, the remainder of the synthesis could be completed. Scheme 40 outlines the overall reaction scheme carried out, with corresponding compound numbers and yields for each step highlighted in Table 2.



Scheme 40: Overall synthetic route to 3-aryl imidazo[1,2-*a*]pyrazines

Coupling of the amino alcohol with 2,3-dichloropyrazine,⁵⁷ afforded the pyrazinyl-amino alcohols in good yields, which was then followed by a Swern oxidation¹⁴⁶ to give the amino ketones, also in good yields. Initially trimethylamine sulphur trioxide complex was used for the oxidation in a variation of the Parikh-Doering oxidation.⁵⁷ However lower yields were observed (25% for **176** cf. 57% for Swern conditions) and so these conditions were not used for the remaining analogues.

Acid-induced cyclisation⁵⁷ furnished the core imidazo[1,2-*a*]pyrazine with the aromatic moiety in the 3-position. For all of the analogues, the reaction was carried out in toluene, but in the case of **183** (18%), the use of CH₂Cl₂ (20%) and concentrated conditions (4%) were also explored, suggesting that CH₂Cl₂ could be a viable alternative reaction solvent.

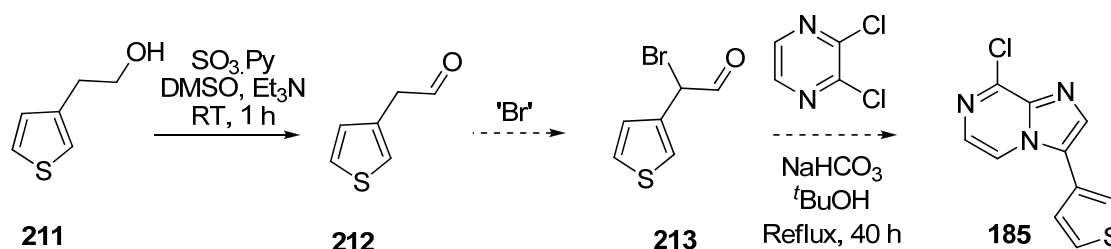
	Aromatic Moiety				
	Naphthalene	Phenoxyphenyl	Dimethoxy	Dimethyl	Thiophene
Aryl Ketone	190	191	192	193	194
α-Bromo Aryl Ketone	195	196 (84%)	197 (52%)	198 (50%)	199 (49%)
α-Azido Aryl Ketone	200 (100%)	201 (95%)	202 (98%)	203 (100%)	204 (99%)
α-Amino Aryl Ketone	205 (100%)	206 (97%)	207 (88%)	208 (99%)	209 (91%)
α-Amino Aryl Alcohol	166 (99%)	167 (100%)	168 (100%)	169 (97%)	170 (100%)^a
Pyrazinyl Alcohol	171 (63%)	172 (64%)	173 (58%)	174 (66%)	175 (46%)
Pyrazinyl Ketone	176 (57%)	177 (64%)	178 (78%)	179 (90%)	180 (76%)
8-Chloro Imidazo[1,2-<i>a</i>]pyrazine	181 (46%)	182 (100%)	183 (20%)	184 (25%)	185 (33%)
8-Sulfonamide Imidazo[1,2-<i>a</i>]pyrazine	4 (41%)	5 (26%)	6 (31%)	7 (67%)	-
8-Aniline Imidazo[1,2-<i>a</i>]pyrazine	12 (35%)	-	-	-	13 (18%)

Table 5: Corresponding compound numbers and reaction yields for Scheme 40. ^a Requires 3 bar H₂ for 5 h

In order to install the sulfonamide or aniline-sulfonamide groups, a Buchwald-Hartwig coupling approach was used.^{147,148} Pd(dba)₂ pre-catalyst and ^tBu-XPhos ligand¹⁴⁹ produced the final compounds in low to good yields. Non-catalytic conditions were also briefly explored: Et₃N in DMF for **5** gave no desired product and K₂CO₃ in MeCN in a sealed tube gave 16% **7** (cf. 67% for palladium conditions). Further optimisation of this final coupling step for a model system is discussed in Section 2.4.1. All of the analogues synthesised were screened in a biochemical assay, described in Section 2.5.1.1.

2.2.5 Alternative Approach to 3-Aryl Imidazo[1,2-*a*]pyrazines

In light of the lengthy synthetic scheme required to synthesise the 3-aryl imidazo[1,2-*a*]pyrazines, an alternative approach utilising the aromatic aldehyde was briefly explored (Scheme 41). The thienyl analogue was first investigated due to initial problems with formation of the amino alcohol. Oxidation under Parikh-Doering conditions¹⁵⁰ proved successful but purification proved to be extremely difficult with the highly sensitive and reactive aldehyde **212** and as such material could only be isolated with major impurities. Swern conditions¹⁴⁶ showed no signs of the desired product, with possible polymerisation evident.

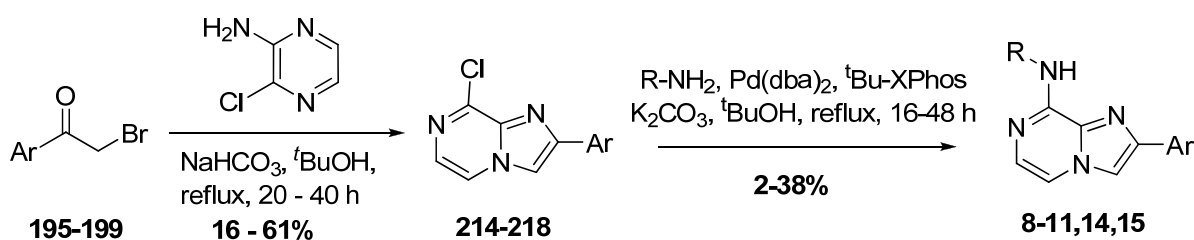


Scheme 41: Alternative synthetic scheme to access 3-aryl imidazo[1,2-*a*]pyrazines.

Unfortunately no further work was carried out trying to optimise the oxidation reaction; if successful, however, bromination followed by condensation is envisaged to produce **185**.

2.3 Synthesis of 2-Aryl Imidazo[1,2-*a*]pyrazines

The targeted 2-aryl imidazo[1,2-*a*]pyrazines can be accessed *via* a shorter 2-/3-step synthesis (Scheme 42). Starting from the α -bromoketones used in the synthesis of the 3-aryl isomers, condensation with 2-amino-3-chloropyrazine results in the core imidazo[1,2-*a*]pyrazine heterocycle with the aromatic moiety exclusively present in the desired 2-position. This is due to the more nucleophilic endocyclic nitrogen initiating the reaction by displacing the α -bromine and thus setting up the correct regiochemistry.



Scheme 42: Synthetic route to access 2-aryl imidazo[1,2-*a*]pyrazines

	Naphthalene	Phenoxyphenyl	Dimethoxy	Dimethyl	Thiophene
α-Bromo Aryl Ketone	195	196	197	198	199
8-Chloro Imidazo[1,2-<i>a</i>]pyrazine	214 (39%)	215 (32%)	216 (61%)	217 (41%)	218 (32%)
8-Sulfonamide Imidazo[1,2-<i>a</i>]pyrazine	8 (38%)	9 (13%)	10 (2%)	11 (29%)^a	-
8-Aniline Imidazo[1,2-<i>a</i>]pyrazine	14 (14%)	-	-	-	15 (8%)

Table 6: Corresponding compound numbers for Scheme 42. ^a Pd(dppf)Cl₂ (2 mol%), Cs₂CO₃, PhMe used.

The regiochemistry of the 2- and 3- substituted imidazo[1,2-*a*]pyrazines was confirmed by 2D NMR. In the case of the 2-substituted imidazo[1,2-*a*]pyrazines, NOE spectroscopy showed coupling through space between the protons in the 3- and 5- positions of the imidazo[1,2-*a*]pyrazine ring. HMBC (Figure 18) also showed correlation between the protons and carbons of these positions. Conversely, with the 3-substituted imidazo[1,2-*a*]pyrazines neither coupling nor correlation was observed between the protons in the corresponding 2- and 5- positions.

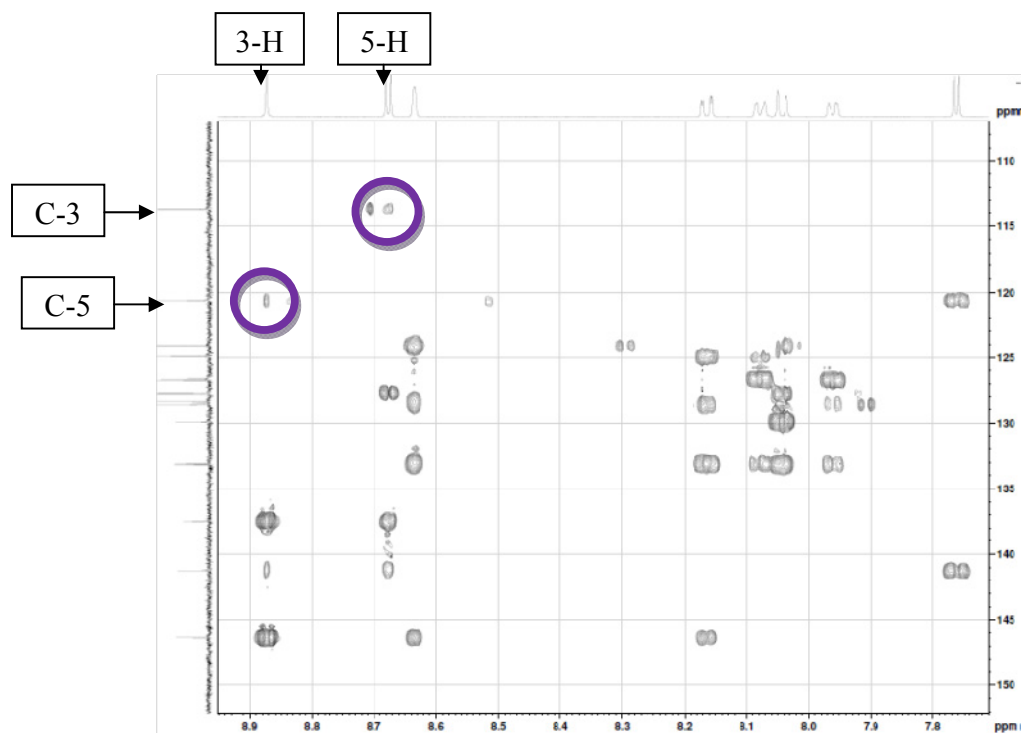


Figure 18: HMBC spectrum of 214, indicating coupling of H₃ with C₅ and H₅ with C₃

The final step involves the same Buchwald-Hartwig coupling conditions employed in the synthesis of the 3-aryl imidazo[1,2-*a*]pyrazines. In the case of **11**, no desired product was isolated using Pd(dba)₂ and ^tBu-XPhos and so a different catalyst system: Pd(dppf)Cl₂ was used. All of the analogues synthesised were screened in a biochemical assay, as described in Section 2.5.1.1.

2.3.1 Optimisation of Condensation Reaction

Table 7 details the different reaction conditions used for the condensation between 2-(bromoacetyl)naphthalene, **195**, and 2-amino-3-chloropyrazine to form 8-chloro-2-(2-naphthyl)imidazo[1,2-*a*]pyrazine, **214**. Initially yields of approximately 20% were observed when using NaHCO₃ and EtOH solvent, which could be increased to 38% when switching to Et₃N and 1,4-dioxane; however, an extremely messy reaction profile followed by a difficult purification meant these conditions were undesirable. Keeping the NaHCO₃ base constant, a solvent screen was carried out, with the crude reaction mixture analysed by LCMS. A comparison of the yields by UV and ES⁺ TIC could then be made, and it can be seen that using ^tBuOH or IPA led to increased yields. Scaling of these conditions resulted in observed yields of 39% and 26% respectively (Entries 10 & 11).

Entry	Method	Base/Acid	Solvent	Temp/°C	Time/h	Yield/%
1	A	NaHCO ₃	EtOH	Reflux	6	20 ^a
2	A	Et ₃ N	Dioxane	Reflux	40	38
3	A	NaHCO ₃	EtOH	Reflux	16	16
4	A	NaHCO ₃	EtOH	Reflux	22	22
5	B	NaHCO ₃	MeOH	Reflux	20	2/0
6	B	NaHCO ₃	EtOH	Reflux	20	70/22 ^b
7	B	NaHCO ₃	IPA	Reflux	20	100/45 ^b
8	B	NaHCO ₃	^t BuOH	Reflux	20	92/50 ^b
9	B	NaHCO ₃	DMF	Reflux	20	37/36 ^b
10	A	NaHCO ₃	^t BuOH	Reflux	40	39
11	A	NaHCO ₃	IPA	Reflux	40	26
12	A	KO ^t Bu	DMF	70	16	0
13	A	KO ^t Bu	NMP	100	16	0
14	A	Cs ₂ CO ₃	MeCN	70	16	8
15	C	HCl (cat.)	H ₂ O/THF	85	40	17
16	D	NaHCO ₃	^t BuOH	160	10 min	1
17	D	NaHCO ₃	MeCN	160	10 min	5
18	D	NaHCO ₃	DMF	160	10 min	4

Table 7: Optimisation of the condensation reaction to form 214. ^a NaHCO₃ still present; ^b Not isolated yield: values are % peak area by ES+/UV respectively from LCMS; Methods A: Basic condensation reaction; Method B: Solvent screening and analysing crude mixtures by LCMS; Method C: Acidic condensation; Method D: Microwave irradiation (20 bar pressure limit)

Briefly exploring other solvent and base combinations, acid-catalysed condensation conditions and microwave irradiation had no beneficial effect on the reaction yields. With compound **214**,

purification was difficult and the flash columns utilised are extremely time consuming due to the polar nature of the heterocycle. However, it should be noted that this compound is extremely insoluble and as such, isolation can be partially achieved by taking the crude mixture (after extraction), adding CH₂Cl₂ and MeOH (~1:1) and filtering off the remaining precipitate. The resulting solid was confirmed as the desired product with high purity, whereas the filtrate contains starting materials, side-products and a proportion of the product. Further purification of the filtrate by flash chromatography can then be used to isolate the remaining product.

2.3.2 Tautomerisation of Imidazo[1,2-*a*]pyrazines

In the ¹H-NMR spectra of the imidazo[1,2-*a*]pyrazine compounds the H₅ and H₆ protons are defined doublets (Figure 19) with a coupling constant value of approximately 4.5 Hz for the 8-chloro precursors, and 5.4 Hz for the 8-aniline final compounds. However, for compounds **4-11** where the sulfonamide is bound directly to the imidazo[1,2-*a*]pyrazine in the 8-position, tautomerisation is observed and as a result the doublets are less defined and become broad (Figure 20).

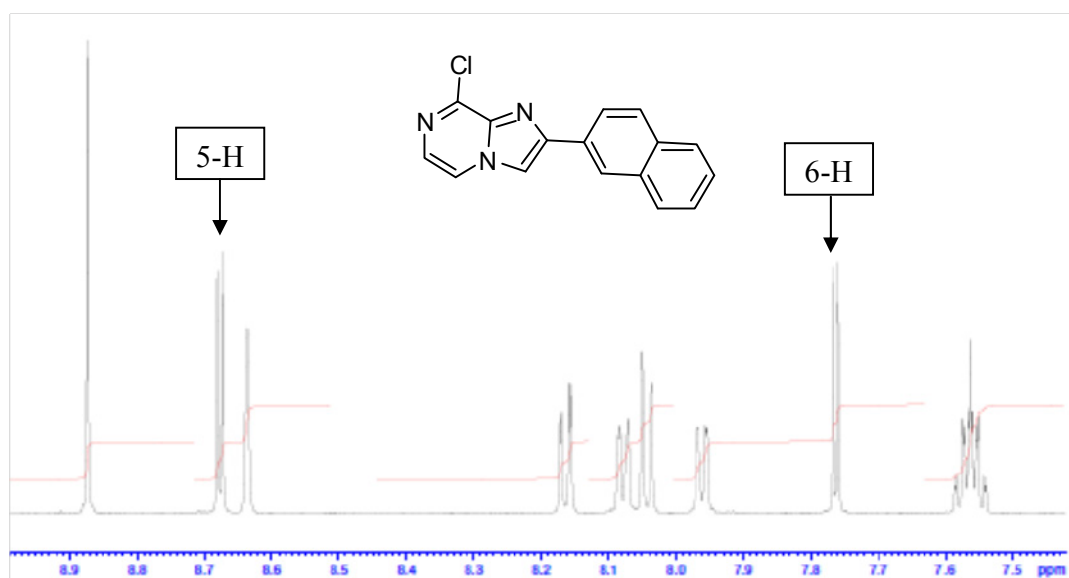


Figure 19: ¹H NMR (600 MHz, (CD₃)₂SO) spectra of compound 214 showing sharp doublet signals for H₅ and H₆

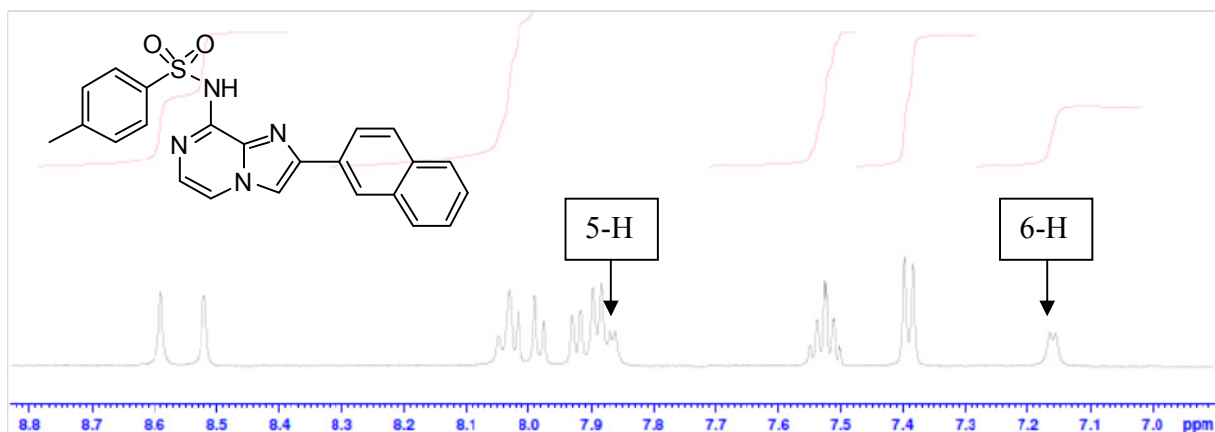


Figure 20: ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$) spectra of compound 8 showing broad doublet signals for H_5 and H_6

In some cases where the sulfonamide is bound directly to the pyrazine ring, H_6 is actually observed as a triplet (Figure 21), which couples to both H_5 and the broad doublet NH signal of H_7 (Figure 22 and Figure 23).

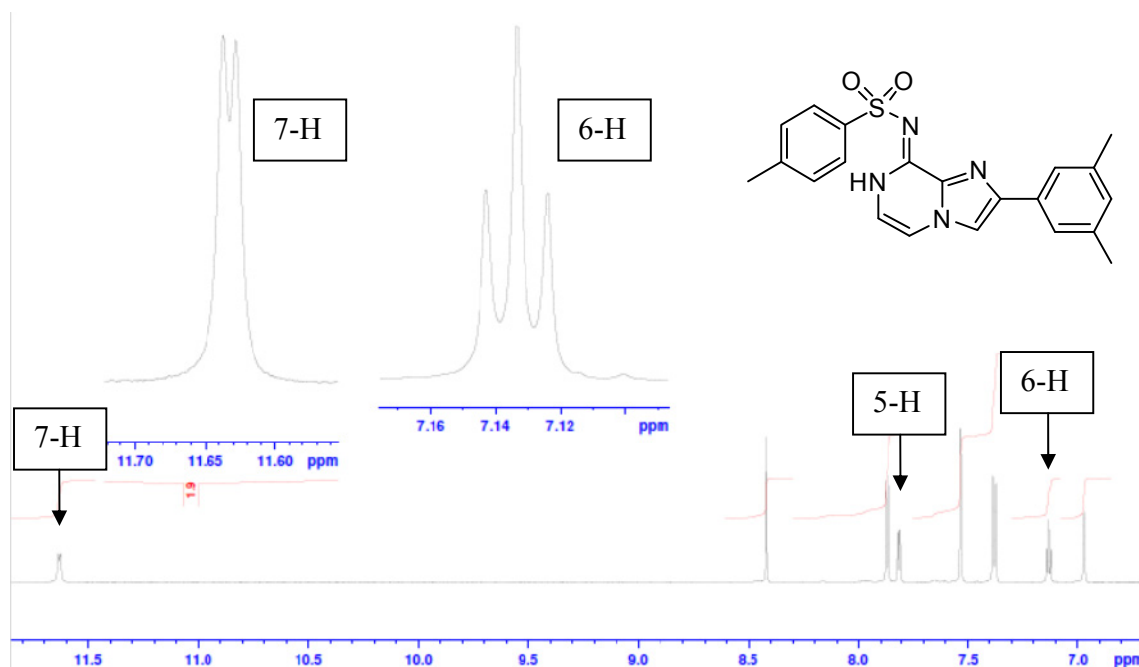


Figure 21: ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$) spectra of compound 11 showing triplet signal of H_6 and broad doublet signals of H_7

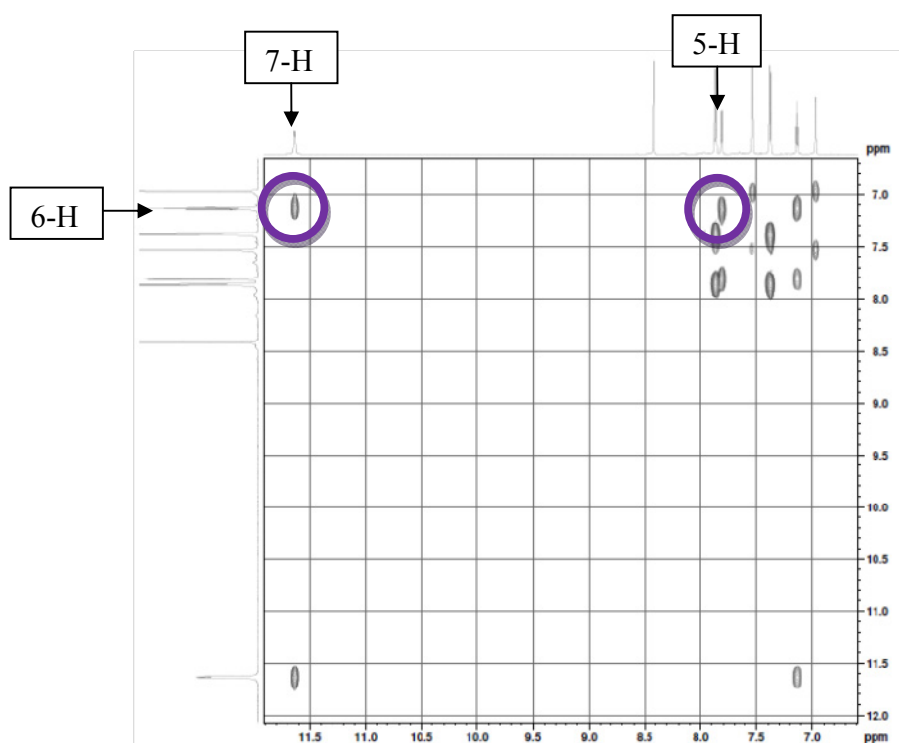


Figure 22: COSY spectrum of compound 11 showing coupling of H₆ with H₇ and H₅

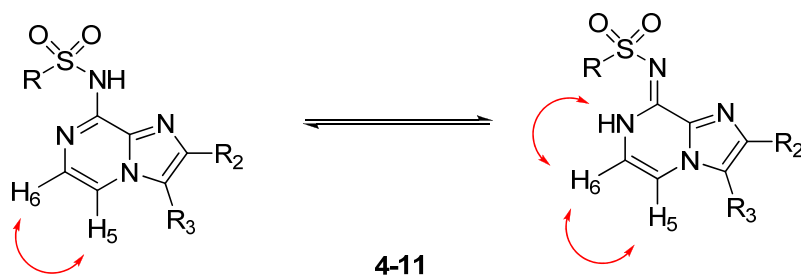
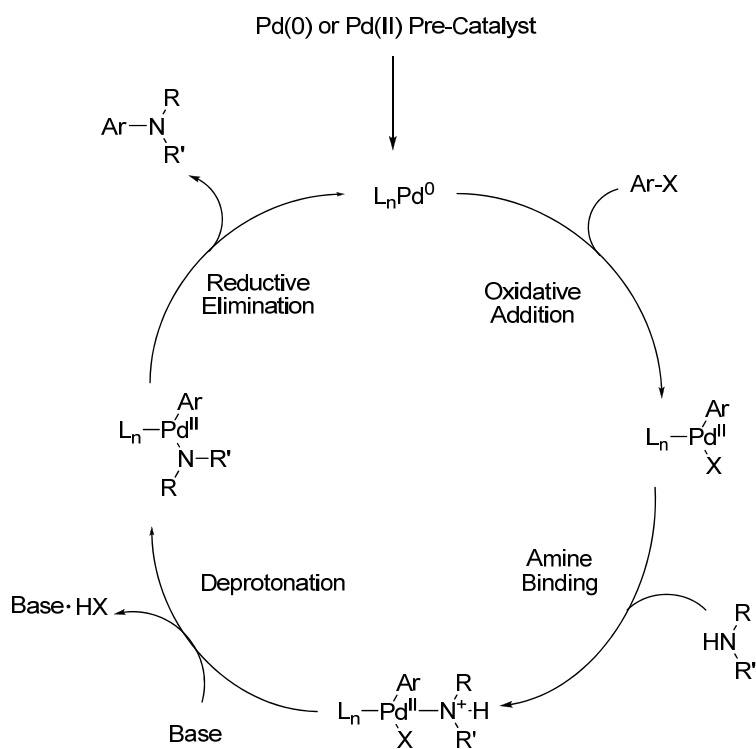


Figure 23 Tautomers of imidazo[1,2-*a*]pyrazines, indicating the coupling protons

2.4 Buchwald-Hartwig Cross Coupling

Buchwald-Hartwig cross coupling is the palladium-catalysed reaction between aryl halides or pseudohalides and primary or secondary amines to form aryl amines.¹⁴⁸ The generally accepted mechanism of this widely used transformation is given in Scheme 43.¹⁴⁸ Oxidative addition of the aryl halide and palladium species is followed by binding of the amine species. Deprotonation then occurs, followed by reductive elimination to yield the reaction product; or by β -hydride elimination if a β -hydride is present in the amine.



Scheme 43: Generalised catalytic cycle for Buchwald-Hartwig cross coupling¹⁴⁸

Each of the different reaction components used are factors which determine the outcome of the reaction. The choice of aryl halide potentially influences the rate of the different catalytic steps and the electronic and steric properties of the amine influence its rate of binding, deprotonation and reductive elimination.¹⁴⁸ The choice of strong and weak bases influences the rate, functional group tolerance and by-product formation;¹⁴⁸ the palladium source will impact that formation of the active catalytic species; the solvent will determine substrate solubility; and temperature influences the rate of coupling and formation of by-products.¹⁴⁸

Prior to the development of the Buchwald-Hartwig cross coupling, formation of aryl amines through C-N bond forming reactions relied on high temperature Ullman-type copper mediated procedures,¹⁵¹ non-regiospecific aryne chemistry,¹⁵² or the highly reducing $\text{S}_{\text{RN}}1$ reaction.¹⁵³ The first instance of Pd-catalysed aryl amine formation involved coupling an aryl bromide with tin amides.¹⁵⁴

Hartwig was then able to suggest a catalytic cycle through isolating and obtaining crystal structures of Pd(0) complexes and intermediates,¹⁴⁷ which was then altered with the discovery of

tin free aryl-amine couplings.^{155,156} Ligand development has led to a greater mechanistic understanding as well as milder conditions.

Much attention in past years has focused on optimising conditions for various substrates, with particular attention to ligand design. These include chelating diphenylphosphino ligands such as BINAP,¹⁵⁷ dppf¹⁵⁸ and Xantphos;¹⁵⁹ electron rich chelating phosphines such as JosiPhos;¹⁶⁰ *N*-heterocyclic carbenes;¹⁶¹ and tri-alkylphosphines.¹⁶² Dialkylbiaryl phosphines, as first introduced by Buchwald,¹⁶³ compare favourably, with a versatile family of structurally related ligands now in place to complete the transformations with a variety of substrates.¹⁴⁸

Optimal ligand and other reaction parameters can vary depending on substrate combinations and the following section will investigate the optimisation of the Buchwald-Hartwig cross coupling between the chloro-imidazo[1,2-*a*]pyrazines and 4-toluenesulfonamide and *N*-(4-aminophenyl)-4-methylbenzenesulfonamide.

2.4.1 Optimisation of Buchwald-Hartwig Cross Coupling Reaction of Imidazo[1,2-*a*]pyrazines

From looking at Scheme 40 and Scheme 42, it is evident that the final Buchwald-Hartwig coupling requires much optimisation. In light of data recorded from the biochemical assay (Section 2.5.1.1) and the relative cost of the lead compounds, it was decided to carry out all optimisation using the naphthalene-containing analogues.

The conditions used in the optimisation of **8** are detailed in Table 8. Initially the ferrocene derived ligand 1,2,3,4,5-pentaphenyl-1'-(di-*tert*-butylphosphino)ferrocene 'Q-Phos',¹⁶⁴ (Figure 24) was used with bis(dibenzylideneacetone)palladium(0), Pd(dba)₂ pre-catalyst, but in both toluene and THF, no product was observed. Switching the ligand to 2-di-*tert*-butylphosphino-2',4',6'-triisopropylbiphenyl '*tert*-butyl XPhos' (*t*Bu-XPhos)¹⁴⁹ resulted in no product, but when changing from THF to *t*BuOH a yield of 38% was observed.

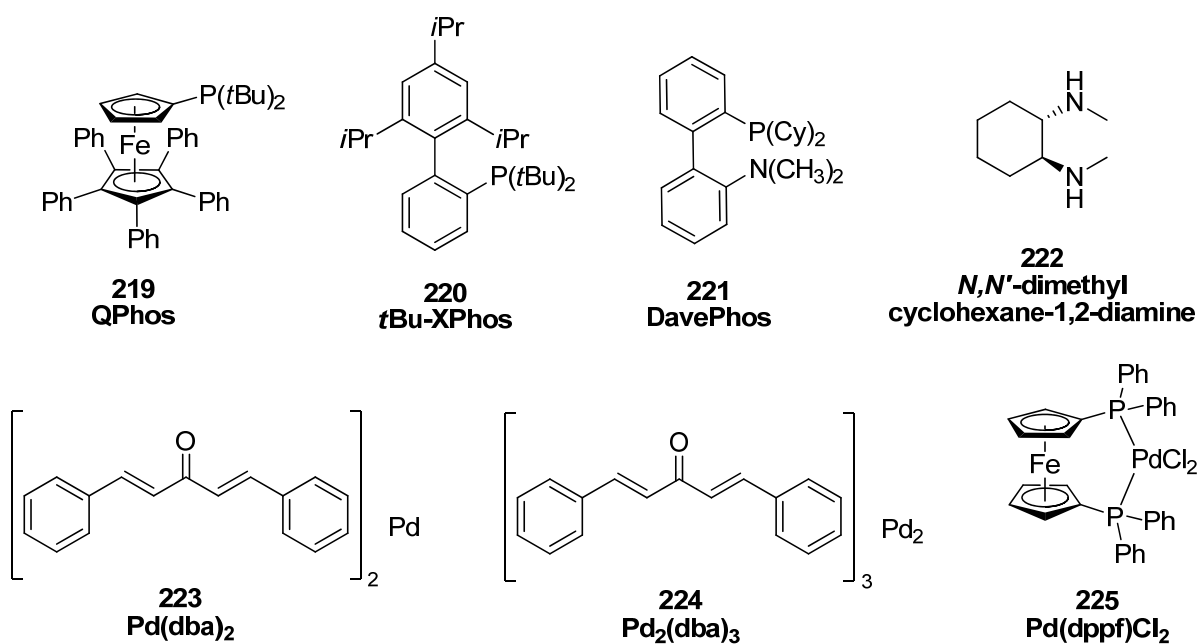
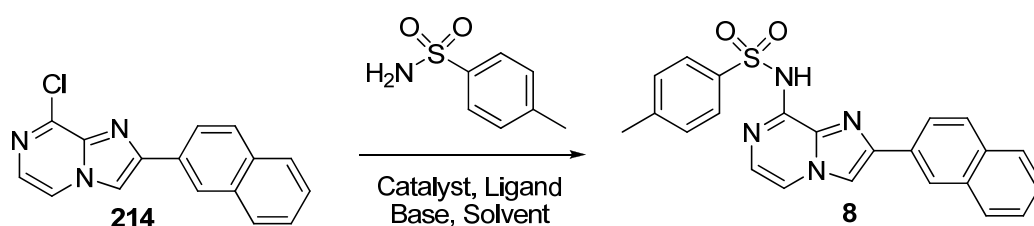


Figure 24: Structures of various catalysts and ligands used in Buchwald-Hartwig coupling

Keeping the solvent and catalyst constant and switching the base to Cs_2CO_3 and the ligand to 2-dicyclohexylphosphino-2'-(*N,N*-dimethylamino)biphenyl, 'DavePhos'¹⁶³ had no positive impact (Entry 6). However, when altering the solvent to dioxane¹⁶⁵ a dramatic increase in yield to 66% was achieved (Entry 7). A similar yield was observed when changing again to NaO^tBu and Toluene (Entry 11), as was the case with $\text{Pd}(\text{OAc})_2$ and NaO^tBu /Toluene (Entry 15); however, if Cs_2CO_3 is used, the yield observed is only 12% (Entry 10). This illustrates that certain combinations of solvents and bases are favoured over others.

With these two systems in mind, the pre-catalyst was switched to $\text{Pd}_2(\text{dba})_3$ and the yields were further boosted to 81% and 93% for Cs_2CO_3 /dioxane and NaO^tBu /toluene respectively (Entry 12 and 13). These results give two possible systems to be used depending on the solubility of the reacting species. The use of microwave irradiation was briefly investigated; however, no positive effect on the outcome of any of the systems used was observed, but a more vigorous screening process could improve the yields.



Entry	Method	Catalyst	Ligand	Base	Solvent	Temp/°C	Time/h	Yield/%
1	A	Pd(dba) ₂ (1 mol%)	Q-Phos (2 mol%)	NaO ^t Bu (1.5 eq)	Toluene	80	22	0
2	A	Pd(dba) ₂ (1 mol%)	Q-Phos (2 mol%)	NaO ^t Bu (1.5 eq)	THF	50	72	0
3	A	Pd(dba) ₂ (1 mol%)	^t Bu-XPhos (5 mol%)	K ₂ CO ₃ (1.2 eq)	THF	Reflux	24	0
4	A	Pd(dba) ₂ (1 mol%)	^t Bu-XPhos (5 mol%)	K ₂ CO ₃ (1.2 eq)	^t BuOH	Reflux	24	38
5	B	Pd(dba) ₂ (1 mol%)	^t Bu-XPhos (5 mol%)	K ₂ CO ₃ (1.2 eq)	^t BuOH	120	30 min	11
6	A	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4 eq)	^t BuOH	Reflux	24	5
7	A	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4 eq)	Dioxane	Reflux	24	66
8	A	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4 eq)	Dioxane	105 ^a	24	17
9	B	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4 eq)	Dioxane	130	30 min	1
10	A	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4 eq)	Toluene	Reflux	16	12
11	A	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.4 eq)	Toluene	Reflux	24	67
12	A	Pd ₂ (dba) ₃ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4 eq)	Dioxane	Reflux	24	81
13	A	Pd ₂ (dba) ₃ (1 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.4 eq)	Toluene	Reflux	24	93
14	B	Pd ₂ (dba) ₃ (1 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.4 eq)	Toluene	160	10 min	46
15	A	Pd(OAc) ₂ (0.5 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.2 eq)	Toluene	Reflux	21	64
16	A	Pd(OAc) ₂ (0.5 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.2 eq)	Toluene	120 ^a	21	28
17	B	Pd(OAc) ₂ (0.5 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.2 eq)	Toluene	95	30	0

Entry	Method	Catalyst	Ligand	Base	Solvent	Temp/°C	Time/h	Yield/%
18	B	Pd(PPh ₃) ₄ (5 mol%)	-	NaO ^t Bu (1.4 eq)	Toluene	160	10 min	8
19	B	Pd(dppf)Cl ₂ (5 mol%)	-	NaO ^t Bu (1.4 eq)	Toluene	160	10 min	38
20	A	CuI (15 mol%)	Diamine ^b (30 mol%)	K ₂ CO ₃ (2 eq)	DMF	100	72	3
21	C	-	-	DIPEA (5 eq)	NMP	140	24	0
22	C	-	-	DIPEA (3.8 eq)	Dioxane	100	21	0
23	C	-	-	NaH (2 eq)	DMSO	RT	21	0
24	C	-	-	NaH (2 eq)	DMSO	100	24	18
25	C	-	-	NaH (2 eq)	DMF	100	24	22
26	D	-	-	DIPEA (1.5 eq)	MeCN	160	10	0

Table 8: Optimisation of the coupling reaction to form compound 8. ^aReaction carried out in sealed tube; ^b *N,N'*-dimethylcyclohexane-1,2-diamine; Method A: Buchwald-Hartwig conditions; Method B: Microwave (20 bar pressure limit) Buchwald-Hartwig conditions; Method C: Solvent/base conditions; Method D: Microwave (20 bar pressure limit) solvent/base conditions

The use of copper provides an alternative to palladium chemistry in the Buchwald-Hartwig coupling.^{166,167} The system of CuI and *N,N'*-dimethylcyclohexane-1,2-diamine¹⁶⁶ only afforded 3% product (Entry 20) and in light of the success with the palladium conditions, no further investigations were carried out.

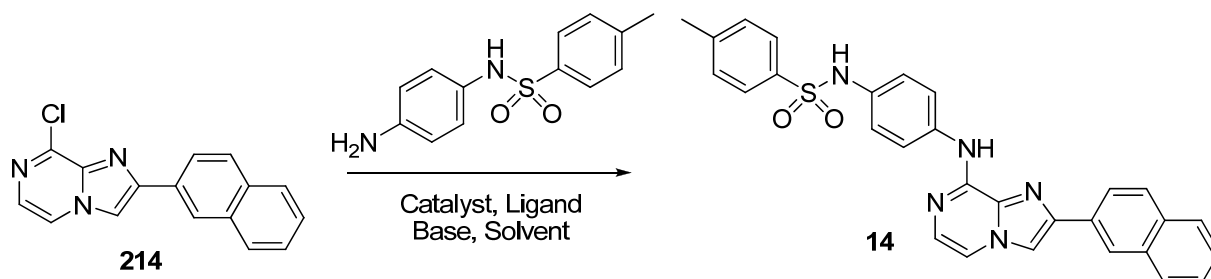
Non-catalytic coupling conditions to **214** were also investigated (Entries 21-26). The use of DIPEA with NMP and dioxane¹⁰⁴ gave no product yield, and the use of a stronger base (NaH) with DMSO¹²⁹ only resulted in product when the reaction vessel was heated.

The optimised conditions of Pd₂(dba)₃, DavePhos, NaO^tBu and toluene was also applied to the repeated synthesis of **5** and **13** with yields of 14% and 31% observed respectively, compared to the original conditions of 26% and 18% respectively. Partially-optimised conditions (Pd(dba)₃, DavePhos, Cs₂CO₃ and dioxane) were also applied to **9** and **10** and yields of 14% (from 13%) and 24% (from 2%) were observed respectively.

The other amine that has been used in the 8-position is *N*-(4-aminophenyl)-4-methylbenzenesulfonamide and so some of the conditions used in the synthesis of **8** have been applied in the synthesis of **14** (Table 9).

Purification of **14** is extremely difficult, with co-elution with either of the starting materials often observed. This is possibly due to numerous π -stacking interactions, and separation was best achieved using toluene as eluent. This purification issue is a reason for the lower yields observed compared with **8**. However a direct comparison should not be made as the chemistry of the sulfonamide differs from that of the aniline and therefore a more comprehensive screening of catalysts and ligands that specialise in aniline coupling reactions is recommended.

One of the optimum conditions for **8**, Cs_2CO_3 and dioxane, only gives small quantities of product **14** and this could be due to the relative solubilities of the amines used. The use of NaO^tBu and toluene does, however, give the desired product. Interestingly, the use of microwave irradiation does enhance the reaction yield from 22% to 47% and so further screening of conditions here is recommended.



Entry	Method	Catalyst	Ligand	Base	Solvent	Temp/°C	Time/h	Yield/%
1	A	$\text{Pd}(\text{dba})_2$ (1 mol%)	$^t\text{Bu-XPhos}$ (5 mol%)	K_2CO_3 (1.2 eq)	$^t\text{BuOH}$	Reflux	24	14
2	A	$\text{Pd}(\text{dba})_2$ (1 mol%)	DavePhos (3 mol%)	Cs_2CO_3 (1.4)	Dioxane	Reflux	40	6
3	A	$\text{Pd}_2(\text{dba})_3$ (1 mol%)	DavePhos (3 mol%)	NaO^tBu (1.4 eq)	Toluene	Reflux	24	22
4	B	$\text{Pd}_2(\text{dba})_3$ (1 mol%)	DavePhos (3 mol%)	NaO^tBu (1.4 eq)	Toluene	160	10 min	47
5	B	$\text{Pd}(\text{PPh}_3)_4$ (5 mol%)	-	NaO^tBu (1.4 eq)	Toluene	160	10 min	23

Entry	Method	Catalyst	Ligand	Base	Solvent	Temp/°C	Time/h	Yield/%
6	B	Pd(dppf)Cl ₂ (5 mol%)	-	NaO ^t Bu (1.4 eq)	Toluene	160	10 min	4
7	C	-	-	Et ₃ N (1.2 eq)	DMF	Reflux	40	0
8	C	-	-	DIPEA (3.8 eq)	Dioxane	100	20	0
9	C	-	-	DIPEA (5 eq)	NMP	140	20	0
10	C	-	-	DIPEA (2.5 eq)	<i>n</i> -BuOH	120	16	0
11	C	-	-	NaH (2 eq)	DMSO	100	24	0
12	C	-	-	NaH (2 eq)	DMF	100	24	0
13	D	-	-	DIPEA (1.5 eq)	MeCN	160	10 min	0

Table 9: Optimisation of the coupling reaction to form compound 14. Method A: Buchwald-Hartwig conditions; Method B: Microwave (20 Bar pressure limit) Buchwald-Hartwig conditions; Method C: Solvent/base conditions; Method D: Microwave (20 bar pressure limit) solvent/base conditions

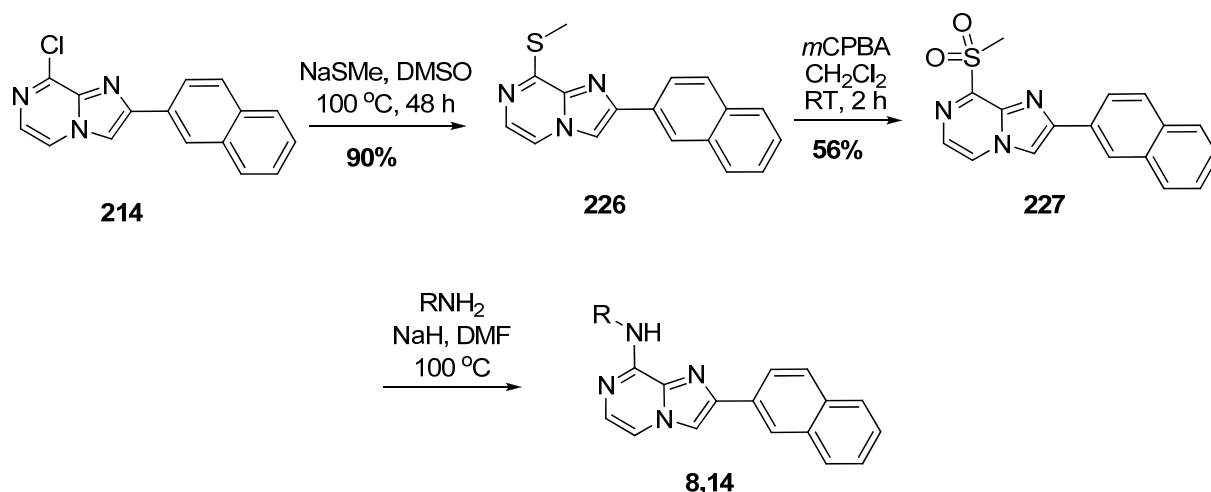
2.4.2 Displacement of Methyl Sulfone Moiety of Imidazo[1,2-*a*]pyrazines as an Alternative to Buchwald-Hartwig Cross Coupling Reaction

As discussed in Section 1.2.2.2.2 methyl sulfones have been used to attach a variety of nucleophiles onto imidazo[1,2-*a*]pyrazine rings.^{86,87} The methyl sulfone leaving group provides an alternative to the Buchwald-Hartwig coupling conditions and therefore provides an opportunity to introduce sulfonamides containing palladium sensitive moieties.

Scheme 44 outlines the synthesis of the heterocycle primed with the methyl sulfone. Nucleophilic displacement of the chloride is achieved in high yields using sodium thiomethoxide in DMSO. Oxidation to the sulfone is accomplished using *m*CPBA, although an excess is required to avoid partial oxidation to the sulfoxide (**228**).

Table 10 shows that displacement of 4-toluenesulfonamide occurs at RT, albeit in low yields. The use of NaH with either DMSO or DMF at 100 °C results in high yields of desired product (Entries 2 and 3), compared with the low yielding corresponding reaction with the chloro leaving group (Table 8, Entries 24 and 25). A very brief exploration in the use of microwave irradiation

to assist the nucleophilic displacement of the methyl sulfone showed no product observed (Entry 4). When switching the amine to *N*-(4-aminophenyl)-4-methylbenzenesulfonamide, no displacement was observed (Entries 5-7).

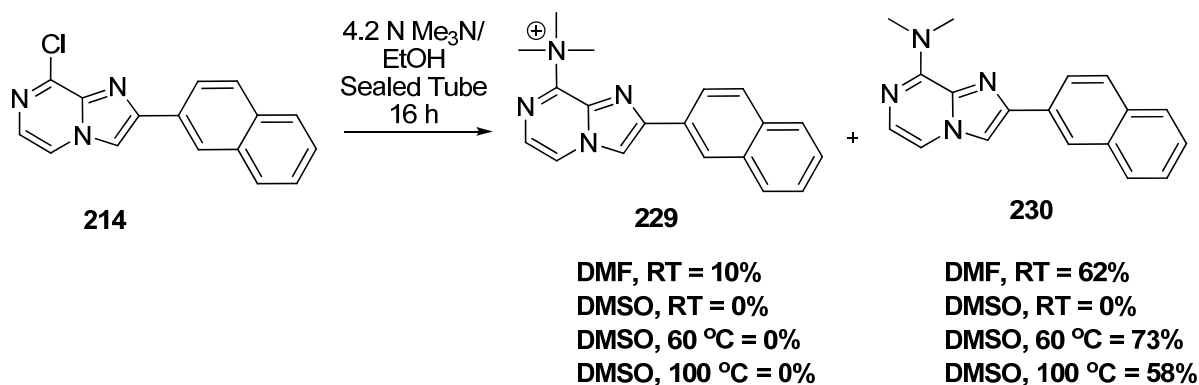


Scheme 44: Synthesis of 227 and the ensuing methyl sulfone displacement reaction

Entry	Amine	Method	Base	Solvent	Temp/°C	Time/h	Yield/%
1	4-toluene sulfonamide	A	NaH (2 eq)	DMSO	RT	20	18
2	4-toluene sulfonamide	A	NaH (2 eq)	DMSO	100	20	62
3	4-toluene sulfonamide	A	NaH (2 eq)	DMF	100	20	72
4	4-toluene sulfonamide	B	DIPEA (2 eq)	MeCN	160	10	0
5	<i>N</i> -(4-aminophenyl)-4-methylbenzenesulfonamide	A	NaH (2 eq)	DMSO	RT	18	0
6	<i>N</i> -(4-aminophenyl)-4-methylbenzenesulfonamide	A	NaH (2 eq)	DMSO	100	18	0
7	<i>N</i> -(4-aminophenyl)-4-methylbenzenesulfonamide	B	DIPEA (2 eq)	MeCN	160	10 min	0

Table 10: Conditions used for methyl sulfone displacement of 227 using 4-toluene sulfonamide and *N*-(4-aminophenyl)-4-methylbenzenesulfonamide. Method A: Solvent/base; Method B: Microwave (20 bar pressure limit) solvent/base

A trimethylammonium leaving group was also explored as an alternative to the methyl sulfone;¹⁶⁸ however, its synthesis proved to be difficult (Scheme 45). When DMF is used as a co-solvent and the reaction is carried out at RT the desired product is isolated in just 10% yield with dimethylamino (**230**) by-product forming as the major product.

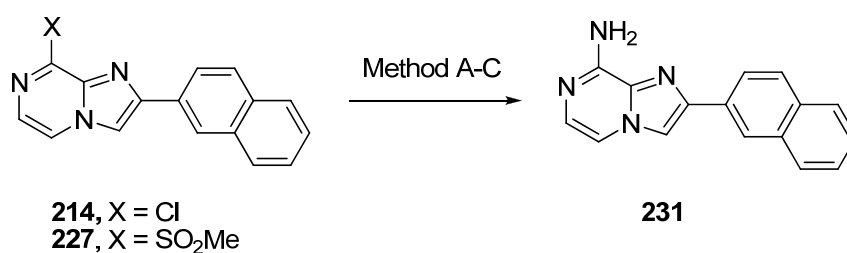


Scheme 45: Synthesis of **229**

2.4.3 Exploring the Synthesis of 2-Aryl Imidazo[1,2-*a*]pyrazin-8-amine

Substituting the chloro or methyl sulfone for an NH₂ group would provide the opportunity for the coupling reaction to be carried out with either the sulfonyl chloride or aryl chloride. Some initial studies were carried out to access **231** (Table 11), including the use of palladium catalyst with ligand (Entries 8 & 9),¹⁶⁹ but low yields were obtained and work into the ensuing coupling step was not investigated due to time constraints.

It is evident that the desired product is easier to make when using **227** compared with **214**. Heating in a sealed tube is required, and yields of 20 and 27% are observed (Entries 1-3). It should also be noted that the use of a non-nucleophilic solvent should be used, because when 7.0 M NH₃/MeOH was used, methoxide displacement occurred to give **232** as the major product (Figure 25).



Entry	Method	Catalyst	Ligand	Base	Solvent	Temp/ °C	Time/ h	Yield/ %
1	A	-	-	-	2 M NH ₃ /IPA	RT	16	0
2	A	-	-	-	2 M NH ₃ /IPA	100	16	20
3	A	-	-	-	7 M NH ₃ /MeOH	80	16	27
4	B	-	-	-	2 M NH ₃ /EtOH	120	24	20
5	B	-	-	-	NH ₄ OH (Sat.Aq.)	90	16	7
6	B	-	-	NH ₄ OH (sat.aq.)	IPA	90	16	Trace
7	B	-	-	NH ₄ OAc	2 M NH ₃ /IPA	90	72	Trace
8	B	Pd(dba) ₂ (1 mol%)	DavePhos (1.2 mol%)	LHMDS (3 eq)	Dioxane	100	48	0
9	B	Pd(dba) ₂ (0.5 mol%)	^t Bu-XPhos (1.2 mol%)	NHMDS (1.2 eq)	THF	65	16	0
10	C	-	-	LiNH ₂ (1 eq)	THF	Reflux	16	0

Table 11: Variation in reaction conditions for formation of compound 231. Method A: Sealed tube with 227; Method B: Sealed tube with 214; Method C: Reflux with 214.

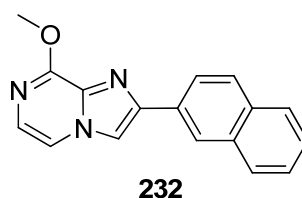


Figure 25: Major product isolated in the reaction between 227 and 7.0 M NH₃/MeOH

2.5 Evaluation of Target Compounds

2.5.1 Biochemical Assay

In order to determine whether a compound inhibits an enzyme, an assay is required to measure its activity. There are many different types of assays that can be used,^{170,171} including spectrophotometric, fluorometric, calorimetric and chemiluminescent. An ATPase assay kit from Innova Biosciences was used as a colorimetric (spectrophotometric) assay to determine the inhibition of the library of imidazo[1,2-*a*]pyrazines synthesised. A P_iColorLock™ Gold reagent, developed for measuring activity of any P_i-generating enzymes in microplates, provides an alternative to more hazardous radioactive methods and other less sensitive colorimetric assays. It is an improved Malachite Green formulation that gives a stable end-point signal and is not prone to precipitation. By binding to free phosphate generated in the hydrolysis of ATP, a colour change from orange to green is observed (Figure 26).

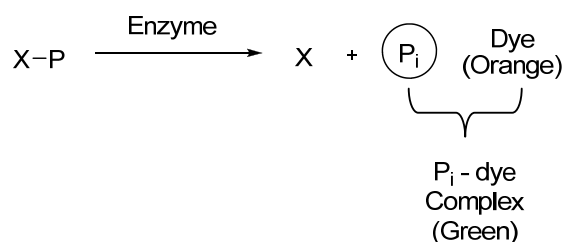


Figure 26: Principle of Innova Biosciences ATPase assay kit

Initially, ATP (125 μM), HP0525 (0.053 μM) and the potential inhibitors (500 μM) were incubated at 37°C in a 96-well plate. After 30 min, the reaction was quenched by the addition of the Gold Mix (P_iColorLock™ Gold and Accelerator) followed by stabiliser after 2 min. After a further 30 min the plate was read at a wavelength of 620 nm and absorbance measured (the plate was read twice and an average recorded). By subtracting the absorbance of the negative control (protein added after the Gold Mix and so corrects for all free P_i not produced by enzyme during incubation), the percentage of absorbance relative to non-inhibited HP0525 (positive control) was calculated. Hits were then validated by repeating the procedure for inhibitor concentrations of 5, 50 and 500 μM , and selected compounds were assayed again at additional concentrations ranging between 0.5 and 200 μM to determine IC₅₀ values.

IC₅₀ is used universally as an indication of how potent an inhibitor is and is defined as the concentration of a compound required to inhibit an enzyme by 50%.¹⁷⁰ By obtaining the IC₅₀ values for the imidazo[1,2-*a*]pyrazine compounds synthesised, comparisons can be made and conclusions can start to be drawn as to the structure activity relationship of the inhibitors.

To establish IC₅₀ values, the measuring points were optimized so that they cover the range to fit a sigmoidal dose response curve. Each compound was screened on 3 separate plates and a mean was calculated for each concentration. The data was normalised by subtracting the negative control (0% active) and relating it to the positive control (100% active). The software GraphPad Prism 5 was used to generate 2 dose-response curves and the IC₅₀ values were calculated from each.¹⁷²

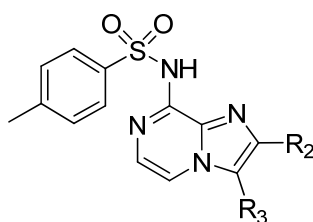
1. Log [inhibitor] vs. normalised response (Standard Slope)
2. Log [inhibitor] vs. normalised response (Variable Slope)

2.5.1.1 Biochemical Assay Data

The hit validation data and IC₅₀ values^b for the imidazo[1,2-*a*]pyrazine compounds synthesised are illustrated in Table 12 and Table 13. Selected dose response curves are illustrated in Figure 71 (Appendix, Section 7.1).

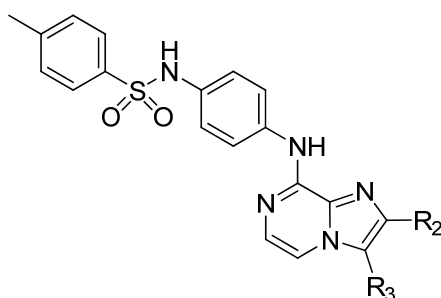
Looking at the 500 µM column for the hit validation results, it is evident there are high values even though the compound clearly inhibits at a lower concentration. This is due to precipitation of the compound at the high concentration and as a result is adsorbing the visible light at 620 nm. The data clearly shows that all of the imidazo[1,2-*a*]pyrazine compounds are inhibitors of HP0525, with some more potent than others. From looking at the IC₅₀, the most potent compound is **14** with an IC₅₀ of 7 µM (Table 13, Entry 1).

^b IC₅₀ experiments carried out by Dr K. Wallden



Entry	Compound	R2	R3	Hit Validation % Absorbance ^a			IC ₅₀ (μM) ^b	cLogP ^c	LogS
				500 μM	50 μM	5 μM			
1	8		H	83 (8)	106 (7)	98 (8)	109 (88/ <u>131</u>)	4.5	-6.7
2	9		H	36 (11)	22 (2)	94 (1)	82 (82/82)	4.7	-7.1
3	10		H	61 (35)	88 (1)	93 (2)	163 (167/158)	3.1	-5.4
4	11		H	78 (18)	95 (2)	98 (1)	134 (133/135)	4.1	-6.1
5	4	H		96 (5)	76 (2)	108 (4)	77 (77/77)	4.4	-6.7
6	5	H		69 (48)	95 (2)	113 (1)	97 (<u>124</u> /70)	4.7	-7.1
7	6	H		37 (3)	102 (4)	112 (4)	135 (136/134)	3.1	-5.4
8	7	H		-4 (0)	76 (3)	100 (1)	146 (147/146)	4.1	-6.1

Table 12: Hit validation screen and IC₅₀ values for 4-methyl-N-(aryl-imidazo[1,2-a]pyrazin-8-yl)benzenesulfonamides. ^a Error values in brackets; ^b Value given is an average of two methods used. The individual values are given in brackets (standard slope first, variable slope second). Where a value is underlined, this is the preferred method as suggested by the program GraphPad Prism 5; ^c cLogP and LogS values determined using AlogP 2.1 from the virtual computational chemistry laboratory¹⁷²



Entry	Compound	R2	R3	Hit Validation % Absorbance ^a			IC ₅₀ (μM) ^b	cLogP ^c	LogS
				500 μM	50 μM	5 μM			
1	14		H	79 (11)	2 (2)	78 (2)	7 (<u>7</u> /7)	5.9	-7.6
2	15		H	0 (0)	40 (5)	91 (1)	108 (99/117)	4.6	-6.3
3	12	H		99 (55)	5 (7)	81 (3)	20 (21/ <u>20</u>)	5.9	-7.6
4	13	H		68 (5)	119 (0)	128 (3)	54 (48/ <u>60</u>)	4.5	-6.3

Table 13: Hit validation screen and IC₅₀ values for 4-methyl-*N*-(4-(aryl-imidazo[1,2-*a*]pyrazin-8-ylamino)phenyl)benzenesulfonamides. ^a Error values in brackets; ^b Value given is an average of two methods used. The individual values are given in brackets (standard slope first, variable slope second). Where a value is underlined, this is the preferred method as suggested by the program GraphPad Prism 5; ^c cLogP and LogS values determined using AlogP 2.1 from the virtual computational chemistry laboratory¹⁷²

From looking at the data, some concluding points can be made. There does not seem to be a preference for the position of the aromatic moiety, with both 2- and 3-regioisomers giving similar results. Compounds with a larger aromatic moiety seem to be more potent, as do the compounds with the aniline bound in the 8-position.

2.5.1.2 Compound **14** is a competitive inhibitor of ATP^c

The first observation from the dose-response curves generated (Figure 27) is that the compounds synthesised are HP0525 antagonists in that they stop the hydrolysis of ATP, inhibiting the signal produced by the HP0525-ATP coupling.

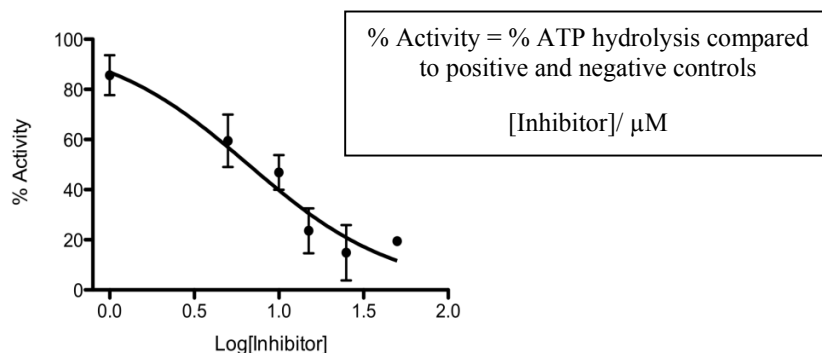


Figure 27: Dose response curve for **14**

Inhibitors can either be reversible or irreversible and binding can either occur at the substrate active site (nucleotide binding site in this case) or at an allosteric site.^{170,171} Since there are no apparent allosteric sites, and no covalent bonds can form between the inhibitor and enzyme, the imidazo[1,2-*a*]pyrazine series are reversible inhibitors.

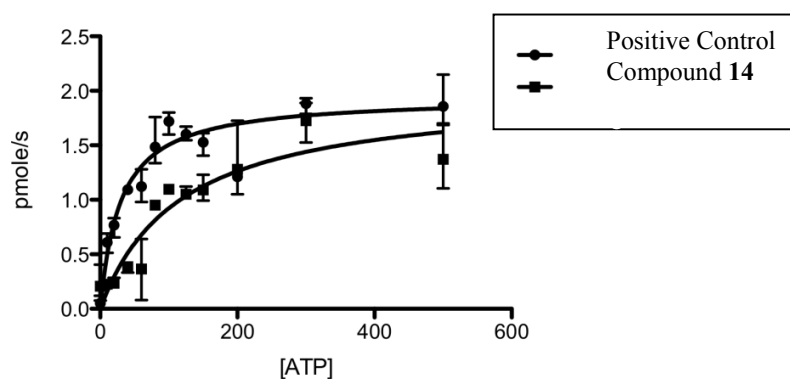
Unfortunately all attempts to co-crystallise the lead compound **14** with HP0525 have proved unsuccessful. Therefore, in order to rely on computational docking to study interactions within the active site and for the project to proceed, it was important to ascertain whether or not the imidazo[1,2-*a*]pyrazine compounds inhibit competitively, non-competitively or uncompetitively.

By using Michaelis-Menten kinetics,¹⁷³ and measuring the reaction rate of ATP hydrolysis with respect to ATP concentration (0-500 μM) with and without (positive control) inhibitor **14** (10 μM), an indication of the type of inhibition can be derived (Figure 28).

The Michaelis Constant (K_m) indicates the substrate concentration at which the reaction rate is half its maximum value ($\frac{1}{2}V_{\text{max}}$). The maximal rate (V_{max}) represents the maximum rate achieved by the system at maximum (saturating) concentrations. The kinetic data of **14** is characteristic of competitive inhibitors whereby K_m is increased and V_{max} remains approximately the same.^{170,171}

^c Experiments Carried out by Dr K.Wallden

With non-competitive inhibitors, K_m stays the same and V_{max} decreases; and for uncompetitive inhibitors both K_m and V_{max} decrease. Therefore **14** is a competitive inhibitor of ATP, and it has been assumed that the remaining imidazo[1,2-*a*]pyrazine compounds are also competitive inhibitors.



	V_{max} (pmole/s)	K_m (μM)
No Inhibitor	1.947	29.42
With 14	2.001	116.9

Figure 28: Michaelis-Menten plot for rate of ATP hydrolysis with and without 14, with relative V_{max} and K_m values

2.5.2 Molecular Modelling

An important tool in developing lead drug compounds is through studying their interactions with the target in question. One method of visualising the position of a molecule within an enzyme active site is through generating crystal structures of the protein soaked with the compound, and then using various modelling programs to view the data. In the absence of a co-crystallisation, but with the aid of the enzyme crystal structure, computational docking programs can be used as an alternative technique.

Docking is an automated computer algorithm that determines how a compound may bind in the active site of the protein.¹⁷⁴ Through scanning the different conformations and orientations the algorithm finds the lowest-energy conformation and hence suggests the favourable location and orientation of the substrate in the active site.

It is the one of the most heavily used tool in computational drug design¹⁷⁴ as it can offer a fast, accurate method for predicting whether a compound will be a good inhibitor of the protein in question. The protein studied in this work is the VirB11 ATPase HP0525 and currently there are five protein structures of HP0525 available from the Protein Data Bank:¹⁷⁵

- 1NLZ – *apo*-HP0525 (hexamer, no substrate);
- 1NLY – ATP γ S-HP0525 (dimer with non hydrolysable ATP γ S);
- 1G6O – ADP-HP0525 (dimer with ADP);
- 1OPX – Sulfate-HP0525 (dimer with sulfate);
- 2PT7 – HP0525 with inhibitory regulator HP1451.

Structural studies showed the active site of ADP-HP0525 and ATP γ S-HP0525 to be very similar,^d whereas the conformation of the *apo* form is variable. Therefore, for docking purposes, any of the four chains (two from ADP-HP0525 and two from ATP γ S-HP0525) could be used. The protein file chosen to run the docking experiments was ADP-HP0525. All heteroatoms (molecules that are not part of the protein, such as PEG chains and H₂O molecules), and the crystal ADP were removed from the file prior to docking.

The docking program AutoDock was used to carry out the docking, and it is designed to predict how small molecules bind to a receptor of known 3D structure. There are currently 2 generations of software: AutoDock 4¹⁷⁶ and AutoDock Vina.¹⁷⁷ AutoDock 4 was initially used but it has its disadvantages: it takes a long time to set up the docking, the set-up used also requires LINUX to run the docking, and the actual docking was very time-consuming.

Therefore the use of AutoDock Vina was investigated. It achieves significant improvements in the average accuracy of the binding mode predictions and is also orders of magnitude faster than AutoDock 4 (8.41 min compared with 521.85 min per complex).¹⁷⁷ Initial comparisons of the data of the imidazo[1,2-*a*]pyrazines with AutoDock 4 showed similar binding modes, and therefore all docking presented herein is generated from Vina.

^d Experiments carried out by Dr Paul Gane; Data not shown.

In order to run Vina the following information is required:

- Target Protein – the pdb file needs to be converted from .pdb to .pdbqt (AutoDock Tools, ADT,¹⁷⁶ used to perform this conversion);
- Ligand to be docked – the file needs to be in .pdbqt format (ADT used to perform this conversion);
- Docking File: this contains the protein and ligand files and search parameters, including the target grid for Docking. This is a grid box drawn around the nucleotide binding site which encompasses all residues involved in binding, and directs the docking of the ligands to this site. Coordinates used were the same as those used for AutoDock 4 (See Section 6.4 for details).

AutoDock Vina makes use of the windows command prompt (cmd) in order run, and will dock each ligand separately. Runs cannot be queued and, much like AutoDock 4, a separate docking file needs to be written for each ligand. It is sufficient to use if investigating a small number of ligands, but if screening of a library of ligands was desired, the process would be very time consuming. Therefore a program called PyRx¹⁷⁸ that runs using Vina was investigated.

Using this program, you can input all the different ligand files (in .pdb or .mol2 format) and it will automatically convert them to .pdbqt format, and run the docking into the specified target protein. Results will then be outputted in an excel format as well as individual ligand .pdbqt formats for analysis. The only disadvantage of using PyRx is that, at the time of screening, you cannot input the coordinates for the grid box. Each time you run the program, you have to manually draw the box in the area you want docking to be directed which affects the reproducibility. In the majority of the cases studied, comparisons of the docking poses showed only small differences between the two methods.

Therefore, in the work carried out herein, all ligands were docked, as part of a library, using PyRx, and selected compounds were also screened using the cmd function. All images generated are the lowest energy conformation from cmd, unless stated otherwise and are visualised using PyMOL.¹⁷⁹

2.5.2.1 Results of Molecular Modelling

Figure 29 shows the lead compound **14** in the active site of ATP γ S-HP0525, illustrating its orientation with respect to the crystal ligand ATP γ S. As can be seen, the naphthalene moiety would appear to occupy the purine region, the core imidazo[1,2-*a*]pyrazine, the ribose region and the aniline-sulfonamide moiety, the phosphate region.

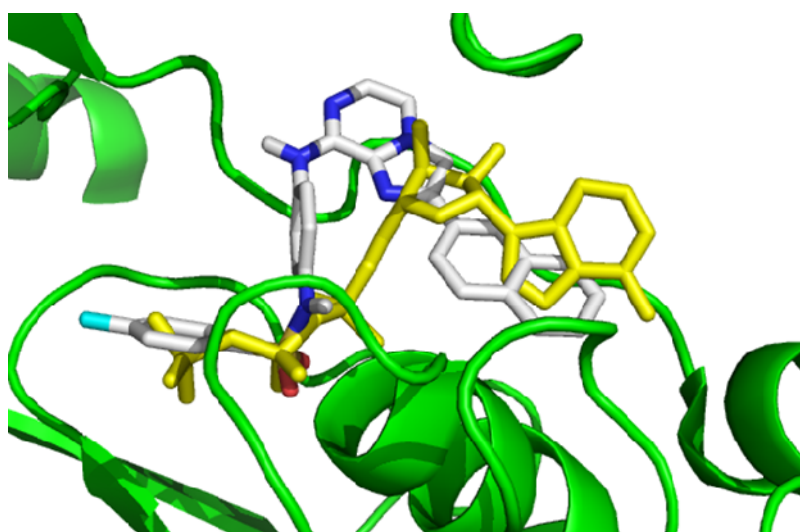


Figure 29: Image of **14** and ATP γ S (Yellow) in the crystal structure of ATP γ S-HP0525. PDB: 1NLY. Image generated using PyMOL

From studying the orientation and the H-bonding interactions of **14** (Figure 30), it is possible to suggest alternative compounds to synthesise. The sulfone moiety interacts with Gly-181, Lys-184 and Thr-185, all of which are involved with the binding of the phosphates of in ADP and ATP γ S.³⁶ Therefore by introducing bioisosteres of phosphates in place of the sulfonamide moiety in position 8 enhanced activities could be achieved. In addition by introducing heteroatoms into the naphthalene ring of **14**, and in doing so, increasing the similarity with purine, increased binding could be observed.

The purine in ADP and ATP γ S interacts *via* π -stacking with Tyr-140 within the nucleotide binding site. It is also evident that in the purine pocket there are numerous other hydrophobic residues where the naphthalene moiety of **14** could also interact. Figure 31 illustrates that there is

further room within this pocket for the aromatic moiety to extend. Therefore, alternative compounds to synthesise could include bi-aryl or even tri-aryl moieties.

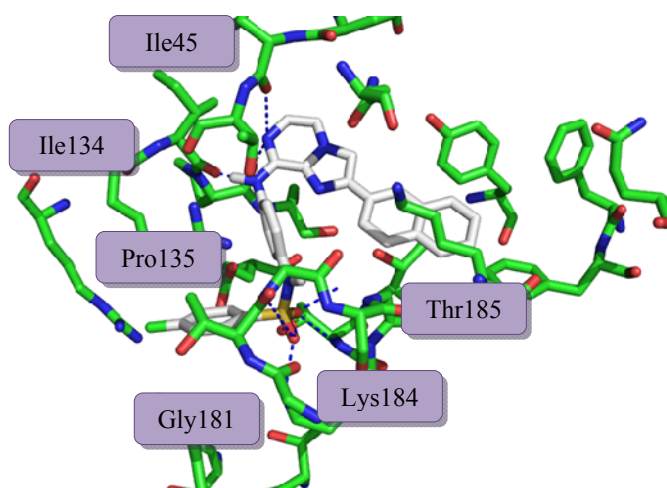


Figure 30: Image showing H-bonding interactions of **14** within the nucleotide binding site of ATP γ S-HP0525. PDB: 1NLY. Image generated using PyMOL

Investigating the surface of the nucleotide binding site with the docked **14** (Figure 31) a potential area for extension of the imidazo[1,2-*a*]pyrazine core could be in either the 5- or 6- positions ('R5/6 position'). Addition of a small moiety could improve binding and therefore potency of the inhibitor.

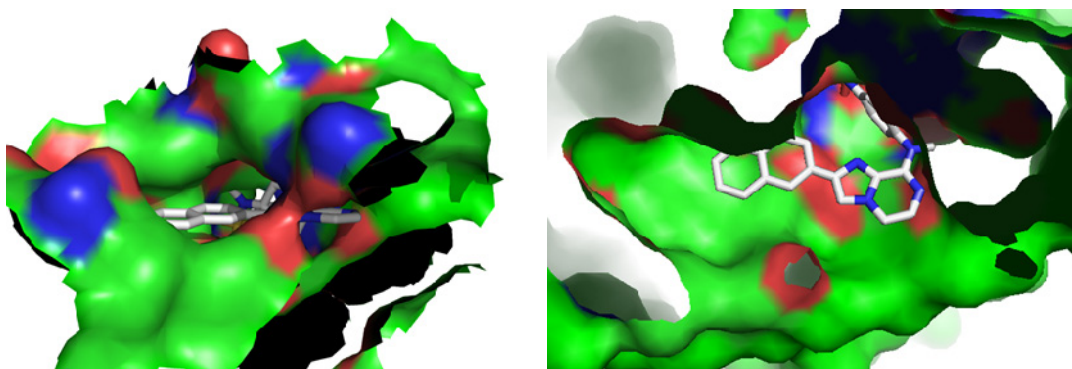


Figure 31: Images indicating the surface of the nucleotide binding site with **14** in the crystal structure of ATP γ S-HP0525. PDB: 1NLY. Images generated using PyMOL

It is evident that there is not too much difference between the activities of the 2- and 3-aryl regioisomers. Comparing **14** and **12** (naphthalene regioisomers) very similar orientations within the nucleotide binding site are observed (Figure 32). The sulfonamide of **14** aligns with the β -phosphate of ATP γ S and that of **12** aligns with the γ -phosphate and this strong binding would be responsible for the similar IC₅₀'s observed.

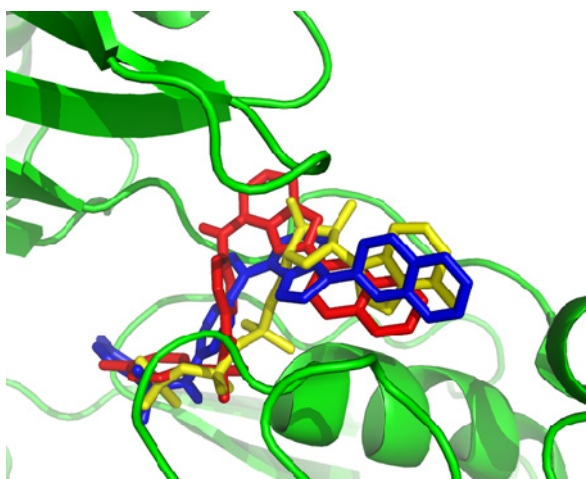


Figure 32: Overlay of **14** (Red) and **12** (Blue) with ATP γ S (Yellow) in ATP γ S-HP0525. PDB: 1NLY.
Image generated using PyMOL

It is also apparent that the larger *N*-(4-aminophenyl)-4-methylbenzenesulfonamide in the 8-position seems to be more potent than where the sulfonamide is directly bound to the heterocycle. Figure 33 and Figure 34 illustrate the difference in binding modes between **8** and **14**; and **4** and **12** respectively. As can be seen, compounds where the sulfonamide is directly bound adopt a conformation where the sulfonamide points away from the phosphate region and out into the extra pocket in the 'R5/6 position' of **14**. This would lead to a difference in binding affinity and therefore potency.

This orientation is also observed with other analogues where the sulfonamide is bound directly to the imidazo[1,2-*a*]pyrazine. There are poses generated, however, where the sulfonamide does point into the phosphate pocket. It would be necessary to synthesise longer-sulfonamide analogues of **9**, **10**, **11**, **5**, **6** and **7** to further enhance the above claim. Due to the relative activities of these compounds, it would be preferential to start with **9**.

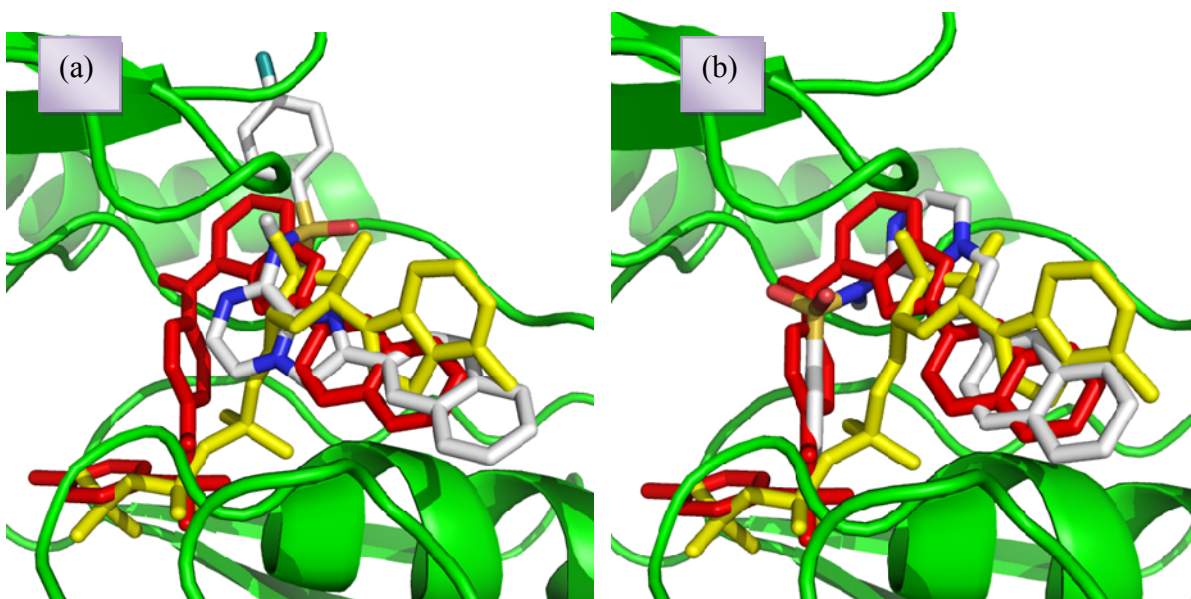


Figure 33: Overlay of 8 and 14 (Red) with ATP γ S (Yellow) in ATP γ S-HP0525. PDB: 1NLY. (a) Lowest energy pose of 8 from cmd (b) 5th lowest energy pose of 8 from cmd. Image generated using PyMOL

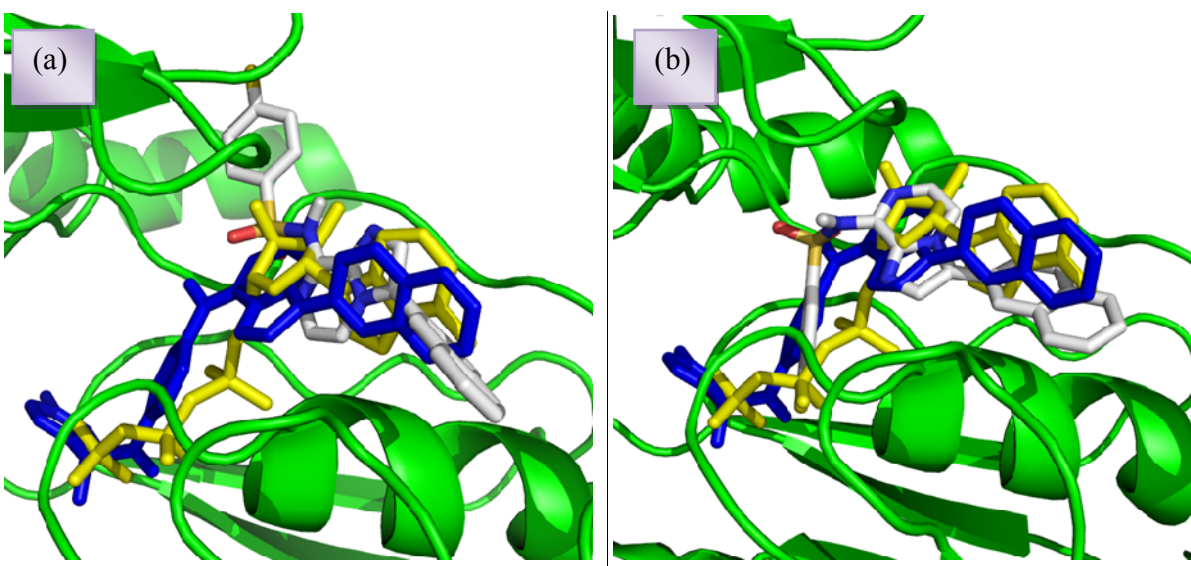


Figure 34: Overlay of 4 and 12 (Blue) with ATP γ S (Yellow) in ATP γ S-HP0525. PDB: 1NLY. (a) Lowest energy pose of 4 from cmd (b) 2nd lowest energy pose of 4 from cmd. Image generated using PyMOL

2.6 Concluding Remarks to Chapter

This chapter has illustrated the synthesis of the target imidazo[1,2-*a*]pyrazines, with two synthetic routes employed to access both 2- and 3-regioisomers. Optimisation of both the condensation reaction and the Buchwald-Hartwig coupling reaction were carried out and yields have been improved, especially for the coupling of 8-chloroimidazo[1,2-*a*]pyrazines and 4-toluene sulfonamide. Further investigations into the coupling with *N*-(4-aminophenyl)-4-methylbenzenesulfonamide would be required given that this amine behaves significantly different to the sulfonamide.

Biological evaluation of the compounds synthesised have shown IC₅₀'s to be in the region of 7-150 μ M, and the lead compound, **14**, has also been shown to be a competitive inhibitors of ATP. *In silico* studies have prompted the design of alternative structures with the aim of improving potency (Figure 35). Some of the suggestions will be further evaluated in Chapter 3.

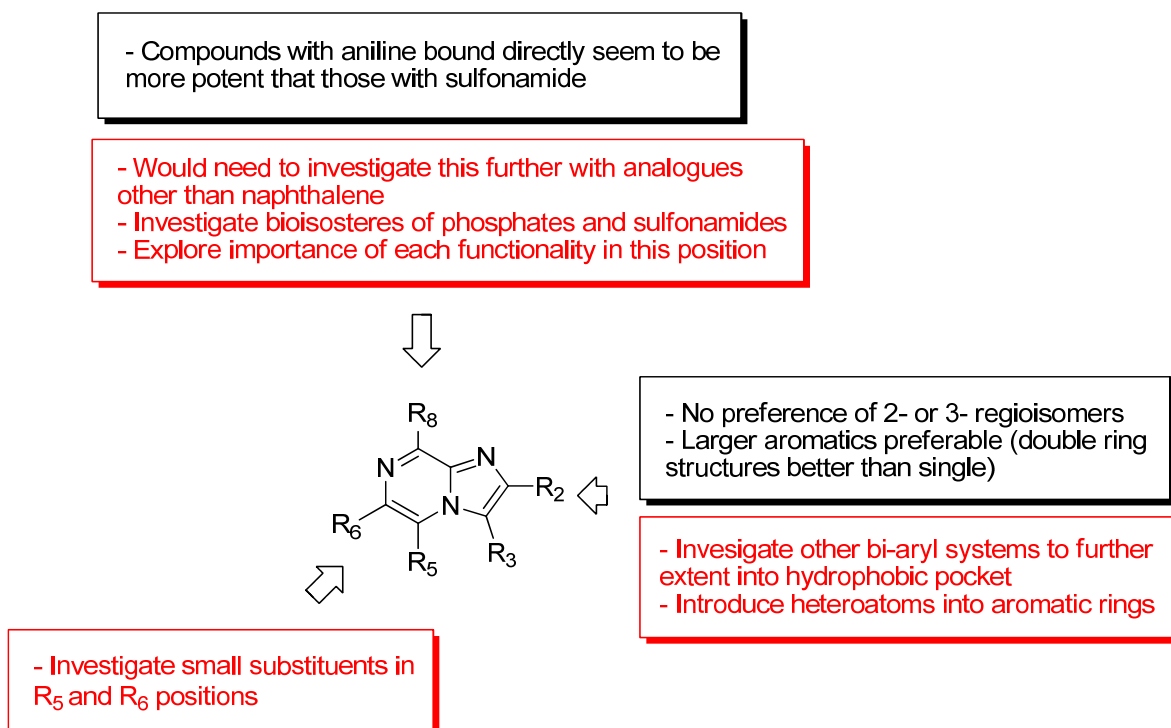


Figure 35: Summary of SAR of target imidazo[1,2-*a*]pyrazines, and suggestions to improve potency. Black indicates current observations, Red indicates suggestions made

There are, however, limitations of the imidazo[1,2-*a*]pyrazine compounds, most notably, their solubility. As a measure of solubility, Table 12 and Table 13 indicate the predicted *cLogP* values of the imidazo[1,2-*a*]pyrazines to be between 3.1 and 5.9 and the predicted *LogS* values between -5.4 and -7.6 indicating the hydrophobic nature and poor aqueous solubility of these compounds.

Therefore it is envisaged that improving the solubility of these compounds would not only enhance pharmacokinetic properties but also the likelihood of achieving co-crystallisation with the enzyme. In doing so, this would provide the binding mode of the inhibitors, and aid with drug design efforts.

Chapter 3: Results & Discussion: 2nd Generation Compounds

3.1 Imidazo[1,2-*a*]pyrazine Analogues

From studying the *in silico* interactions of the lead compound, **14** (Section 2.5.2.1) within the nucleotide binding site, a 2nd generation of compounds was designed. Two areas were investigated (Figure 36) with the aim of improving potency and solubility and also to developing an understanding of the SAR of the imidazo[1,2-*a*]pyrazine inhibitors.

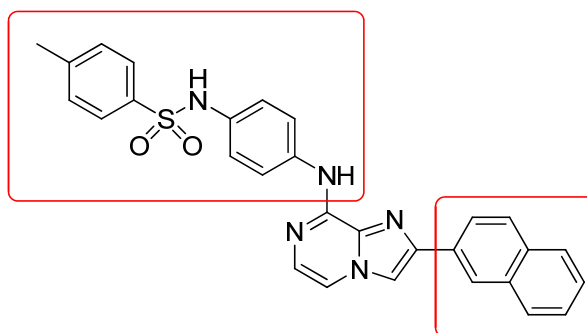


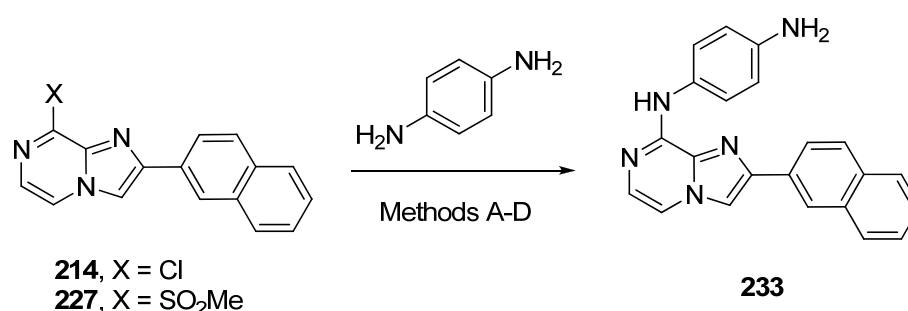
Figure 36: Compound 14, highlighting the two areas that were investigated in order to improve potency and develop an understanding of the SAR of the imidazo[1,2-*a*]pyrazine inhibitors

Variations at position 8 investigated the importance of the sulfonamide and the presence of both aromatic rings. Removal of either of these rings would also potentially improve the solubility of the inhibitor. The amino linker was also investigated by changing to an ether, and in doing so, altering the 3D structure of the compound as well as its H-bonding/accepting nature.

Since the naphthalene moiety is predicted to occupy the purine region of the nucleotide binding site, it was envisaged that introducing heteroatoms could improve potency. In addition, the importance of having a group in this position, and ascertaining whether larger bi-aryl systems are desired was also investigated.

3.1.1 Variations at Position 8

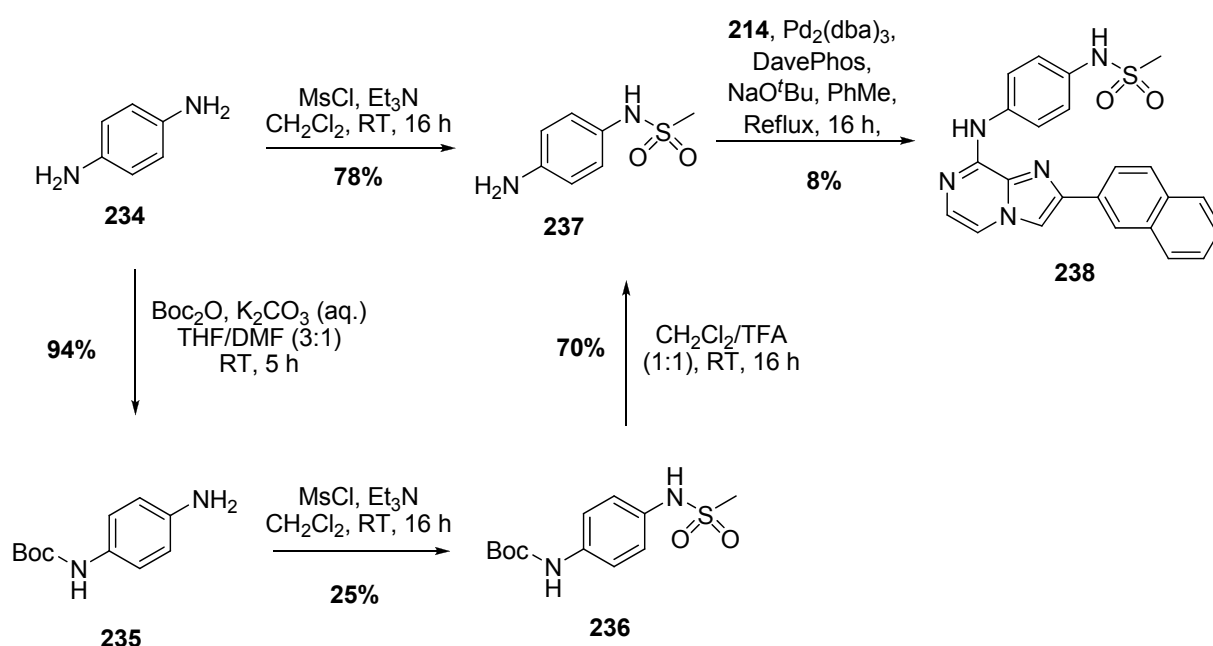
With the sulfonamide occupying the phosphate binding region of the nucleotide binding site, it is also important to look at alternative moieties to this group in the 8-position. Firstly the synthesis of **233** will give an indication of the importance the sulfonamide plays in the inhibitor activity. As can be seen from Table 14, **233** can only be obtained, albeit in low yields, using palladium catalysed Buchwald-Hartwig conditions. A maximum yield of 27% is observed using the optimised conditions, which can be further increased to 32% under microwave irradiation.



Exp	Method	Catalyst	Ligand	Base	Solvent	Temp/°C	Time/h	Yield/%
1	A	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4 eq)	Dioxane	Reflux	40	17
2	A	Pd ₂ (dba) ₃ (1 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.4 eq)	Toluene	Reflux	16	27
3	B	Pd ₂ (dba) ₃ (1 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.4 eq)	Toluene	160	10	32
4	B	Pd(PPh ₃) ₄ (5 mol%)	-	NaO ^t Bu (1.4 eq)	Toluene	160	10	0
5	B	Pd(dppf)Cl ₂ (5 mol%)	-	NaO ^t Bu (1.4 eq)	Toluene	160	10	20
6	C	-	-	DIPEA (1.5 eq)	MeCN	160	10	0
7 ^a	D	-	-	DIPEA (2 eq)	MeCN	160	10	0

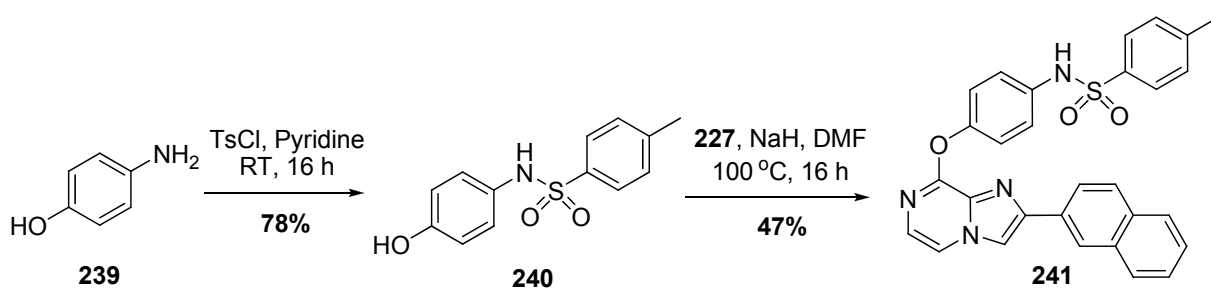
Table 14: Reaction conditions used for the formation of **233**. ^a 1.4 eq diaminobenzene used; Method A: Buchwald-Hartwig conditions with **214**; Method B: Microwave Buchwald-Hartwig conditions with **214**; Method C: Microwave solvent and base only with **214**; Method D: Microwave solvent and base only with **227**

Replacing the tosyl group with a mesyl group will inform us of the importance of the terminal toluoyl moiety. Starting with a 5-fold excess of 1,4-diaminobenzene, mono-mesylation using mesyl chloride is achieved in high yields (Scheme 46). Extraction with EtOAc is required due to compound solubility (a yield of 14% is observed when extracting with CH₂Cl₂). **237** can also be obtained *via* mono-Boc protection of the di-amine, protecting the remaining amine with mesyl chloride and deprotection of the Boc group under acidic conditions. However, this longer route is not necessary in the synthesis.

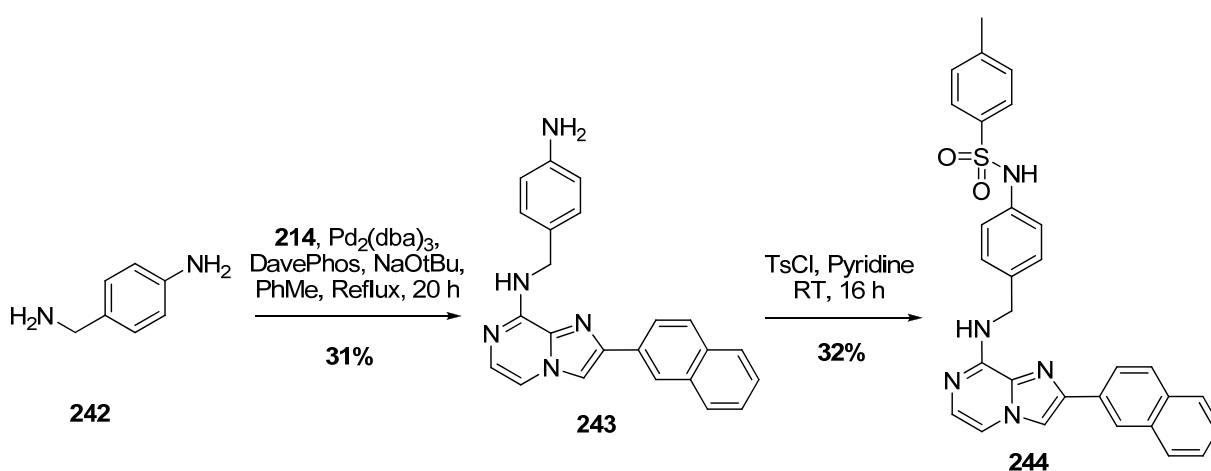


Scheme 46: Synthesis of compound **23**

The next moiety to investigate is the amine that links the sulfonamide moiety to the imidazo[1,2-*a*]pyrazine. Scheme 47 and Scheme 48 highlight the synthesis of ether (**241**) and benzylamine (**244**) linked heterocycles. **241** can be accessed by tosylating 4-aminophenol in pyridine¹⁸⁰ and coupling to the core heterocycle using **227**, all in good yields. Subjecting 4-(aminomethyl)aniline to the optimised Buchwald-Hartwig coupling conditions gives compound **243**, the regioselectivity of which was confirmed by 2D-NMR (HMBC coupling between the benzyl CH₂ protons and the quaternary C₈ carbon). Tosylation in pyridine proceeded, albeit in lower yields to before.

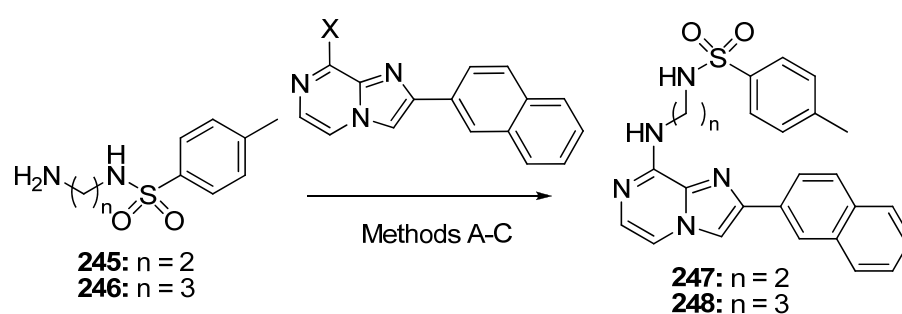


Scheme 47: Synthesis of the ether linked imidazo[1,2-*a*]pyrazine, 241



Scheme 48: Synthesis of the benzylamine linked imidazo[1,2-*a*]pyrazine, 244

It is also recommended to synthesise the thioether and methylene linked heterocycles to obtain a more complete picture of the SAR. The final area investigated is importance of the phenyl closest to the core heterocycle. By replacing this moiety with an alkyl chain, not only could the solubility of the inhibitor be enhanced, but it could be easier to access synthetically (Table 15).

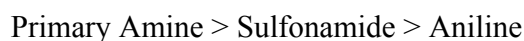


Exp	Method	n	X	Yield/%
1	A	2	SO ₂ Me	21
2	B	2	Cl	8
3	C	2	Cl	6
4	A	3	SO ₂ Me	33

Table 15: The synthesis of **247** and **248**. Method A: **227**, Amine (2 eq), DIPEA (2 eq), DMF, 100 °C, 16 h; Method B: **214**, Amine (1.2 eq), Pd₂(dba)₃ (1 mol%), DavePhos (3 mol%), NaO^tBu (1.4 eq), PhMe, Reflux, 16 h; Method C: **214**, Amine (1.5 eq), DIPEA (2.5 eq), ⁿBuOH, 80 °C, 16 h

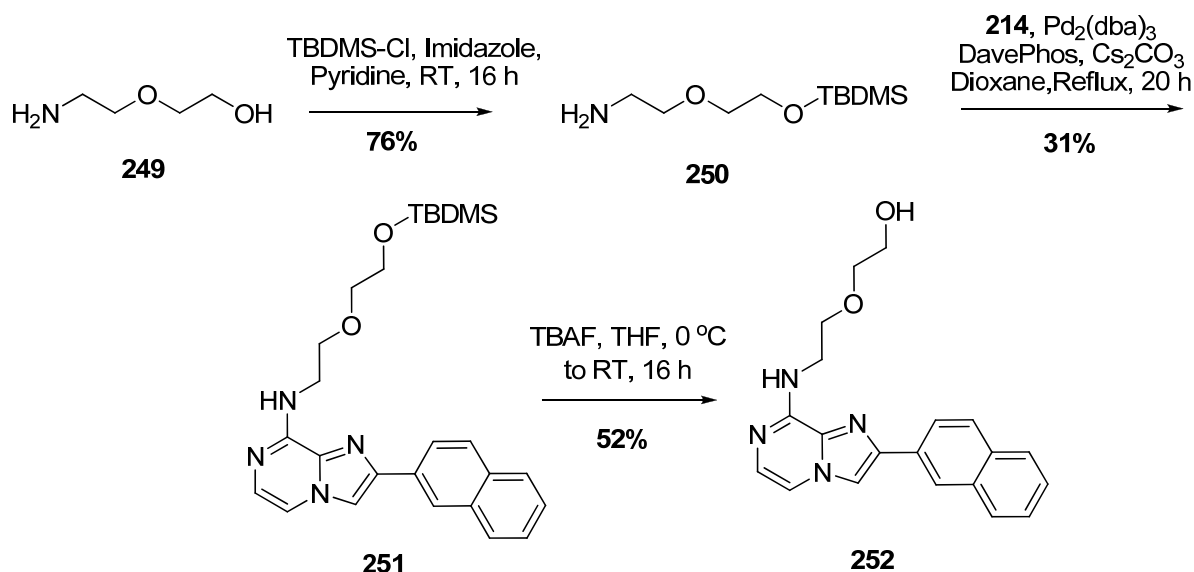
Compounds **247** and **248** can be synthesised using either Pd-catalysed Buchwald-Hartwig conditions (Method B) or solvent/base conditions with either **214** (Method C) or **227** (Method A). It is advised to use method B or C, even though lower yields are observed, as the reaction is easier to purify compared with Method A, where there is poor separation between the product and **227**.

Entry 3 (Method C) indicates that the primary amine of **245** can displace the chloro in **214** using DIPEA as base, something that cannot be achieved when reacting a sulfonamide or aniline. It is therefore not surprising that the following reactivity is observed for the chloro-heterocycle:



Looking at Table 8 (Section 2.4.1), it can be seen that a strong base (NaH) is required for the reaction between **214** and a sulfonamide, and therefore increasing the strength of the DIPEA base here could improve the reaction yield. Applying NaH and DMF to entries 1 and 4, would provide the opportunity to study and compare the reactivity of the methyl sulfone imidazo[1,2-*a*]pyrazines.

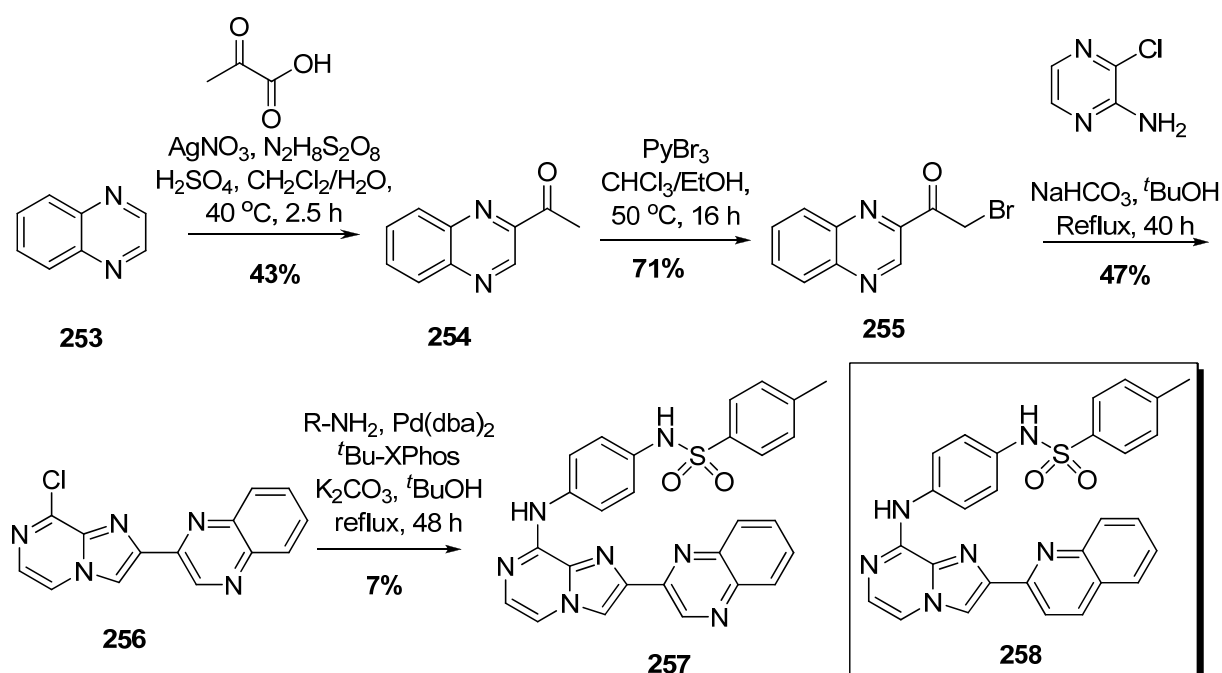
In addition, it was also decided to synthesise the sulfonamide-free PEGylated imidazo[1,2-*a*]pyrazine **252** as illustrated in Scheme 49. Silyl protection of 2-(2-aminoethoxy)ethanol¹⁸¹ was achieved in high yield and the ensuing Buchwald-Hartwig coupling and silyl deprotection¹⁸² also proceeded in good yields.



Scheme 49: Synthesis of PEGylated imidazo[1,2-*a*]pyrazine **252**

3.1.2 Variations with the Aromatic Group in Position 2

According to the *in silico* data, the naphthalene moiety of **14** occupies the purine region of the nucleotide binding site. Therefore by introducing heteroatoms into the aromatic ring, it was envisaged that extra H-bonding interactions could enhance the activity. Scheme 50 outlines the synthesis of the quinoxaline derivative of **14** and relies on the same chemistry employed previously. The aryl ketone can be accessed *via* homolytic acylation of quinoxaline with acyl radicals, which are produced from the silver catalysed decarboxylation of α -keto acids by persulfate.¹⁸³ When the reaction was carried out over 2.5 h, the yield obtained is 43%; leaving for 16 h increases it to just 47%. Bromination and condensation proceeds in good yields, but the Buchwald-Hartwig coupling was low yielding. Applying the optimised conditions discussed in Section 2.4.1 should improve the yield.

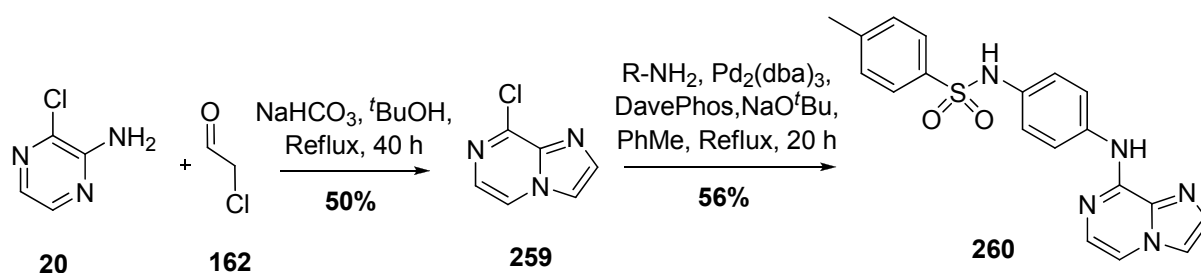


Scheme 50: Synthesis of the quinoxaline derivative of the lead compound 14: 257, and the structure of compound 258

The quinoline equivalent, **258** (Scheme 50), has also been synthesised within the group,^e using the same method described above. In order to establish an SAR of these inhibitors, it is also vital to establish the importance of the aromatic moiety in the 2- or 3- positions. Scheme 51 outlines the synthesis of **260**, a derivative of compound **14** with no aromatic group present. Using the previously described optimised conditions, **260** can be obtained in good yields. The results of the *in vitro* testing of these compounds are given in Section 3.3.1.

The intermediate **259** could also act as a starting material in an alternative synthesis of 3-aryl imidazo[1,2-*a*]pyrazines. Bromination using NBS should afford the 3-bromo-8-chloro substituted heterocycle, providing the opportunity for carrying out a Suzuki coupling reaction at the 3-position.

^e Synthesis carried out by H.Koss



Scheme 51: Synthesis of 260

3.2 Maybridge Analogues

In addition to the imidazo[1,2-*a*]pyrazines originally synthesised and tested (Chapter 2), 500 fragments from a Maybridge chemical library were screened utilising the same biochemical assay as discussed in section 2.5.1. As with the imidazo[1,2-*a*]pyrazine compounds initial screening at 500 μM was followed by hit validation at 5, 50 and 500 μM giving rise to 5 fragments which displayed activity at 50 μM , and are illustrated in Figure 37.

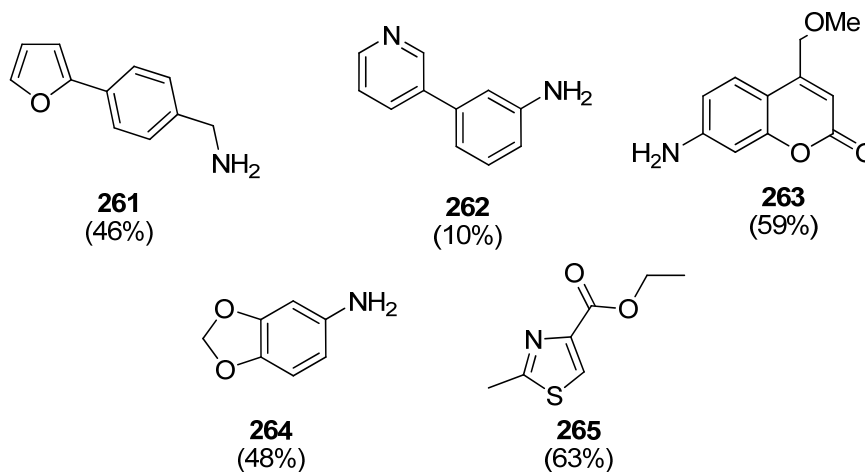


Figure 37: Structures of the hit Maybridge fragments, with the % absorbance relative to positive control at 50 μM given in brackets

Establishing fragments that bind to a target is used in fragment-based drug discovery, FBDD.¹⁸⁴ It is the process of identifying small molecules, which may only weakly bind to the biological

target, and growing them, or combining them, to create a larger compound with a higher binding affinity.

The process relies on obtaining an X-ray crystal structure of the fragment with the enzyme active site. Chemical intuition can then be used to grow a fragment in order to increase the number of key interactions, or to link fragments, that are found in different binding pockets, together. Unfortunately all attempts to co-crystallise the hit fragments with HP0525^f were unsuccessful and so this approach could not be utilised.

An alternative is to use NMR spectroscopy and by observing shifts in any of the amide signals, the point of binding can be determined. This method, however, relies on solving the NMR structure of the enzyme, which to date has not been completed. Therefore an alternative method was used to try and improve the potency of these fragments.

3.2.1 Maybridge Fragment Substructure Search

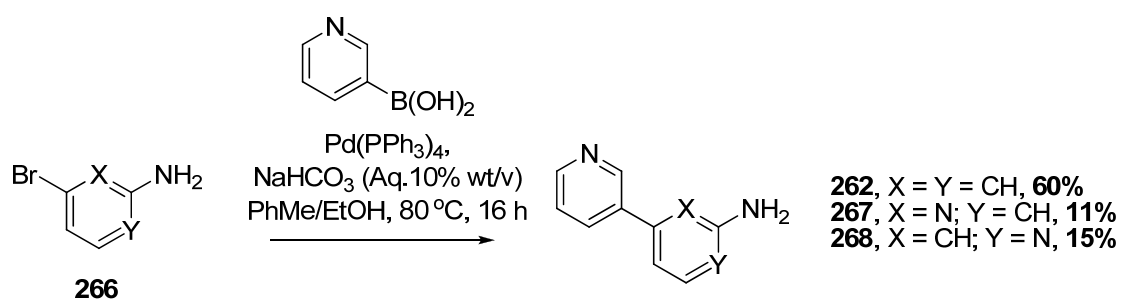
Running a sub-structure search of the lead fragment **262** using the Reaxys online chemical search engine,¹⁸⁵ resulted in a list of compounds containing the **262** moiety. These compounds, together with suggestions made,^g were then docked using the docking program Fred.^h Compounds that use **262** as a starting material were chosen for synthesis and these will now be discussed.

Owing to the commercial cost of **262**, it was decided to carry out its synthesis on a larger scale so that the further compounds could be synthesised. Scheme 52 shows the Suzuki coupling reaction between pyridine-3-ylboronic acid and 2-bromoaniline proceeds with moderate yields.¹⁸⁶ However, when carrying out the reaction with 2-aminochloropyridines the yield was very low.

^f Experiments carried out by Dr K. Wallden

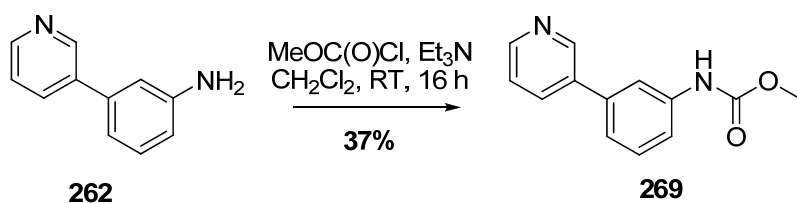
^g Suggestions made by Prof D. Selwood

^h Work Carried out by Dr P. Gane

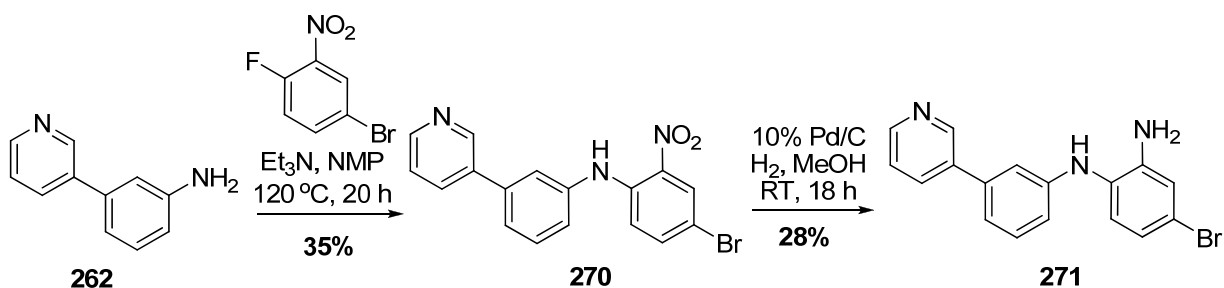


Scheme 52: Synthesis of 262 and 2 heteroaromatic derivatives

The synthesis for introducing a carbamate moiety into the fragment is illustrated in Scheme 53 and comprises reacting **262** with methyl chloroformate.¹⁸⁷ The isolated yield quoted was low due to purification difficulties in separating the product from the starting material. Compound **271** can be synthesised by displacing the fluorine in 4-bromo-1-fluoro-2-nitrobenzene with **262**,¹⁸⁸ followed by reduction of the nitro group under standard hydrogenation conditions (Scheme 54). The reduction was low yielding owing to the formation of the de-brominated species as a side product.

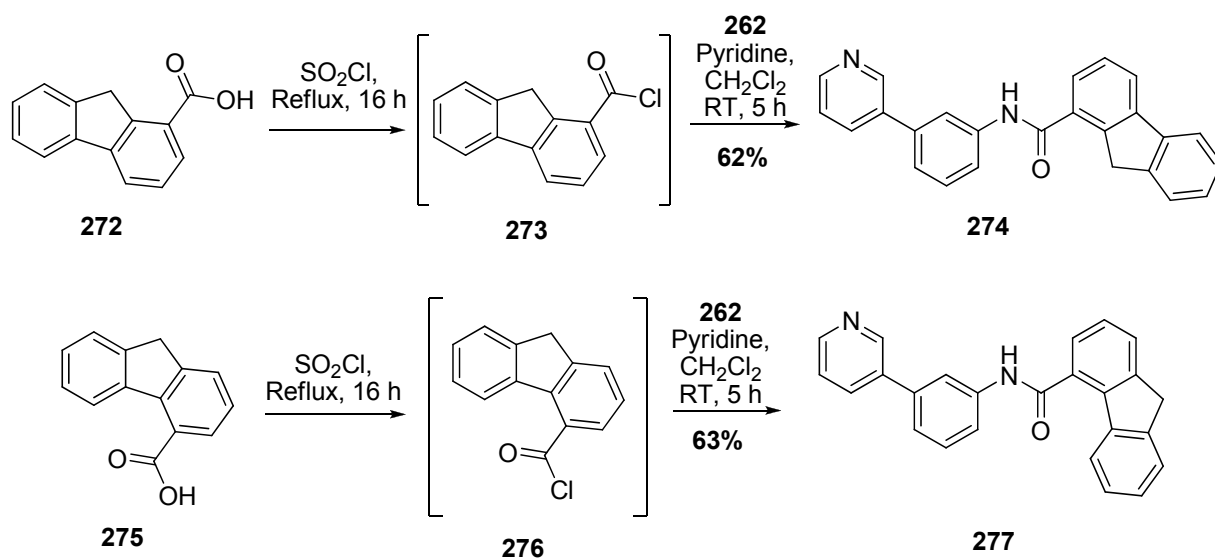


Scheme 53: Synthesis of 269



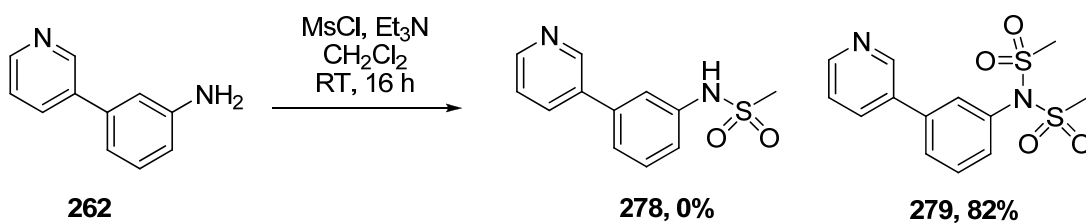
Scheme 54: Synthesis of 271

Scheme 55 illustrates the synthesis of the fluorene compounds **274** and **277**. Starting from either the 1- or 4-carboxylic acid (**272** or **275**), the acyl chloride was formed *in situ* using thionyl chloride,¹⁸⁹ which is then used, as crude, in the reaction with **262**.¹⁹⁰



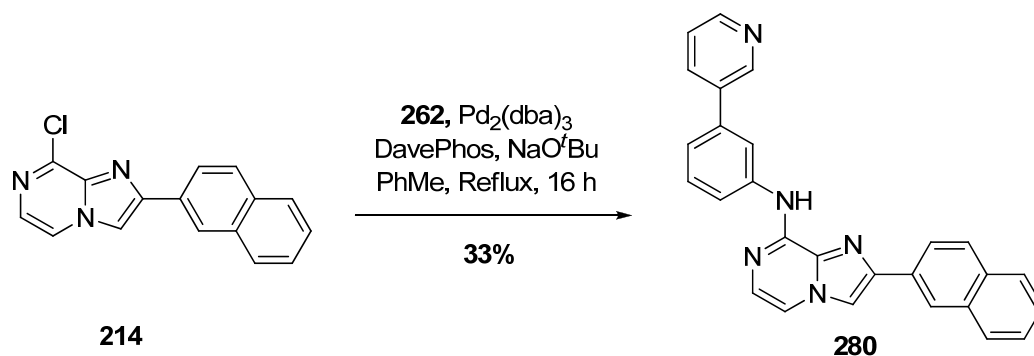
Scheme 55: Synthesis of fluorene compounds **274** and **277**

The final Maybridge compound synthesised was the mesylated version of **262** (Scheme 56). All attempts resulted in no formation of **278**, but instead 82% conversion to the di-mesylated species **279**. Monitoring the reaction by TLC showed that the undesired product was formed after just 5 minutes with no other signs of desired product.

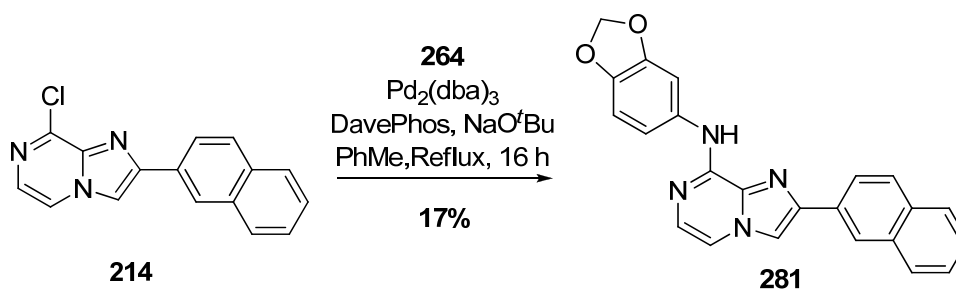


Scheme 56: Mesylation of **262**

It was also decided to incorporate two of the Maybridge fragments into the imidazo[1,2-*a*]pyrazine inhibitors. Scheme 57 and Scheme 58 show the synthesis of **280** (using **262**) and **281** (using **264**) respectively using previously optimised Buchwald-Hartwig conditions.



Scheme 57: Synthesis of **280**



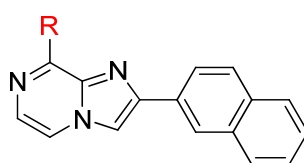
Scheme 58: Synthesis of **281**

3.3 Compound Analysis

Each of the 2nd generation compounds synthesised were screened employing the same colorimetric assay as used in Section 2.5.1. Selected dose response curves are illustrated in Figure 71 (Appendix, Section 7.1).

3.3.1 Imidazo[1,2-*a*]pyrazines

Table 16 indicates the IC₅₀ values when varying the group in the 8-position of **14**. Firstly it is evident that inhibition is diminished when removing either the 4-toluenesulfonamide or *N*-(4-aminophenyl)-4-methylbenzenesulfonamide groups, indicating their importance in the activity (Entries 2-5). Removing the aniline (**8**, Entry 7) results in a drop in inhibition indicating desired position of the sulfonamide moiety should be further away from the core heterocycle.



Entry	Compound	R	IC ₅₀ (μM) ^a	cLogP ^b	LogS
1	14 (Lead)		7 (7/7)	5.9	-7.6
2	214		8974 (141/17807)	3.9	-5.5
3	231		1703 (308/3098)	3.2	-5.2
4	227		315 (168/462)	2.7	-5.1
5	228		183 (213/153)	2.7	-4.1
6	252		107 (85/130)	3.7	-5.2
7	8		109 (88/131)	4.5	-6.7

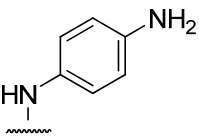
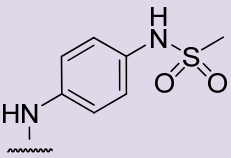
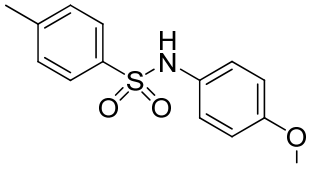
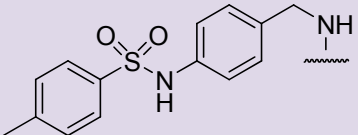
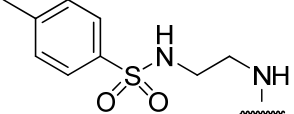
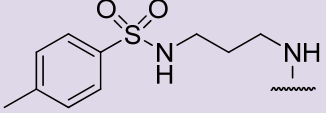
Entry	Compound	R	IC ₅₀ (μM) ^a	cLogP ^b	LogS
8	233		36 (40/ <u>33</u>)	4.4	-6.5
9	238		73 (<u>63</u> /83)	4.3	-7.0
10	241		22 (<u>21</u> /22)	5.9	-8.1
11	244		84 (80/88)	5.9	-7.5
12	247		16 (16/ <u>16</u>)	4.4	-6.4
13	248		153	4.8	-6.6

Table 16: IC₅₀ data comparing substituents in the 8-position of lead compound **14**. ^a Value given is an average of two methods used. The individual values are given in brackets (standard slope first, variable slope second). Where a value is underlined, this is the method suggested by the program GraphPad Prism 5; ^b cLogP and LogS values determined using AlogP 2.1 from the virtual computational chemistry laboratory¹⁷²

Interestingly, compound **252** (Entry 6) has a similar potency to **8**, indicating that the benzene sulfonamide moiety is perhaps not required. In addition, compound **233** (Entry 8) displays good activity and when comparing to the lead compound **14**, it suggests the sulfonamide moiety is not a vital component.

Replacing the tosyl with a mesyl reduced the inhibition (**238**, Entry 9), suggesting presence of the terminal toluoyl moiety is desired but not vital, whilst, solubility calculations¹⁷² suggest **238** to be more soluble. Changing the type of linker from amine to ether (**241**) results in a very similar activity, but inserting methylene (**244**) reduces it. Figure 38 shows the docking images of these three compounds, all following the same profile as **14**, with the extended position of the

sulfonamide in **244** possibly accounting for the reduced activity. According to predictions, neither compounds show any improvement in solubility.

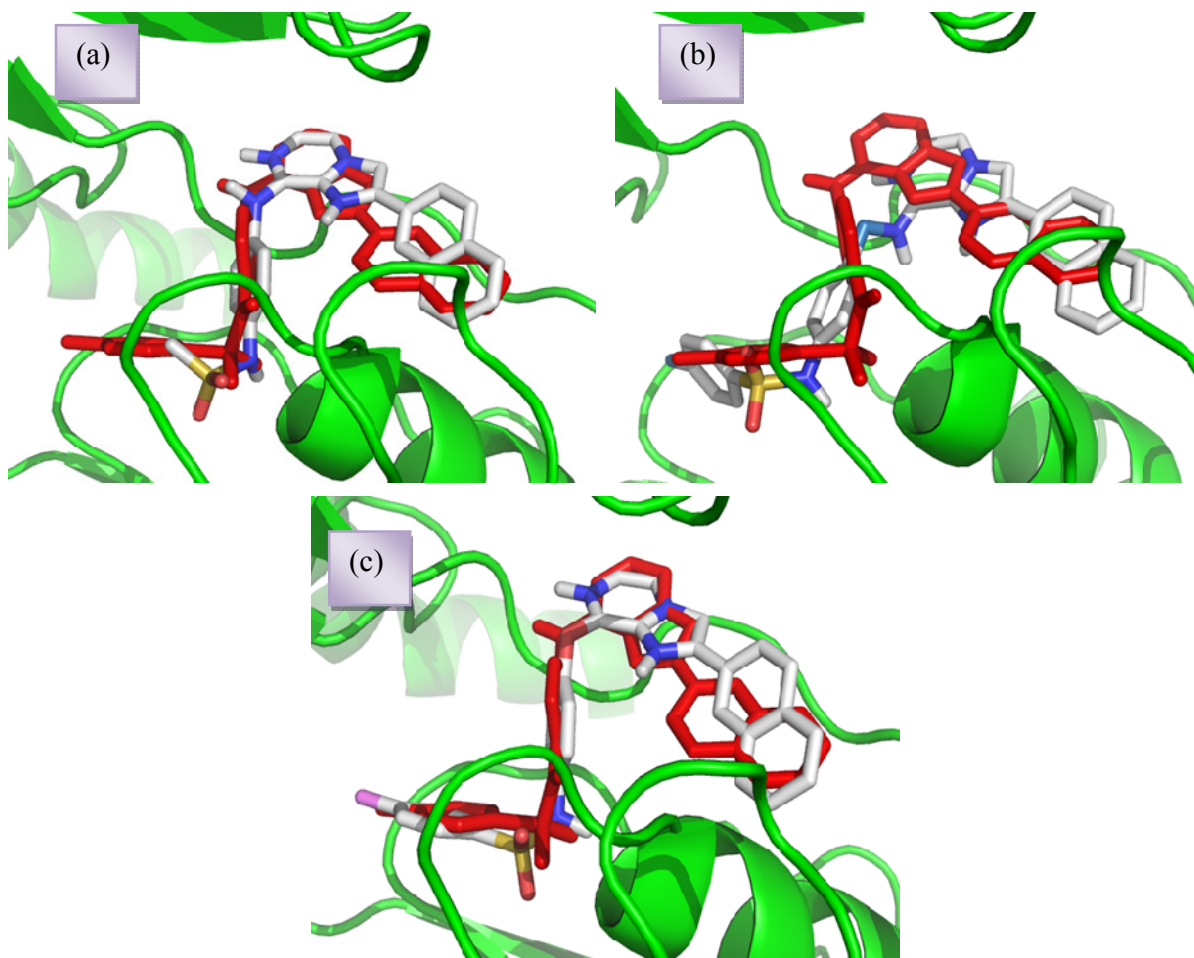
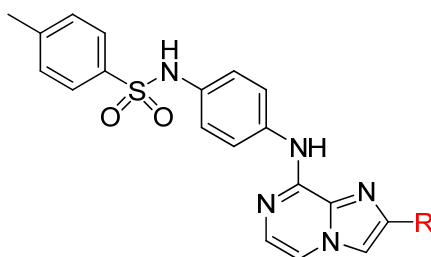


Figure 38: Images of 238 (a), 244 (b) and 241 (c) with 14 (Red) in ATPγS-HP0525. PDB: 1NLY. All images correspond to the lowest energy pose from PyRx, and are generated using PyMOL

Interestingly, replacing the initial phenyl for an ethyl chain (**247**, Entry 12) maintains the potency of the inhibitor, showing that it is not a requirement and provides an alternative lead compound. However, when a propyl chain is used, the inhibition drops considerably. Figure 39 shows that **247** and **248** have identical conformations and both align with **14**. The sulfonamide in **248** is further away however, which could result in lower binding and therefore lower potency. Further investigation into chain length would be required for future work. It would also be interesting to see whether potency is maintained with **247** if using a terminal mesyl as opposed to tosyl. In

addition, predicted solubility calculations suggest **247** has more favourable pharmacokinetics compared with **14**.

Table 17 shows the IC₅₀ values for analogues of the lead compound **14** comparing substituents in the 2-position.



Entry	Compound	R	IC ₅₀ (μM) ^a	cLogP ^b	LogS
1	14 (Lead)		7 (7/7)	5.9	-7.6
2	15		108 (88/117)	4.6	-7.6
3	257		28 (29/28)	4.4	-6.2
4	258^c		67 (65/69)	5.2	-6.9
5	282		19 (18/19)	6.3	-8.1
6	260		1389 (739/1941)	3.3	-5.3

Table 17: IC₅₀ Data comparing substituents in the 2-position of lead compound **14**. ^a Value given is an average of two methods used. The individual values are given in brackets (standard slope first, variable slope second). Where a value is underlined, this is the method suggested by the program GraphPad Prism 5; ^b cLogP and LogS values determined using AlogP 2.1 from the virtual computational chemistry laboratory;¹⁷² ^c Sample contains impurities

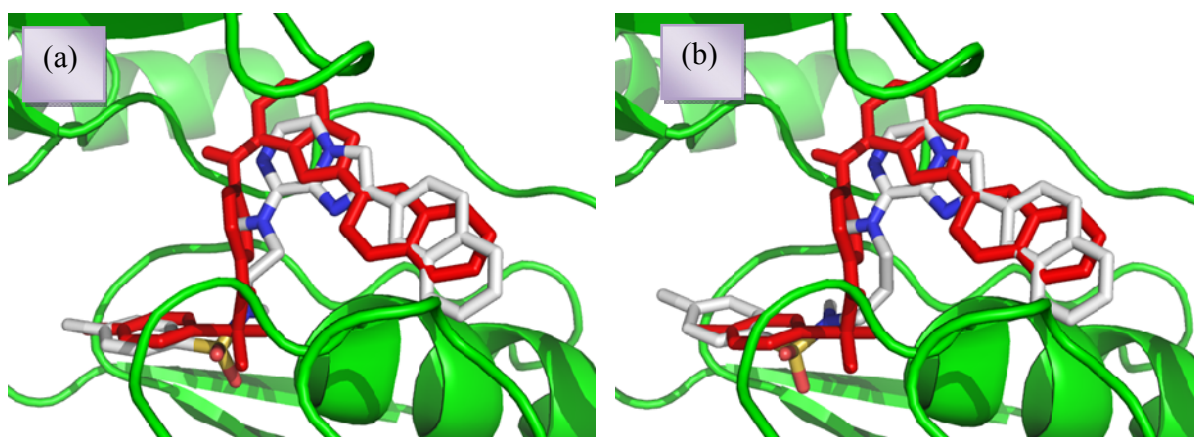


Figure 39: Images of 247 (a) and 248 (b) with 14 (Red) in ATP γ S-HP0525. PDB: 1NLY. Images generated using PyMOL

Firstly it is evident that a substituent needs to be present in this position, since when it is absent (**260**, Entry 6), there is little inhibition observed. Introducing nitrogen atoms into the naphthalene ring (quinoxaline **257** and quinoline **258**ⁱ), whilst improving the predicted solubility, does not improve the inhibition. Figure 40 shows that **257** and **258** align in very similar conformations, but slightly different to **14**, meaning that the sulfonamide now lies close to the γ -phosphate of ATP γ S, as with **12**, perhaps leading to slightly lower activity.

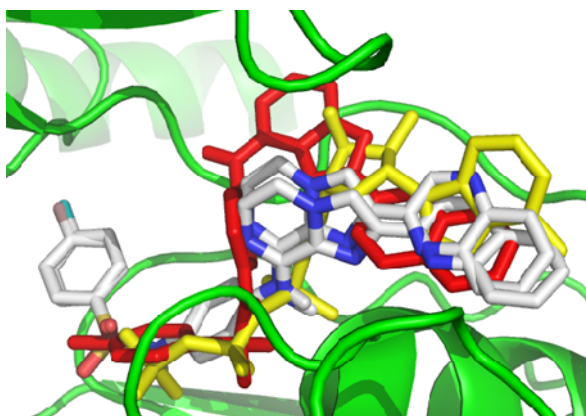


Figure 40: *In silico* docking image of 257, 258, and 14 (Red) and ATP γ S (Yellow) in ATP γ S-HP0525. PDB: 1NLY. Image generated using PyMOL

ⁱ Synthesised by H.Koss

As mentioned in Section 2.6, it is advised to synthesise further analogues, investigating bi-aryl systems in the 2- position, as these would possibly be able to further extend into and interact with the hydrophobic pocket containing possible pi-stacking interactions. Also, substituting for non-aromatic cyclic systems would clarify if an aromatic group is required in this position.

Compound **282**^j (Entry 5) can be compared with **9** (Section 2.5.1.1, Table 12, Entry 2) as it contains the same phenoxy phenyl substituent in the 2-position (Figure 41). The lower IC₅₀ value here further enhances the original claim that imidazo[1,2-*a*]pyrazines containing the longer *N*-(4-aminophenyl)-4-methylbenzenesulfonamide are more potent than those directly bound to the sulfonamide.

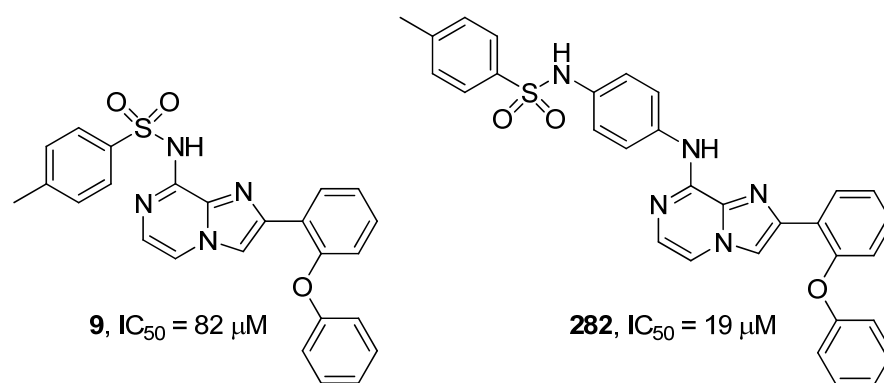


Figure 41: Structures of compounds **9** and **282**

Figure 42 shows the different poses of **282** generated using both the cmd format and PyRx program of AutoDock Vina. As can be seen, the phenoxy phenyl moiety is located in the purine pocket and the sulfonamide points towards the phosphate. The lowest energy conformations of cmd and PyRx (shown in red and blue respectively) have similar conformations, but slightly different alignments, which is an example where there are discrepancies between the two formats. Poses coloured yellow and blue (2nd lowest energy conformation from cmd and lowest energy from PyRx respectively) show a similar binding mode to that of **14**.

^j Synthesised by H.Koss



Figure 42: Image of the different poses of 282. Red: Lowest energy pose from cmd. Yellow: 2nd lowest energy pose from cmd. Blue: Lowest energy pose from PyRx. PDB: 1NLY. Images generated using PyMOL

As previously mentioned, compounds, such as **9**, where the sulfonamide is bound directly to the imidazo[1,2-*a*]pyrazine, the orientation of the lowest binding mode is such that the sulfonamide moiety points away from the phosphate region, and into the ‘R5/6 position’ (Figure 43, (a)). There are binding modes where the components of the compound align with the nucleotide and **14** (Figure 43 (b)) and these differences could correspond with the differences in potencies observed.

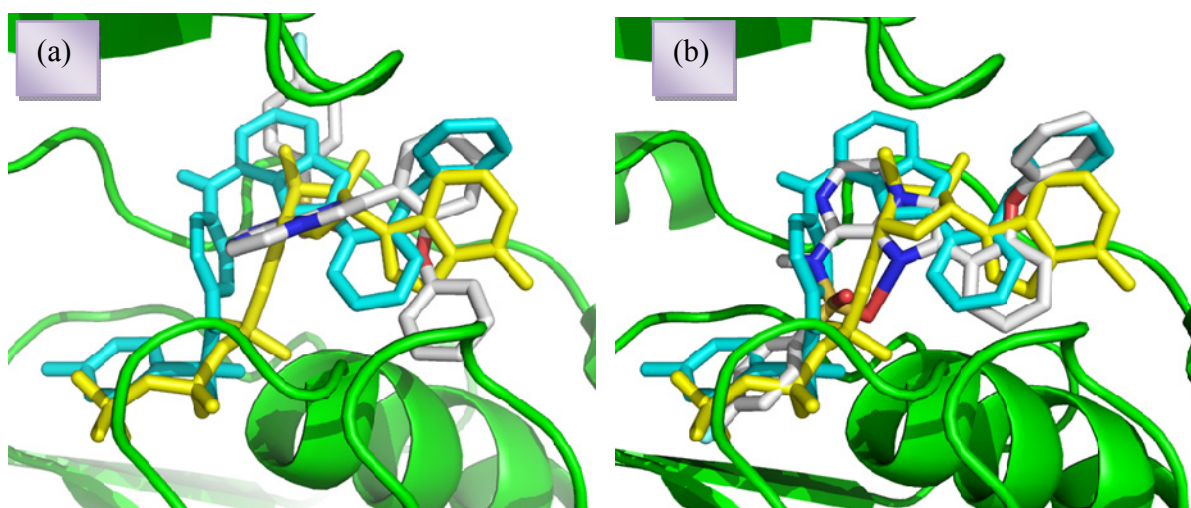


Figure 43: Images of **9** with 282 (2nd Lowest Energy Pose from cmd, Cyan) and ATPγS (Yellow) in ATPγS-HP0525. PDB: 1NLY. (a) Lowest energy pose of **9** from cmd (b) 3rd lowest energy pose of **9** from cmd. Images generated using PyMOL

3.3.2 Maybridge Compounds

Each of the Maybridge fragment analogues synthesised were initially screened at 250, 50 and 5 μM with IC_{50} determined on the hits produced (Table 18).

Entry	Compound	Structure	% Absorbance			$\text{IC}_{50}/\mu\text{M}^a$
			250 μM	50 μM	5 μM	
1	262		95	89	102	332 (503/161)
2	283		102	115	118	-
3	267		65	95	97	357 (283/431)
4	268		78	70	87	497 (330/665)
5	269		85	91	86	-
6	274		75	92	86	11 (11/11)
7	277		63	86	96	117 (77/156)
8	271		90	100	93	-

Entry	Compound	Structure	% Absorbance			IC ₅₀ /μM ^a
			250 μM	50 μM	5μM	
9	280		-	-	-	4 (<u>4</u> /3)
10	281		-	-	-	92 (<u>66</u> /118)

Table 18: Initial screening and selected IC₅₀ values of the Maybridge compounds synthesised. ^a Value given is an average of two methods used. The individual values are given in brackets (standard slope first, variable slope second). Where a value is underlined, this is the method suggested by the program GraphPad Prism 5

None of the ‘simple’ Maybridge fragment analogues showed any improved IC₅₀ activities (Entries 2-5); however, when attaching a fluorene moiety, the inhibition dramatically improved (Entries 6 & 7). Interestingly, there is a difference in the activity when looking at the position of attachment of the fluorene. This can be attributed to the different lowest energy poses of **274** and **277** (Figure 44).

Entry 9 shows that when combining the **262** fragment with the imidazo[1,2-*a*]pyrazine core, the resulting compound displays a similar potency to the lead compound **14**. The lowest energy *in silico* pose of **280** (Figure 45 a) is similar to that of the aniline substituted **233** (Figure 45 b) where the fragment or aniline is in the ‘R5/6’ position. This gives the possibility synthesising tri-substituted systems with groups at C₅ or C₆ extending into the phosphate region. Interestingly, there are no conformations that align with **14**, but there are conformations where the aromatic substituents have switched and the naphthalene occupies the phosphate and **262** the purine. It should be noted, however, that **280** requires re-synthesis in order to validate the IC₅₀ result.

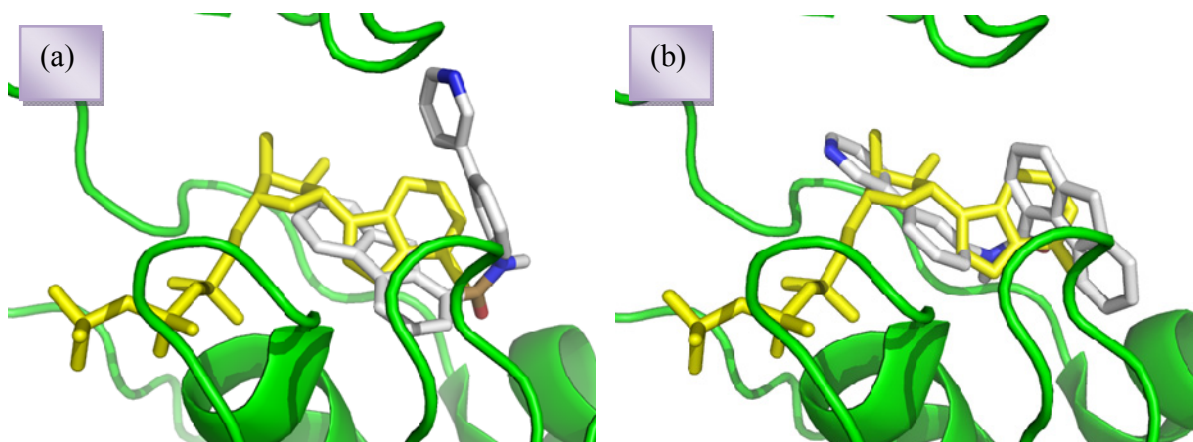


Figure 44: Images of (a) 274 and (b) 277 with ATP γ S (Yellow) in ATP γ S-HP0525. PDB: 1NLY Images generated using PyMOL

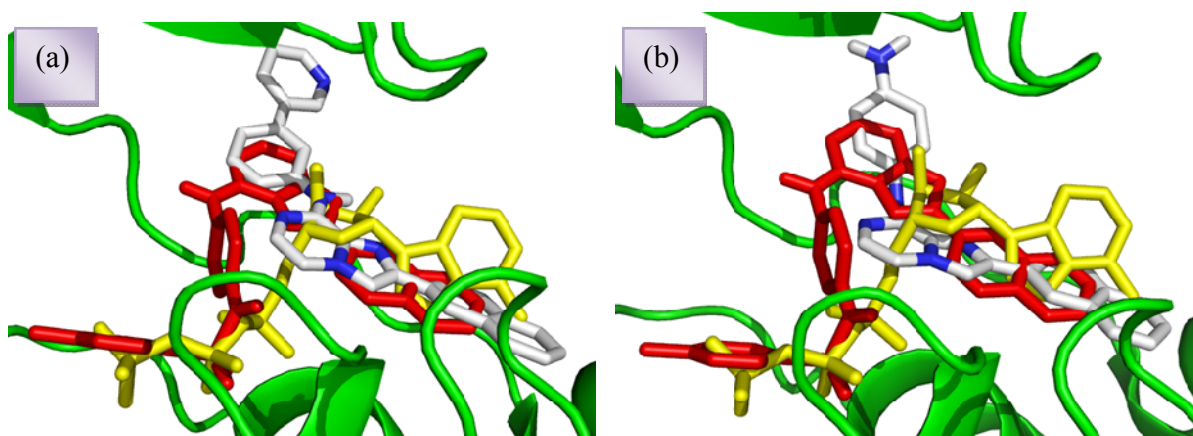


Figure 45: Images of (a) 280 and (b) 233 with 14 (Red) and ATP γ S (Yellow) in ATP γ S-HP0525. PDB: 1NLY Images generated using PyMOL

3.3.3 BioFocus Compounds

To aid with the development of the imidazo[1,2-*a*]pyrazine inhibitors and Maybridge fragments, it was decided to purchase compounds from an external source, BioFocus. By searching through their online library for the imidazo[1,2-*a*]pyrazine core and each of the 5 Maybridge fragment hits, a number of compounds were returned. Each of these compounds was docked using PyRx, ranked according to their binding affinities and a selected number were purchased with the aim of covering as many different types of scaffolds as possible (Table 19). For example, the imidazo[1,2-*a*]pyrazine yielded scaffolds of 5-substituted, 3,8-disubstituted (x2), 6,8-disubstituted and 2,3,6-trisubstituted.

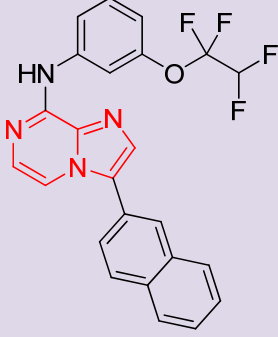
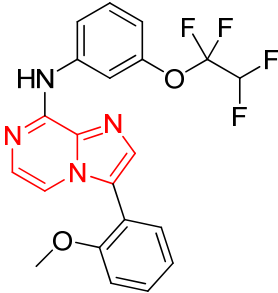
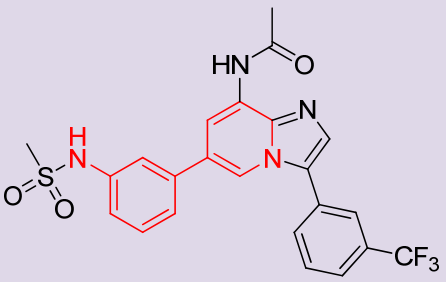
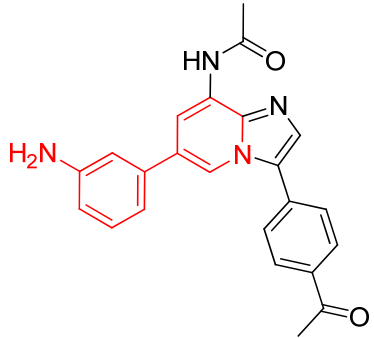
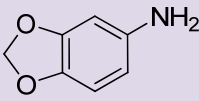
Entry	Compounds	Hits Returned	No. of Types of Scaffolds	No. of Compounds Purchased
1	Imidazo[1,2- <i>a</i>]pyrazine	24	5	10
2	261	0	-	-
3	262	45	4	10
4	263	0	-	-
5	264	89	7	16
6	265	0	-	-

Table 19: Results of BioFocus compound search indicating the number of hits returned and the number of compounds purchased

The same biochemical assay was then carried out on the BioFocus compounds purchased screening at concentrations of 250, 50 and 5 μ M with IC₅₀ values determined for selected compounds (Table 20). Studying the BioFocus compounds purchased, initially looking at those based on the imidazo[1,2-*a*]pyrazine core, there is no improvement on the activity of the lead compound **14**, but introducing fluorine atoms into the compound (Entries 1 & 2) maintains activity when compared to some of the other lower scoring inhibitors in Section 2.5.1.1.

Fluorine is often used in medicinal chemistry as an isostere of hydrogen, since it is virtually the same size, but its increased electronegativity can be used to vary the electronic properties of a compound.¹⁷⁰

Compounds based on **262** also show inhibition albeit no improvement on **14**. Alternative central cores are evident, providing an insight into potentially changing the imidazo[1,2-*a*]pyrazine core. Two of the compounds based on **264** do, however, show comparable levels of inhibition with **14** (Entries 7 & 8). They consist of a 5-azaindole (pyrrolo[3,2-*c*]pyridine) core, providing an alternative to the imidazo[1,2-*a*]pyrazine core. As can be seen from Figure 46 (a), when a similar ‘2 and 8- substituted’ system is docked (**290**), the orientation is similar to that of the imidazo[1,2-*a*]pyrazines, with the alternative core occupying the ribose region.

Entry	Compound	Structure	% Absorbance			IC50/ μ M ^a
			250 μ M	50 μ M	5 μ M	
1	284		50	74	76	103
2	285		72	63	69	122 (102/142)
3	286		83	68	74	220 (235/205)
4	287		24	57	69	142 (160/124)
5	264		-	-	-	140

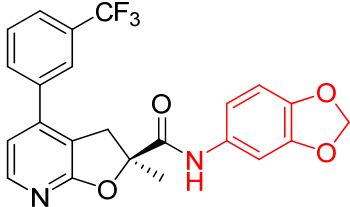
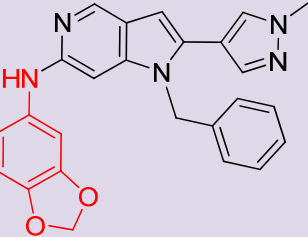
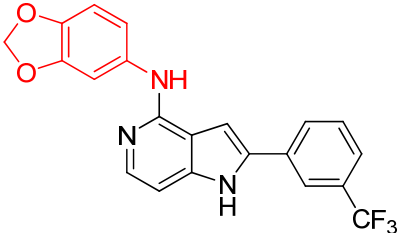
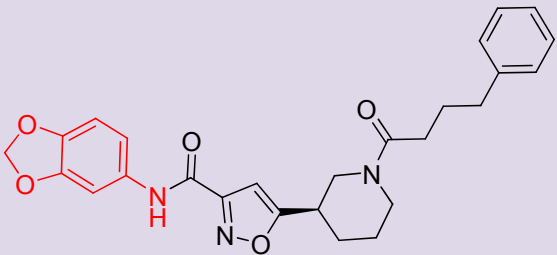
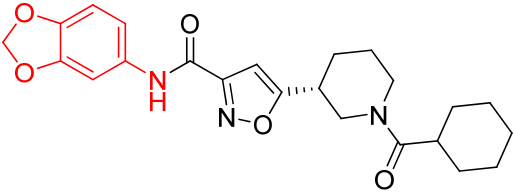
Entry	Compound	Structure	% Absorbance			IC ₅₀ /μM ^a
			250 μM	50 μM	5μM	
6	288		75	71	78	729
7	289		55	60	77	18 (<u>19</u> /18)
8	290		36	44	93	14 (<u>14</u> /14)
9	291		73	52	73	1031 (1867/196)
10	292		82	69	81	1304

Table 20: Initial screening and IC₅₀ values of selected BioFocus compounds purchased. Imidazo[1,2-*a*]pyrazine core, 262 and 264 fragment in **RED**; ^a Value given is an average of two methods used. The individual values are given in brackets (standard slope first, variable slope second). Where a value is underlined, this is the method suggested by the program GraphPad Prism 5

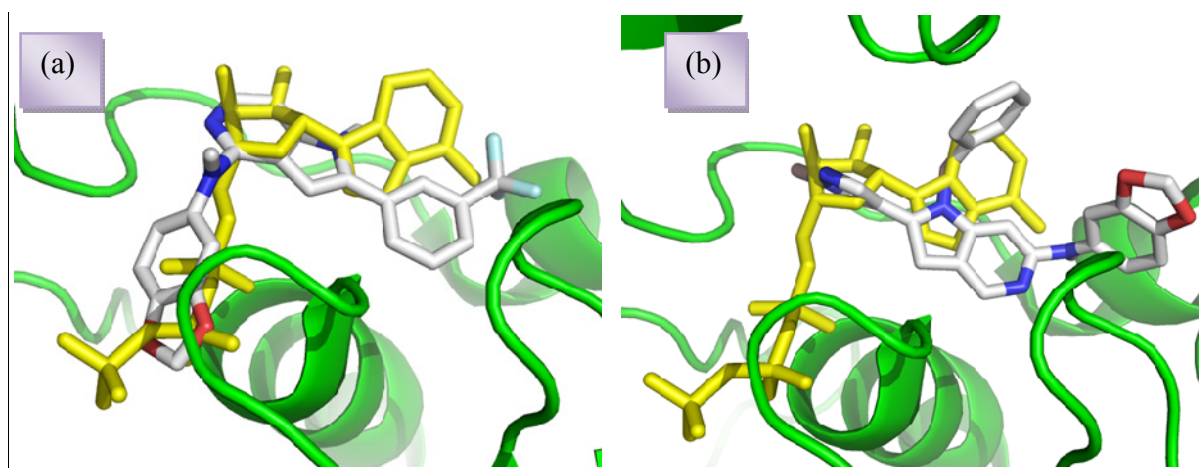


Figure 46: Images of (a) **290** and (b) **289** with ATP γ S (Yellow) in ATP γ S-HP0525. PDB: 1NLY. Images generated using PyMOL

When a tri-substituted system (**289**) is docked, the entire orientation shifts so to occupy the extended hydrophobic pocket. Compound **290** also contains the **264** fragment in a position similar to the 8-position of imidazo[1,2-*a*]pyrazines, hence the reason for the synthesis of **281**. It is suggested that a direct analogue of **290** be synthesised with the imidazo[1,2-*a*]pyrazine instead of pyrrolo[3,2-*c*]pyridine to see if the alternative core heterocycle affects potency.

3.4 Concluding Remarks to Chapter

Through investigations into both the 2- and 8-positions of the lead compound **14**, SAR trends have started to be established (Figure 48).

It is evident that the IC₅₀ still needs improvement, but the use of the ethyl chain in **247** provides a viable alternative to **14**. The inhibitory effect of **233** and **280** shows that the sulfonamide is not vital for the activity (although further validation of **280** is still required), and their orientations in the nucleotide binding site suggest incorporating further substituents in the C₅ and C₆ positions. Since **252** displays inhibition, one possibility could be to insert a flexible PEG chain into one of these positions to form tri-substituted imidazo[1,2-*a*]pyrazines.

Recent work within the laboratory has incorporated a bromine in position 6 of **14** (**293**, Figure 47),^k and this results in the activity being maintained, with an IC₅₀ of 6 μ M observed. This

^k Compound synthesised by Dr T. Pesnot

synthetic handle would provide the opportunity to explore further functionalities in this position. Further work into bi-aryls in the 2-position is also currently being explored.

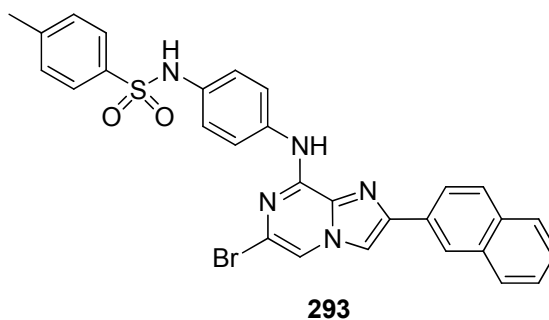


Figure 47: Structure of compound 293, a variant of 14 with bromine in the 6-position

Initial studies into the use of fragments in drug design have also been explored. Since no crystal structure was available, a substructure search using an online search engine and the BioFocus database yielded a number of potential compounds that displayed inhibitory effects. Certain functionalities within these compounds can be incorporated into the synthesis of future imidazo[1,2-*a*]pyrazine compounds.

- Amine or ether linked functionalities will inhibit
- The 1st aromatic is not necessary and can be replaced by an ethyl chain
- The sulfonamide needs to be a certain distance away from heterocyclic core for good activity (as opposed to bound directly)
- Sulfonamide moiety not vital for activity

- Need to investigate the optimum distance of the sulfonamide from the core heterocycle
- Study the relative position of the sulfonamide: $\text{-NHSO}_2\text{-}$ or $\text{-SO}_2\text{NH-}$
- Investigate bioisosteres of phosphates and sulfonamides including acyl sulfonamides
- Investigate heteroaromatic rings in place of toluoyl moiety
- Incorporate fluorine atoms

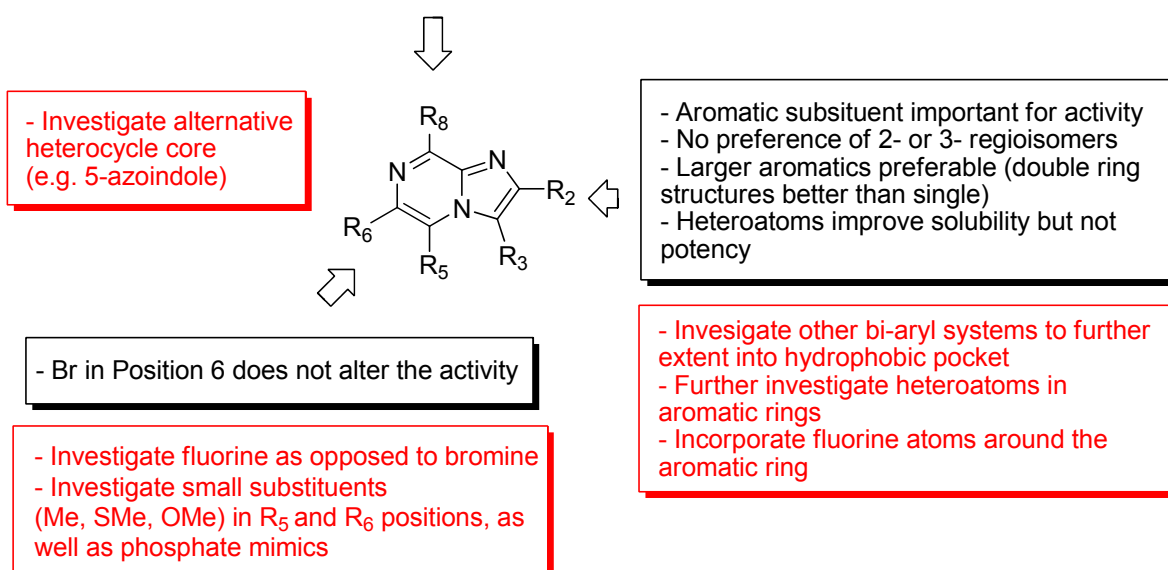


Figure 48: Summary of SAR of all imidazo[1,2-a]pyrazines synthesised, and suggestions to improve potency. Black indicates current observations, Red indicates suggestions made

Chapter 4: Results & Discussion: Peptide-Small Molecule Bivalent Reagents

4.1 Introduction to Bivalent Inhibitors

Affinity and specificity of an inhibitor can be greatly enhanced by taking advantage of hydrophobic/hydrophilic features near the enzyme active site. By linking an active site binding compound to a moiety that interacts with these features on the enzyme surface, it is possible to differentiate among enzymes.¹⁹¹ The resulting compounds are known as bivalent inhibitors.

Closely related to bivalent inhibitors are bisubstrate inhibitors, the objective of which is to mimic two natural substrates/ligands and therefore bind simultaneously to two areas of a given target.¹⁹² The process can be thought of as applying the principles of fragment based drug discovery in that you are linking two ligands together.¹⁹³ The first example of a bisubstrate inhibitor dates back to 1973, and was applied to the adenylate kinase.¹⁹⁴ Since then many different approaches of using bisubstrates as protein kinase inhibitors have been reported.

Peptide-peptide interactions play crucial roles in a number of biological processes, therefore, as a result their disruption can lead to novel therapeutic agents.¹⁹⁵ Many of the reported bivalent inhibitors will target these interactions as well as substrate binding sites.

Other than protein kinases, bivalent inhibitors have many other applications. These include the BiFunctional Fusion Inhibitor, CD4-BFFI, which consists of an anti CD4 monoclonal antibody with a fusion inhibitor and has applications as a HIV-1 entry inhibitor,¹⁹⁶ and bifunctional chimeric proteins containing a type 1 ribosome-inactivating protein and a serine protease inhibitor have enhanced cytotoxic activity against SVT2 cells.¹⁹⁷ There has also been a great deal of interest in conjugating peptides to PNAs (Peptide Nucleic Acids) in order to achieve enhanced properties of the RNA such as water solubility and cell membrane permeability.¹⁹⁸

4.1.1 Peptide-Small Molecule Bivalent Inhibitors

Bivalent molecules comprising a small molecule and a peptide have been extensively studied throughout the literature, the majority of which target protein kinases. These are enzymes that catalyse the phosphorylation of Tyr, Thr and Ser residues in many proteins by the transfer of the γ -phosphoryl group of ATP.¹⁹² Therefore many of these bivalent inhibitors target the ATP-binding site as well as the protein-protein interactions of a protein substrate binding site (bisubstrate inhibitors) as illustrated in Figure 49. A few examples of these will now be discussed.

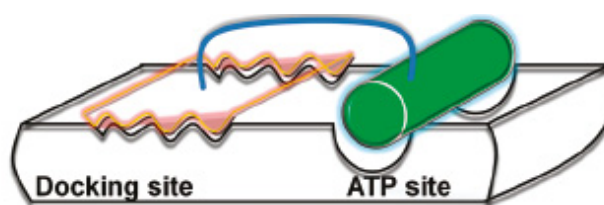


Figure 49: Schematic representation of a bivalent inhibitor approach which targets both ATP- and protein-binding sites.¹⁹³ Reproduced with permission.

Parang *et al* designed and synthesised a potent and selective bisubstrate inhibitor of the insulin receptor protein tyrosine kinase (IRK).¹⁹⁹ The design was made based upon observations on the phosphoryl transfer mechanism of the dissociative transition state. It was predicted that the reaction coordination distance between the nucleophilic oxygen (of Ser/Thr/Tyr) and the attacked γ -phosphoryl group should be ≥ 4.9 Å. Therefore they linked the non-hydrolysable ATP γ S to a peptide sequence, based upon the well characterised IRS727 peptide substrate,²⁰⁰ *via* a spacer that ensured the distance between the Tyr nucleophilic atom and γ -phosphate was 5 Å. During the native mechanism, the tyrosine hydroxyl serves as a hydrogen bond donor and therefore to conserve this interaction whilst being incorporated into the bisubstrate inhibitor, the Tyr oxygen was replaced with a nitrogen (Figure 50).

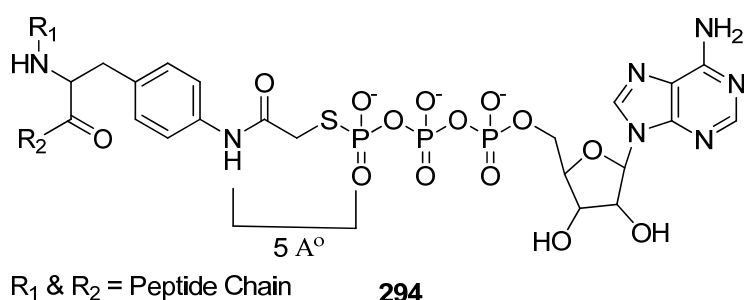


Figure 50: ATP-peptide bisubstrate inhibitor of insulin receptor protein tyrosine kinase (IRK)¹⁹⁹

Analysis revealed that the bisubstrate was a competitive inhibitor versus both the nucleotide and peptide substrates. In addition, the group also obtained a crystal structure of the inhibitor bound to IRK in which the mechanism-based design was validated.

The same group applied a similar ATP-peptide bisubstrate for the inhibition of the serine/threonine kinase protein kinase A (PKA).²⁰¹ The bisubstrate consisted of ATP γ S linked, *via* an acetyl bridge, to aminoalanine (acting as the serine analogue) with Kemptide, a well defined substrate for protein kinase A,²⁰² chosen as the substrate peptide. Results indicated that the inhibitor was competitive versus ATP, while non-competitive versus peptide substrate. In addition, a 20-fold selectivity versus protein kinase C and protein tyrosine kinase Csk was observed, highlighting the specificity of these bisubstrate compounds.

By merging bisubstrate analogue technology with the method of expressing protein ligation, it is possible to synthesis a protein-ATP conjugate.²⁰³ Shen *et al* targeted Csk, a protein tyrosine kinase responsible for site-specific tail phosphorylation of Src and Src family members. The group synthesised an ATP-conjugated peptide derived from the Src tail sequence, and fused this to the Src protein by expressed protein ligation to generate a semi-synthetic Src-ATP protein. This protein displayed high affinity and selectivity towards Csk.

Schneider *et al*²⁰⁴ covalently linked the high-affinity non selective kinase inhibitor K252a^{204,205} (a staurosporine-like natural product) to a miniature protein based on the protein kinase inhibitor protein (PKI). PKI selectively recognises PKA and inhibits its function, and from observation of the structure of the catalytic subunit in complex with the active portion of PKI, the miniature protein was designed. An octamethylene chain links this to K252a to generate the bivalent

conjugate (Figure 51), which was shown to be a highly potent (IC_{50} of 3.65 nM) and selective inhibitor of PKA.

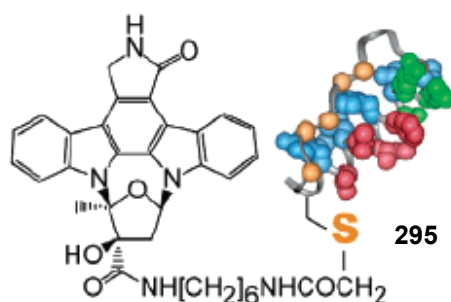


Figure 51: Miniature protein-K252a conjugate for the selective inhibition of PKA.²⁰⁴ Reproduced with permission.

Stebbins *et al* designed and synthesised a dual ATP- and substrate-competitive inhibitor of the serine/threonine protein kinase c-Jun-*N*-terminal kinase (JNK).¹⁹³ JNKs bind substrates and scaffold proteins, such as JIP-1, and the binding pocket for such substrates is in close proximity to the ATP binding site. The peptide part of the inhibitor consisted of a seven amino acid sequence based upon a sequence of JIP-1, and the ATP mimetic was based upon a previously reported potent but non-selective JNK inhibitor SP600125.²⁰⁶ Results indicated inhibition of JNK with an IC_{50} of 0.7 nM compared to 14 μ M and >50 μ M for the ATP mimic and unconjugated peptide respectively.

Other examples include adenosine-arginine conjugates ('ARC') linked *via* a 6-aminohexanoic acid linker chain. These have been reported as inhibitors of cAMP dependent protein kinases,²⁰⁷ with a crystal structure of an ARC inhibitor bound to PKA also reported.²⁰⁸ Studies in which the adenosine moiety has been replaced have also been investigated, including the 2-pyrimidyl-5-amidothiophene fragment,²⁰⁹ isoquinolinesulfonamide moiety,^{207,210} and the carbocyclic adenosine mimic.²¹¹

Thus far, the examples explored have been bisubstrate inhibitors which target both nucleotide and protein binding sites of kinases. Other bivalent inhibitors have been reported that target the nucleotide site and a secondary binding domain. Examples of this have been illustrated by Maly and co-workers,^{212,213,214} who have employed a chemical genetic method to synthesise protein-small molecule conjugate inhibitors.

Firstly, they investigated the two closely related non-receptor tyrosine kinases Src and Abl.^{212,213} Both contain the catalytic SH1 domain (containing the ATP and protein substrate binding sites) and the SH2 and SH3 domains which are responsible for the regulation, substrate selection and localisation of SH1. The synthesised bivalent inhibitor targets both the ATP binding site in the SH1 domain and a secondary binding domain in the SH3 domain (Figure 52).

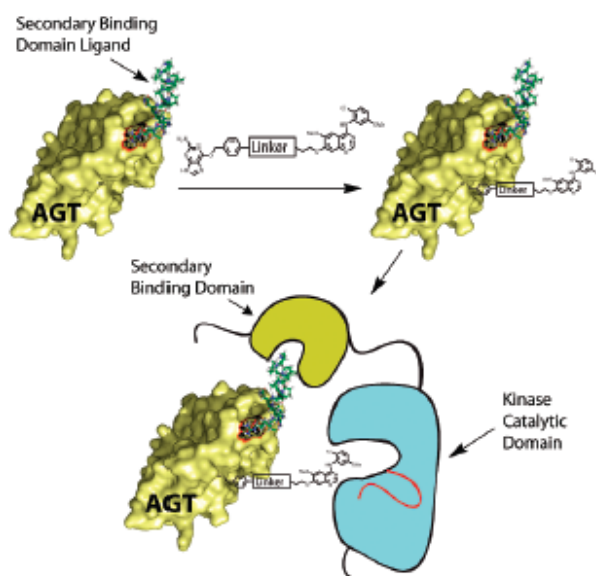


Figure 52: Representation of an AGT linked bivalent inhibitor of Src and Abl.²¹² Reproduced with permission.

The ATP inhibitor is a 4-anilinoquinazoline derivate which is then linked to *O*⁶-benzylguanine (BG). This can selectively be transferred to the active site cysteine of *O*⁶-alkylguanine-DNA alkyltransferase (AGT) which is primed with the SH3 domain ligand: short polyproline (PP) motifs. Src and Abl have distinct PP motif binding preferences in their SH3 domains and therefore selectivity between the two kinases can be achieved by using slightly different PP ligands.

A similar approach utilising an engineered form of AGT (SNAP-tag), was applied to a further three unrelated protein kinases: Pim1, p38 α and EGFR.²¹⁴ Each of the three kinases have different reported ATP-competitive inhibitors, which are linked to *O*⁴-benzyl-2-chloro-6-aminopyridine (CLP). This is then able to chemoselectively label the active site of SNAP-tag,

which has been fused to a peptide ligand that binds to the signalling interaction site of the targeted kinase.

Similarly, dipeptide inhibitors of Src kinase have been reported, consisting of an SH2 domain recognition sequence bound *via* a flexible GABA-based tether to an active site directed inhibitory peptide.²¹⁵

Whilst the aforementioned examples provide excellent potency and selectivity, a potential drawback is that they rely on structural information of the targeted kinase. A bivalent tethering approach adopted by Ghosh and co-workers,²¹⁶ requires no prior knowledge of the structure of the kinase or the protein substrate (Figure 53). It combines small molecule targeting and biological selection, by utilising a phage display cyclic peptide library. Phage display involves producing libraries of peptides displayed on phage, these can contain 10^{10} different peptides.²¹⁷

The active-site directed small molecule used was the inhibitor staurosporine, which is tethered to the ‘zipper’ domain of Jun. The cyclic peptide library is tethered to the zipper domain of Fos, which is able to form a coiled-coil heterodimer with Jun. When Fos and Jun come together the cyclic peptides are in close proximity to the small molecule. These conjugates (still bound to phage) have been screened against PKA²¹⁶ and Aurora Kinase A.²¹⁸

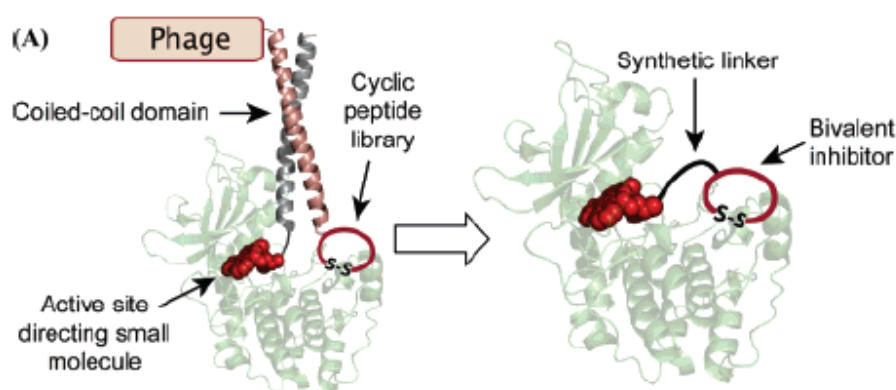


Figure 53: Non-covalent tethering approach of small molecule with a phage-display peptide library through a coiled-coil heterodimer for targeting PKA. Selected peptide can then be conjugated to the small molecule to create a bivalent kinase inhibitor.²¹⁶ Reproduced with permission.

This small warhead-guided phage display selection would potentially afford cyclic peptide motifs with affinity for the kinase surface adjacent to the active site. The selected peptides can then be conjugated to the small molecule to create a bivalent kinase inhibitor, with affinities higher than that of the staurosporine small molecule.^{216,218}

Work carried out in the laboratory of Ed Tate,²¹⁹ has previously looked at novel peptide-based inhibitors with a small-molecule binding partner (or ‘Trojan horse’) that target the active site of NMT (*N*-myristoyl transferase) (Figure 54).

The ‘Trojan horse’ is attached to a randomised peptide sequence, generating a library of small-molecule/peptide chimeras. This one-bead one-peptide library was constructed using a split-and-mix protocol, producing an even distribution of 19⁴ peptides. This protocol involves adding each amino acid onto a linker attached polystyrene/PEG copolymer resin in 19 pots; mixing the resin and splitting each pot into 19 portions and loading the next amino acid and then repeating this mix and split process. A terminal amino acid is added (1:1 aminooxy acetic acid and Glycine) allowing linkage to a myristoyl binding partner.

A high-throughput on-bead assay was then used to select peptides which enhance binding by making one or more contacts with nearby epitopes. This selection was based on staining the assayed library and resulted in 130 distinct compounds (0.1% hit rate). Five of the beads were then chosen and were Edman sequenced²²⁰ and re-synthesised for inhibition assays with the lowest IC₅₀ observed being 65 μ M.

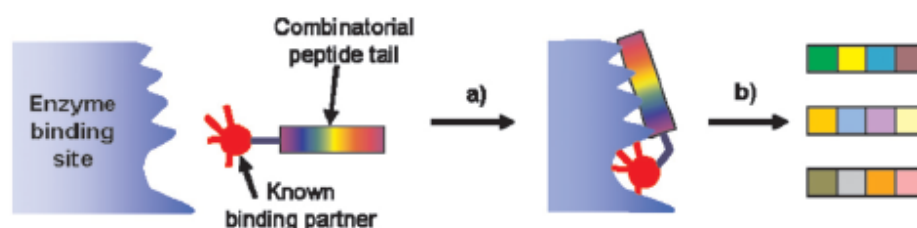


Figure 54: Schematic representation of the ‘Trojan horse’ approach for the generation of peptide aptamers targeted to a specific protein binding site. (a) Trojan horse is used to target a combinatorial peptide to the binding site of the protein; (b) High throughput on-bead assay selects for peptides which enhance binding.²¹⁹ Reproduced with permission.

The research presented herein incorporates the bivalent inhibitor approach discussed above. The imidazo[1,2-*a*]pyrazine compounds previously synthesised target the ATP binding site of HP0525, whilst a peptide sequence has been rationally designed to disrupt the peptide-peptide interactions of the subunit-subunit interface, and in doing so, disrupting hexamer formation.

4.1.2 Conjugation Techniques and Protein Modification

Posttranslational modification (PTM) is the natural chemical modification of a protein after its translation.²²¹ Folded proteins are subjected to specific enzyme-catalysed covalent modifications on their side chains (phosphorylation, acylation, alkylation, glycosylation and oxidation) or covalent cleavage of their backbone (by proteases or autocatalytic cleavage).²²¹

Chemical modification of proteins is a powerful tool for expanding a proteins functional capacity.^{222,223} Attachment of fluorescent/radioactive probes allows proteins to be tracked and imaged, in real time, *in vitro* and *in vivo*.^{224,225} Tethering of antigens to protein carriers has applications in synthetic vaccine candidates for HIV,²²⁶ cancer²²⁷ and malaria.²²⁸

For a reaction to be considered for protein modification, it must be site selective, efficient under conditions compatible with proteins; i.e. in aqueous media, at low to ambient temperatures and at or near to neutral pH; and be able to tolerate salts and surfactants used to stabilise protein structures.²²³

Most strategies rely on the nucleophilic side chains of residues of lysine, cysteine, aspartic acid and glutamic acid. However, there have been extensive studies into the use of transition-metal-catalysed reactions to carry out site-selective protein modifications using unnatural amino acids, especially with recent advances in transition metal-catalysed reactions that can be carried out in aqueous media.^{229,230}

Figure 55 gives examples of transition-metal-catalysed reactions used in protein modifications.^{223,231} These include tryptophan alkylation,²³² allylation of tyrosine,²³³ Heck and Sonogashira reactions of *p*-iodophenylalanine,^{234,235} Suzuki cross-coupling with *p*-boronophenylalanine,²³⁶ azide-alkyne [3+2] cycloaddition^{237,238,239,240} and olefin metathesis.^{223,241}

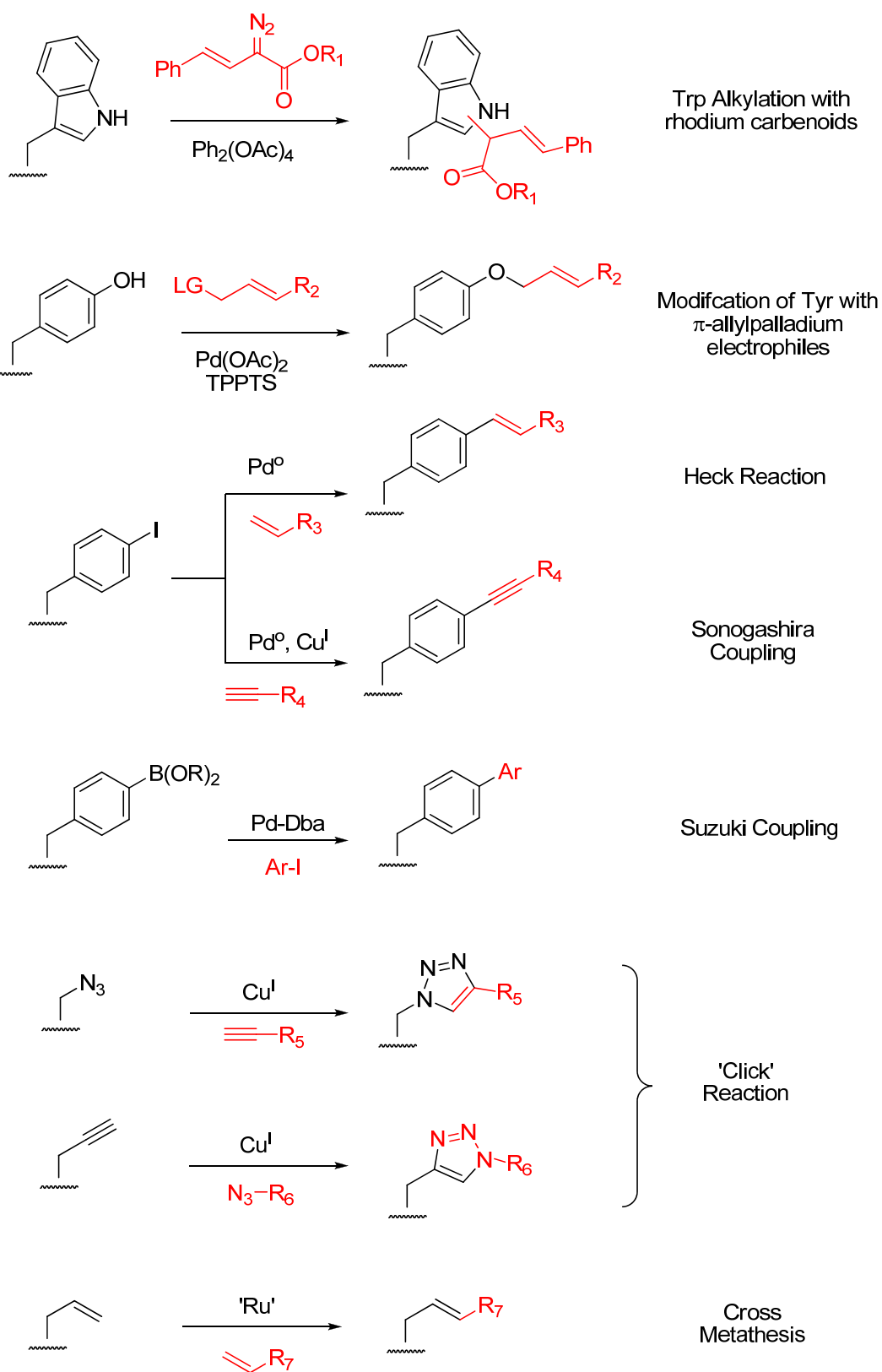
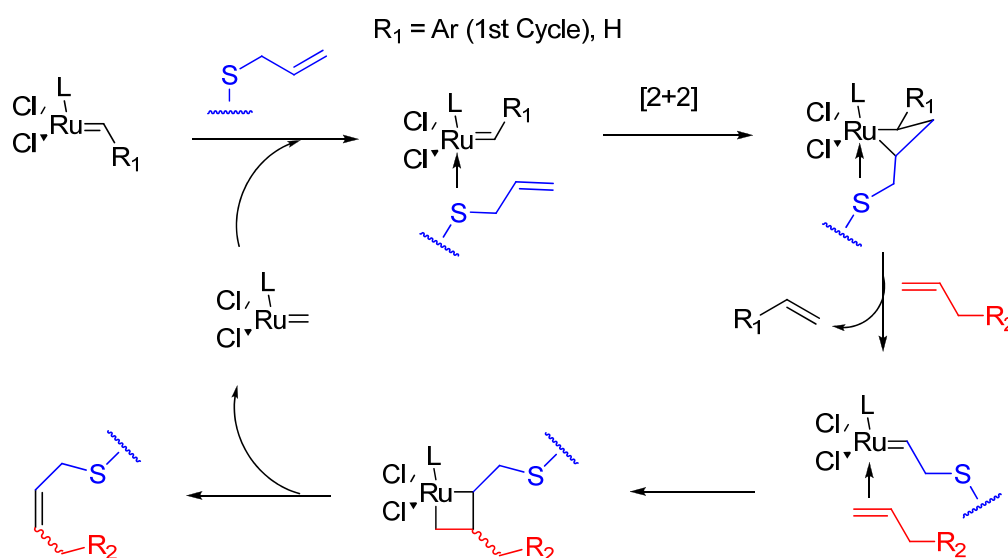


Figure 55: Examples of transition-metal-catalysed reactions used in protein modification²²³

Olefin metathesis has been used to introduce non-labile C-C bonds in order to:

- Stabilise peptide secondary structure, including cross-linking α -helices²⁴² and substituting carbon equivalent of disulfides^{243,244} and thioether links,²⁴⁵
- Introduce post-synthetic labelling with functional tags.^{182,223,241}

Allyl amines, ethers and sulfides have been examined in the cross metathesis with allyl alcohol, and allyl sulfides have been found to be the most reactive in aqueous media and therefore most compatible when considering modification of proteins.¹⁸² The enhanced activity of allyl sulfides can be attributed to sulfur coordination to the ruthenium centre as shown in the suggested mechanism for sulfur-assisted cross metathesis (Scheme 59).^{182,223}

Scheme 59: Sulfur-assisted Cross Metathesis of allyl sulfides^{182,223}

Different methods of incorporating allyl sulfides into proteins have been investigated²⁴¹ and these include conjugate addition of allyl thiol to dehydroalanine (Dha) (installed by the oxidative elimination of cysteine using *O*-mesitylenesulfonylhydroxylamine (MSH)); direct allylation of cysteine residues (achieved using both the amino acid and on the protein surface); and desulfurization of allyl disulfide. It has also been shown that *S*-allyl cysteine can be genetically incorporated into proteins as a methionine surrogate.²²³

MgCl₂ has also been used as a mild Lewis acid additive to prevent non-productive chelation of the metal centre to other side chain groups.¹⁸² Hoveyda-Grubbs second generation catalyst²⁴⁶ (Figure 56) has been selected as it is phosphine-free and therefore more likely to be compatible with protein disulfides than other catalysts.

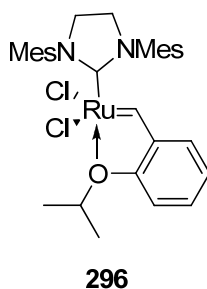


Figure 56: Structure of Hoveyda-Grubbs Second Generation Catalyst²⁴⁶

Click chemistry describes chemistry that is used to generate substances quickly and reliably by joining small units together.²⁴⁷ A number of criteria must be met for a reaction to be deemed a ‘Click’ Reaction including being modular, wide in scope, high yielding, stereo-specific, simple reaction conditions, readily available starting materials and simple product isolation.²⁴⁷

Click reactions achieve their characteristics by having a high thermodynamic driving force and are therefore thought of as being “spring-loaded”.²⁴⁷ Carbon-heteroatom bond forming reactions are the most prevalent and include cycloadditions, nucleophilic substitutions, carbonyl chemistry and addition of C-C bonds.²⁴⁷

Azides are ideal bioorthogonal chemical tags as they are not found in biological systems and are inert to such conditions.²⁴⁸ They are easy to install, small and can react either as an electrophiles in the Staudinger Ligation,^{249,250} or as 1,3-dipoles in [3+2] cycloadditions.^{247,251} Copper catalysed Azide-Alkyne Huisgen [3+2] cycloadditions have numerous applications in bioconjugation chemistry,^{237,238,239,240} and there are also instances of Cu-free reactions using strained cyclooctyne probes.^{252,253,254}

In addition, there are numerous methods of non-transition metal catalysed bioconjugations, especially when applied to PEG containing substrates.^{255,256} Typical reactive amino acids include

lysine, cysteine, histidine, arginine, aspartic acid, glutamic acid, serine, threonine, tyrosine, *N*-terminal amino group and the *C*-terminal carboxylic acid.

Various groups used for conjugation to amine residues^{255,256} include dichlorotriazine, tresylate, succinimidyl carbonate, benzotriazole carbonate, *p*-nitrophenyl carbonate, carbonyl imidazole and succinimidyl succinate. Those used for cysteine conjugation^{255,256} include maleimide, bromo-maleimide,²⁵⁷ vinyl sulfone, iodoacetamide and *o*-pyridyl disulfide.

It is evident that there are many different approaches to achieving selective modification of proteins and peptide. This chapter investigates some of the different conjugation techniques discussed above and their application into the synthesis of bivalent inhibitor reagents.

4.2 Bivalent Inhibitor Reagents as Potential ATPase Inhibitors

As previously mentioned, one of the aims of this project are to synthesise bivalent inhibitor reagents. These consist of a small molecule inhibitor, based on imidazo[1,2-*a*]pyrazine, that will bind to the nucleotide binding site, linked (*via* a PEG chain) to a peptide, based on the α F helix of HP0525, that will interact with the adjacent subunit (Figure 57).



Figure 57: Generic representation of bivalent inhibitor reagents

A number of factors need to be considered when designing the bivalent inhibitor reagents:

1. What is the nature of the small molecule?
2. How will the small molecule be connected to the PEG chain?
3. What will be the peptide sequence and length?

4. How will the PEG chain be connected to the peptide?
5. What will be the length of the PEG chain?

Firstly, the nature of the small molecule inhibitor needs to be decided as well as the location of the linker to the PEG chain. It should be noted that work commenced on this area prior to the synthesis of the 2nd generation compounds (Chapter 3) and therefore at the time of design, **14** was the lead compound. It requires a shorter synthesis compared to the 3-aryl isomer **12**, and therefore was used as the small molecule part. Analogues of **8** were also used, since it possess the sulfonamide moiety bound directly to the 8-position and is therefore possibly easier to access synthetically.

In silico studies of the lead compound, **14**, have suggested its favoured orientation within the enzyme active site: the naphthalene occupies the purine region towards the solvent side (outside) of the enzyme; and the sulfonamide moiety with the terminal toluoyl group, is in the phosphate region, pointing towards the adjacent subunit and the centre of the hexameric chamber. Therefore it was decided to connect the PEG based linker *via* the toluoyl moiety.

Secondly, it was decided to utilise a carbamate functionality to link the small molecule to the PEG chain. This is because it would be more stable than an ester bond *in vivo*, would provide extra hydrogen bonding possibilities compared with an amide bond and can be considered to be easier to access synthetically. Using the Curtius reaction,²⁵⁸ the carbamate can be formed through the reaction of an acyl azide and a PEG alcohol, which would be more accessible than the corresponding PEG amine required to form an amide bond.

It is important to establish the sequence and the length of the peptide to be synthesised. The strategy employed herein is to synthesise a peptide that resembles the native sequence of the region highlighted in Figure 58 (i.e. the α F helix and neighbouring residues). It is envisaged that by anchoring the small molecule in the nucleotide binding site, the peptide part of the chimera would interact with the subunit-subunit interface, possibly displacing the native segment and in the process disrupting hexamer formation.

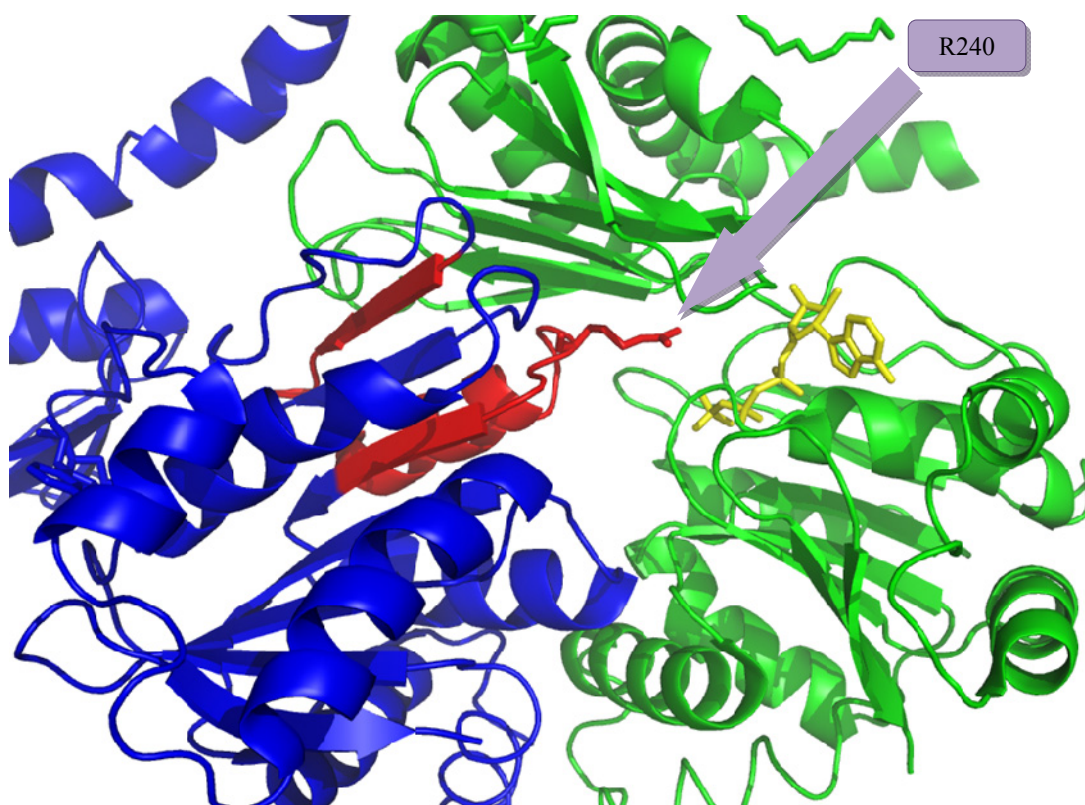


Figure 58: Crystal Structure of ATP γ S highlighting the targeted peptide region at the subunit-subunit interface. ATP γ S is in yellow. Image generated using PyMOL.

The peptide sequence highlighted consists of the β 9 sheet followed by a loop into the α F helix followed by a further loop into the β 10 sheet and is further illustrated in Figure 59. It is unclear what length of peptide would be required to interact with the native peptide region and therefore a small number of peptides with varying lengths were chosen for synthesis:

- α F with loop (14 amino acids): Ser-Ala-Asp-Cys-Leu-Lys-Ser-Cys-Leu-Arg-Met-Arg-Pro-Asp;
- α F and β 10 (18 amino acids): Ser-Ala-Asp-Cys-Leu-Lys-Ser-Cys-Leu-Arg-Met-Arg-Pro-Asp-Arg-Ile-Ile-Leu;
- β 9 and α F and β 10 (28 amino acids): Tyr-Thr-Glu-Leu-Phe-Phe-Gly-Gly-Asn-Ile-Thr-Ser-Ala-Asp-Cys-Leu-Lys-Ser-Cys-Leu-Arg-Met-Arg-Pro-Asp-Arg-Ile-Ile-Leu.

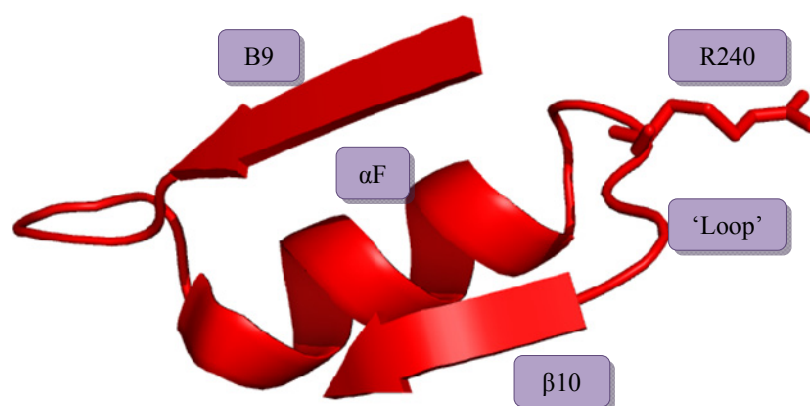


Figure 59: Highlighted peptide region from Figure 58 showing the different secondary structures and the potential first point of attachment for conjugation. Image generated using PyMOL.

Arginine in position 240 (R240) is in close proximity to the nucleotide binding site (Figure 58) and therefore its position is ideal for conjugation to the imidazo[1,2-*a*]pyrazine partner. By substituting this arginine for other amino acids capable of conjugating to other moieties, the bivalent inhibitor reagent could be synthesised. Three different approaches were initially investigated to connect the PEG chain to the peptide (Figure 60):

- Maleimide to conjugate to cysteine, “R240C”;
- Allyl to carry out cross metathesis with *S*-allyl cysteine, “R240SAC”;
- Alkyne to carry out click chemistry with azido lysine, “R240AzLys”;

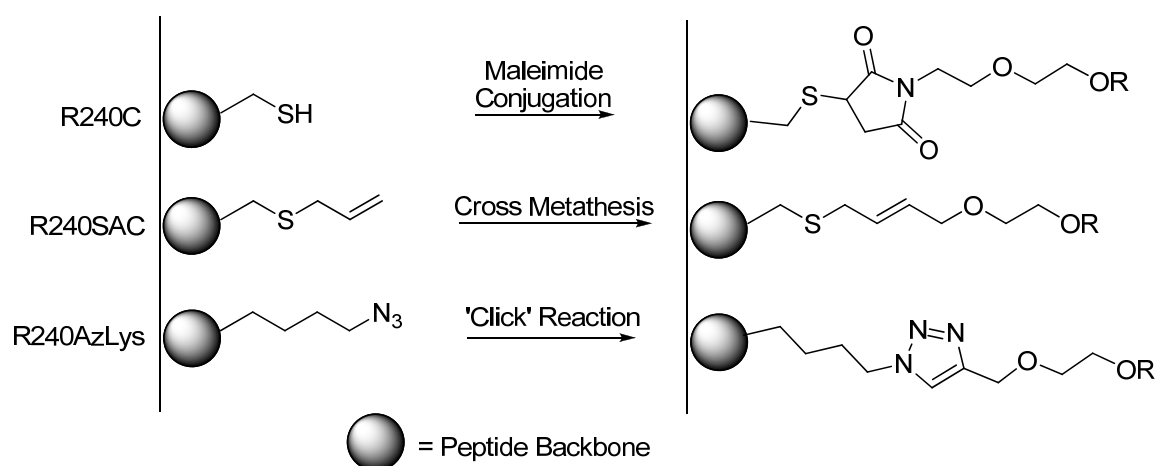


Figure 60: Representation of the each of the four approaches of conjugation and the PEG chain length required for each type of linker

The length of the PEG chain is also an important factor with the design of the bivalent inhibitor reagents. The PEG chain length should be of sufficient length to extend from the carbamate linked to the toluoyl moiety, to the peptide sequence that will make interactions with the adjacent subunit. *In silico* modelling studies, using the modelling programme MOE²⁵⁹ were carried out to determine the distance between the mutated residue in the peptide sequence and **14** (Table 21).

Mutated Residue	Cysteine	S-Allyl Cysteine	Azido Lysine
Distance (Å)	8.36	5.56	6.13

Table 21: Table indicating distances between the mutated residue and **14**, as determined using MOE²⁵⁹

Using MOE,²⁵⁹ by taking each of the mutated residues and extending them atom-by-atom until the chain reaches the nucleotide binding site, an indication of the required chain length can be determined. In all of the cases, in order to reach the phosphate region (i.e. where the PEG chain will be linked to the small molecule) and taking into account the size of the moiety to be conjugated (maleimide, vinyl sulfone, allyl and alkyne), one PEG unit is required. For example, even though the cysteine is further away from **14** compared with *S*-allyl cysteine, when the

maleimide conjugation is carried out the resulting species is similar in length to that after cross metathesis (Figure 60).

Figure 61 illustrates the end product from the step-by-step atom growth from a mutated cysteine, conjugating through a maleimide to a short PEG chain, which in turn is connected to **14** via a carbamate linker. If conjugation was required to a Maybridge fragment, a longer PEG linker would also be required comprising 5 PEG units. This would enable the chain to reach the purine region of the active site, a potential location for the fragments as seen from *in silico* studies.

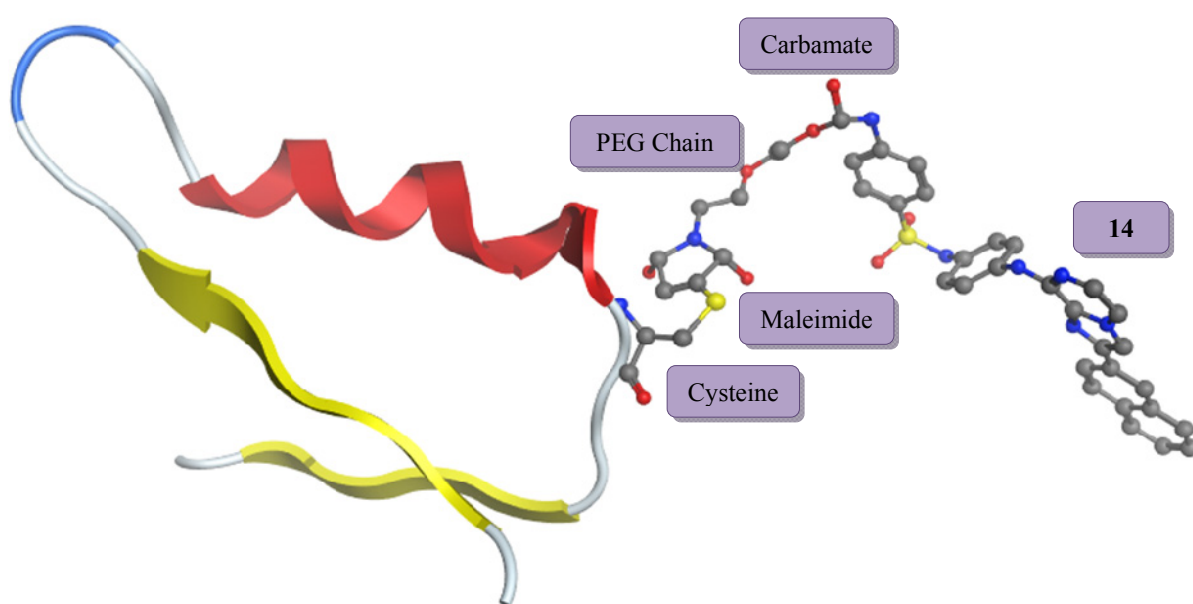


Figure 61: Representation of a bivalent inhibitor reagent: 14 linked *via* carbamate linker to PEG chain, which is linked, through a maleimide to a cysteine residue in the peptide sequence. Image generated using MOE²⁵⁹

There are a number of possible approaches to the synthesis of these bivalent inhibitor reagents. The strategy adopted herein is to synthesise the small molecule bound to the PEG chain primed with the conjugation moiety ('PEGylated-imidazo[1,2-*a*]pyrazine') followed by conjugating to a synthesised peptide. Other options could be to synthesise the peptide, conjugate to the PEG chain and then connect to the imidazo[1,2-*a*]pyrazine; or to synthesise the PEGylated-imidazo[1,2-*a*]pyrazine, conjugate to the relative amino acid and then incorporate into the peptide synthesis.

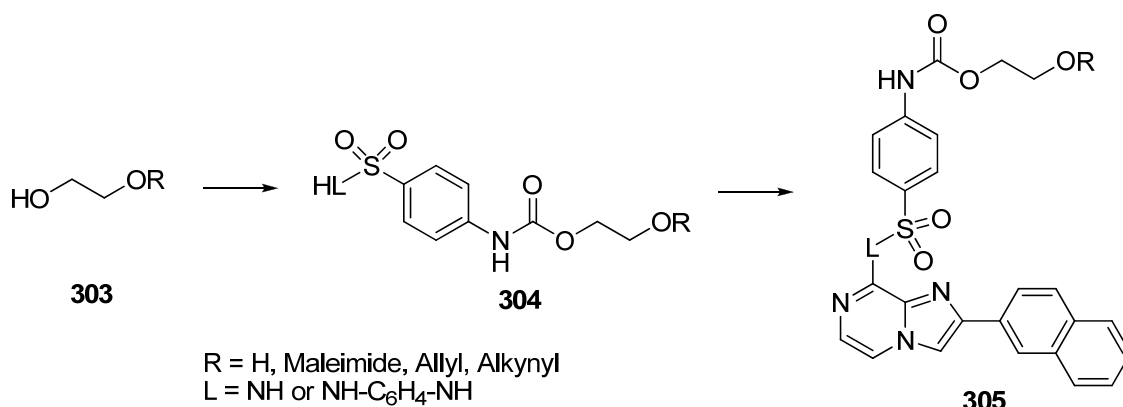
Figure 62 illustrates the target compounds primed with a conjugation moiety that could be conjugated to a synthetic peptide.

R	L	
	-NH-	-NH-C ₆ H ₄ -NH-
	297	300
	298	301
	299	302

Figure 62: Target PEGylated-imidazo[1,2-*a*]pyrazine compounds primed various conjugation moieties

4.3 Synthesis of PEGylated Imidazo[1,2-*a*]pyrazines

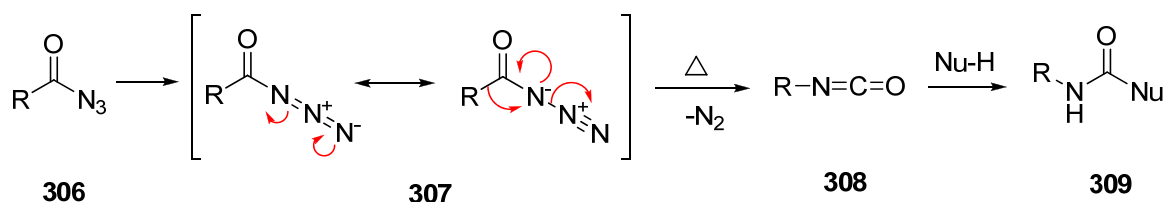
The initial approach of forming the PEGylated-imidazo[1,2-*a*]pyrazines is outlined in Scheme 60. It comprises the synthesis of the PEG alcohol primed with the maleimide, allyl or alkyl moieties, followed by a Curtius reaction to link the sulfonamide moiety and the PEG chain. Finally coupling to the core heterocycle can be carried out using the methods previously described. It was envisaged that both the 4-toluenesulfonamide bonded directly to the imidazo[1,2-*a*]pyrazine and the *N*-(4-aminophenyl)-4-methylbenzenesulfonamide would be investigated and the conjugates could then be compared with **8** and **14** respectively.



Scheme 60: Generic reaction scheme illustrating the order of reactions in the synthesis of the PEGylated imidazo[1,2-*a*]pyrazines

4.3.1 Synthesis of the Carbamate Linker for PEG attachment to Imidazo[1,2-*a*]pyrazine Inhibitors

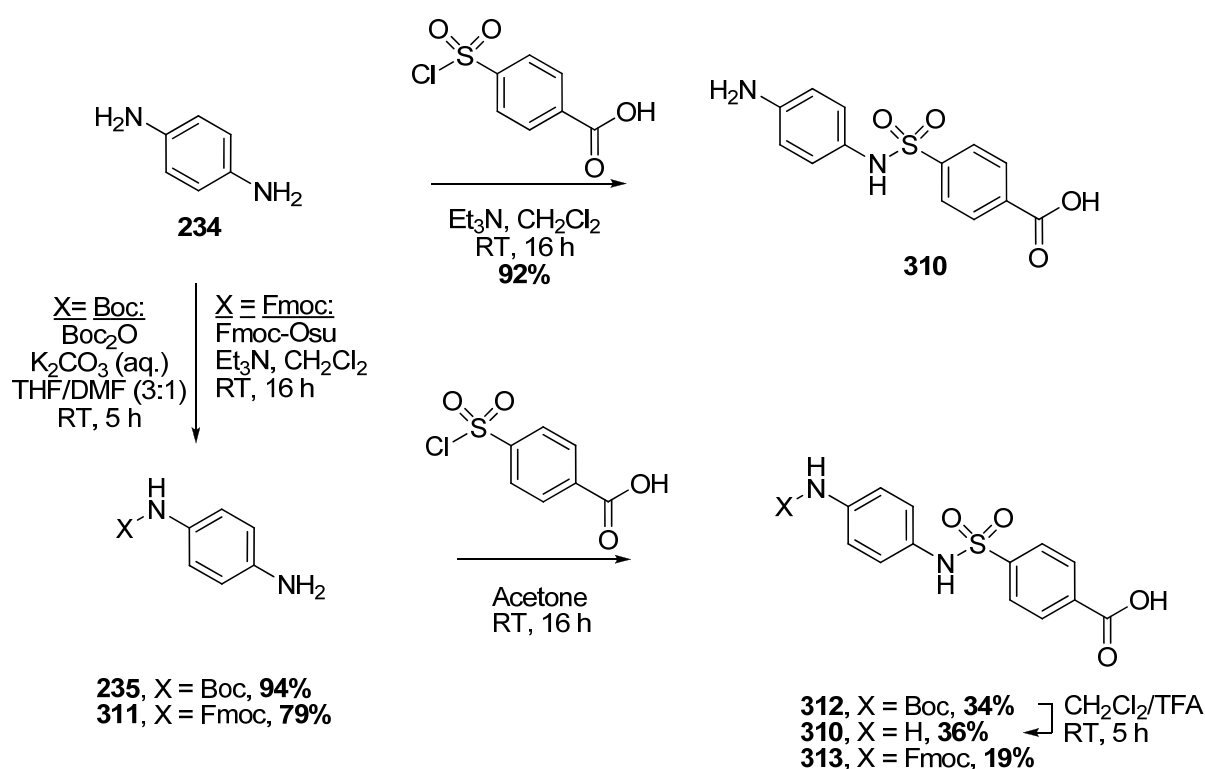
In order to attach the PEG chain to the imidazo[1,2-*a*], a carbamate linker would replace the methyl of the tosyl moiety of **8** and **14**. One method for installing this functionality is to utilise the Curtius reaction which is the thermal decomposition of carboxylic azides to produce an isocyanate.²⁵⁸ These intermediates can then be isolated, or reacted with a variety of nucleophiles to form different functional moieties; for example alcohols and amines will form carbamates and ureas respectively (see Scheme 61). It is important to carry the reaction out under anhydrous conditions as water will react with the isocyanate to form the unstable carbamic acid which will undergo spontaneous decarboxylation to leave the primary amine.



Scheme 61: Generic mechanism for the Curtius reaction²⁵⁸

Scheme 62 outlines the synthesis of the 4-(*N*-(4-aminophenyl)sulfamoyl)benzoic acids, which can then be transformed to the carboxylic azides for the Curtius reaction. 4-Sulfamoylbenzoic

acid is commercially available. **310** can be formed in high yields by reacting 4-(chlorosulfonyl)benzoic acid with a 5-fold excess of 1,4-diaminobenzene. The yield calculated is for the triethylamine salt; however, even though the ethyl peaks are present by NMR, the integration does not correspond to a 1:1 stoichiometry. Elemental analysis also does not match the composition of a triethylamine salt and therefore the compound was probably isolated as a partial triethylamine salt. When using 1,4-dioxane instead of CH₂Cl₂ no reaction was observed and if no Et₃N was used (CH₂Cl₂ only), the product was isolated in 59% yield.



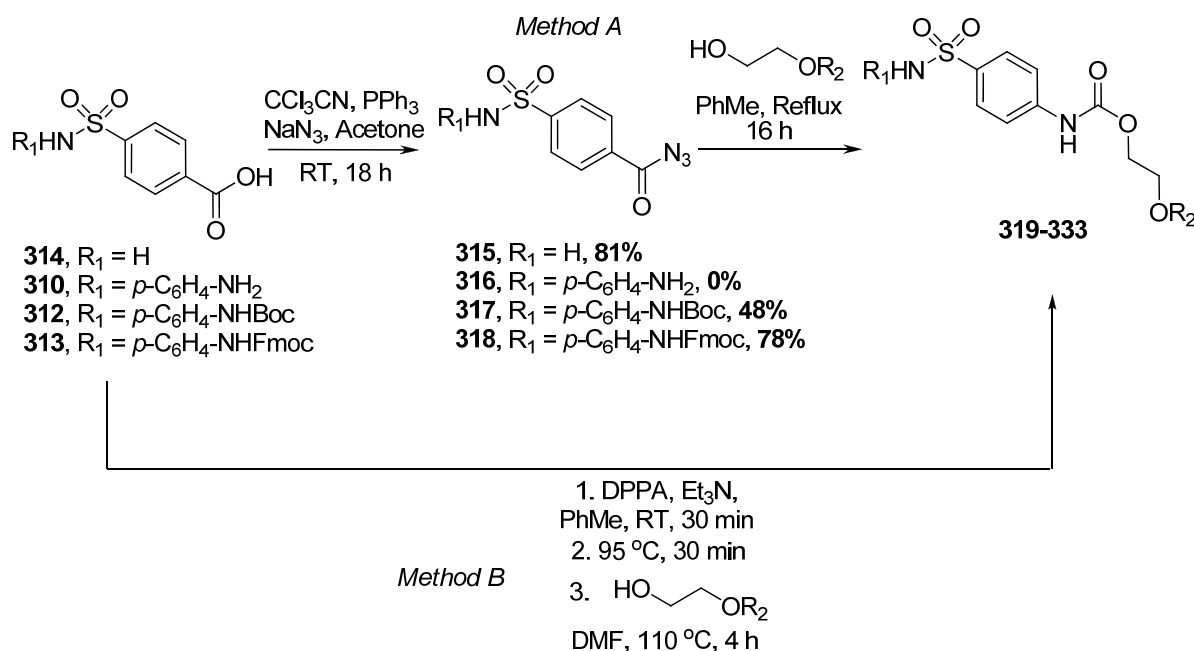
Scheme 62: Synthesis of the carboxylic acids to be used in the Curtius reaction

Both Boc²⁶⁰ and Fmoc protection of 1,4-diaminobenzene were carried out with high yields. Coupling with the 4-(chlorosulfonyl)benzoic acid proved to be problematic and low yielding. When considering the Boc-protected reaction, a 4:1 stoichiometry of the amine to sulfonyl chloride was used and isolation was completed by washing with acid and then precipitating out the product with base.²⁶¹ When a 1:1 stoichiometry was used, purification *via* flash chromatography proved to be extremely difficult due to co-elution with the sulfonyl chloride and

multiple columns were required to isolate **312**. TFA deprotection could afford **310**, but this is unnecessary in light of the high yielding one-step approach.

Purification of **313** also proved to be difficult, with co-elution observed. For both Boc and Fmoc routes, using the same conditions as with the one-pot approach, i.e. 5-fold excess of the amine and Et₃N in CH₂Cl₂ followed by column chromatography, could lead to increased yields. However, care must be taken to avoid Fmoc deprotection under the basic conditions.

Two methods of forming the carbamate from the carboxylic acids synthesised were explored (Scheme 63): Method A involves the formation and isolation of the acyl azide from the corresponding carboxylic acid;²⁶² and method B is a one-pot method where the acyl azide is formed *in situ*.²⁶³ Different R₁ groups were investigated: these are H, for direct sulfonamide bonding to imidazo[1,2-*a*]pyrazines (analogues of **8**); aniline and two protected forms of aniline (analogues of **14**).



Scheme 63: Synthetic route to access carbamate containing sulfonamides

It can be seen that, with the exception of the unprotected aniline **316**, each of the acyl azides can be isolated in good yields. It should, however, be noted that due to their instability at high

temperatures, no product mass was observed using a variety of ionising techniques and so NMR and IR were used for characterisation. Through heating these products with the PEG alcohols in toluene, the Curtius reaction can take place to form the desired products. Table 22 shows the different Curtius reactions carried out using both methods and their yields. It is evident that Method A is the more consistent route, with no reaction often observed when employing the one-pot method. However, the reactions that failed in these cases used the unprotected aniline, which as previously explained proved to be problematic when forming and isolating its acyl azide using Method A. When the one-pot method did work, it gave the product in a lower yield than using the 2-step approach (Entries 1 and 2). Each of the individual reactions will be discussed in the following sections.

Entry	Product	R ₁	R ₂	Method	Yield/%
1	319	H	TBDMS	A	50
2	319	H	TBMDS	B	11
3	320	H	CH ₂ CH ₂ TBDMS	A	37
4	321	H	(CH ₂ CH ₂) ₂ TBDMS	A	46
5	322	H	CH ₂ CH ₂ Mal	A	0
6	322	H	CH ₂ CH ₂ Mal	B	0
7	323	H	CH ₂ CH ₂ NHBoc	A	77
8	324	H	CH ₂ CH=CH ₂	B	23
9	325	H	CH ₂ C≡CH	A	40
10	326	<i>p</i> -C ₆ H ₄ -NH ₂	TBDMS	B	0
11	327	<i>p</i> -C ₆ H ₄ -NH ₂	CH ₂ CH ₂ Mal	B	0
12	328	<i>p</i> -C ₆ H ₄ -NH ₂	CH ₂ CH=CH ₂	B	0
13	329	<i>p</i> -C ₆ H ₄ -NHBoc	TBMDS	A	87

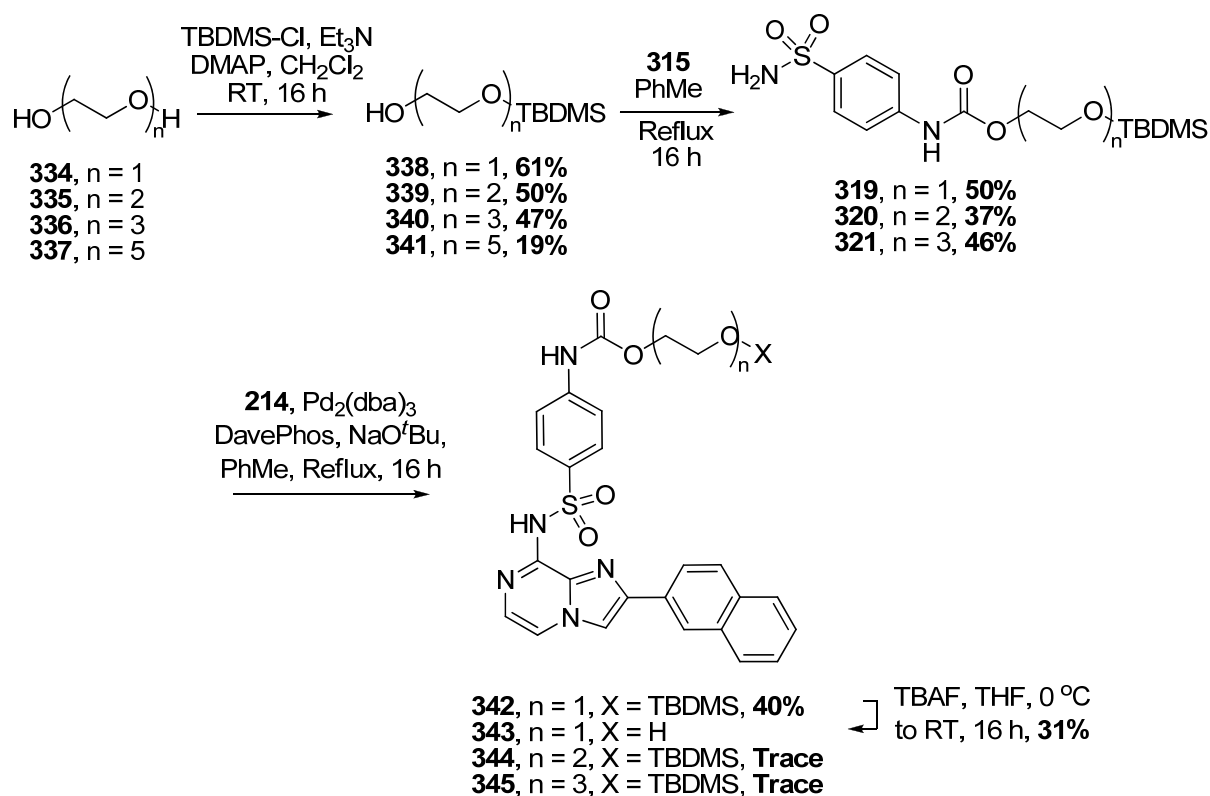
14	330	<i>p</i> -C ₆ H ₄ -NHBoc	CH ₂ CH ₂ TBDMS	A	65
15	331	<i>p</i> -C ₆ H ₄ -NHBoc	CH ₂ CH ₂ NHBoc	A	74
16	332	<i>p</i> -C ₆ H ₄ -NHBoc	CH ₂ CH=CH ₂	A	61
17	333	<i>p</i> -C ₆ H ₄ -NHFmoc	CH ₂ CH ₂ NHBoc	A	20 ^a

Table 22: Table displaying the yields for the different Curtius reactions carried out. ^aReaction time of 2 h. Method A: *via* isolation of acyl azide; Method B: one-pot reaction from carboxylic acid

4.3.2 Synthesis of PEGylated Free Hydroxyl Imidazo[1,2-*a*]pyrazines

4.3.2.1 PEGylated-Benzenesulfonamide-bound Imidazo[1,2-*a*]pyrazines

Considering the poor solubility of the imidazo[1,2-*a*]pyrazine inhibitors, it was decided to incorporate a PEG chain with a free terminal hydroxyl. Scheme 64 outlines the synthesis of these compounds based on the **8** inhibitor. Mono silyl ether protection of different length ethylene glycols was achieved in moderate yields using *tert*-butyldimethylsilyl chloride (TBDMS-Cl), Et₃N and DMAP additive.²⁶⁴ It should be noted that the relatively low yields here are due to a slight excess of the TBDMS-Cl used and therefore expected di-protection. Using an excess of the n-ethylene glycol should avoid this and boost the yield. The use of imidazole in place of the Et₃N and DMAP for **338** was also investigated,¹⁸² and resulted in a yield of 34%.

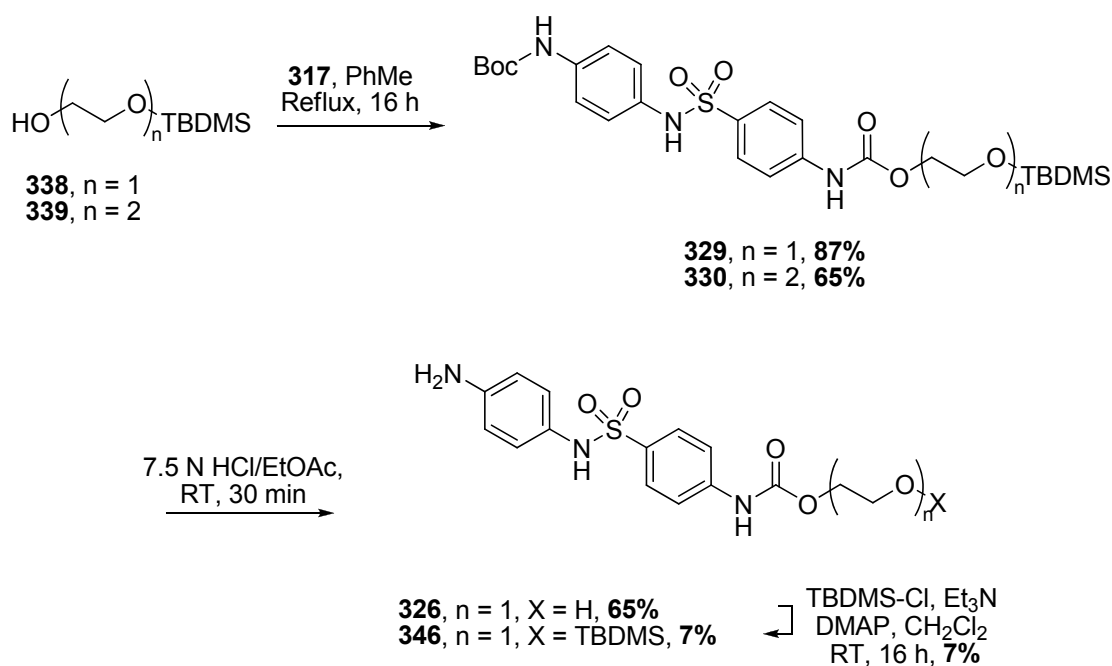


Scheme 64: Synthesis of PEGylated free hydroxyl benzenesulfonamide-bound imidazo[1,2-*a*]pyrazines

Curtius reaction using the acyl azide **315** proceeded in moderate yields to give the sulfonamides with carbamate linker. An uncharacterised side product, thought to be a methyl ester, did however form in the reactions involving **339** and **340**, further indicating the instability of the acyl azide and the need for reagents to be pure and dry. The products were then coupled to the core heterocycle using the optimised Buchwald-Hartwig conditions with varying success. **319** coupled with 40% yield but the two longer PEG lengths, **320** and **321** only coupled with trace amounts of product visible. It is suggested that using the alternative Cs_2CO_3 /dioxane conditions are attempted as there could be a problem with the solubility of the PEG compound. Deprotection of **342** was accomplished using standard TBAF conditions in THF with a yield of 31%.¹⁸¹ There were, however, problems with purification, and the final compound isolated was not completely pure.

4.3.3.2 Attempted Synthesis of PEGylated-*N*-(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2-*a*]pyrazines

The synthesis of the **14** analogue proved to be more problematic and in fact no desired product was able to be synthesised. Scheme 65 shows the attempted synthesis of the *N*-(4-aminophenyl)benzenesulfonamide containing the carbamate linker. Curtius reaction with the Boc-protected aniline acyl azide **317** proceeded in high yields; however, problems arose when attempting the selective deprotection of the Boc group over the TBDMS group.²⁶⁵ In the case attempted, the only product isolated was the doubly deprotected species **326**. All subsequent attempts to re-protect the hydroxyl²⁶⁶ resulted in extremely low yields, even when using 4 equivalents of TBDMSCl and so no further work was carried out on this route. The next stage would have involved Buchwald-Hartwig cross coupling to 8-chloroimidazo[1,2-*a*]pyrazine.

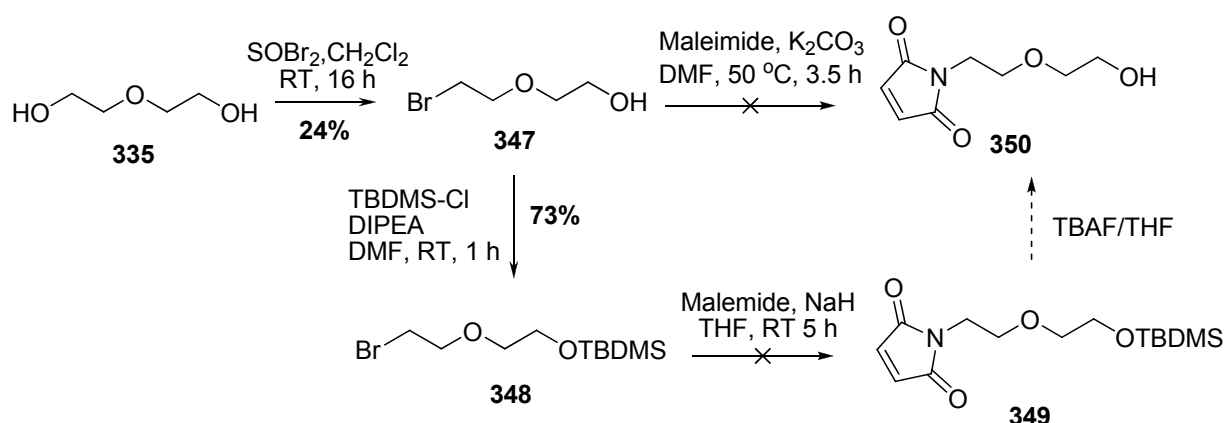


Scheme 65: Attempted synthesis of PEGylated free hydroxyl *N*-(4-aminophenyl)benzenesulfonamide-bound imidazo[1,2-*a*]pyrazines

4.3.3 Synthesis of PEGylated Maleimide Linked Imidazo[1,2-*a*]pyrazines

4.3.3.1 PEGylated-Benzenesulfonamide-bound Imidazo[1,2-*a*]pyrazines

The initial approach to synthesising PEGylated-imidazo[1,2-*a*]pyrazines primed with a maleimide was to synthesise the PEG chain with maleimide and then carry out the Curtius reaction (as previously discussed in Scheme 60). Two methods to synthesise the PEGylated-maleimide were investigated; the first involved mono-bromination of diethylene glycol and hexaethylene glycol using thionyl bromide (Scheme 66).²⁶⁷ Both reactions were low yielding due to formation of the di-brominated species and difficulties with purification. Attempts to directly react **347** with maleimide using K_2CO_3 ²⁶⁸ proved unsuccessful, as did the reaction with the protected hydroxyl **348**²⁶⁹ and the use of the stronger base NaH.

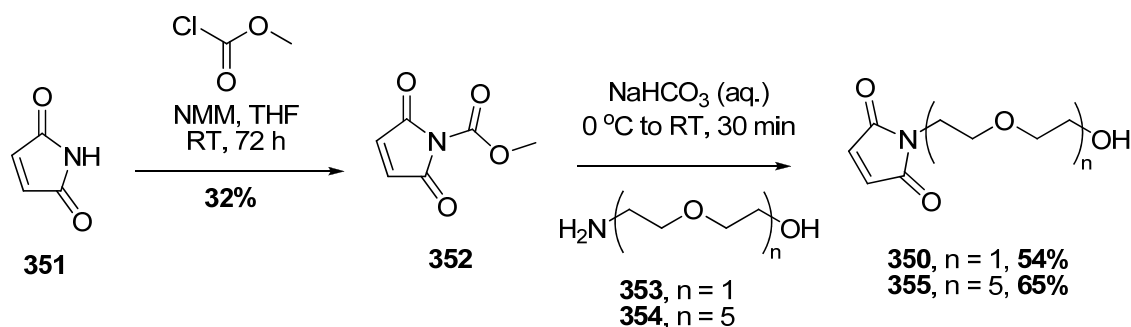


Scheme 66: Attempted synthesis of PEGylated-maleimide *via* reaction between the brominated PEG and maleimide

A possibility that was not explored is the use of the Mitsunobu reaction in forming **350**. Mono-protection of diethylene glycol may be required, but it should be possible to react the PEG alcohol with maleimide using DEAD and PPh_3 in THF.²⁷⁰

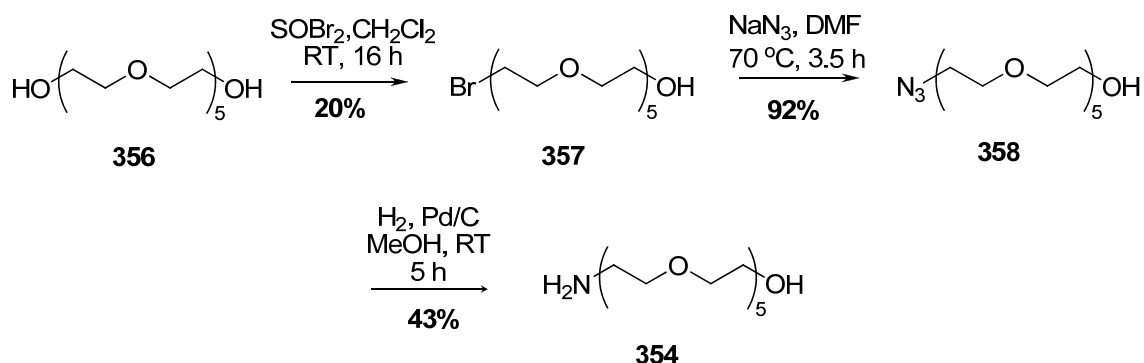
An alternative method involved the synthesis of the activated maleimide carbamate, **352**, which is able to react with a PEG amine to form the desired PEGylated-maleimide under basic aqueous conditions (Scheme 67).²⁷¹ The reaction between maleimide and methyl chloroformate proceeds in either EtOAc (23%) or THF (32%) but due to the instability of the product in solution and on silica, the isolated yield is very low. This also contributes to the moderate yields observed when

reacting with the PEG amines, and it is suggested that using an excess of the activated maleimide carbamate could enhance the yields. In fact a trial stability test was carried out by stirring **352** in the coupling media (sat. aq. NaHCO_3), and it was observed that after 30 minutes no material remained.



Scheme 67: Synthesis of PEGylated-maleimide *via* reaction between amino-PEG compounds and activated PEG carbamate

Out of the two reacting PEG amines, compound **353** is commercially available, but the longer hexaethylene amine, **354**, required synthesis (Scheme 68). Brominating hexaethylene glycol,²⁶⁷ followed by substituting the bromo for an azide²⁷² and then reducing to the amine using standard hydrogenation conditions, affords the desired product. The reason for the low yield in this final step is due to difficulties with removing the extremely polar product from the silica column.



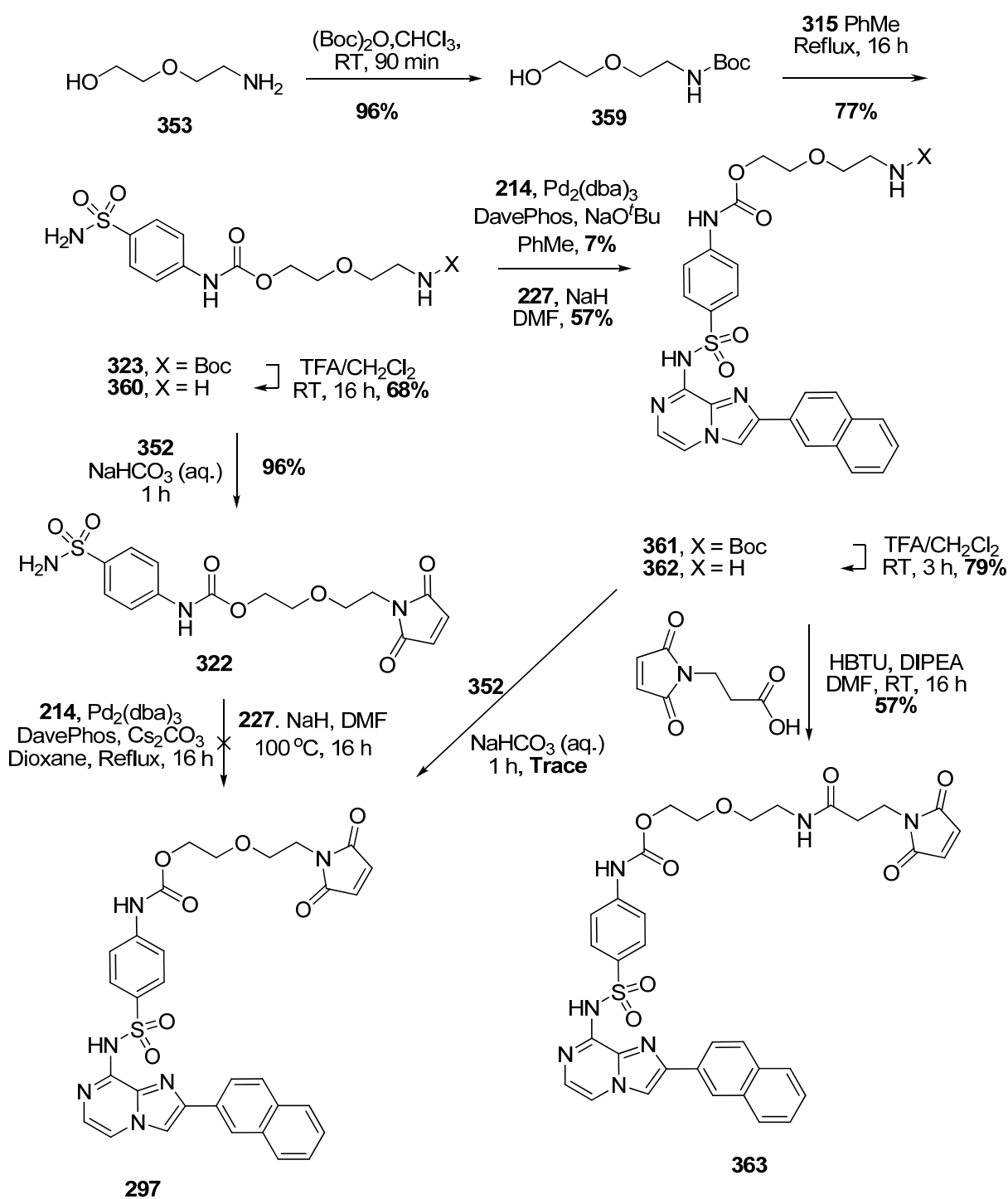
Scheme 68: Synthesis of PEG amine **354**

As can be seen from Table 22, no reaction was observed when **350** was subjected to the Curtius reaction with both **315** (Entries 5 and 6) and **271** (Entry 11), using both methods A and B. When reacting with **315** (Method A), the PEGylated maleimide would not dissolve, even under reflux conditions. It took on the appearance of an extremely viscous oil that was stuck to the reaction vessel. It was also observed that **350** took on this appearance after a period of time of drying, suggesting that the compound may have polymerised. Due to the non-reactivity of this route and potential stability problems of the intermediates and products, an alternative approach was devised.

Scheme 69 outlines the two alternative methods attempted for the synthesis of the PEGylated-maleimido-benzenesulfonamide-bound imidazo[1,2-*a*]pyrazines. Both involve successfully carrying out the Curtius reaction with **315** and the Boc-protected 2-(2-aminoethoxy)ethanol, **359**,²⁷³ to give **323** in high yields. Boc-deprotection was then carried out, followed by successful coupling with the activated maleimide carbamate (using excess **352**), but attempts to couple **322** to the core heterocycle failed. Both Buchwald-Hartwig coupling conditions to **214** and solvent/base conditions with **227** resulted in no signs of starting material left, but no desired product observed, suggesting that **322** and in particular the maleimide moiety is unstable to either Pd-catalysed conditions, strong bases or high temperatures.

Instead, coupling of **323** to the heterocycle was first carried out. Both sets of coupling conditions were attempted, and it was evident that using **227** with NaH and DMF was the preferred method, consistently giving yields of 52-57%. Boc deprotection proceeded in high yields; but **362** should be left as the TFA salt since decomposition was observed when attempting a basic work up. Elemental analysis also indicated 4 molecules of TFA present in the salt. Coupling with the activated maleimide carbamate **352** produced trace quantities of the desired product **297** using previously reported conditions. A possible explanation for this could be poor solubility of the reacting species, but when using Et₃N and DMF, the reaction profile by TLC was extremely messy and no product was observed by LCMS or NMR.

It was then decided to couple *N*-maleoyl- β -alanine using standard amide bond forming conditions. The low yield can be attributed to loss of material during reaction work up and also purification using column chromatography. Using excess amine (at least 1.5 eq) is advised due to potential separation issues with the acid. When using slight excess of the acid, reverse phase preparative HPLC was required to purify the product, resulting in a yield of 43%.

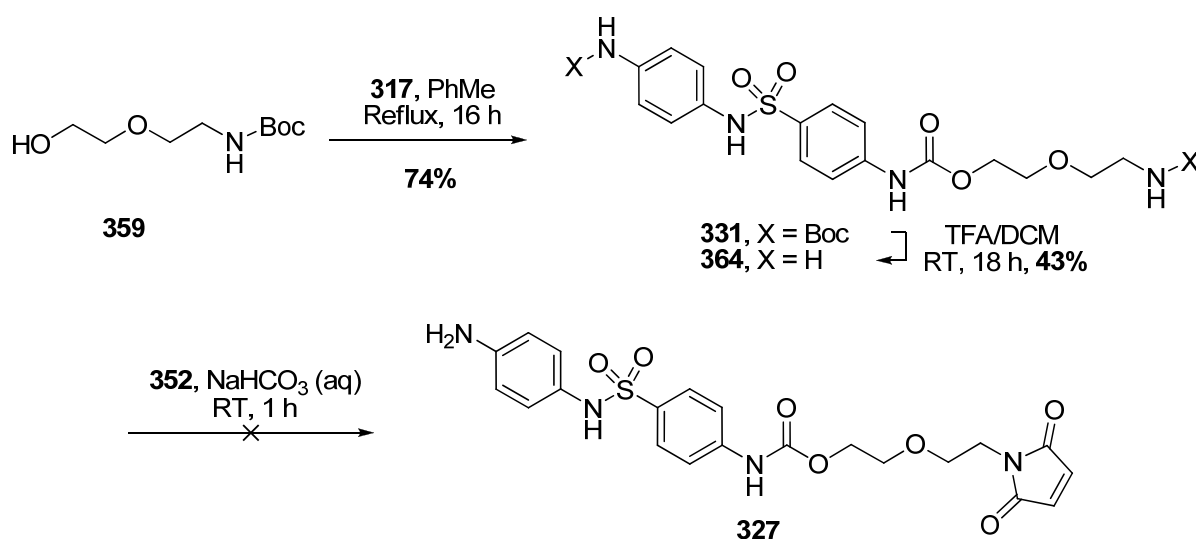


Scheme 69: Synthesis of the PEGylated-maleimido-benzenesulfonamide-bound imidazo[1,2-*a*]pyrazine, **363**

4.3.3.2 Approaches to PEGylated-*N*-(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2-*a*]pyrazines

A similar approach was initially adopted when attempting to incorporate the maleimide moiety into the PEGylated-*N*-(4-aminophenyl)benzenesulfonamide-bound imidazo[1,2-*a*]pyrazines (Scheme 70). The Curtius reaction between **317** and **359** was achieved with a high yield, but double Boc deprotection was low yielding, owing to possible decomposition during reaction work up.

Reaction of **364** with the activated maleimide carbamate failed, which was surprising considering the high yield observed with the corresponding reaction of **360**. An explanation for this failure could be solubility problems of the free amine compared to the TFA salt used with **360**, and therefore, it is advised to isolate **364** as the TFA salt and continue the reaction.



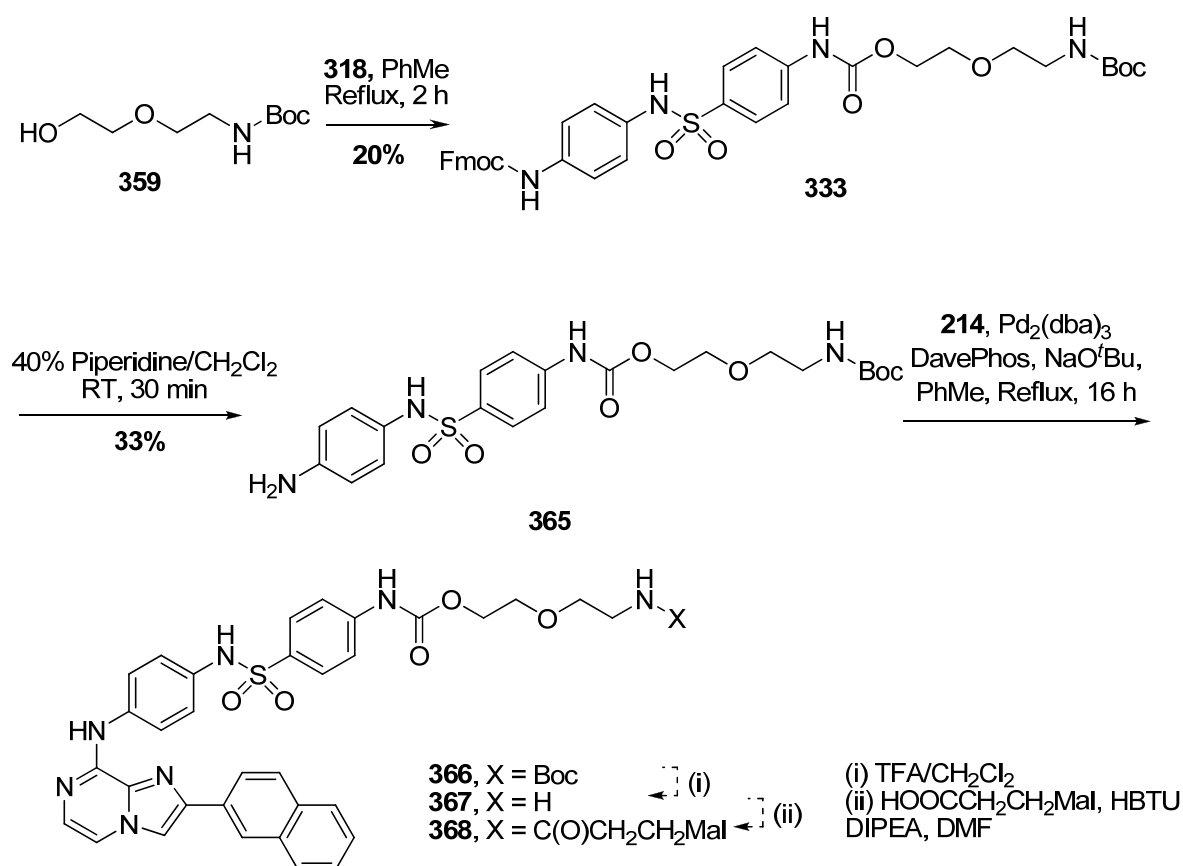
Scheme 70: Attempted synthesis of the *N*-(4-aminophenyl)benzenesulfonamide PEGylated-maleimido-imidazo[1,2-*a*]pyrazine

The next step in the sequence (to form **300**) would have involved a Buchwald-Hartwig coupling with **214**. However, in light of the issues experienced in Section 4.3.3.1 with the stability of the maleimide moiety towards Pd-catalysed coupling conditions, even if **327** was successfully synthesised, problems with the final coupling are envisaged.

Therefore, the route would have to be altered whereby **364** is coupled to the heterocycle first followed by the conjugation of the maleimide. However, it is likely that chemoselectivity issues would arise with the coupling of **364** due to the more nucleophilic primary alkyl amine out-competing the aniline amine in the reaction.

Therefore, the use of Fmoc in place of the Boc protecting group has been trialled as illustrated in Scheme 71. Curtius reaction of the Fmoc protected aniline acyl azide **318** with **359** results in the orthogonally protected species **333**. The low yield is due to the shorter reaction time of 2 h compared with 16 h for all other analogues and co-elution of the product with PEG alcohol (2:1 ratio by NMR). However, the reaction was continued, without further purification to see if this route proved to be more successful.

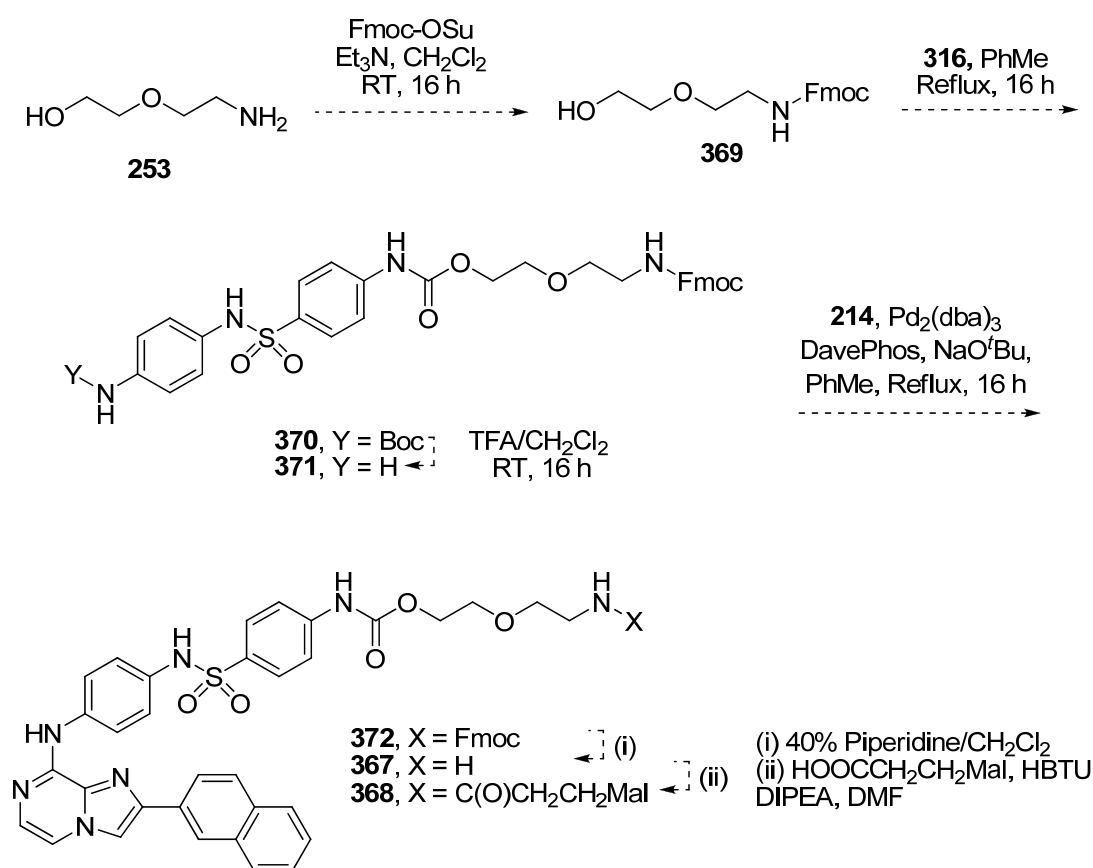
Selective Fmoc deprotection was achieved using 40% piperidine in CH₂Cl₂, but the product **365** was still contaminated with **359** (approximately 2:1 by NMR). Nonetheless, the mixture was subjected to the optimised Pd-catalysed coupling conditions on a small scale and by LCMS, the desired product mass was observed. Due to the small scale and contamination of sample, no further work was carried out, but these results suggest this as a viable approach for the synthesis of the *N*-(4-aminophenyl)benzenesulfonamide-bound imidazo[1,2-*a*]pyrazine primed with maleimide moiety. The next stage would involve Boc deprotection and subsequent coupling with the *N*-maleoyl- β -alanine using conditions previously stated.



Scheme 71: Trial reaction scheme involving the use of Fmoc protecting group to deliver the PEGylated-maleimido-*N*-(4-aminophenyl)benzenesulfonamide-bound imidazo[1,2-*a*]pyrazines

This route could also be employed for the synthesis of the free hydroxyl PEGylated imidazo[1,2-*a*]pyrazines, whereby the different TBDMS protected PEG alcohols undergoes the Curtius reaction with **318**, followed by subsequent Fmoc-deprotection, coupling and silyl-deprotection steps.

An alternative Fmoc route to access the maleimide containing compounds is outlined in Scheme 72, whereby the Fmoc and Boc protecting groups are switched. Fmoc protection of 2-(2-aminoethoxy)ethanol could be followed by the Curtius reaction with **317**, which in turn would be subjected to Boc deprotection, coupling with the core heterocycle, Fmoc deprotection and maleimide addition. The potential issue here is the unknown efficiency of the Fmoc deprotection step and consequent amide bond formation.



Scheme 72: Reaction Scheme suggesting an alternative use of Fmoc protecting group to deliver the PEGylated-maleimido-*N*-(4-aminophenyl)benzenesulfonamide-bound imidazo[1,2-*a*]pyrazines

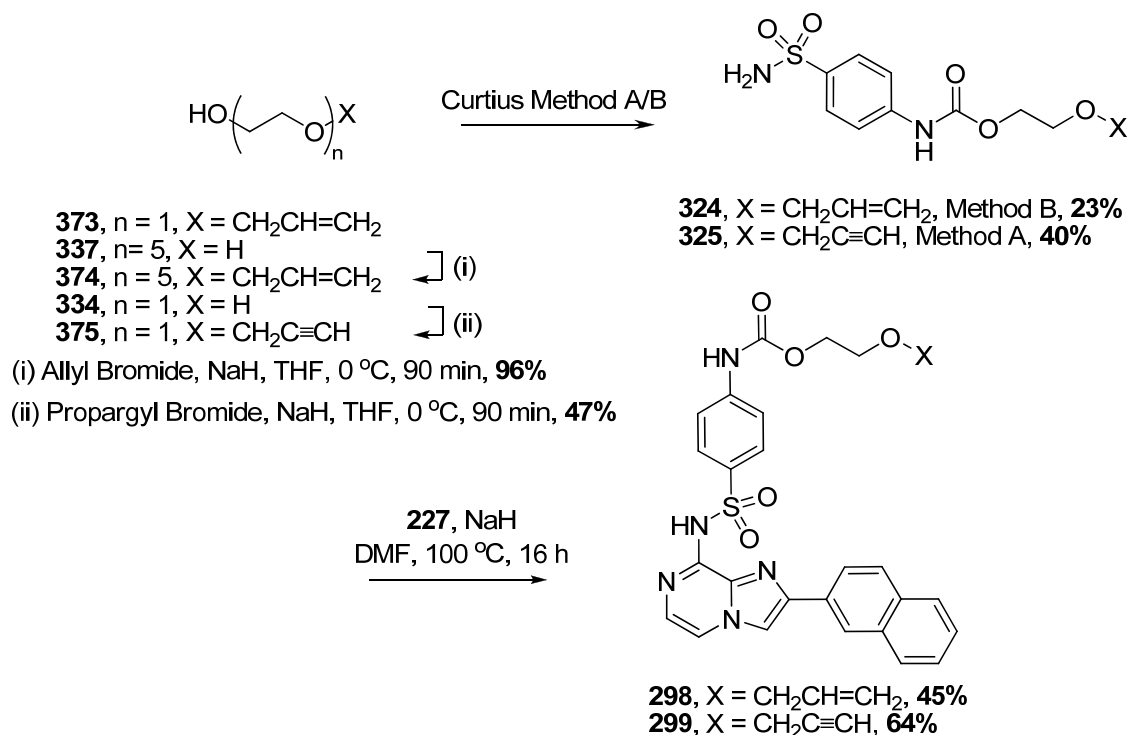
4.3.4 Synthesis of PEGylated Allyl and Alkyne Linked Imidazo[1,2-*a*]pyrazines

4.3.4.1 PEGylated-Benzenesulfonamide-bound Imidazo[1,2-*a*]pyrazines

The synthesis of the allyl and alkyne linked imidazo[1,2-*a*]pyrazines relies on similar synthetic methodology (Scheme 73). The allyl and propargyl PEG alcohols can be synthesised where necessary using the diol (in excess), NaH and allyl and propargyl bromide respectively.^{182,274} The low yield observed for **375** is due to difficulties in removing the product from the aqueous layer during work up (an acid wash should have been carried out here), and also formation of the di-alkyne species.

The shorter PEG chains (*n*=1), **373** and **375** were then subjected to the Curtius reaction using Method B and A respectively. Coupling of the resulting products using solvent/base conditions with **227** afforded the final products **298** and **299** ready for peptide conjugation. The Buchwald-

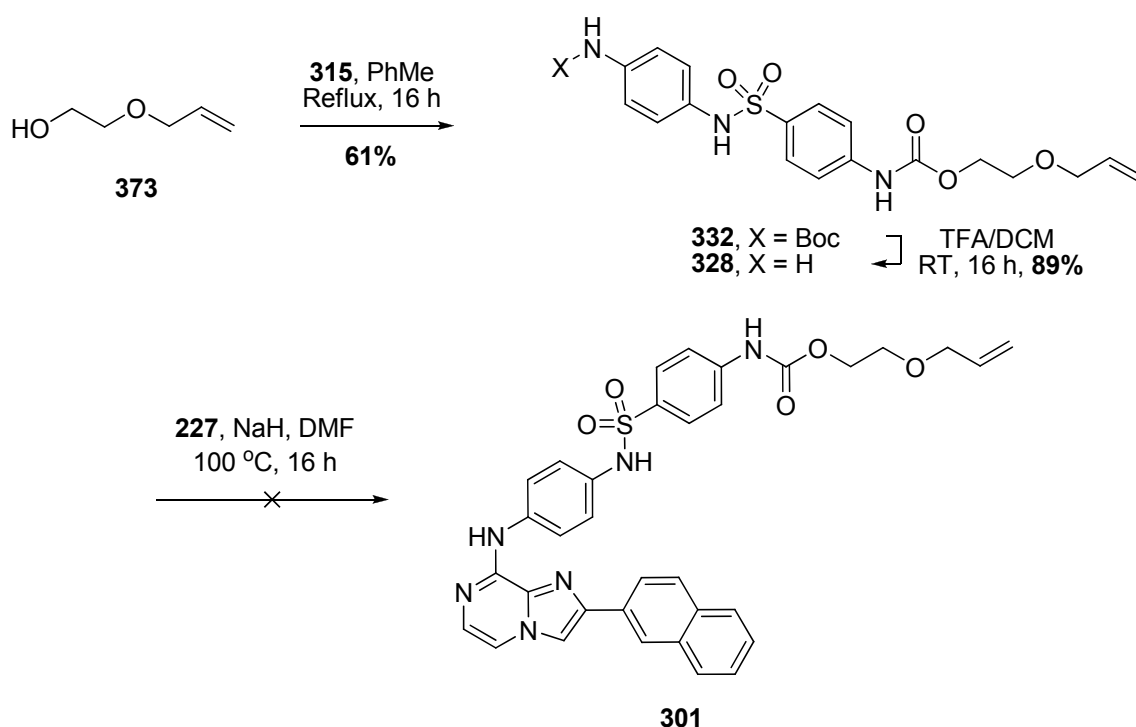
Hartwig conditions were not attempted in this case due to the possibility of competing Heck and Sonogashira coupling reactions with the allyl and alkyne moieties.



Scheme 73: Synthetic route to access the PEGylated allyl and alkynyl benzenesulfonamide-bound imidazo[1,2-*a*]pyrazine

4.3.4.2 Approaches to PEGylated-*N*-(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2-*a*]pyrazines

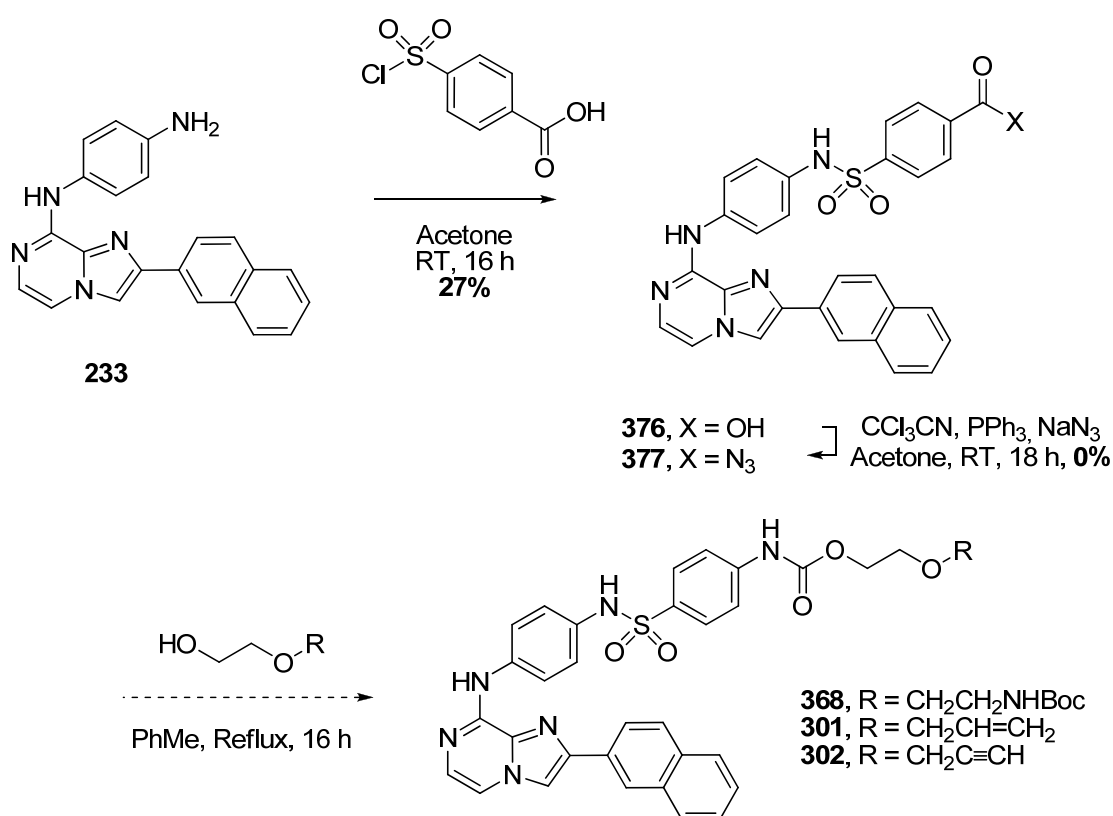
Scheme 74 outlines the attempted synthesis for priming **14** with the allyl moiety. As with the other analogues, the Curtius reaction proceeds well with **317**, but not with **310**, and the Boc deprotection also works in high yields. Instead of leaving **328** as the TFA salt, a basic wash was carried out to isolate the free amine and unlike **362**, the compound appears to be stable. Coupling with **227** under solvent/base conditions failed, which is not surprising considering the analogous reaction to make **14** also failed.



Scheme 74: Attempted synthesis of the PEGylated allyl-*N*-(4-aminophenyl)benzenesulfonamide-bound imidazo[1,2-*a*]pyrazines

Since Buchwald Hartwig conditions cannot be employed when the allyl moiety is already in place and the solvent/base approach using **227** does not work, the allyl group would have to be added after the aniline is coupled to the core heterocycle. This would rule out using the Fmoc route (Scheme 71), and instead an alternative can be suggested (Scheme 75). Starting with **233**, which to date can be formed with a maximum yield of 32% (Section 3.1.1), reaction with 4-(chlorosulfonyl)benzoic acid (1 equivalent) gives **376** in low yields. When attempting the coupling with 1:1 stoichiometry and Et₃N/CH₂Cl₂, the product could not be isolated due to co-elution with a variety of impurities. In order to increase the yield, it is suggested that an excess of the amine is used with Et₃N and CH₂Cl₂ (as with **310**).

Attempts to form the acyl azide resulted in the isolation of only PPh₃=O with no signs of desired product. A repeat of these conditions would be required to see if **377** can be isolated, and if so it is thought that ensuing Curtius reaction should proceed without complications. Otherwise it might be necessary to attempt a one-pot Curtius reaction with the *in situ* formation of the acyl azide. The other PEG containing analogues could also be synthesised using this shortened method but it is important to optimise the steps leading to the formation of **377**.



Scheme 75: Suggested alternative synthesis to access the PEGylated-*N*-(4-aminophenyl)benzenesulfonamide-bound imidazo[1,2-*a*]pyrazines

An alternative method could be to utilise an amide bond as opposed to the carbamate and carry out a coupling reaction between the relative acid and the PEG amines. This would, however, require extensive studies into the synthesis of the PEGylated amine compounds.

4.4 Peptide Synthesis

4.4.1 Synthesis of *N*-Fmoc protected *S*-Allyl Cysteine

In order to conjugate the allyl containing imidazo[1,2-*a*]pyrazine to a peptide, the unnatural amino acid, *S*-allyl cysteine (SAC) needs to be synthesised.¹⁸² Our laboratory utilises Fmoc solid phase peptide synthesis (SPPS) and therefore SAC was required with an Fmoc protected amine and a free acid, a cysteine derivative that to our knowledge, has not previously been synthesised. Scheme 76 outlines the different methods taken towards its synthesis.

Initially, *tert*-butyl protection of Fmoc-Cys(Trt)-OH was carried out using *tert*-butyl 2,2,2-trichloroacetimidate (TBTA)²⁷⁵ or SiCl₄²⁷⁶ in yields of 20% and 2% respectively (Route A). The resulting compound **379** was then stirred in 10% TFA with 5% TES scavenger in order to selectively remove the trityl protecting group,²⁷⁷ which resulted in another poor yield of 33%. Care must be taken with removal of the reaction solvent because if the TFA becomes too concentrated, deprotection of the *tert*-butyl group is also observed.

Due to the low yielding steps an alternative route was sought with a view to making the procedure scalable. The *tert*-butyl protection of commercially available cystine to form **380**,¹ can be achieved using perchloric acid and *tert*-butyl acetate in high yields (90%).²⁷⁸ Both Boc and Fmoc protection routes (Routes B and C) were explored in parallel.

Fmoc protection was carried out using standard literature conditions with Fmoc-Cl.²⁷⁸ A low yield of 43% was observed (literature yield reported is 81%), which was due to contamination of the reagent. The use of a fresh batch, or the use of Fmoc-OSu, which has been previously used in this report, may give a higher yield here. Boc protection was carried out with a yield of 48%.

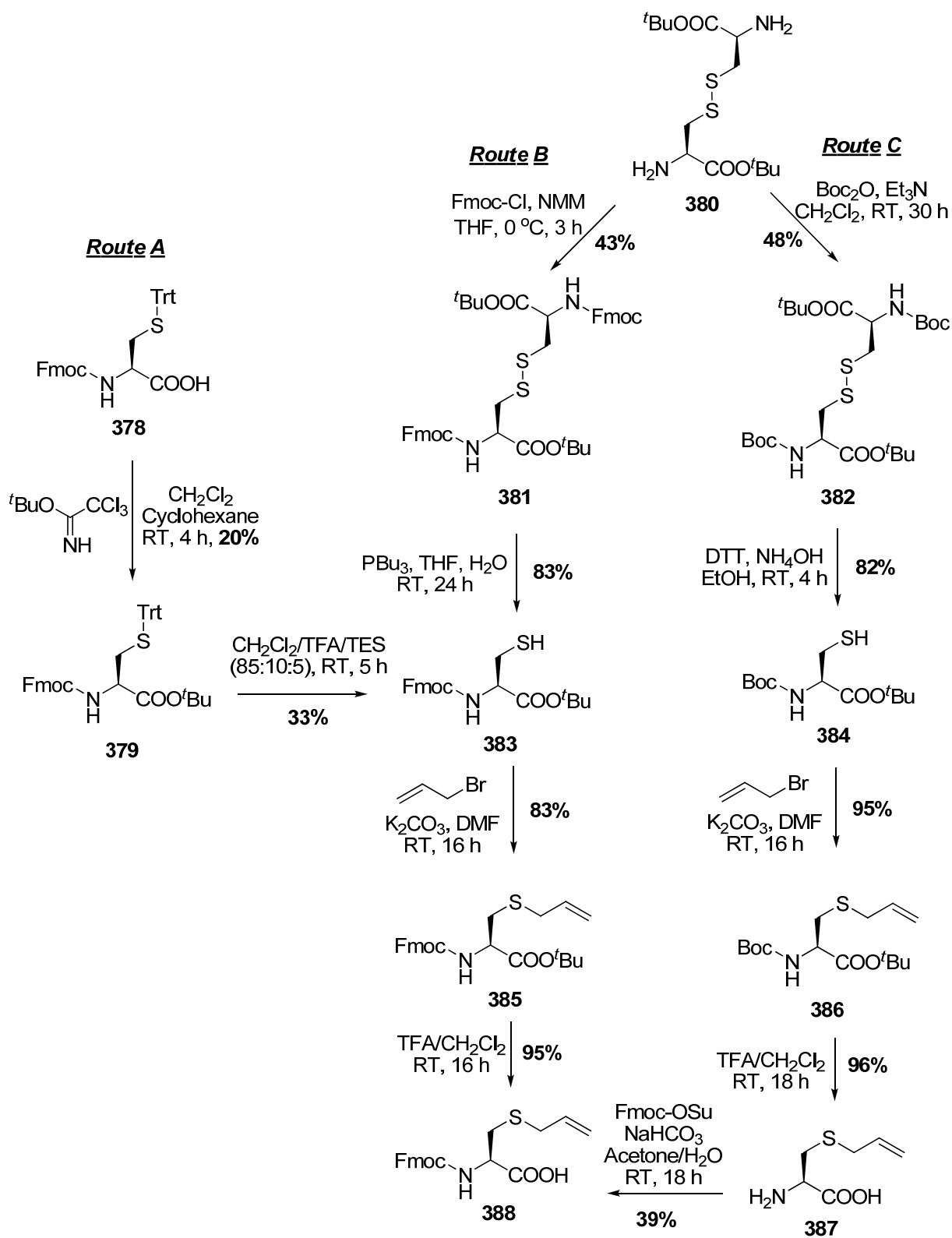
The next stage involved cleavage of the disulfide using PBu₃ for the Fmoc protected cystine²⁷⁸ and dithiothreitol (DTT, Clelands' reagent) under basic conditions for the Boc protected version.²⁷⁹ Both proceed well in high yields.

Using excess allyl bromide, the free thiol in both the Boc and Fmoc protected cysteines can be allylated in high yields.¹⁸² It was initially thought that the use of base could lead to Fmoc deprotection but Table 21 shows that using 2.5 equivalents of K₂CO₃ gives a high yield of **385**.

Entry	Base	Eq	Yield/%
1	CS ₂ CO ₃	0.5	19
2	CS ₂ CO ₃	1.0	67
3	K ₂ CO ₃	2.5	82

Table 23: Comparison of bases and equivalents for the formation of **385**

¹ Synthesis carried out by T. Tran



Scheme 76: Various synthetic routes used for the synthesis of *S*-allyl cysteine

The next step in the Fmoc route involves the deprotection of the *tert*-butyl group and this is achieved in high yields with TFA²⁷⁷ to give the final product Fmoc-Cys(SAC)-OH, **388**, ready for incorporation into SPPS. Route C involved universal deprotection of **386** followed by Fmoc protection of the resulting free amine,²⁸⁰ which proceeded in only moderate yields. When making a comparison of the two routes, Route B is the suggested procedure for the formation of Fmoc-Cys(SAC)-OH

4.4.2 Peptide Synthesis

With the required amino acids in hand, we aimed to use Fmoc SPPS to make the peptide subunits incorporating either cysteine or *S*-allyl cysteine in the R240 position. In addition the peptides corresponding to the native sequence were also synthesised (i.e. R240 position unchanged), in order to establish any secondary structures. Table 24 indicates the amino acid sequences for each of the nine peptides synthesised.

Peptide	Sequence
α F-loop	SAD ^{RED} CLKS ^{GREEN} CLRM ^{YELLOW} RPD
α F- β 10	SAD ^{RED} CLKS ^{GREEN} CLRM ^{YELLOW} RPDRIL ^{BLUE}
β 9- α F- β 10	YTQLFFGGNITSAD ^{RED} CLKS ^{GREEN} CLRM ^{YELLOW} RPDRIL ^{BLUE}

Table 24: Table indicating the amino acid sequence for each of the peptides synthesised. Letters in **RED** indicate an α -helix; **BLUE** indicates a β -sheet; **BLACK** indicates turns and loops; Highlighted R in **Yellow** indicates R240 in which cysteine or *S*-allyl cysteine are incorporated; C in **Green** are cysteine residues which need orthogonal protecting groups in the case of R240C

The process of SPPS is illustrated in Figure 63. The C-terminus amino acid is pre-loaded on a solid supported resin, and for the peptides synthesised, Fmoc-Asp(OtBu)-NovaSyn®TGT and Fmoc-Leu-NovaSyn®TGT were used (Figure 64). The TGT resin is a composite of polyethylene glycol and a low-cross linked polystyrene gel-type resin. They are 90 μ m beads and are derivatised with the extremely acid sensitive 4-carboxytrityl linker (0.5% TFA/CH₂Cl₂ required for cleavage from the resin).

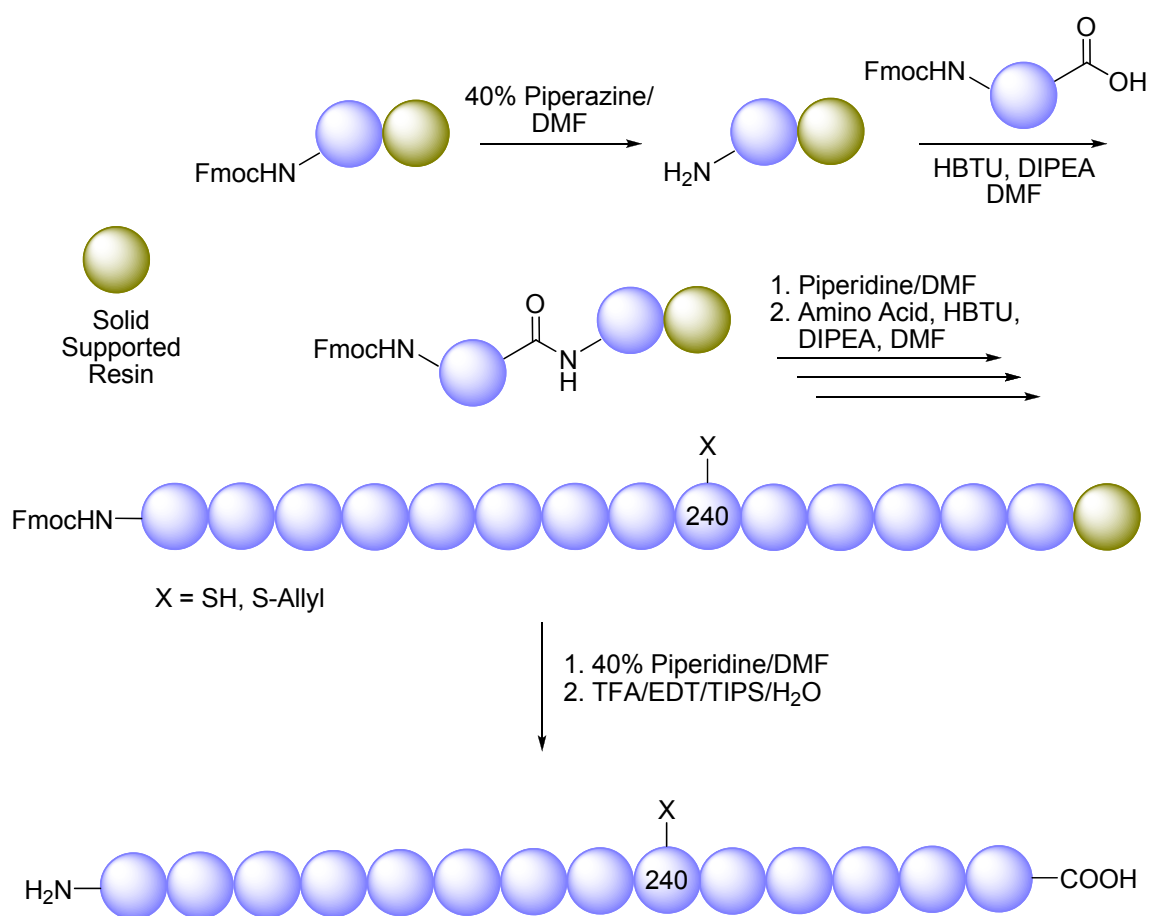


Figure 63: Generic representation of the process of solid phase peptide synthesis

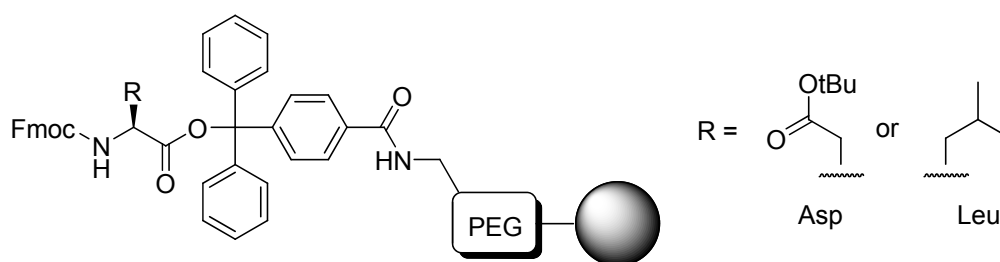


Figure 64: Structure of the Fmoc-Asp(OtBu)-NovaSyn®TGT and Fmoc-Leu-NovaSyn®TGT resins

The peptide synthesiser cycles through processes of Fmoc deprotection (40% Piperidine/DMF) and amino acid coupling (HBTU, DIPEA, DMF) from the C-terminus to the N-terminus of the sequence. A cleavage cocktail (TFA/EDT/H₂O/TIPS; 88:5:5:2) was then used to cleave the peptide from the resin and also remove the acid-labile side chain protecting groups.

It is important to note in the case of R240C peptides that a non-acid labile protecting group was used on the other cysteine residues so that after deprotection and cleavage from the resin, the peptide produced has only one thiol free for maleimide conjugation. There are two potential alternatives to Fmoc-Cys(Trt)-OH: Fmoc-Cys(Acm)-OH and Fmoc-Cys(^tBu)-OH. Both protecting groups are stable to the cleavage cocktail and can be removed using mercury (II) acetate or thallium (III) trifluoroacetate.²⁸¹ Fmoc-Cys(Acm)-OH was used in the R240C peptide synthesised herein and Fmoc-Cys(Trt)-OH for the wild type and R240Sac peptides.

Reverse phase preparative HPLC was then employed to purify the peptides and characterisation was completed using reverse phase analytical HPLC and ES⁺ or MALDI mass spectrometry. Table 25 details the nine peptides synthesised, including the native wild type (WT) peptides.

Variant	α F-loop	α F- β 10	β 9- α F- β 10
WT	389	392	395
R240C	390	393	396
R240SAC	391	394	397

Table 25: Table detailing the compound numbers for each of the peptides synthesised

4.5 Peptide-Imidazo[1,2-*a*]pyrazine Conjugation to give Bivalent Inhibitor Reagents

To re-cap, three PEGylated-imidazo[1,2-*a*]pyrazines were successfully synthesised and ready to conjugate to a variety of peptides (Figure 65).

Before any conjugation was carried out, it was important to establish the methods necessary for successful reactions. Due to the limited quantities of peptide available, model systems were employed for the trial reactions.

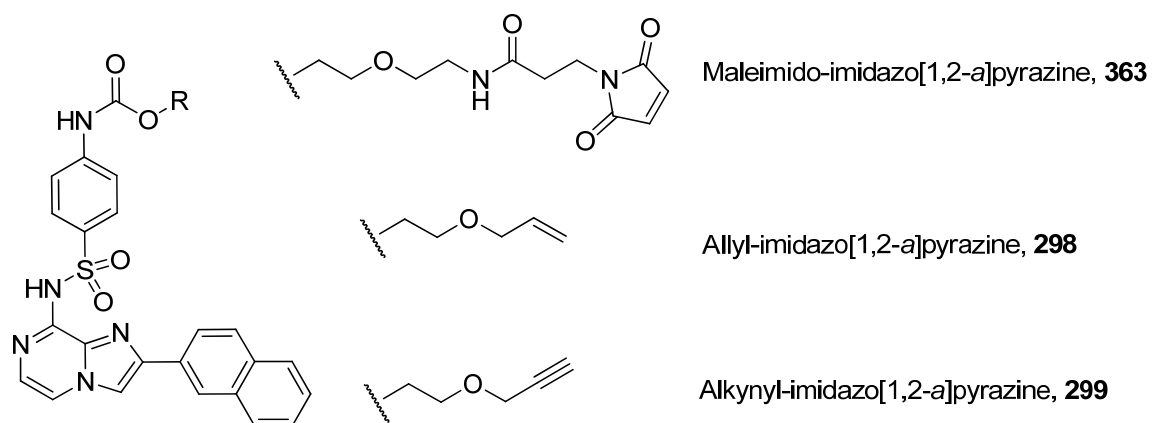
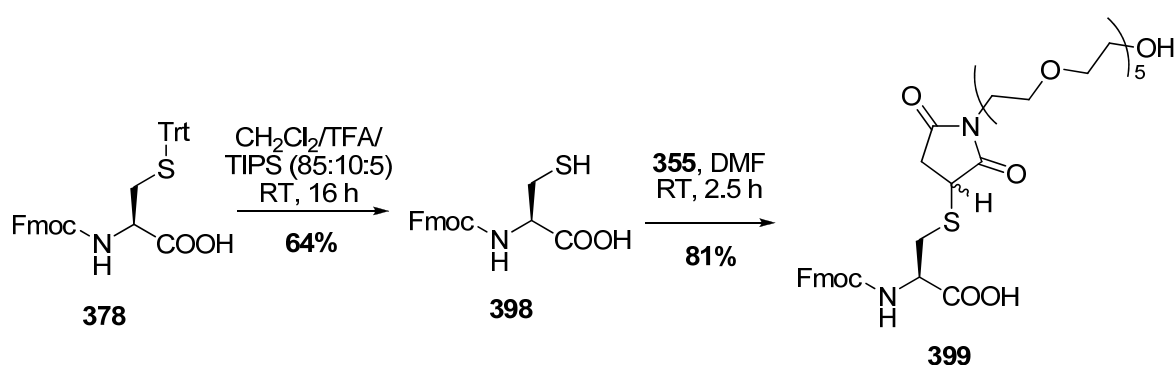


Figure 65: Structures of the PEGylated imidazo[1,2-a]pyrazines ready for peptide conjugation

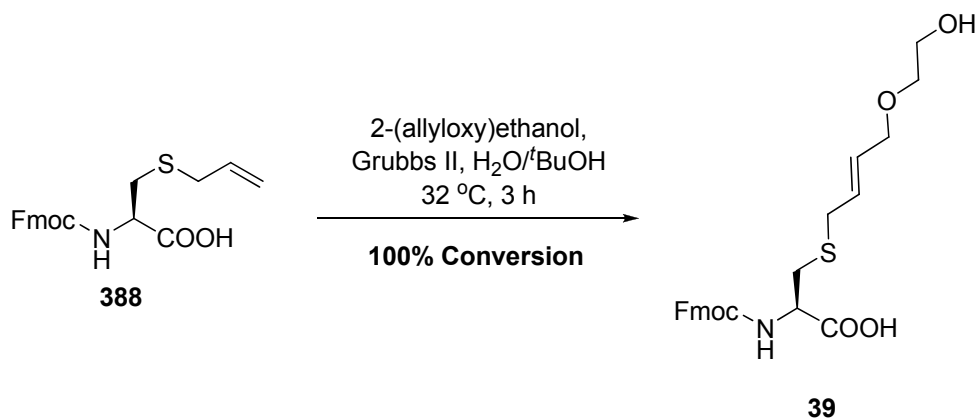
4.5.1 Trial Reactions

Trityl deprotection of the commercially available Fmoc-Cys(Trt)-OH gave **398** in moderate yields, and reaction with **355** in DMF gave 81% of desired maleimido conjugated product (Scheme 77). The use of a 50 mM phosphate buffer (pH 6.5) was also investigated as there is literature precedent for slightly acidic conditions catalysing the reaction,²⁵⁵ but this gave the product in only 29% yield. If the conjugation was to be carried out with a large macromolecule protein, the use of buffer would be advisable, but since relatively small peptides are being used, it was decided to use DMF only for the conjugation.



Scheme 77: Trial maleimide conjugation between Fmoc-Cys-OH (**398**) and **355**

The conditions for the cross metathesis¹⁸² were also briefly explored using the model system of *S*-allyl cysteine and 2-(allyloxy)ethanol (Scheme 78). Using Grubbs' 2nd generation catalyst,²⁴⁶ the reaction proceeded with 100% conversion as indicated by LCMS. No purification was carried out at this stage, and the conditions used were deemed efficient for use with the peptide and **298**.



Scheme 78: Trial cross metathesis between *S*-allyl cysteine and 2-(allyloxy)ethanol

4.5.2 Solution Based Conjugation of Peptides and PEGylated Imidazo[1,2-*a*]pyrazines

Table 26 shows the results of the conjugation reactions with peptides in solution. Maleimide conjugation was carried out between **390**, **393** and **396** with **363**, by stirring in DMF at RT for 3 h. MALDI analysis on each of the crude samples showed that the desired product mass was present. Purification was carried out and the peptide-small molecule conjugates were isolated.

ES+ for **401** showed the desired product with 3+ and 4+ ions, including the [M+K+3H]⁴⁺ ion; **402** displayed the 3+ and 4+ ions, as well as signs of the non-conjugated **393**; **403** showed the 3+, 4+ and 5+ ions as well as the [M+K+4H]⁵⁺ and [M+K+5H]⁶⁺ ions. The desired mass was present in the MALDI spectra of **401**, but not for **402** and **403**. In all cases un-reacted **363** was isolated.

With all three peptide conjugates in place, the Acn group on the remaining cysteine required removal. However, it was decided not to carry out any further work due to the minute quantity of material and harsh conditions required.

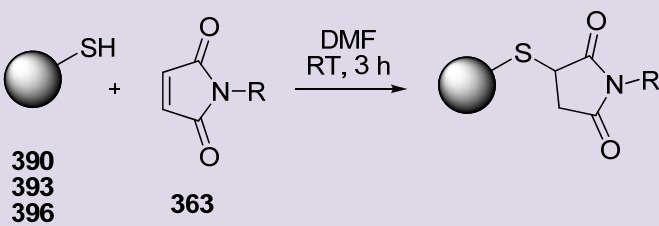
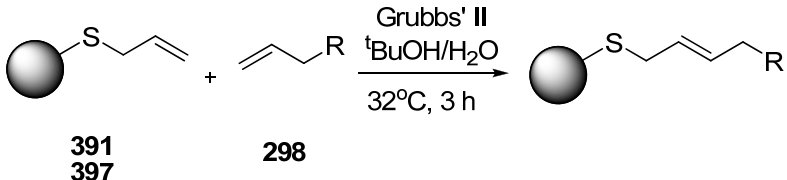
Reaction		α F-loop	α F- β 10	B9- α F- β 10
		401 ✓	402 ✓	403 ✓
		404 ✗	-	405 ✗

Table 26: Table illustrating the results of the peptide conjugations carried out in solution

Cross metathesis between **391**, **397** and **298** (10 eq) was carried out by stirring with Grubbs' II catalyst (6 mol%) in *t*BuOH/H₂O at 32 °C for 3 h. MALDI analysis of the crude samples showed possible signs of product in **404** but not in **405**. Nonetheless, purification was carried out on both, but difficulties with poor solubility were encountered. Since **298** is used in excess it proved difficult to dissolve the crude material and a substantial quantity of DMF was required. After purification it was evident that no reaction product was present in any of the fractions collected. None of the un-conjugated peptides were evident, nor any products of self-metathesis.

In light of the failures and draw-backs with solution-phase conjugation encountered, it was decided to change the approach and carry out the conjugation whilst the peptide is still bound to the resin.

4.5.3 Resin-Bound Conjugation of Peptides and PEGylated Imidazo[1,2-*a*]pyrazines

To carry out peptide conjugation on resin there are three possible approaches:

- 1 Synthesise the entire sequence and then carry out the conjugation reaction (Figure 66);
- 2 Synthesise the peptide up to R240, carry out the conjugation reaction and then complete the peptide sequence;

- 3 Carry out conjugation of the amino acid with the PEGylated-imidazo[1,2-*a*]pyrazine coupling partner and then incorporate the resulting imidazo[1,2-*a*]pyrazinyl amino acid into the SPPS.

It was decided to carry out the order according to the first approach because it was unknown how the conjugated species would respond to repeated deprotection and coupling conditions; whilst avoiding any potential solubility issues of the imidazo[1,2-*a*]pyrazinyl amino acid.

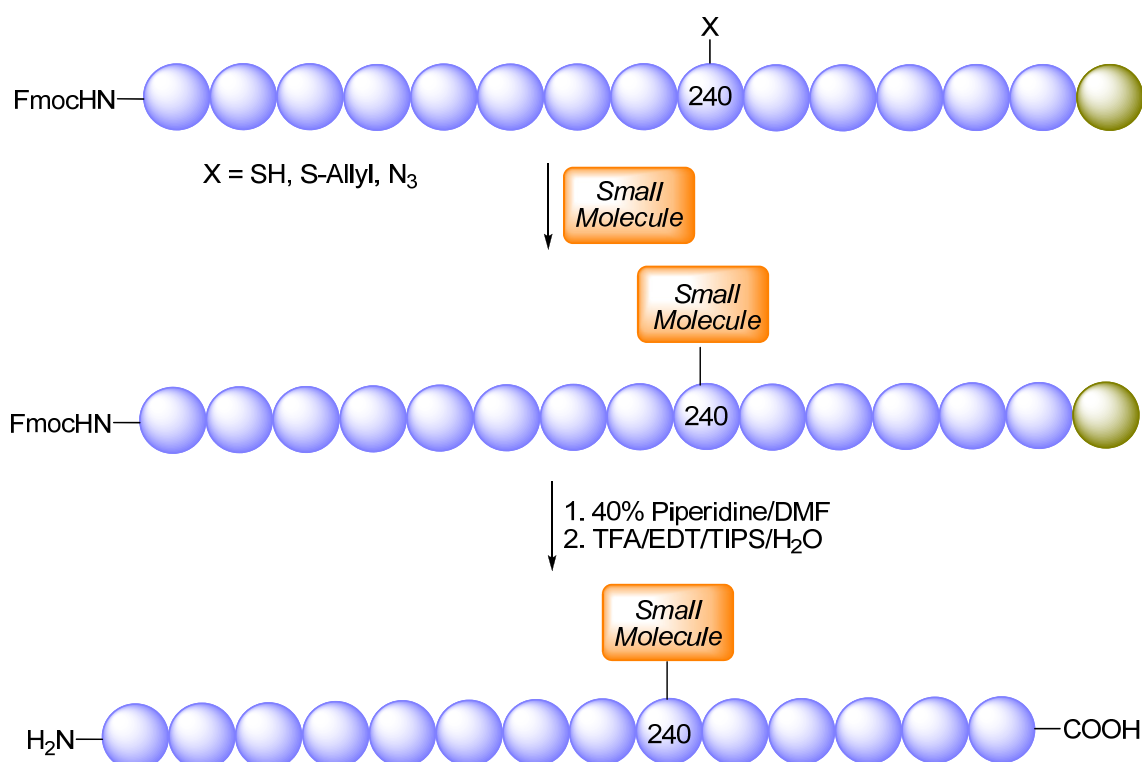


Figure 66: Generic representation for the sequence of events used in the conjugation of resin-bound peptides to PEGylated imidazo[1,2-*a*]pyrazine compounds

Peptides were synthesised with the terminal Fmoc protecting group left on and the amino acid residues Fmoc-Cys(*S*^tBu)-OH, Fmoc-Cys(allyl)-OH (**388**) or azido lysine²⁸² (previously synthesised by the group)^m were substituted in position R240 to give peptides R240C, R240SAC and R240AzLys respectively. All other remaining residues in the sequence consisted of amino acids with standard side chain protecting groups.

^m The synthesis of azido lysine was completed by Dr F. Campbell

Once the peptides were synthesised, the three different conjugation chemistries could be carried out (Table 27), and after each conjugation reaction, the resins were washed, the terminal Fmoc group removed and the peptides cleaved from the resin. The crude peptides were then purified by reverse phase HPLC to give the peptide-imidazo[1,2-*a*]pyrazine conjugates.


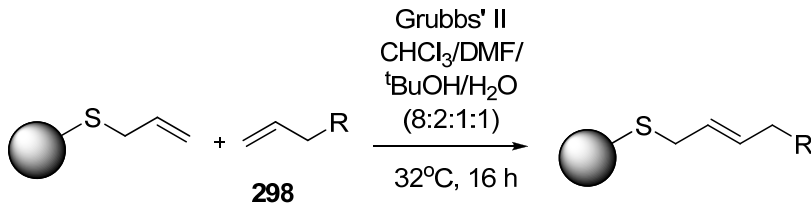
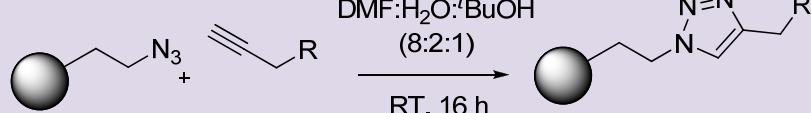
Reaction	α F-loop	α F- β 10	B9- α F- β 10
 <p>363</p>	406 ✓	407 ✗	408 ✗
 <p>298</p>	409 ✗	-	-
 <p>299</p>	410 ✓	411 ✓	412 ✗

Table 27: Table illustrating the different peptide conjugations carried out on resin

Prior to conjugation of R240C peptides to the maleimido-imidazo[1,2-*a*]pyrazine compound **363**, selective deprotection of the *tert*-butylthio group on the R240 cysteine was required. This was achieved by agitating the resin-bound peptide with PBu₃ in EtOH/CH₂Cl₂/H₂O (4:6:1) for 3 h at RT.²⁸³ The trityl protecting groups on the other cysteine residues remain intact under these conditions. Conjugation of the free cysteine to **363** can then be carried out by agitating in DMF at RT for 16 h.

The cross metathesis conditions were slightly altered from those used in the solution-based conjugation in order to make them more compatible with the use of the resin.ⁿ This was achieved

ⁿ Conditions determined by H.Koss

by the additional use of chloroform and DMF. The reaction between R240SAC and **298** (4 eq) with Grubbs' 2nd generation catalyst (6 mol%) in CHCl₃/DMF/H₂O/^tBuOH (8:2:1:1) was carried out by agitating the mixture at 37 °C for 16 h.

There is extensive literature precedent for the click reaction when applied to peptide synthesis, with selected examples using sodium ascorbate and a source of copper, either in the form of CuI,²⁸⁴ CuBr,²⁸⁵ CuSO₄²⁸⁶ or Cu(OAc)₂.²⁸⁷ Reactions can be carried out in DMF^{284,285} or in H₂O with an alcohol (EtOH or ^tBuOH).^{286,287} It was decided to carry out the reaction between R240AzLys peptides and **299** by agitating with excess CuI (20 eq) and sodium ascorbate (40 eq) in DMF/H₂O/^tBuOH (8:2:1) at RT for 16 h.

As can be seen from Table 27, for the shortest length peptide (α F-loop) both the maleimide conjugation (**406**) and click reaction (**410**) worked well; the 2+ and 3+ ions were visible with ES+ and the M+ ion present in MALDI analysis for both conjugates. However, the cross metathesis failed and so it was decided not to carry out the cross metathesis on the two longer peptide lengths. In order to optimise this reaction, it would be necessary to carry out more extensive trials using **298** instead of a model system. The use of MgCl₂ could aid the reaction by hindering chelation of the soft metal centre to the side chains.¹⁸²

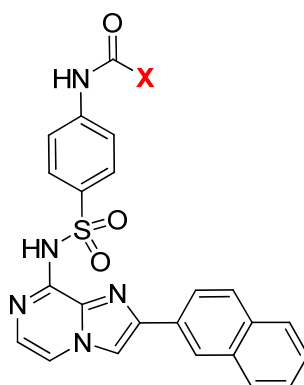
Of the 4 remaining peptide conjugations, only the α F- β 10 R240AzLys-Click (**411**) worked. It was possible **407** also worked, since product mass appeared to be present in the crude MALDI sample. However, problems with freeze drying the purified sample resulted in its appearance altering from a fluffy white solid to a hygroscopic green solid. No product mass was evident, indicating signs of possible decomposition.

A plausible explanation for the conjugation failing for the longer length peptides could be the accessibility of the relevant amino acid. As can be seen from the sequences in Table 24, the R240 amino acid is close to the C-terminus and therefore close to the resin. Therefore, the thiol, allyl or azide moieties might be considerably "buried" from their conjugation partner. In order to overcome this problem, it might be necessary to synthesis the peptide up to position R240, carry out the conjugation, and then continue the sequence.

4.6 Compound Evaluation

4.6.1 PEGylated-Imidazo[1,2-*a*]pyrazines

Each of the PEGylated-imidazo[1,2-*a*]pyrazines products were tested for inhibition of HP0525 using the same colorimetric assay as previously used. Table 28 shows the IC₅₀ data for a selection of the PEGylated-imidazo[1,2-*a*]pyrazines synthesised. By comparing these values with the IC₅₀ for **8** (109 µM), conclusions can be drawn as to the effectiveness of employing a PEG chain on the activity. Selected dose response curves are illustrated in Figure 71 (Appendix, Section 7.1).



Entry	Compound	PEG Chain (X)	IC ₅₀ (µM)
1	8	Carbamate replaced by CH ₃	109 (88/ <u>131</u>)
2	343		192 (<u>241</u> /142)
3	362		137 (<u>146</u> /128)
4	363		18 (19/ <u>18</u>)
5	298		46 (<u>48</u> /43)
6	299		61 (69/ <u>54</u>)

Table 28: IC₅₀ data for the PEGylated imidazo[1,2-*a*]pyrazines synthesised

The three PEGylated-imidazo[1,2-*a*]pyrazines ready for conjugation, **363**, **298** and **299**, show improved inhibition of HP0525 than the parent compound **8**. This is perhaps not surprising given the extra H-bonding possibilities provided by the carbamate moiety and in the case of **363**, the

amide and maleimide. When studying their *in silico* lowest energy binding modes within the nucleotide binding site, they appear to adopt a conformation similar to that of **14** rather than **8**. When looking at **363** (Figure 67), the molecule may shift so that the carbamate mimics the same sulfonamide interactions as in **14**; the sulfonamide is now in the ribose region and the imidazo[1,2-*a*]pyrazine is located in the purine region. This means that the naphthalene moiety extends further into the enzyme active site, possibly picking up the further π -stacking interactions earlier discussed.

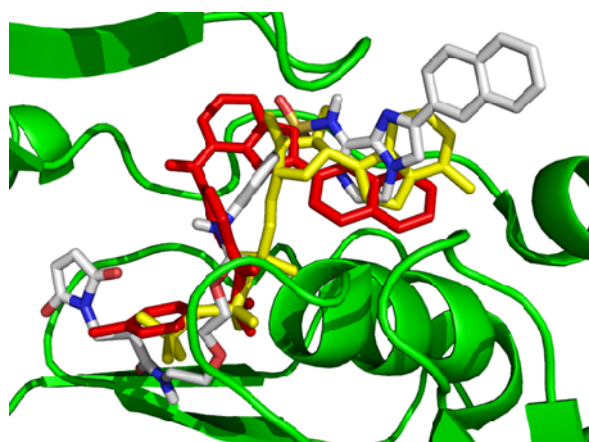


Figure 67: *In silico* image of 363 with 14 (Red) and ATP γ S (Yellow) in ATP γ S-HP0525. PDB: 1NLY.
Image generated using PyMOL

The free PEGylated version of **8** (**343**) does not seem to improve the activity, but as previously mentioned, the sample prepared was not very pure and so repetition with a purified sample would be advised. **298** and **299** have similar IC₅₀ values and this can be attributed to similar conformations observed within the nucleotide binding site (Figure 68 a and b). It is interesting that **362** does not reduce the enzyme activity because *in silico* studies indicate similar conformations as **298** and **299** (Figure 68 c) and therefore a similar activity might be expected.

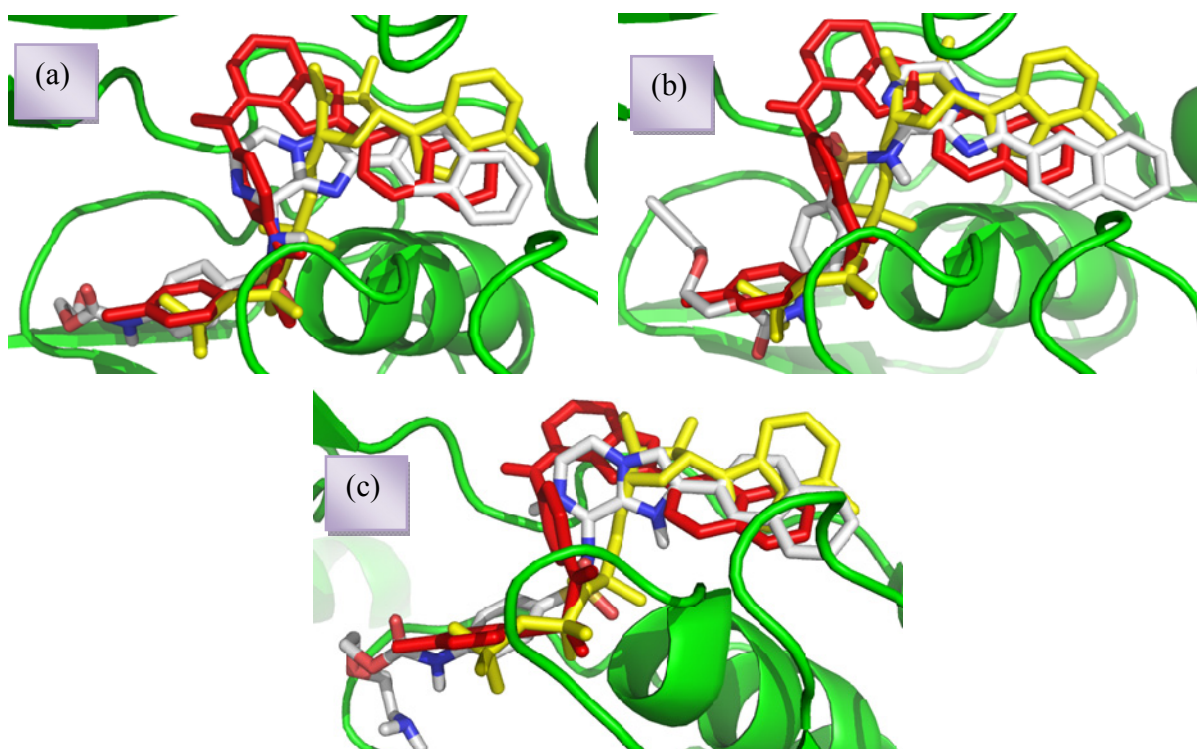


Figure 68: *In silico* docking image of 14 (Red), ATPγS (Yellow) and (a) 298, (b) 299 and (c) 362. PDB: 1NLY. Images generated using PyMOL

In addition, intermediate compound **376** was tested against HP0525 and showed an IC_{50} value of 52 μ M. *In silico* docking indicated an exact alignment with **14** (Figure 69).

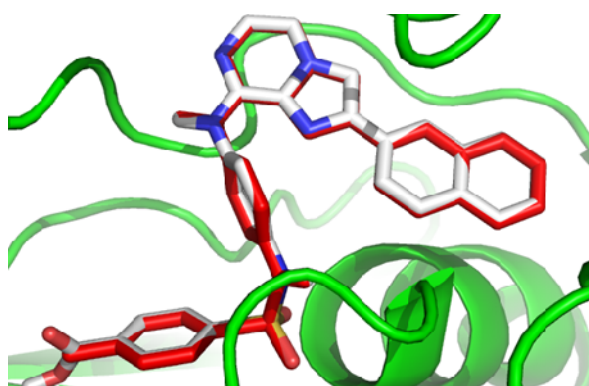


Figure 69: *In silico* docking image of 376 and 14 (Red). PDB: 1NLY. Images generated using PyMOL

4.6.2 Wild-Type Peptides

The stability of the peptide fold might be important for both activity and reactivity. *In silico* stability testing on the three WT peptides using Molecular Dynamics^o showed only the $\beta 9$ - αF - $\beta 10$ peptide was stable to the simulations (data not shown); the αF peptide unfolded immediately and the αF - $\beta 10$ unfolded, but at a slower rate. Circular dichroism (C.D.) spectroscopy,^o however, indicated that none of the WT peptides synthesised form α -helices (helical contents: αF : 6-7%, αF - $\beta 10$: 9-10%, $\beta 9$ - αF - $\beta 10$: 8-9%).

4.6.3 Bivalent Inhibitor Reagents

The three peptide-imidazo[1,2-*a*]pyrazine conjugates were tested at concentrations of 5, 50 and 500 μM and the % absorbance analysed to give an indication of their inhibitory effect Table 29.^p The results indicate that the peptide conjugates only inhibit weakly at a high concentration.

Entry	Peptide Conjugate	% Absorbance		
		500 μM	50 μM	5 μM
1	406	93	100	100
2	410	91	100	100
3	411	90	100	100

Table 29: % Absorbance values for each of the peptide conjugates at 500, 50 and 5 μM

4.7 Concluding Remarks to Chapter

Chapter 4 has looked at using *in silico* modelling to design bivalent reagents. Successful synthesis of PEGylated-benzenesulfonamide-bound imidazo[1,2-*a*]pyrazines was carried out, although numerous synthetic hurdles were encountered, especially with the maleimide based compounds. Inhibition studies of these PEGylated-imidazo[1,2-*a*]pyrazines show that they are more potent than the parent compound **8**.

^o Experiments carried out by H.Koss

^p Experiments carried out by Dr K. Wallden

Unfortunately, the synthesis of the longer PEGylated-*N*-(4-aminophenyl)benzenesulfonamide-bound imidazo[1,2-*a*]pyrazines could not be completed, but important strides were taken to ensure the likelihood of their synthesis in future attempts.

The synthesis of the novel amino acid: Fmoc-protected *S*-allyl cysteine (Fmoc-Cys(allyl)-OH) has been carried out and its successful incorporation into a peptide has been reported. However, no cross metathesis to PEGylated allyl-imidazo[1,2-*a*]pyrazines were observed and further optimisation would be required.

The maleimido- and alkynyl-imidazo[1,2-*a*]pyrazines did undergo successful conjugation to peptides bearing cysteine and azido lysine coupling partners respectively. A small percentage of inhibition was observed with the resulting conjugates; however, this was at a high concentration (500 μ M). Therefore the conjugates synthesised do not show any improvement on using the parent imidazo[1,2-*a*]pyrazine **8**.

C.D. experiments show that the native peptides have no maintained helical structure, and this could be a possible explanation as to the lack of activity of the peptide-imidazo[1,2-*a*]pyrazine conjugates. Another potential explanation could be due to the steric bulk of these conjugates and possible lack of flexibility of the protein. Even though the small molecule would be able to access the ATP binding site, it remains to be seen whether the peptide would be able to then access the subunit-subunit interface. Therefore alternative designs of the peptide sequence and target are encouraged.

Chapter 5: Conclusions

This thesis has investigated the potential of imidazo[1,2-*a*]pyrazines as inhibitors of the *Helicobacter pylori* VirB11 ATPase. Structural elucidation of the crystal structure of HP0525, a VirB11 homologue, instigated a virtual high throughput screen, which identified the imidazo[1,2-*a*]pyrazine core as a potential inhibitor.

Chapter 2 looked at the synthesis of 2- and 3-aryl regioisomers of this heterocycle, including optimisation of various key transformations. The compounds were evaluated for their potential to inhibit HP0525 in an *in vitro* colorimetric biochemical assay.

14 was identified as the lead compound with an IC₅₀ of 7 μM and studies utilising Michaelis-Menten kinetics revealed the compound to be a competitive inhibitor of ATP. Considering the absence of a co-crystal structure, this information is vital as all proceeding work relied on *in silico* observations within the ATP binding site.

Initial trends in the structure-activity-relationship of imidazo[1,2-*a*]pyrazine were identified, and with the aid of computational docking, second generation compounds were designed.

Chapter 3 investigated the synthesis and evaluation of these compounds and identified **247**, a closely related analogue of **14**, as an alternative lead inhibitor, with potentially more favourable pharmacokinetics.

In addition, initial studies were undertaken looking at the use of fragments from a Maybridge library as inhibitors of HP0525. All attempts to obtain co-crystallisation of these compounds, and therefore continue with the process of fragment-based drug design, failed. However, alternative compounds were synthesised and interestingly, when a Maybridge fragment was attached to the imidazo[1,2-*a*]pyrazine core (compound **280**), a comparable potency to **14** was observed. An alternative *in silico* docking conformation was evident, giving rise to the opportunity of tri-substituted systems.

The additional testing of imidazo[1,2-*a*]pyrazine- and Maybridge fragment- related compounds purchased from BioFocus has shown the potential of tri-substituted heterocycles, whilst also providing an insight into an alternative heterocyclic core.

With the lead compound, **14**, showing competitive inhibition of ATP, attention was switched to the design of bivalent compounds. Using molecular modelling techniques, the different components of the peptide-small molecule conjugate were established. It was envisaged that by synthesising peptides of varying length, based upon the native sequence of the α F helix, they would displace this native segment and disrupt the subunit-subunit interface and therefore hexamer formation.

A series of PEGylated benzenesulfonamide-bound imidazo[1,2-*a*]pyrazines, primed with a maleimide, alkene and alkyne, were then successfully synthesised. Comparing to their parent compound, **8**, improved inhibition of these compounds was observed, indicating optional moieties that are tolerated by the active site.

After the limited success with solution based conjugation of these compounds to the various synthesised peptides, resin-based conjugation was achieved with both the maleimide and alkyne analogues. Even though cross metathesis to *S*-allyl cysteine failed, the preparation of this novel Fmoc-protected amino acid was successfully completed and reported in Chapter 4.

Initial evaluation of these bivalent compounds showed them to display limited inhibition at 500 μ M, indicating that further work is required into their design.

5.1 Future Directions

In order for the project to continue forward, it is imperative that co-crystallisation of HP0525 with an imidazo[1,2-*a*]pyrazine inhibitor or Maybridge fragment is achieved. This would provide actual data of the conformation within the active site, instead of relying heavily on computational predictions.

Other than this need, both sections of the bivalent compounds require attention. Firstly, improvements to the ATPase inhibitor are sought, not only for potency, but also pharmacokinetic properties. Already mentioned are the suggestion of tri-substituted imidazo[1,2-*a*]pyrazines. Work within the group has recently incorporated a bromine into position 6 of compound **14**. The

IC₅₀ of compound **293**^q has been calculated to be 6 μM, indicating that inserting a bromine does not affect the activity. This synthetic handle would provide the opportunity to explore further functionalities around the core heterocycle and prepare tri-substituted systems.

Keeping the ring substituents constant but using alternative core heterocycles should also be investigated to ascertain the importance of the imidazo[1,2-*a*]pyrazine. Pyrrolo[3,2-*c*]pyridine is a suggested alternative since it showed levels of inhibition with the BioFocus compounds.

Additionally, a modelling program called Brood²⁸⁸ can be used as an alternative method to generate new target compounds. The user splits the lead compound into different groups, and then the program takes each group in turn and inputs alternative fragments from an *in silico* library using shape, chemistry and electrostatic similarity, to generate alternative compounds.

Each of these compounds can then be re-docked into the enzyme active site and their binding energies ranked and compared with the parent compound. Any promising new compounds with docking scores above the parent compound can then be suggested for synthesis. This approach has been carried out with the imidazo[1,2-*a*]pyrazine compounds and the Maybridge fragments,^r and the synthesis of potential compounds is currently under investigation within the laboratory.

There is also the possibility of incorporating ATPγS as the small molecule part of the bivalent compound and in doing so, ensuring an extremely potent active site binder.

In order to make improvement with the peptide section of the bivalent compound, there are different approaches that are suggested. Firstly, the lack of helical structure requires attention. This can be addressed by adopting a helix-stabilisation approach,²⁸⁹ such as incorporating staple amino acids into the peptide sequence, as described by Verdine *et al.*^{280,290,291,292} By inserting amino acids at the *i* and (*i*+4) positions, intramolecular cross metathesis has been shown to induce helical folding (Figure 70).

^q Synthesised by Dr T. Pesnot

^r Brood programming carried out by Dr Paul Gane

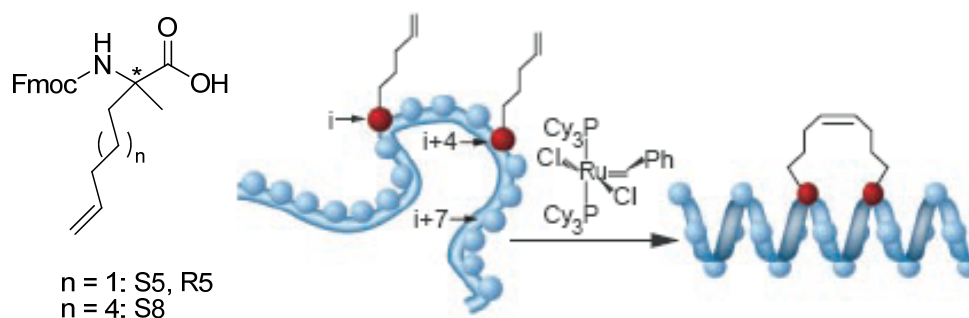


Figure 70: Representation of stapled amino acid to aid helical folding as described by Verdine *et al.*²⁹¹
Reproduced with permission.

It may also be necessary to target a different area of the protein, for example, mimicking interactions of the inhibitory protein HP1541. The peptide sequence could be based upon the sequence of the native protein that is known to make contact with HP0525 through peptide-peptide interactions.

An alternative approach could be to adopt the work carried out by Ed Tate²¹⁹ and synthesise a random combinatorial bivalent library looking for interactions at random epitopes on the protein surface. Similarly, the PEGylated small molecule inhibitor could be covalently bound to the active site, and a random combinatorial library of peptides added to the protein. *In situ* conjugation could then be achieved and comparisons made to find a suitable bivalent inhibitor.

The suggestions made should provide enough optimism for the synthesis of a more potent small molecule ATPase inhibitor, whilst hopefully ensuring that inhibition is also evident with the design of new bivalent compounds.

Chapter 6: Experimental

6.1 Compound Synthesis

6.1.1 General Considerations

Melting points (Mpt) were recorded on a Gallenkamp Melting Point Apparatus and are uncorrected. Proton nuclear magnetic resonance (^1H NMR) were recorded using Bruker AV400 (400 MHz), AV500 (500 MHz) and AV600 (600 MHz) spectrometers as indicated. Carbon nuclear magnetic resonance (^{13}C NMR) were recorded using Bruker AV400 (100 MHz), AV500 (125 MHz) and AV600 (150 MHz) spectrometers as indicated. Spectra were obtained using CDCl_3 , CD_2Cl_2 , CD_3OD , CD_3CN and $(\text{CD}_3)_2\text{SO}$ as solvents and chemical shifts are quoted on the δ scale in units of ppm using TMS as an internal standard. Coupling constants (J) are reported in Hz with the following splitting abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), dd (doublet of doublets), bs (broad singlet), dt (doublet of triplets), td (triplet of doublets), tt (triplet of triplets) and ddd (doublet of doublets of doublets). Compounds were characterised with the aid of 2D spectra (COSY, HSQC, HMBC and NOESY).

Infra-Red (IR) spectroscopy was carried out using a PerkinElmer Spectrum 100 FT-IR Spectrometer using thin films. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}).

High Performance Liquid Chromatography (HPLC) was performed using a Varian ProStar instrument; A Chiralpak AD column for normal phase analytical HPLC; DiscoveryBIO wide pore C18-10 (25 cm x 4.6 mm, 10 μm) for reverse phase analytical HPLC; and a DiscoveryBIO wide pore C18 (25 cm x 21.2 mm, 10 μm) column for reverse phase preparative HPLC. Each solvent used contained 0.1% TFA buffer.

Liquid Chromatography Mass Spectrometry (LCMS) was carried out using SQD-Waters Acquity UPLC/SQD with C18 (50 mm x 2.1 mm, 1.7 μm) column. A total run time of 5 minutes and flow rate of 0.6 mL/min was used with gradient elution: 95% H_2O / 5% MeCN (0 min), 5% H_2O / 95% MeCN (3 min), 95% H_2O / 5% MeCN (4.5 min). Each solvent contained 0.1% formic acid buffer. LRMS refers to low resolution mass spectrometry and HRMS refers to high resolution mass spectrometry. Electron Impact/ Chemical Ionisation (EI/CI) MS was carried out using

MAT900XP (Thermo Finnigan) instrument and electrospray ionization (ESI) accurate mass was determined using Waters LCT Premier XE instrumentation.

α_D was measured using PerkinElmer Model 343 Polarimeter. Three values were read at 589 nm and an average of these taken. Concentrations are given in units of g mol^{-1} and α_D in $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$.

Elemental analysis was obtained on a CE-440 CHN analyser, supplied by Exeter Analytical Instruments.

Microwave irradiation was carried out in either a CEM 150 W microwave reactor or a Biotage 120 W Initiator & Alstra automated microwave peptide synthesiser.

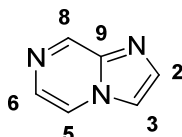
Thin layer chromatography (TLC) was carried out using Fluka aluminium backed sheets coated with 60F₂₅₄ silica gel. Visualisation of the silica plates was achieved using a UV lamp ($\lambda_{\text{max}} = 245 \text{ nm}$) and/or potassium permanganate (KMnO_4 in 1M NaOH with 5% K_2CO_3). Flash chromatography was carried out using either Geduran (Merck) or ZEOprep (Apollo) Si60 40-63 μm silica gel.

Solvents and reagents were obtained from commercial sources and were used as received unless otherwise stated. Dry solvents were dried over anhydrous columns.²⁹³ Moisture levels were usually <15 ppm by Karl Fischer titration. Brine refers to a saturated solution of sodium chloride. Anhydrous magnesium sulfate (MgSO_4) or sodium sulfate (Na_2SO_4) were used as drying agents after reaction workup, as indicated. Pet. ether refers to the fraction of light petroleum ether boiling in the range 40-60 °C.

6.1.2 Synthesis of Target Compounds

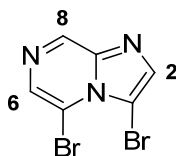
6.1.2.1 Initial Synthetic Approach

Imidazo[1,2-*a*]pyrazine (**115**)



2-Aminopyrazine (4.00 g, 0.042 mol) and chloroacetaldehyde (aq. 50% wt/v; 7.04 mL, 0.055 mol) were stirred in MeOH (60 mL) under reflux for 24 h. The reaction was cooled on ice for 2 h to give a very dark red solution. Excess Et₂O was added, the resulting precipitate was filtered and the Et₂O was removed *in vacuo*. The resulting residue was re-dissolved in the minimum quantity of MeOH and excess Et₂O added once again. The precipitate was filtered, combined with that of the previous washes, dried, and purified by flash chromatography (applied in MeCN; eluted 5% MeOH/MeCN) to give the title compound as a deep yellow precipitate (4.89 g, 0.041 mmol, 98%) with the NMR consistent with literature values.⁴⁷ Mpt: Decomposed before melting; *R_f* = 0.21 (5% MeOH/MeCN); IR (ν_{max} /cm⁻¹, thin film): 3319 (aromatic C-H stretch), 1486 (aromatic C=N and C=C stretches), 1328; ¹H NMR (500 MHz, CD₃OD): δ_{H} = 7.83 (s, 1H, **3-H**), 7.88 (d, *J* = 4.7 Hz, 1H, **5-H**), 8.06 (s, 1H, **2-H**), 8.52 (dd, *J* = 4.7, 1.5 Hz, 1H, **6-H**), 9.00 (s, 1H, **8-H**); ¹³C NMR (125 MHz, CD₃OD): δ_{C} = 115.1 (**C-2**), 120.7 (**C-6**), 129.0 (**C-5**), 134.9 (**C-3**), 141.8 (**C-9**), 142.6 (**C-8**); LRMS *m/z* (EI⁺): 119 [M]⁺.

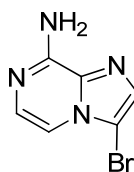
3,5-Dibromoimidazo[1,2-*a*]pyrazine (**136**)^{47,52}



Br₂ (0.650 mL, 12.6 mmol) in AcOH (0.5 mL) was added dropwise to a solution of **115** (0.300 g, 2.52 mmol) dissolved in AcOH (3.5 mL), and the reaction was heated under reflux for 1 h. AcOH and residual Br₂ were removed *in vacuo* to leave an orange oil, which was taken up in NaHCO₃ (sat. aq. 75 mL) and extracted with CH₂Cl₂ (3 x 50 mL). The organic extracts were

combined, dried (Na₂SO₄), filtered and solvent removed *in vacuo* to give an orange solid. The regioisomers were purified by flash chromatography (applied in hexane; eluted 3:1 hexane/Et₂O) to give an off white solid (120 mg, 0.433 mmol, 17%). Normal phase chiral analytical HPLC (hexane/IPA 97:3) and reverse phase analytical HPLC indicated 2 regioisomers were present. Preparative reverse phase column (10% to 20% MeCN over 30 min) afforded the separated isomers. The solvent was removed and the samples were freeze dried. Recrystallisation from hot H₂O gave the title compound as needle like crystals, with the NMR consistent with literature values.^{47,52} *R_f* = 0.11 (3:1 hexane/Et₂O); ¹H NMR (500 MHz, CDCl₃): δ_H = 7.78 (s, 1H, **2-H**), 7.98 (s, 1H, **6-H**), 8.99 (s, 1H, **8-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = 110.7 (**C-3**), 130.2 (**C-5**), 134.3 (**C-6**), 138.9 (**C-2**), 142.1 (**C-9**), 142.9 (**C-8**); LRMS *m/z* (EI): 279 [M(⁸¹Br₂)]⁺, 277 [M(^{79/81}Br₂)]⁺, 275 [M(⁷⁹Br₂)]⁺, 198, 197, 196 [M-Br], 119 [M-Br₂].

Attempted formation of 8-amino-3-bromoimidazo[1,2-*a*]pyrazine (**163**)^{51,52}

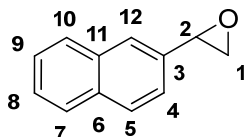


Compound **136** (50.0 mg) was dissolved in 2.0 M NH₃/EtOH (2.5 mL) and the mixture was heated in a sealed tube at 120 °C for 5 h. After the reaction was cooled to RT, the mixture was transferred to a round bottom flask and the solvent removed *in vacuo*. The solid was recrystallised from hot ethanol. With the crude di-bromo species, a brown/black solid (45.9 mg) was obtained. When the pure di-bromo heterocycle was used, a pale brown fluffy and static solid (32.0 mg) resulted. In both cases, no product was evident by MS or NMR and so no further work was carried out.

6.1.2.2 Synthesis of 3-Aryl Imidazo[1,2-*a*]pyrazines

6.1.2.2.1 Formation of Amino Alcohol Moiety via Epoxide

2-(2-Naphthyl)oxirane (188)



Method A:¹³¹

Purification of the 2-vinylnaphthalene was carried out by flash chromatography (hexane; 60:1 hexane/EtOAc). To a stirred solution of 2-vinylnaphthalene (1.54 g, 0.010 mol) in $\text{CH}_2\text{Cl}_2/\text{NaHCO}_3$ (sat. aq.) (1:1; 200 mL) at 0 °C, was added *m*CPBA (1.73g, 0.010 mol) in small portions over a period of 10 min. The mixture was then stirred at RT for 4 h, cooled to 0 °C, a further quantity of *m*CPBA (1.73 g, 0.010 mol) added over a 10 min period and continued to stir at RT for a further 4 h. The organic layer was extracted, washed with $\text{Na}_2\text{S}_2\text{O}_3$ (sat. aq. 4 x 25 mL) and dried (Na_2SO_4). The mixture was filtered and solvent removed *in vacuo* to give a deep orange/red oil. The crude epoxide was purified by flash chromatography (applied in hexane; eluted 60:1 to 40:1 to 30:1 hexane/EtOAc) to give a white solid (0.524 g, 3.08 mmol, 31%) consistent with NMR literature values.²⁹⁴ R_f = 0.46 (6:1 hexane/EtOAc); $\nu_{\text{max}}/\text{cm}^{-1}$ (thin film): 3060 (aromatic C-H stretch); ^1H NMR (500 MHz, CDCl_3): δ_{H} = 2.91 (dd, J = 5.4, 2.6 Hz, 1H, **1-H**), 3.23 (dd, J = 5.4, 4.1 Hz, 1H, **1-H**), 4.03 (dd, J = 4.1, 2.6 Hz, 1H, **2-H**), 7.32-7.34 (m, 1H, **4-H**), 7.48-7.51 (m, 2H, **8,9-H**), 7.80-7.84 (m, 4H, **5,7,10,12-H**); ^{13}C NMR (125 MHz, CDCl_3): δ_{C} = 51.4 (**C-1**), 52.7 (**C-2**), 122.7 (**C-4**), 126.2 (**C-8**), 126.4 (**C-9**), 125.2, 127.8, 128.5 (**C-5,7,10,12**), 133.3 (**C-6**), 133.4 (**C-11**), 135.1 (**C-3**); LRMS m/z (EI): 170 $[\text{M}]^+$, 154 $[\text{M}-\text{O}]^+$, 141 $[\text{M}-29]^+$, 115, 127.

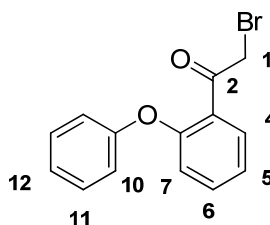
Method B:

All glassware was flushed with Ar prior to use. The sulfur ylide was prepared *in situ* by refluxing a mixture of NaH (0.123 g, 8.53 mmol) and trimethyloxosulfonium iodide (1.88 g, 8.53 mmol) in dry THF (10 mL) for 1.5 h in a 3-necked round bottom flask, under Ar. The resulting milky – white solution was cooled to 55 °C and a solution of 2-naphthaldehyde (1.00 g, 6.40 mmol) in dry THF (10 mL) added dropwise over a period of 30 min with stirring. The mixture

immediately turned yellow on addition of the 2-naphthaldehyde and then to orange. The mixture was stirred at 55 °C for 1 h and was then quenched with H₂O (15 mL) and extracted with Et₂O (3 x 20 mL). The combined extracts were dried (Na₂SO₄), filtered, and concentrated eluted to give a dark yellow oil. Initial purification by flash chromatography (CH₂Cl₂) afforded a light yellow oil (0.653 g, 3.84 mmol, 60%), which consisted of the starting aldehyde and product in 2:1 ratio.

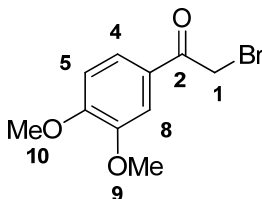
6.1.2.2.2 Synthesis of α -bromo aryl ketones

2-Bromo-1-(2-phenoxyphenyl)ethanone (196)



1-(2-Phenoxyphenyl)ethanone (2.00 g, 9.42 mmol) was dissolved in CHCl₃/EtOH (1:1; 120 mL). Pyridinium tribromide (7.50 g, 23.6 mmol) was added and the reaction was stirred at 50 °C for 16 h. The reaction mixture was cooled to RT and the solvents removed *in vacuo*. The resulting orange slurry was suspended in H₂O (30 mL) and extracted with EtOAc (4 x 30 mL). The combined organic extracts were washed with H₂O (2 x 20 mL) and brine (1 x 20 mL), dried (Na₂SO₄), filtered and concentrated *in vacuo* to give a yellow oil. Flash chromatography was carried out (applied in pet. ether; eluted 0% to 10% to 33% CH₂Cl₂) to afford the title compound as a pale yellow oil (2.30 g, 7.90 mmol, 84%). *R*_f = 0.68 (CH₂Cl₂); IR (ν_{max} /cm⁻¹, thin film): 1677 (C=O stretch), 1598, 1574, 1475, 1446 (aromatic C=C stretches), 1223 (aromatic C-O stretch); ¹H NMR (500 MHz, CDCl₃): δ_{H} = 4.65 (s, 2H, **1-H**), 6.86 (d, *J* = 8.4 Hz, 1H, **7-H**), 7.09 (d, *J* = 7.7 Hz, 2H, **10-H**), 7.17 (t, *J* = 7.6 Hz, 1H, **5-H**), 7.22 (t, *J* = 7.3 Hz, 1H, **12-H**), 7.40-7.47 (m, 3H, **6,11-H**), 7.92 (dd, *J* = 7.6, 1.5 Hz, 1H, **4-H**); ¹³C NMR (125 MHz, CDCl₃): δ_{C} = 36.8 (**C-1**), 117.6 (**C-7**), 119.5 (**C-10**), 123.0 (**C-5**), 124.4 (**C-12**), 126.2 (**C-3**), 129.9 (**C-11**), 131.3 (**C-4**), 134.2 (**C-6**), 155.0 (**C-9**), 156.5 (**C-8**), 191.6 (**C-2**); LRMS *m/z* (EI⁺): 292 [M (⁸¹Br)]⁺, 290 [M (⁷⁹Br)]⁺, 212 [M-Br]⁺, 197 [M-CH₂Br]⁺; HRMS *m/z* (EI⁺): Found 289.9940 [M(⁷⁹Br)]⁺; C₁₄H₁₁BrO₂ requires 289.9937.

2-Bromo-1-(3,4-dimethoxyphenyl)ethanone (197)¹⁴⁰



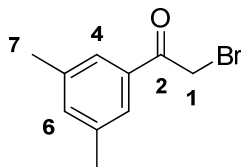
Method A:

3,4-Dimethoxyacetophenone (1.00 g, 5.55 mmol) was dissolved in CHCl₃/EtOH (1:1; 60 mL). Pyridinium tribromide (4.46 g, 13.9 mmol) was added and the mixture was stirred at 50 °C for 16 h. The reaction was cooled to RT and solvents removed *in vacuo* to give a sticky orange solid, which was then dissolved in H₂O (30 mL), and organics extracted with EtOAc (3 x 20 mL) and washed with H₂O (2 x 20 mL) and brine (20 mL). The organics were dried (MgSO₄), filtered and concentrated *in vacuo* to give an orange oil containing a mixture of mono- and di-bromated species (approx 1.5:1). Flash chromatography (CH₂Cl₂ isocratic) afforded the title compound as an off-white solid (752 mg, 2.90 mmol, 52%). Spectroscopic data was consistent with that previously reported.¹⁴⁰ Mpt: 62 °C [Lit.¹⁴⁰ 67-70 °C]; *R*_f = 0.12 (CH₂Cl₂); IR (ν_{max}/cm⁻¹, thin film): 2940 (C-H stretch), 1679 (C=O stretch), 1585, 1512, 1465, 1418 (aromatic C=C stretches), 1241 (aromatic C-O stretch); ¹H NMR (500 MHz, CDCl₃): δ_H = 3.93 (s, 3H, **9-H**), 3.95 (s, 3H, **10-H**), 4.40 (s, 2H, **1-H**), 6.90 (d, *J* = 8.4 Hz, 1H, **5-H**), 7.53 (d, *J* = 2.1 Hz, 1H, **8-H**), 7.60 (dd, *J* = 8.4, 2.1 Hz, 1H, **4-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = 30.5 (**C-1**), 56.1 (**C-9**), 56.2 (**C-10**), 110.2 (**C-5**), 110.9 (**C-8**), 123.9 (**C-4**), 127.1 (**C-3**), 149.4 (**C-7**), 154.1 (**C-6**), 190.2 (**C-2**); LRMS *m/z* (EI⁺): 260 [M(⁸¹Br)]⁺, 258 [M(⁷⁹Br)]⁺, 165 [M-CH₂Br]⁺; HRMS *m/z* (EI⁺): Found 257.9887 [M(⁷⁹Br)]⁺; C₁₀H₁₁BrO₃ requires 257.9886.

Method B:

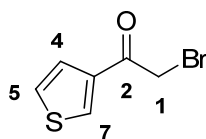
3,4-Dimethoxyacetophenone (1.00 g, 5.55 mmol) was dissolved in glacial acetic acid (1 mL) and the mixture was stirred on ice. Br₂ (2.76 mL, 5.55 mmol), dissolved in AcOH (1 mL), was added to the reaction *via* a dropping funnel over a period of 45 min. The reaction mixture solidified on addition of the Br₂ and so further AcOH (3 mL) was added. The reaction was stopped after 15 min, poured into crushed ice and extracted with EtOAc (3 x 20 mL). The organics were dried (Na₂SO₄), filtered and solvent removed *in vacuo* to give a red oil. NMR showed that no product was evident and so no further work was carried out.

2-Bromo-1-(3,5-dimethylphenyl)ethanone (198)



3,5-Dimethyl acetophenone (1.00 g, 6.75 mmol) was dissolved in $\text{CHCl}_3/\text{EtOH}$ (1:1; 80 mL). Pyridinium tribromide (6.47 g, 20.2 mmol) was added and the reaction was stirred at 50 °C for 17 h. The reaction mixture was cooled to RT and the solvents removed *in vacuo*. The resulting orange slurry was suspended in H_2O (30 mL) and extracted with EtOAc (4 x 30 mL). The combined organic extracts were washed with H_2O (2 x 20 mL) and brine (1 x 20 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give a yellow oil. Flash chromatography was carried out (applied in pet. ether; eluted pet. ether to 20:1 to 2:1 pet. ether/ CH_2Cl_2) to afford the title compound as a pale yellow oil (760 g, 3.34 mmol 50%). Spectroscopic data was consistent with that previously reported.²⁹⁵ R_f = 0.31 (1:1 pet. ether/ CH_2Cl_2); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 2919 (C-H stretch), 1683 (C=O stretch); ^1H NMR (500 MHz, CDCl_3): δ_{H} = 2.38 (s, 6H, **7-H**), 4.44 (s, 2H, **1-H**), 7.24 (s, 1H, **6-H**), 7.58 (s, 2H, **4-H**); ^{13}C NMR (125 MHz, CDCl_3): δ_{C} = 21.3 (**C-7**), 31.3 (**C-1**), 126.7 (**C-4**), 134.2 (**C-3**), 135.7 (**C-6**), 138.7 (**C-5**), 191.7 (**C-2**); LRMS m/z (EI^+): 228 [$\text{M}(^{81}\text{Br})^+$], 226 [$\text{M}(^{79}\text{Br})^+$], 133 [$\text{M}-\text{CH}_2\text{Br}^+$]; HRMS m/z (EI^+) [$\text{M}(^{79}\text{Br})^+$]: Found 225.9985; $\text{C}_{10}\text{H}_{11}\text{BrO}$ requires 225.9988.

2-Bromo-1-(3-thienyl)-1-ethanone (199)

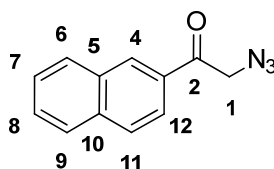


1-(3-Thienyl)ethanone (2.00 g, 15.9 mmol) was dissolved in $\text{CHCl}_3/\text{EtOH}$ (1:1; 200 mL). Pyridinium tribromide (10.1 g, 31.7 mmol) was added and the reaction was stirred at 50 °C for 18 h. The reaction mixture was cooled to RT and the solvents removed *in vacuo*. The resulting orange slurry was suspended in H_2O (40 mL) and extracted with EtOAc (4 x 50 mL). The combined organic extracts were washed with H_2O (2 x 30 mL) and brine (1 x 30 mL), dried (MgSO_4), filtered and concentrated *in vacuo* to give an amber liquid. Flash chromatography was

carried out (pet. ether; 3:1 pet. ether/CH₂Cl₂) to afford the title compound as a white solid (1.61 g, 7.81 mmol, 49%). Spectroscopic data was consistent with that previously reported.²⁹⁶ Mpt: 59-60 °C [Lit.²⁹⁶ 60-65 °C]; *R_f* = 0.48 (1:1 pet. ether/ CH₂Cl₂); IR (ν_{max}/cm⁻¹, thin film): 3095 (C-H stretch), 1689 (C=O stretch), 1508, 1415, 1400, 1393, 1380 (aromatic C=C stretches), 1181; ¹H NMR (600 MHz, CDCl₃): δ_H = 4.34 (s, 2H, **1-H**), 7.36 (dd, *J* = 5.2, 2.9 Hz, 1H, **5-H**), 7.58 (dd, *J* = 5.2, 1.3 Hz, 1H, **4-H**), 8.17 (dd, *J* = 2.9, 1.3 Hz, 1H, **7-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 31.7 (**C-1**), 127.0 (**C-5**), 127.4 (**C-4**), 133.9 (**C-7**), 138.9 (**C-3**), 185.7 (**C-2**); LRMS *m/z* (CI⁺): 207 [M(⁸¹Br)]⁺, 205 [M(⁷⁹Br)]⁺.

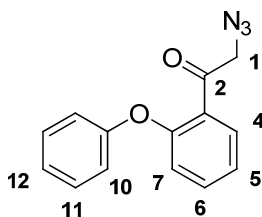
6.1.2.2.3 Synthesis of α-azido aryl ketone

2-Azido-1-(2-naphthyl)ethanone (200)



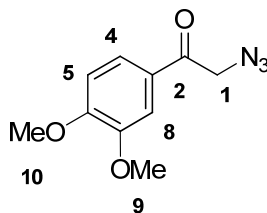
2-(Bromoacetyl)naphthalene (2.00 g, 8.03 mmol) was dissolved in DMSO (10 mL) and the mixture was cooled on ice such that the temperature was kept below 10 °C. NaN₃ (0.630 g, 9.64 mmol) was added in one portion and the reaction was stirred under Ar at RT for 90 min. The reaction was quenched with H₂O (20 mL), and extracted with EtOAc (3 x 30 mL). The organic layers were combined, washed with H₂O, dried (Na₂SO₄) and filtered. The solvent was removed *in vacuo* to give the title compound as a brown/orange oil (1.69 g, 8.01 mmol, 100%) with NMR consistent with literature values.²⁹⁷ *R_f* = 0.63 (5:1 pet. ether/EtOAc); IR (ν_{max}/cm⁻¹, thin film): 2105 (-N=N⁺=N⁻ stretch), 1690 (C=O stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 4.73 (s, 2H, **1-H**), 7.59-7.62 (m, 1H, **7-H**), 7.65-7.68 (m, 1H, **8-H**), 7.91 (d, *J* = 8.1 Hz, 1H, **9-H**), 7.95 (d, *J* = 8.6 Hz, 1H, **11-H**), 7.98-8.01 (m, 2H, **6,12-H**), 8.42 (s, 1H, **4-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 55.0 (**C-1**), 123.3 (**C-12**), 127.2 (**C-7**), 127.9 (**C-9**), 129.0 (**C-11**), 129.1 (**C-8**), 129.6 (**C-6**), 129.8 (**C-4**), 131.7 (**C-5**), 132.4 (**C-10**), 136.0 (**C-3**), 193.2 (**C-2**); LRMS *m/z* (EI⁺): 211 [M]⁺, 155 [M-CH₂N₃]⁺, 127 [Naphthalene]⁺.

2-azido-1-(2-phenoxyphenyl)ethanone (201)



2-Bromo-1-(2-phenoxyphenyl)ethanone (3.07 g, 10.5 mmol) was dissolved in DMSO (15 mL) and the mixture was cooled on ice. NaN₃ (824 mg, 12.7 mmol) was added in one portion and the reaction was stirred under Ar at RT for 5 h. An extra portion of NaN₃ (200 mg) was added and left to stir at RT. After 15 h, the reaction was quenched with H₂O (30 mL), and extracted with EtOAc (4 x 40 mL). The combined organic layers were washed with H₂O (5 x 20 mL), dried (Na₂SO₄) and filtered. The solvent was removed *in vacuo* to give the title compound as a brown oil (2.52 g, 9.96 mmol, 95%). *R_f* = 0.4 (CH₂Cl₂); IR (*v*_{max}/cm⁻¹, thin film): 3071 (C-H stretch), 2100 (-N=N⁺=N⁻ stretch), 1685 (C=O stretch), 1599, 1475, 1448 (aromatic C=C stretches), 1225 (aromatic C-O stretch); ¹H NMR (500 MHz, CDCl₃): δ_H = 4.60 (s, 2H, **1-H**), 6.87 (dd, *J* = 8.3, 0.9 Hz, 1H, **7-H**), 7.04-7.06 (m, 2H, **10,14-H**), 7.18-7.23 (m 2H, **5,12-H**), 7.40-7.43 (m, 2H, **11,13-H**), 7.45-7.49 (m, 1H, **6-H**), 7.92 (dd, *J* = 7.8, 1.5 Hz, 1H, **4-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = 58.9 (**C-1**), 118.0 (**C-7**), 119.2 (**C-10**), 123.2 (**C-5**), 124.4 (**C-12**), 126.2 (**C-3**), 130.0 (**C-11**), 130.8 (**C-4**), 134.5 (**C-6**), 155.0 (**C-9**), 157.0 (**C-8**), 193.6 (**C-2**); LRMS *m/z* (CI⁺): 198 [M-CH₂N₃]⁺, 85 [COCH₂N₃]⁺.

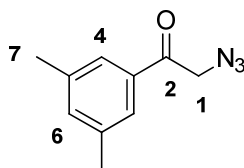
2-Azido-1-(3,4-dimethoxyphenyl)ethanone (202)



197 (2.33 g, 8.61 mmol) was dissolved in anhydrous DMSO (10 mL) and the mixture was cooled on ice. NaN₃ (671 mg, 10.3 mmol) was added in one portion (an immediate colour change of yellow to orange was observed) and the reaction was stirred under Ar at RT for 2 h. The reaction was then quenched with H₂O (30 mL) and extracted with EtOAc (4 x 40 mL). The combined

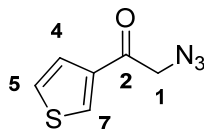
organic layers were washed with H₂O (5 x 20 mL) and brine (20 mL), dried (MgSO₄) and filtered. The solvent was removed *in vacuo* to give the title compound as a sticky orange solid (1.87 g, 8.46 mmol, 98%) with NMR data comparable with literature values.²⁹⁸ R_f = 0.22 (CH₂Cl₂); IR (ν_{\max} /cm⁻¹, thin film): 2106 (-N=N⁺=N⁻ stretch), 1682 (C=O stretch), 1595, 1515 (aromatic C=C stretches), 1264 (aromatic C-O stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 3.94 (s, 3H, **9-H**), 3.96 (s, 3H, **10-H**), 4.52 (s, 2H, **1-H**), 6.90 (d, J = 8.5 Hz, 1H, **5-H**), 7.47 (dd, J = 8.5, 2.1 Hz, 1H, **4-H**), 7.52 (d, J = 2.1 Hz, 1H, **8-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 54.5 (**C-1**), 56.1 (**C-9**), 56.2 (**C-10**), 110.0 (**C-8**), 110.1 (**C-5**), 122.5 (**C-4**), 127.5 (**C-3**), 149.4 (**C-7**), 154.1 (**C-6**), 191.8 (**C-2**); LRMS m/z (CI⁺): 222 [M+H]⁺, 165 [M-CH₂N₃]⁺; HRMS m/z (CI⁺): Found 222.0878 [M+H]⁺; C₁₀H₁₂N₃O₃ requires 222.0879.

2-Azido-1-(3,5-dimethylphenyl)ethanone (**203**)



198 (3.44 g, 15.1 mmol) was dissolved in anhydrous DMSO (15 mL) and the mixture was cooled on ice. NaN₃ (1.18 g, 18.2 mmol) was added in one portion and the reaction was stirred under Ar at RT for 16 h. An extra portion of NaN₃ (200 mg) was added and the reaction was left to stir for a further 1 h. A colour change from yellow to deep orange was observed. The reaction was then quenched with H₂O (30 mL), and extracted with EtOAc (4 x 40 mL). The combined organic layers were washed with H₂O (5 x 20 mL) and brine (20 mL), dried (MgSO₄) and filtered. The solvent was removed *in vacuo* to give the title compound as a sticky orange solid (2.85 g, 15.1 mmol, 100%). R_f = 0.59 (CH₂Cl₂); IR (ν_{\max} /cm⁻¹, thin film): 2920 (C-H stretch), 2105 (-N=N⁺=N⁻ stretch), 1692 (C=O stretch), 1604 (aromatic C=C stretch); ¹H NMR (500 MHz, CDCl₃): δ_H = 2.35 (s, 6H, **7-H**), 4.52 (s, 2H, **1-H**), 7.20 (s, 1H, **6-H**), 7.40 (s, 2H, **4-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = 21.3 (**C-7**), 55.0 (**C-1**), 125.7 (**C-4**), 134.5 (**C-3**), 135.9 (**C-6**), 138.8 (**C-5**), 193.6 (**C-2**); LRMS m/z (CI⁺): 132 [M-CH₂N₃]⁺.

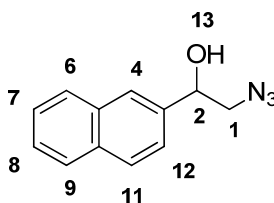
2-Azido-1-(3-thienyl)ethanone (204)



2-Bromo-1-(3-thienyl)-1-ethanone (573 mg, 2.80 mmol) was dissolved in DMSO (3 mL) and the mixture was cooled on ice. NaN₃ (218 mg, 3.35 mmol) was added in one portion and the reaction was stirred under Ar at RT for 5 h. The reaction was quenched with H₂O (20 mL), and extracted with EtOAc (3 x 30 mL). The organic layers were combined, washed with H₂O (5 x 20 mL) and brine (20 mL), dried (Na₂SO₄) and filtered. The solvent was removed *in vacuo* to give the title compound as a brown/orange oil (460 mg, 2.75 mmol, 98%). *R*_f = 0.27 (CH₂Cl₂); IR (ν_{max}/cm⁻¹, thin film): 3105 (C-H stretch), 2097 (-N=N⁺=N⁻ stretch), 1679 (C=O stretch), 1508, 1409, 1231, 1177; ¹H NMR (500 MHz, CDCl₃): δ_H = 4.43 (s, 2H, **1-H**), 7.38 (dd, *J* = 5.1, 2.9 Hz, 1H, **5-H**), 7.55 (dd, *J* = 5.1, 1.3 Hz, 1H, **4-H**), 8.10 (dd, *J* = 2.9, 1.3 Hz, 1H, **7-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = 55.5 (**C-1**), 126.6 (**C-5**), 127.2 (**C-4**), 132.7 (**C-7**), 139.1 (**C-3**), 187.6 (**C-2**); LRMS *m/z* (ES⁺): 185 [M+18]⁺, 168 [M+H]⁺; HRMS *m/z* (ES⁺): Found 168.0235 [M+H]⁺; C₆H₆N₃OS requires 168.0232.

6.1.2.2.4 Synthesis of α-azido aryl alcohols

2-Azido-1-(2-naphthyl)ethanol (205)



Method A:

200 (2.11 g, 10.0 mmol) was dissolved in anhydrous MeOH (100 mL) and cooled on ice. NaBH₄ (568 mg, 15.0 mmol) was added portionwise and the mixture was stirred on ice under Ar for 1 h. The solvent was removed and the resulting residue was taken up in CH₂Cl₂ (100 mL) and carefully washed with H₂O (2 x 60 mL) followed by brine (60 mL). The organic extracts were dried (Na₂SO₄), filtered and concentrated *in vacuo* to give the title compound as a brown oil (2.14

g, 10.0 mmol, 100%). Spectroscopic data was consistent to that previously reported.²⁹⁹ R_f = 0.65 (3:1 pet. ether/EtOAc); IR (ν_{\max} /cm⁻¹, thin film): 3398 (O-H stretch), 2100 (-N=N⁺=N⁻ stretch); ¹H NMR (500 MHz, CDCl₃): δ_H = 2.70 (bs, 1H, **13-H**), 3.46-3.58 (m, 2H, **1-H**), 5.02 (dd, J = 8.1, 3.9 Hz, 1H, **2-H**), 7.44 (dd J = 8.4, 1.6 Hz, 1H, **11-H**), 7.49-7.52 (m, 2H, **7,8-H**), 7.83-7.86 (m, 4H, **4,6,7,12-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = 58.1 (**C-1**), 73.6 (**C-2**), 123.7 (**C-12**), 125.1 (**C-7**), 126.4 (**C-9**), 126.5 (**C-11**), 127.8 (**C-8**), 128.1 (**C-6**), 128.6 (**C-4**), 133.3 (overlapping signals, **C-5,10**), 138.0 (**C-3**); LRMS m/z (EI⁺): 221, 157 [M-CH₂N₃]⁺, 147, 129.

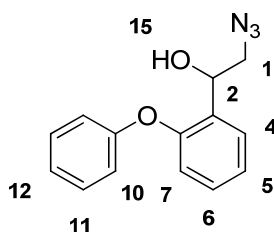
Method B:

200 (1.70 g, 8.06 mmol) was dissolved in anhydrous Et₂O (70 mL). Activated neutral alumina (7.00 g) and NaBH₄ (609 mg, 16.11 mmol) were added and the suspension was stirred at RT under Ar. After 15 h the reaction mixture was filtered and washed with Et₂O. The resulting filtrate was washed with H₂O (2 x 20 mL) and brine (2 x 20 mL). The combined organic extracts were dried (Na₂SO₄), filtered and concentrated *in vacuo* to give the title compound as an orange/yellow oil (962 mg, 4.52 mmol, 56%).

Method C:

Epoxide **188** (50.0 mg, 0.294 mmol) was taken up in anhydrous MeCN (2 mL), molecular sieves (4Å, 100 mg) were added and the vessel was evacuated and charged with Ar. NaN₃ (28.7 mg, 0.441 mmol) was added and the mixture was stirred under Ar for 24 h. No reaction was indicated by TLC and so no further work was carried out.

2-Azido-1-(2-phenoxyphenyl)ethanol (**206**)



Method A:

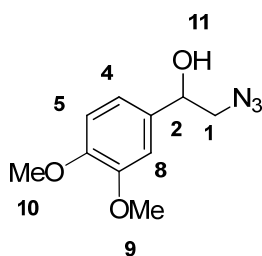
201 (2.52 g, 9.96 mmol) was dissolved in anhydrous MeOH (50 mL) and cooled on ice. NaBH₄ (565 mg, 14.9 mmol) was added portionwise and the mixture was stirred on ice under Ar for 1 h.

The solvent was removed and the resulting residue was taken up in CH₂Cl₂ (50 mL) and carefully washed with H₂O (2 x 40 mL). Re-extraction of the aqueous layers with CH₂Cl₂ (3 x 30 mL), followed by washing the combined organic extracts with brine, drying (Na₂SO₄), filtering and concentrating *in vacuo* gave the title compound as a brown oil (2.47 g, 9.69 mmol, 97%). *R*_f = 0.19 (CH₂Cl₂); IR (ν_{max}/cm⁻¹, thin film): 3413 (O-H stretch), 3039 (C-H stretch), 2102 (-N=N⁺=N⁻ stretch), 1583, 1483, 1452 (aromatic C=C stretches), 1230 (aromatic C-O-C stretch); ¹H NMR (500 MHz, CDCl₃): δ_H = 2.54 (bs, 1H, 15-H), 3.50 (dd, *J* = 12.5, 8.0 Hz, 1H, **1-H**), 3.58 (dd, *J* = 12.5, 3.5 Hz, 1H, **1-H**), 5.23 (dd, *J* = 8.0, 3.5 Hz, 1H, **2-H**), 6.84 (dd, *J* = 8.2, 1.1 Hz, 1H, **7-H**), 6.97-7.00 (m, 2H, **10-H**), 7.13 (tt, *J* = 7.6, 1.1 Hz, 1H, **5-H**), 7.17 (td, *J* = 7.5, 1.0 Hz, 1H, **12-H**), 7.24-7.27 (m, 1H, **6-H**), 7.34-7.37 (m, 2H, **11-H**), 7.58 (dd, *J* = 7.6, 1.7 Hz, 1H, **4-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = 56.5 (**C-1**), 68.7 (**C-2**), 118.0 (**C-7**), 118.3 (**C-10**), 123.4 (**C-5**), 123.5 (**C-12**), 127.1 (**C-4**), 129.6 (**C-6**), 129.6 (**C-11**), 130.8 (**C-3**), 153.5 (**C-9**), 156.3 (**C-8**); LRMS *m/z* (CI⁺): 200 [M-CH₂N₃]⁺, 182 [M-OH,CH₂N₃]⁺.

Method B:

201 (660 mg, 2.61 mmol) was dissolved in anhydrous Et₂O (30 mL). Activated neutral alumina (3.00 g) and NaBH₄ (197 mg, 5.21 mmol) were added and the suspension was stirred at RT under Ar. After 14 h the reaction mixture was filtered and washed with Et₂O. The resulting filtrate was washed with H₂O (2 x 20 mL), followed by brine (2 x 20 mL). The combined organic extracts were dried (Na₂SO₄), filtered and concentrated *in vacuo* to give the title compound as an orange oil (394 mg, 1.54 mmol, 59%).

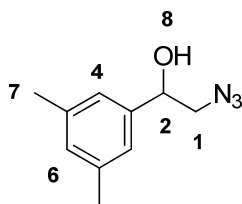
2-Azido-1-(3,4-dimethoxyphenyl)ethanol (**207**)



202 (1.86 g, 8.40 mmol) was dissolved in anhydrous Et₂O (80 mL). Activated neutral alumina (8.00 g) and NaBH₄ (635 mg, 16.8 mmol) were added and the suspension was stirred at RT under Ar. After 16 h the reaction mixture was filtered and washed with Et₂O. The resulting filtrate was

washed with H₂O (2 x 30 mL), followed by brine (2 x 30 mL). The combined organic extracts were dried (Na₂SO₄), filtered and concentrated *in vacuo* to give the title compound as a yellow oil (1.64 g, 7.37 mmol, 87%). *R_f* = 0.65 (2:1 CH₂Cl₂/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3497 (O-H stretch), 2938, 2839 (C-H stretch), 2106 (-N=N⁺=N⁻ stretch), 1516 (aromatic C=C stretch), 1263 (aromatic C-O-C stretch); ¹H NMR (500 MHz, CDCl₃): δ_{H} = 2.67 (bs, 1H, **11-H**) 3.36 (dd, *J* = 12.6, 3.8 Hz, 1H, **1-H**), 3.49 (dd, *J* = 12.6, 8.3 Hz, 1H, **1-H**) 3.84 (s, 3H, **9-H**), 3.85 (s, 3H, **10-H**), 4.78 (dd, *J* = 8.3, 3.8 Hz, 1H, **2-H**), 6.82 (d, *J* = 8.2 Hz, 1H, **5-H**), 6.86 (dd, *J* = 8.2, 1.9 Hz, 1H, **4-H**), 6.89 (d, *J* = 1.9 Hz, 1H, **8-H**); ¹³C NMR (125 MHz, CDCl₃): δ_{C} = 55.6 (**C-9,10**), 57.7 (**C-1**), 72.9 (**C-2**), 108.6 (**C-8**), 110.8 (**C-5**), 117.9 (**C-4**), 132.0 (**C-3**), 148.6 (**C-7**), 148.8 (**C-6**); LRMS *m/z* (EI⁺): 223 [M]⁺, 167 [M-CH₂N₃]⁺, 139 [M-CH(OH)CH₂N₃]⁺.

2-Azido-1-(3,5-dimethylphenyl)ethanol (208)



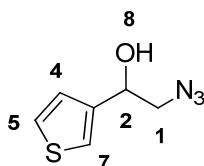
Method A:

203 (2.85 g, 15.1 mmol) was dissolved in anhydrous MeOH (60 mL) and cooled on ice. NaBH₄ (855 mg, 22.6 mmol) was added portionwise and the mixture was stirred on ice under Ar for 1 h. The solvent was removed and the resulting orange oil was taken up in CH₂Cl₂ (60 mL) and carefully washed with 2.0 M HCl (40 mL), H₂O (30 mL) and brine (30 mL). The combined organic extracts were dried (MgSO₄), filtered and concentrated *in vacuo* to give the title compound as an orange oil (2.85 g, 14.9 mmol, 99%). *R_f* = 0.27 (CH₂Cl₂); IR (ν_{max} /cm⁻¹, thin film): 3395 (O-H stretch), 2918 (C-H stretch), 2099 (-N=N⁺=N⁻ stretch); ¹H NMR (500 MHz, CDCl₃): δ_{H} = 2.32 (s, 6H, **7-H**), 3.40 (dd, *J* = 12.6, 3.8 Hz, 1H, **1-H**), 3.47 (dd, *J* = 12.6, 8.4 Hz, 1H, **1-H**), 4.79 (dd, *J* = 8.4, 3.8 Hz, 1H, **2-H**), 6.96 (s, 1H, **6-H**), 6.97 (s, 2H, **4-H**); ¹³C NMR (125 MHz, CDCl₃): δ_{C} = 21.2 (**C-7**), 58.2 (**C-1**), 73.5 (**C-2**), 123.8 (**C-4**), 130.0 (**C-6**), 138.4 (**C-5**), 140.7 (**C-3**); LRMS *m/z* (CI⁺): 192 [M+H]⁺, 132 [M-OH-N₃]⁺.

Method B:

203 (707 mg, 3.74 mmol) was dissolved in anhydrous Et₂O (30 mL). Activated neutral alumina (3.00 g) and NaBH₄ (283 mg, 7.48 mmol) were added and the suspension was stirred at RT under Ar. After 18 h the reaction mixture was filtered and washed with Et₂O. The resulting filtrate was washed with H₂O (2 x 30 mL), followed by brine (2 x 30 mL). The combined organic extracts were dried (Na₂SO₄), filtered and concentrated *in vacuo* to give the title compound as an orange oil (419 mg, 2.18 mmol, 58%).

2-Azido-1-(3-thienyl)ethanol (209)



Method A:

204 (278 mg, 1.67 mmol) was dissolved in anhydrous MeOH (10 mL) and cooled on ice. NaBH₄ (94.5 mg, 2.45 mmol) was added portionwise and the mixture was stirred on ice under Ar for 1 h. The solvent was removed and the resulting residue was taken up in CH₂Cl₂ (30 mL) and carefully washed with H₂O (2 x 20 mL) followed by brine (20 mL). The organic extracts were dried (Na₂SO₄), filtered and concentrated *in vacuo* to give the title compound as a yellow oil (257 mg, 1.52 mmol, 91%). *R_f* = 0.8 (1:1 pet. ether/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3372 (O-H stretch), 3105 (C-H stretch), 2096 (-N=N⁺=N⁻ stretch); ¹H NMR (500 MHz, CDCl₃): δ_{H} = 3.51-3.54 (m, 2H, **1-H**), 4.98 (dd, *J* = 4.7, 1.9 Hz, 1H, **2-H**), 7.08 (dd, *J* = 5.0, 1.3 Hz, 1H, **4-H**), 7.29-7.30 (m, 1H, **7-H**), 7.34 (dd, *J* = 5.0, 3.0 Hz, 1H, **5-H**); ¹³C NMR (125 MHz, CDCl₃): δ_{C} = 57.5 (**C-1**), 69.9 (**C-2**), 122.0 (**C-7**), 125.4 (**C-4**), 126.7 (**C-5**), 142.0 (**C-3**); LRMS *m/z* (CI⁺): 152 [M-OH]⁺, 127 [M-N₃]⁺, 113 [M-CH₂N₃]⁺; HRMS *m/z* (CI⁺): Found 152.0286 [M-OH]⁺; C₆H₆N₃S requires 152.0282.

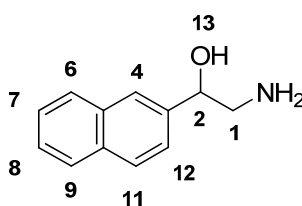
Method B:

204 (500 mg, 2.99 mmol) was dissolved in anhydrous Et₂O (20 mL). Activated neutral alumina (2.00 g) and NaBH₄ (226 mg, 5.99 mmol) were added and the suspension was stirred at RT under

Ar. After 14 h the reaction mixture was filtered and washed with Et₂O. The resulting filtrate was washed with H₂O (2 x 20 mL), followed by brine (2 x 20 mL). The combined organic extracts were dried (Na₂SO₄), filtered and concentrated *in vacuo* to give the title compound as an orange oil (299 mg, 1.77 mmol, 59%).

6.1.2.2.5 Synthesis of α -amino aryl alcohols

2-Amino-1-(2-naphthyl)ethanol (166)



Method A:

205 (2.18 g, 10.2 mmol) was dissolved in anhydrous MeOH (50 mL) and 10% Pd/C (218 mg, 10% wt/wt) was added. The vessel was evacuated and purged with Ar (3x), and under static vacuum a balloon of H₂ was added. The reaction was stirred at RT under H₂ atmosphere until completion as determined by TLC and disappearance of N₃ peak by IR. After 3½ h, the H₂ was carefully released, the vessel evacuated and purged Ar (3x), and the reaction mixture was filtered through Celite (pre-washed with MeOH). Solvent removal *in vacuo* gave the title compound as a orange oil (1.91 mg, 10.2 mmol, 100%). Spectroscopic data was consistent with that previously reported.²⁹⁹ *R_f* = 0.06 (5:1 EtOAc/MeOH); IR (ν_{max} /cm⁻¹, thin film): 3290 (O-H stretch), 3054 (C-H stretch), 2916 (N-H stretch), 1599 (N-H bend); ¹H NMR (500 MHz, CDCl₃): δ_{H} = 2.82 (m, 2H, **1-H**), 4.59-4.75 (m, 1H, **2-H**), 7.36-7.40 (m, 3H, **7,8,9-H**), 7.74-7.76 (m, 4H, **4,6,11,12-H**); ¹³C NMR (125 MHz, CDCl₃): δ_{C} = 49.8 (**C-1**), 74.3 (**C-2**), 123.8 (**C-12**), 124.5 (**C-7**), 125.7 (**C-9**), 126.0 (**C-11**), 127.5 (**C-8**), 128.1 (**C-4**), 132.8 (**C-5**), 133.1 (**C-10**), 139.7 (**C-3**); LRMS *m/z* (ES⁺): 229.2 [M+MeCN]⁺, 211.2 [M+Na]⁺, 188.1 [M+H]⁺, 170 [M-OH]⁺.

Method B:

Compound **188** (250 mg, 1.41 mmol) was dissolved in EtOH (2 mL) and 2.0 M NH₃/EtOH (8 mL) was added and the mixture was heated in a sealed tube at 100 °C for 2 h. A green solid (by-product) was isolated by filtration (105 mg) and the filtrate was concentrated *in vacuo* to give a

yellow solid (91.6 mg). Reverse phase flash chromatography (40% MeCN/60%, 20% w/v NaHCO₃ solution) was carried out on the filtrate, but no product was observed.

Method C:

Compound **188** (50.0 mg, 0.294 mmol) was added to a solution of hexamethylenetetramine (20.6 mg, 0.147 mmol) in EtOH (1 mL). The mixture was stirred at reflux for 7 h, before it was allowed to cool gradually to RT. The solid was re-dissolved in EtOH (1 mL), treated with concentrated HCl (100 μ L) and warmed to 50 °C for 10 min. After cooling to RT, the sample was dissolved in CH₂Cl₂ (10 mL), the pH was adjusted to ~ pH 8 *via* the addition of NaHCO₃ and was washed with brine (3 x 4 mL). The aqueous layers were washed with CH₂Cl₂ (10 mL), before the organic layers were combined and dried (Na₂SO₄), filtered and evaporated to give a light amber coloured oil (32.2 mg). No further work was carried out.

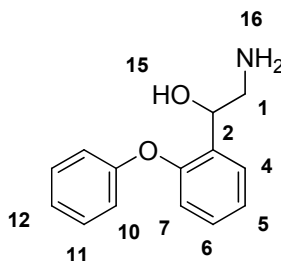
Method D:

Compound **188** (20.0 mg, 0.118 mmol) and ammonium acetate (13.6 mg, 0.177 mmol) were weighed into a microwave reaction tube. The mixture was heated to 100 °C at a power of 300 W, with a ramp time of 30 s and hold time of 20 s. NMR and TLC indicated majority starting material present, with MS showing trace quantities of product mass. No further work was carried out.

Method E (via 2-[(4-methoxyphenyl)amino]-1-(2-naphthyl)ethanol):

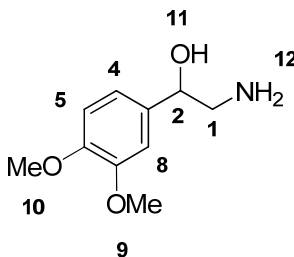
Compound **188** (50.0 mg, 0.294 mmol), 4-methoxybenzylamine (45.7 μ L, 0.352 mmol) and H₂O (600 μ L) were stirred vigorously at RT for 15 h. The organics were extracted using Et₂O (3 x 5 mL), combined, dried (Na₂SO₄), filtered and solvent removed to give a pale yellow solid. MS showed no desired product mass and so no further work was carried out.

2-Amino-1-(2-phenoxyphenyl)ethanol (167)



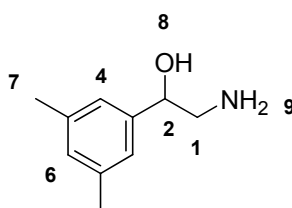
206 (2.46 g, 9.66 mmol) was dissolved in anhydrous MeOH (50 mL) and 10% Pd/C (246 mg, 10% wt/wt) was added. The vessel was evacuated and purged with Ar (3x), and under static vacuum a balloon of H₂ was added. The reaction was stirred at RT under H₂ atmosphere until completion as determined by TLC and disappearance of N₃ peak by IR. After 22 h, the H₂ was carefully released, the vessel evacuated and purged Ar (3x), and the reaction mixture was filtered through Celite (pre-washed with MeOH). Solvent removal *in vacuo* gave the title compound as a brown oil (2.21 g, 9.66 mmol, 100%). *R*_f = 0.0 (CH₂Cl₂); IR (ν_{max}/cm⁻¹, thin film): 3413 (O-H stretch), 3055 (C-H stretch), 2983 (N-H stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 2.17 (bs, 3H, **15,16-H**), 2.84 (dd, *J* = 13.2, 7.8 Hz, 1H, **1-H**), 3.10 (dd, *J* = 13.2, 4.2 Hz, 1H, **1-H**), 4.99 (dd, *J* = 7.8, 3.6 Hz, 1H, **2-H**), 6.82 (dd, *J* = 8.4, 1.2 Hz, 1H, **7-H**), 6.95 (d, *J* = 7.8 Hz, 2H, **10-H**), 7.08-7.11 (m, 1H, **12-H**), 7.13-7.15 (m, 1H, **5-H**), 7.21 (td, *J* = 7.8, 1.8 Hz, 1H, **6-H**), 7.30-7.33 (m, 2H, **11-H**), 7.57 (dd, *J* = 7.8, 1.2 Hz, 1H, **4-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 47.7 (**C-1**), 69.4 (**C-2**), 118.2 (**C-7**), 118.5 (**C-10**), 123.4 (**C-12**), 123.9 (**C-5**), 127.5 (**C-4**), 128.6 (**C-6**), 130.0 (**C-11**), 133.3 (**C-3**), 153.8 (**C-9**), 157.2 (**C-8**); LRMS *m/z* (ES⁺): 230.1 [M+H]⁺ 212.1 [M-OH]⁺, 195.1 [M-OH, NH₂]⁺.

2-amino-1-(3,4-dimethoxyphenyl)ethanol (168)



207 (1.58 g, 7.09 mmol) was dissolved in anhydrous MeOH (30 mL) and 10% Pd/C (158 mg, 10% wt/wt) was added. The vessel was evacuated and purged with Ar (3x), and under static vacuum a balloon of H₂ was added. The reaction was stirred at RT under H₂ atmosphere until completion as determined by TLC and disappearance of N₃ peak by IR. After 2 h, the H₂ was carefully released, the vessel evacuated and purged Ar (3x) and the reaction mixture was filtered through Celite (pre-washed with MeOH). Solvent removal *in vacuo* gave the crude compound as an orange oil. Flash chromatography (100% EtOAc followed by 100% MeOH), followed by re-dissolving in CH₂Cl₂ and filtering to remove silica yielded the title compound as a white solid (880 mg, 4.47 mmol, 63%). On repeating the reaction, with no flash chromatography, the title compound was obtained in quantitative yield. Spectroscopic data was consistent that previously reported.³⁰⁰ *R*_f = 0.30 (1:1 EtOAc/MeOH); IR (ν_{max}/cm⁻¹, thin film): 3362 (O-H stretch), 2938, 2838 (N-H stretch); ¹H NMR (500 MHz, CDCl₃): δ_H = 2.14 (bs, 3H, **11,12-H**) 2.80 (dd, *J* = 12.7, 7.9 Hz, 1H, **1-H**), 2.96 (dd, *J* = 12.6, 4.0 Hz, 1H, **1-H**) 3.86 (s, 3H, **9-H**), 3.88 (s, 3H, **10-H**), 4.56 (dd, *J* = 7.9, 4.0 Hz, 1H, **2-H**), 6.82 (d, *J* = 8.2 Hz, 1H, **5-H**), 6.86 (dd, *J* = 8.5, 1.8 Hz, 1H, **4-H**), 6.91 (d, *J* = 1.8 Hz, 1H, **8-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = 48.9 (**C-1**), 55.5 (**C-9**), 55.6 (**C-10**) 73.8 (**C-2**), 108.7 (**C-8**), 110.7 (**C-5**), 117.8 (**C-4**), 134.8 (**C-3**), 148.1 (**C-7**), 148.7 (**C-6**); LRMS *m/z* (EI⁺): 197 [M]⁺, 167 [M-CH₂NH₂]⁺; HRMS *m/z* (EI⁺): Found: 197.1049 [M]⁺; C₁₀H₁₅NO₃ requires 197.1046.

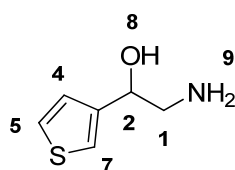
2-Amino-1-(3,5-dimethylphenyl)ethanol (**169**)



208 (2.85 g, 14.9 mmol) was dissolved in anhydrous MeOH (60 mL) and 10% Pd/C (285 mg, 10% wt/wt) was added. The vessel was evacuated and purged with Ar (3x), and under static vacuum a balloon of H₂ was added. The reaction was stirred at RT under H₂ atmosphere until completion as determined by TLC and disappearance of N₃ peak by IR. After 22 h, the H₂ was carefully released, the vessel evacuated and purged Ar (3x) and the reaction mixture was filtered through Celite (pre-washed with MeOH). Solvent removal *in vacuo* gave the title compound as a

sticky green/brown solid (2.37 g, 14.4 mmol, 97%). $R_f = 0.0$ (CH_2Cl_2); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3289 (O-H stretch), 3010 (C-H stretch), 2916, 2861 (N-H stretch); ^1H NMR (600 MHz, CDCl_3): $\delta_{\text{H}} = 2.31$ (s, 6H, **7-H**), 2.81 (dd, $J = 12.6, 7.8$ Hz, 1H, **1-H**), 2.96 (dd, $J = 13.2, 4.2$ Hz, 1H, **1-H**), 4.57 (dd, $J = 7.8, 4.2$ Hz, 1H, **2-H**), 6.91 (s, 1H, **6-H**), 6.96 (s, 2H, **4-H**); ^{13}C NMR (150 MHz, CDCl_3): $\delta_{\text{C}} = 21.5$ (**C-7**), 49.3 (**C-1**), 74.5 (**C-2**), 123.8 (**C-4**), 130.6 (**C-6**), 138.1 (**C-5**), 142.5 (**C-3**); LRMS m/z (EI^+): 224, 135 $[\text{M}-\text{CH}_2\text{NH}_2]^+$, 117 $[\text{M}-\text{CH}_2\text{OHNH}_2]^+$.

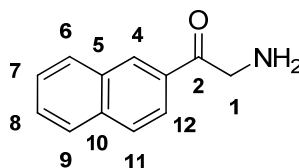
2-Amino-1-(3-thienyl)ethanol (170)



209 (257 mg, 1.52 mmol) was dissolved in anhydrous MeOH (10 mL) and 10% Pd/C (25.7 mg, 10% w/w) was added. The vessel was evacuated and purged with Ar (3x), and under static vacuum the reaction vessel was subjected to 3 bar H_2 for 5 h. After this period the mixture was filtered through Celite and concentrated *in vacuo* to give a pale yellow solid (217 mg, 1.52 mmol, 100%). $R_f = 0.0$ (1:1 $\text{CH}_2\text{Cl}_2/\text{EtOAc}$); $\nu_{\text{max}}/\text{cm}^{-1}$ (thin film): 3194 (O-H stretch), 3091 (N-H stretch), 2921 (C-H stretch); ^1H NMR (600 MHz, CDCl_3): $\delta_{\text{H}} = 1.91$ (bs, 3H, **8,9-H**), 2.88 (dd, $J = 12.6, 7.8$ Hz, 1H, **1-H**), 3.04 (d, $J = 10.2$ Hz, 1H, **1-H**), 4.75 (dd, $J = 6.6, 3.6$ Hz, 1H, **2-H**), 7.08 (d, $J = 5.4$ Hz, 1H, **4-H**), 7.23 (d, $J = 2.4$ Hz, 1H, **7-H**), 7.31 (dd, $J = 4.8, 3.0$ Hz, 1H, **5-H**); ^{13}C NMR (150 MHz, CDCl_3): $\delta_{\text{C}} = 48.4$ (**C-1**), 70.9 (**C-2**), 121.1 (**C-7**), 125.6 (**C-4**), 126.3 (**C-5**), 144.0 (**C-3**); LRMS m/z (CI^+): 177, 159, 127 $[\text{M}-\text{NH}_2]^+$; (EI^+): 131, 127 $[\text{M}-\text{NH}_2]^+$, 119, 114.

6.1.2.2.6 Synthesis of α -amino aryl ketones

2-Amino-1-(2-naphthyl)ethanone hydrochloride (210)



*Method A:*¹⁴²

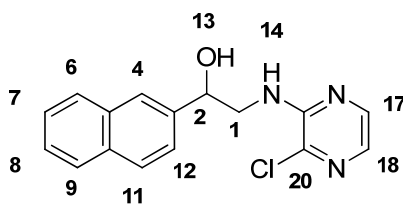
2-Azido-1-(naphthalene-2-yl)ethanone (1.57 g, 7.44 mmol) was dissolved in MeOH (12 mL), and 10% Pd/C (15.7 mg, 1% wt/wt) was added. 2.0 M HCl/ Et₂O (7 mL) was added and the vessel was evacuated and purged with Ar (3x), and under static vacuum a balloon of H₂ was added. The reaction was stirred at RT under H₂ atmosphere. After 24 h a further 1 mol% 10% Pd/C was added and the reaction was topped up with H₂. After 24 h, the H₂ balloon was removed, the vessel evacuated and purged Ar (3x), the reaction mixture was filtered through Celite, washed with MeOH and concentrated *in vacuo*. The residual was then stirred vigorously in EtOAc (25 mL) for 2 h at RT. Filtration of the suspension followed by washing with EtOAc afforded the crude material as a pale yellow solid. Flash chromatography was carried out (pet. ether; 10:1 to 5:1 to 1:1 pet. ether/EtOAc to 10% MeOH/EtOAc) to afford the title compound as a pink/red solid (366 mg, 1.98 mmol, 27%). *R_f* = 0.25 (MeOH); *v*_{max}/cm⁻¹ (thin film): 2939 (C-H stretch), 2837, 2791 (N-H stretch), 1688 (C=O stretch); ¹H NMR (500 MHz, CD₃OD): δ_{H} = 4.74 (s, 2H, **1-H**), 7.63-7.69 (m, 2H, **7,8-H**), 7.96-8.10 (m, 4H, **6,9,11,12-H**), 8.66 (s, 1H, **4-H**); ¹³C NMR (125 MHz, CD₃OD): δ_{C} = 46.2 (**C-1**), 124.0 (**C-12**), 128.4 (**C-7**), 129.0 (**C-9**), 130.0 (**C-11**), 130.5 (**C-8**), 130.9 (**C-6**), 131.8 (**C-4**), 132.3 (**C-5**), 133.9 (**C-10**), 137.7 (**C-3**), 193.2 (**C-2**); LRMS *m/z* (CI⁺): 186 [M+H]⁺, 168 [M-17]⁺.

Method B:

2-Azido-1-(naphthalene-2-yl)ethanone (0.960 g, 4.53 mmol) was dissolved in anhydrous THF (10 mL), and PPh₃ (1.79 g, 6.82 mmol) was added and the reaction mixture was stirred at RT. After a period of 24 h no amine formation was observed and so the reaction was discarded.

6.1.2.2.7 Synthesis of 2-[(3-chloropyrazin-2-yl)amino]-1-(2-aryl)ethanols

2-[(3-Chloropyrazin-2-yl)amino]-1-(2-naphthyl)ethanol (171)



Method A:

166 (289 mg, 1.55 mmol), 2,3-dichloropyrazine (177 μ L, 1.70 mmol) and Et₃N (301 μ L, 2.16 mmol) were dissolved in 1,4-dioxane (3 mL) and the reaction was stirred under reflux, under Ar. After 19 h, the reaction was cooled to RT, and the solvent removed *in vacuo*. The residue was taken up in CH₂Cl₂ (60 mL) and washed with H₂O (3 x 20 mL) and brine (20 mL), dried (Na₂SO₄), filtered and concentrated *in vacuo* to give the crude product as an amber oil. Purification was carried out *via* flash chromatography (applied in CH₂Cl₂; eluted 0% to 33% EtOAc) to afford the title compound as a yellow oil (295 mg, 0.983 mmol, 63%). *R_f* = 0.64 (2:1 CH₂Cl₂/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3419 (O-H stretch), 3054 (C-H stretch), 2922 (N-H stretch), 1523 (N-H bending); ¹H NMR (500 MHz, CDCl₃): δ_{H} = 3.65-3.70 (m, 1H, **1-H**), 3.88 (bs, 1H, **13-H**), 3.92-3.97 (m, 1H, **1-H**), 5.12 (dd, *J* = 7.5, 2.8 Hz, 1H, **2-H**), 5.67 (t, *J* = 5.3 Hz, 1H, **14-H**), 7.47-7.50 (m, 3H, **7,8,12-H**), 7.59 (d, *J* = 2.7 Hz, 1H, **18-H**), 7.81-7.84 (m, 3H, **6,9,11-H**), 7.86 (s, 1H, **4-H**), 7.91 (d, *J* = 2.7 Hz, 1H, **17-H**); ¹³C NMR (125 MHz, CDCl₃): δ_{C} = 49.4 (**C-1**), 73.9 (**C-2**), 123.9 (**C-12**), 124.8 (**C-7**), 126.1 (**C-9**), 126.4 (**C-11**), 127.8 (**C-8**), 128.0 (**C-6**), 128.4 (**C-4**), 131.3 (**C-18**), 133.2 (**C-5**), 133.3 (**C-10**), 135.1 (**C-20**), 139.4 (**C-3**), 140.2, (**C-17**), 151.5 (**C-15**); LRMS *m/z* (ES⁺): 300.1 [M(³⁵Cl)+H]⁺, 284.2 [M(³⁷Cl)-OH]⁺, 282.2 [M(³⁵Cl)-OH]⁺; HRMS *m/z* (ES⁻): Found 298.0731 [M(³⁵Cl)-H]⁻; C₁₆H₁₃ClN₃O requires 298.0747.

Method B (ring opening of epoxide):

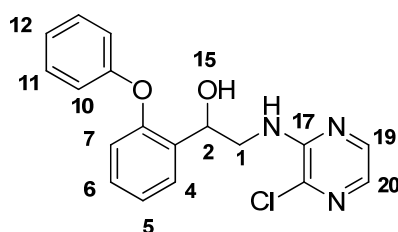
Compound **188** (50.0 mg, 0.294 mmol), 2-amino-3-chloropyrazine (45.7 mg, 0.352 mmol) and H₂O (600 μ L) were stirred vigorously at RT for 15 h. The organics were extracted using Et₂O (3 x 5 mL), combined, dried (Na₂SO₄), filtered and solvent removed *in vacuo* to give a pale yellow solid. MS indicated the correct product mass was present. Flash chromatography (applied in hexane; eluted 30:1 to 20:1 to 10:1 to 1:1 hexane/EtOAc) was carried out, but no product was isolated.

Method C (ring opening of epoxide):

Lithium amide (13.3 mg, 0.579 mmol) dissolved in anhydrous DMF (500 μ L) was added to a solution of 2-amino-3-chloropyrazine (38.1 mg, 0.294 mmol) in anhydrous DMF (500 μ L). The mixture was stirred at RT for 4 h, where the colour changed from milky white to light yellow. The mixture was heated to 80 °C in order to removed NH₃ produced, and the yellow colour

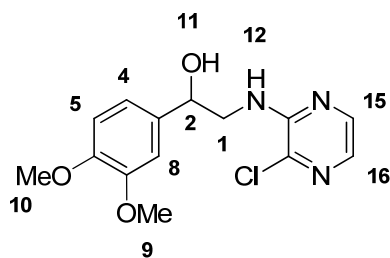
intensified. Compound **188** (50.0 mg, 0.294 mmol) was added portionwise at 90 °C, held at this temperature for 20 min before heating at 110 °C for a total of 20 h. The reaction was cooled to RT and solvent removed *in vacuo*. H₂O (10 mL) was added and organics extracted with CHCl₃ (4 x 5 mL), combined, dried (Na₂SO₄), filtered and solvent removed to give light brown/amber oil. Flash chromatography was carried out (applied in hexane; eluted 30:1 to 20:1 to 10:1 to 1:1 hexane/EtOAc), but no product was isolated.

2-[(3-Chloropyrazin-2-yl)amino]-1-(2-phenoxyphenyl)ethanol (172)



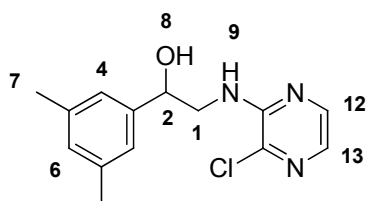
167 (2.21 g, 9.66 mmol), 2,3-dichloropyrazine (1.11 mL, 10.6 mmol) and Et₃N (1.88 mL, 13.5 mmol) were dissolved in 1,4-dioxane (22 mL) and the reaction was stirred under reflux, under Ar. After 17 h, the reaction was cooled to RT, and the solvent removed *in vacuo*. The residue was taken up in CH₂Cl₂ (200 mL) and washed with H₂O (3 x 75 mL) and brine (75 mL). The organic extracts were dried (Na₂SO₄), filtered and concentrated *in vacuo* to give the crude product as a brown oil. Purification was carried out *via* flash chromatography (applied in CH₂Cl₂; eluted 0% to 10% EtOAc) to afford the title compound as an orange oil (2.09 g, 6.15 mmol, 64%). *R_f* = 0.42 (9:1 CH₂Cl₂/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3423 (O-H stretch), 3060 (C-H stretch), 2924 (N-H stretch); ¹H NMR (400 MHz, CDCl₃): δ_{H} = 3.76-3.83 (m, 1H, **1-H**), 3.92-3.98 (m, 1H, **1-H**), 4.04 (bs, 1H, **15-H**), 5.31 (dd, *J* = 7.2, 3.2 Hz, 1H, **2-H**), 5.64 (bs, 1H, **16-H**), 6.87 (dd, *J* = 8.1, 1.1 Hz, 1H, **7-H**), 6.99-7.02 (m, 2H, **10-H**), 7.10-7.18 (m, 2H, **5,12-H**), 7.25 (dd, *J* = 8.1, 1.7 Hz, 1H, **6-H**), 7.33-7.38 (m, 2H, **11-H**), 7.61-7.63 (m, 2H, **4,20-H**), 7.89 (d, *J* = 2.8 Hz, 1H, **19-H**); ¹³C NMR (100 MHz, CDCl₃): δ_{C} = 47.9 (**C-1**), 69.3 (**C-2**), 118.2 (**C-7,10**), 123.2 (**C-5**), 123.5 (**C-12**), 127.2 (**C-4**), 128.6 (**C-6**), 129.6 (**C-11**), 130.8 (**C-20**), 132.2 (**C-3**), 134.8 (**C-22**), 139.2 (**C-19**), 151.0 (**C-17**), 153.4 (**C-9**), 156.6 (**C-8**); LRMS *m/z* (CI⁺): 325 [M(³⁷Cl)-OH]⁺, 323 [M(³⁵Cl)-OH]⁺; HRMS *m/z* (ES⁻): Found 340.0867 [M(³⁵Cl)+H]⁺; C₁₈H₁₅ClN₃O₂ requires 340.0853.

2-[(3-Chloropyrazin-2-yl)amino]-1-(3,4-dimethoxyphenyl)ethanol (173)



168 (827 Mg, 4.20 mmol), 2,3-dichloropyrazine (481 μ L, 4.62 mmol) and Et_3N (781 μ L, 5.88 mmol) were dissolved in 1,4-dioxane (8 mL) and the reaction was stirred under reflux, under Ar. After 16 h, the reaction was cooled to RT, and the solvent removed *in vacuo*. The residual brown oil was taken up in CH_2Cl_2 (60 mL) and washed with H_2O (3 x 30 mL) and brine (20 mL). The organic extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo* to give the crude product as a brown oil. Purification was carried out *via* flash chromatography (applied in CH_2Cl_2 ; eluted 0% to 10% to 20% EtOAc) to afford the title compound as a light orange oil (748 mg, 2.42 mmol, 58%). R_f = 0.27 (2:1 $\text{CH}_2\text{Cl}_2/\text{EtOAc}$); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3377 (O-H stretch), 2934 (N-H stretch); ^1H NMR (500 MHz, CDCl_3): δ_{H} = 3.60-3.66 (m, 1H, **1-H**), 3.83-3.89 (m, 1H, **1-H**) 3.88 (s, 3H, **9-H**), 3.89 (s, 3H, **10-H**), 4.93 (dd, J = 7.6, 3.7 Hz, 1H, **2-H**), 5.61 (s, 1H, **12-H**), 6.87 (d, J = 8.2 Hz, 1H, **5-H**), 6.93 (d, J = 8.2 Hz, 1H, **4-H**), 6.97 (s, 1H, **8-H**), 7.62 (d, J = 1.0 Hz, 1H, **16-H**), 7.93 (d, J = 1.0 Hz, 1H, **15-H**); ^{13}C NMR (125 MHz, CDCl_3): δ_{C} = 49.0 (**C-1**), 55.6 (**C-9,10**), 73.2 (**C-2**), 108.6 (**C-8**), 110.8 (**C-5**), 117.8 (**C-4**), 130.9 (**C-16**), 134.1 (**C-3**), 134.7 (**C-18**), 139.6 (**C-15**), 148.4 (**C-7**), 148.8 (**C-6**), 150.9 (**C-13**); LRMS m/z (Cl^+): 312 [$\text{M}(^{37}\text{Cl})+\text{H}$] $^+$, 310 [$\text{M}(^{35}\text{Cl})+\text{H}$] $^+$, 294 [$\text{M}(^{37}\text{Cl})-\text{OH}$] $^+$, 292 [$\text{M}(^{35}\text{Cl})-\text{OH}$] $^+$; HRMS m/z (Cl^+): Found 310.0956 [$\text{M}(^{35}\text{Cl})+\text{H}$] $^+$; $\text{C}_{14}\text{H}_{17}\text{ClN}_3\text{O}_3$ requires 310.0958

2-[(3-Chloropyrazin-2-yl)amino]-1-(3,5-dimethylphenyl)ethanol (174)



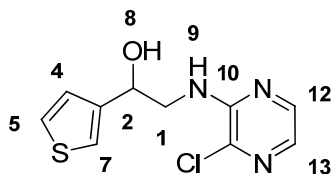
Method A:

169 (2.34 g, 14.2 mmol), 2,3-dichloropyrazine (1.62 mL, 15.6 mmol) and Et₃N (2.76 mL, 19.8 mmol) were dissolved in 1,4-dioxane (24 mL) and the reaction was stirred under reflux, under Ar. After 16 h, the reaction was cooled to RT, and the solvent removed *in vacuo*. The residue was taken up in CH₂Cl₂ (80 mL) and washed with H₂O (3 x 30 mL) and brine (20 mL). The organic extracts were dried (MgSO₄), filtered and concentrated *in vacuo* to give the crude product as a brown oil. Purification was carried out *via* flash chromatography (applied in CH₂Cl₂; eluted CH₂Cl₂ to 30:1 to 10:1 CH₂Cl₂/EtOAc) to afford the title compound as a light orange oil (2.58 g, 9.37 mmol, 65%). *R_f* = 0.37 (9:1 CH₂Cl₂/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3422 (O-H stretch), 2921 (C-H stretch); ¹H NMR (600 MHz, CDCl₃): δ_{H} = 2.33 (s, 6H, **7-H**), 3.27 (s, 1H, **8-H**) 3.61 (ddd, *J* = 13.8, 7.9, 4.9 Hz, 1H, **1-H**), 3.88 (ddd, *J* = 13.8, 4.9, 2.8 Hz, 1H, **1-H**), 4.91 (dd, *J* = 7.9, 2.8 Hz, 1H, **2-H**), 5.62 (bt, *J* = 4.9 Hz, 1H, **9-H**), 6.95 (s, 1H, **6-H**), 7.02 (s, 2H, **4-H**), 7.62 (d, *J* = 3.0 Hz, 1H, **13-H**), 7.93 (d, *J* = 3.0 Hz, 1H, **12-H**); ¹³C NMR (150 MHz, CDCl₃): δ_{C} = 21.5 (**C-7**), 49.5 (**C-1**), 73.8 (**C-2**), 123.7 (**C-4**), 129.7 (**C-6**), 131.2 (**C-13**), 135.3 (**C-15**) 138.4 (**C-5**), 139.7 (**C-12**), 142.5 (**C-3**), 151.3 (**C-10**); LRMS *m/z* (Cl⁺): 280 [M(³⁷Cl)+H]⁺, 278 [M(³⁵Cl)+H]⁺, 262 [M(³⁷Cl)-OH]⁺, 260 [M(³⁵Cl)-OH]⁺; HRMS *m/z* (Cl⁺): Found 278.1059 [M(³⁵Cl)+H]⁺; C₁₄H₁₇ClN₃O requires 278.1060.

Method B:

169 (80.0 mg, 0.485 mmol), 2,3-dichloropyrazine (79.5 μ L, 0.533 mmol) and NaHCO₃ (57.0 mg, 0.679 mmol) were dissolved in ^tBuOH (1 mL) and the reaction was stirred under reflux, under Ar. After 17 h, the reaction was cooled to RT, and the solvent removed *in vacuo*. The residue was taken up in CH₂Cl₂ and washed with H₂O (3 x 30 mL) and brine (20 mL). The organic extracts were dried (MgSO₄), filtered and concentrated *in vacuo* to give the crude product as a yellow oil. Purification was carried out *via* flash chromatography (applied in CH₂Cl₂; eluted CH₂Cl₂ to 50:1 to 10:1 CH₂Cl₂/EtOAc) to afford the title compound as a yellow oil (26.2 mg, 0.094 mmol, 19%).

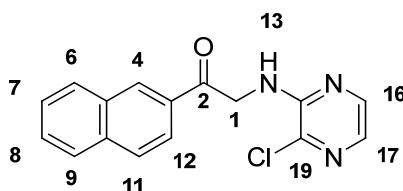
2-[(3-Chloropyrazin-2-yl)amino]-1-(3-thienyl)ethanol (175)



170 (232 mg, 1.63 mmol), 2,3-dichloropyrazine (183 μ L, 1.79 mmol), Et₃N (316 μ L, 2.27 mmol) and 1,4-dioxane (2.5 mL) were stirred under reflux, under Ar for 18 h. The solvent was removed *in vacuo* and the residual was taken up in CH₂Cl₂ (30 mL), washed with H₂O (5 x 10 mL) and brine (10 mL). The organics were dried (MgSO₄), filtered and solvent removed *in vacuo* to give a brown oil. Flash chromatography (applied in CH₂Cl₂; eluted CH₂Cl₂ to 2:1 CH₂Cl₂/EtOAc) afforded the title compound as an orange oil (193 mg, 0.753 mmol, 46%). R_f = 0.46 (2:1 CH₂Cl₂/EtOAc) ; IR (ν_{\max} /cm⁻¹, thin film): 3420 (O-H stretch), 3091 (N-H stretch), 2920 (C-H stretch), 1583 (N-H bend), 1525; ¹H NMR (600 MHz, CDCl₃): δ_H = 3.69-3.74 (m, 1H, **1-H**), 3.96-4.00 (m, 1H, **1-H**), 5.10 (dd, J = 7.2, 3.0 Hz, 1H, **2-H**), 5.72 (bs, 1H, **9-H**), 7.13 (dd, J = 5.1, 1.2 Hz, 1H, **4-H**), 7.31-7.32 (m, 1H, **7-H**), 7.35 (dd, J = 5.1, 3.0 Hz, 1H, **5-H**), 7.65 (d, J = 2.7 Hz, 1H, **13-H**), 7.93 (d, J = 2.7 Hz, **12-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 49.0 (**C-1**), 70.4 (**C-2**), 121.6 (**C-7**), 125.5 (**C-4**), 126.7 (**C-5**), 131.3 (**C-13**), 135.6 (**C-15**), 138.8 (**C-12**), 143.1 (**C-3**), 150.8 (**C-10**); LRMS m/z (ES⁺): 240 [M(³⁷Cl)-OH]⁺, 238 [M(³⁵Cl)-OH]⁺; HRMS m/z (ES⁻): Found 254.0145 [M(³⁵Cl)-H]⁻; C₁₀H₉ClN₃OS requires 254.0155.

6.1.2.2.8 Synthesis of 2-[(3-chloropyrazin-2-yl)amino]-1-(2-aryl)ethanones

2-[(3-Chloropyrazin-2-yl)amino]-1-(2-naphthyl)ethanone (176)



Method A:

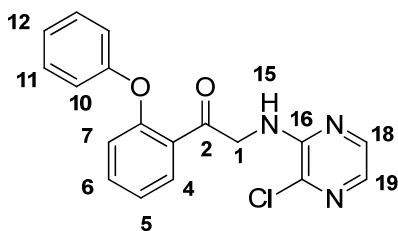
DMSO (982 μ L, 13.9 mmol) was dissolved in anhydrous CH₂Cl₂ (60 mL) and the reaction mixture was cooled to and maintained at -78 °C. Oxalyl chloride (586 μ L, 6.93 mmol) was

added dropwise and the mixture was stirred for 20 min. **171** (1.60 g, 5.33 mmol), dissolved in anhydrous CH₂Cl₂ (40 mL) was added dropwise, and stirred for 20 min. Et₃N (3.54 mL, 26.6 mmol) was added dropwise and the reaction mixture was allowed to warm to RT over a period of 2½ h. The reaction was then quenched with H₂O (50 mL) and organics extracted, which were then washed with 2.0 M HCl (2 x 40 mL), NaHCO₃ (sat. aq. 40 mL), H₂O (40 mL) and brine (40 mL). The organic were dried (MgSO₄), filtered and solvent removed *in vacuo* to give a yellow/orange solid. Flash chromatography (applied in CH₂Cl₂; eluted 100:1 to 30:1 CH₂Cl₂/EtOAc) afforded the title compound as a yellow solid (903 mg, 3.03 mmol, 57%). Mpt: 160 °C; *R_f* = 0.30 (30:1 CH₂Cl₂/EtOAc); IR (ν_{max}/cm⁻¹, thin film): 1680 (C=O stretch); ¹H NMR (500 MHz, CDCl₃): δ_H = 5.10 (d, *J* = 4.3, 2H, **1-H**), 6.54 (s, 1H, **13-H**), 7.58-7.62 (m, 1H, **7-H**), 7.64-7.67 (m, 1H, **8-H**), 7.68 (d, *J* = 6.1 Hz, 1H, **17-H**), 7.91 (d, *J* = 8.0 Hz, 1H, **9-H**), 7.96 (d, *J* = 8.6 Hz, 1H, **11-H**), 8.00-8.02 (m, 2H, **6,16-H**), 8.10 (dd, *J* = 8.6, 1.8 Hz, 1H, **12-H**), 8.62 (s, 1H, **4-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = 48.4 (**C-1**), 123.4 (**C-12**), 127.3 (**C-7**), 128.0 (**C-9**), 129.0 (**C-8**), 129.2 (**C-11**), 129.8 (**C-6**), 130.1 (**C-4**), 131.3 (**C-17**), 131.8 (**C-5**), 132.6 (**C-10**), 136.2 (**C-3**), 139.7 (**C-16**), 193.8 (**C-2**); LRMS *m/z* (ES⁺): 300[M(³⁷Cl)+H]⁺, 298 [M(³⁵Cl)+H]⁺, 282[M(³⁷Cl)-OH]⁺, 280[M(³⁵Cl)-OH]⁺; HRMS *m/z* (ES⁻): Found 296.0591 [M(³⁵Cl)-H]⁻; C₁₆H₁₁³⁵ClN₃O requires 296.0591; Anal. Calcd. for C₁₆H₁₂ClN₃O: C, 64.54; H, 4.06; N, 14.11. Found C, 64.35; H, 3.94; N, 13.82%.

Method B:

171 (474 mg, 1.58 mmol) was dissolved in anhydrous DMSO (2.5 mL) and Et₃N (2.5 mL). Trimethylamine sulfur trioxide (661 mg, 4.75 mmol) was added and the reaction mixture was stirred under Ar at RT. After 16 h, the reaction was heated to 40 °C for 3 h, after which point the reaction was cooled, quenched with CHCl₃ (50 mL) and washed with H₂O (3 x 30 mL). The aqueous layers were washed with CHCl₃ (2 x 20 mL), and the organics were combined, dried (MgSO₄), filtered and concentrated *in vacuo* to give a brown oil. Flash chromatography was carried out (applied in CH₂Cl₂; eluted CH₂Cl₂ to 50:1 to 30:1 CH₂Cl₂/EtOAc) to afford the title compound as a sticky yellow solid (118 mg, 25%).

2-[(3-Chloropyrazin-2-yl)amino]-1-(2-phenoxyphenyl)ethanone (177)

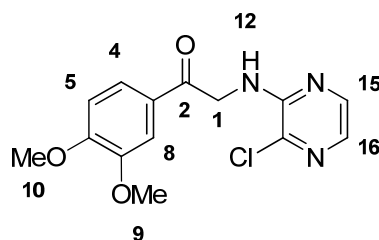


DMSO (1.13 mL, 16.0 mmol) was dissolved in anhydrous CH_2Cl_2 (100 mL) and the mixture was cooled to and maintained at -78°C . Oxalyl chloride (677 μL , 7.99 mmol) was added dropwise and the reaction was stirred for 20 min. **172** (2.10 g, 6.15 mmol), dissolved in CH_2Cl_2 (20 mL), was then added dropwise and after 20 min stirring, Et_3N (4.08 mL, 30.7 mmol) was added dropwise. The reaction was then allowed to slowly warm to RT over a period of 2½ h. The reaction was quenched with H_2O (50 mL) and organics extracted followed by washing with 2.0 M HCl (2 x 40 mL), NaHCO_3 (sat. aq. 40 mL), H_2O (40 mL) and brine (40 mL). Drying (MgSO_4), filtration and concentration *in vacuo* gave a brown/orange oil. Flash chromatography was carried out (applied in CH_2Cl_2 ; eluted 50:1 to 10:1 $\text{CH}_2\text{Cl}_2/\text{EtOAc}$) to afford the title compound as a yellow solid (1.33 g, 3.92 mmol, 64%). Mpt: $76\text{--}78^\circ\text{C}$; $R_f = 0.74$ (9:1 $\text{CH}_2\text{Cl}_2/\text{EtOAc}$); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3423, 3060 (C-H stretch), 2924 (N-H stretch); ^1H NMR (500 MHz, CDCl_3): $\delta_{\text{H}} = 4.95$ (d, $J = 4.8$ Hz, 1H, **1-H**), 6.30 (1H, **15-H**), 6.90 (dd, $J = 8.4$, 0.8 Hz, 1H, **7-H**), 7.11-7.13 (m, 2H, **10-H**), 7.18-7.23 (m, 2H, **5,12-H**), 7.40-7.44 (m, 2H, **11-H**), 7.46-7.49 (m, 1H, **6-H**), 7.59 (d, $J = 2.7$ Hz, 1H, **19-H**), 7.88 (d, $J = 2.7$ Hz, 1H, **18-H**), 8.01 (dd, $J = 7.9$, 1.8 Hz, 1H, **4-H**); ^{13}C NMR (125 MHz, CDCl_3): $\delta_{\text{C}} = 52.0$ (**C-1**), 118.1 (**C-7**), 119.4 (**C-10**), 123.0 (**C-5**), 124.3 (**C-12**), 126.5 (**C-3**), 129.9 (**C-11**), 130.7 (**C-4**), 130.8 (**C-19**), 134.3 (**C-6**), 134.8 (**C-21**), 140.0 (**C-18**), 150.2 (**C-16**), 155.2 (**C-9**), 157.2 (**C-8**), 194.8 (**C-2**); LRMS m/z (ES^+): 342 [$\text{M}(^{37}\text{Cl})+\text{H}$] $^+$, 340 [$\text{M}(^{35}\text{Cl})+\text{H}$] $^+$, 322 [$\text{M}(^{35}\text{Cl})-\text{OH}$] $^+$; HRMS m/z (ES^+): Found 340.0864 [$\text{M}(^{35}\text{Cl})+\text{H}$] $^+$; $\text{C}_{18}\text{H}_{15}\text{ClN}_3\text{O}_2$ requires 340.0853.

Method B:

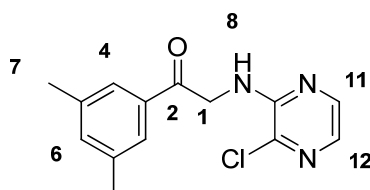
172 (70.0 mg, 0.205 mmol), trimethylamine sulfur trioxide (85.6 mg, 0.615 mmol), DMSO (0.5 mL) and Et_3N (0.5 mL) were stirred under Ar at RT for 16 h, followed by heating at 40°C for 4½ h. After this time period, no product mass was evident by LCMS and experiment was discarded.

2-[(3-Chloropyrazin-2-yl)amino]-1-(3,4-dimethoxyphenyl)ethanone (178)



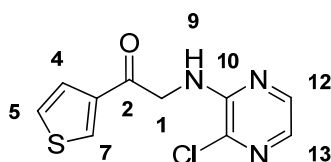
DMSO (417 μ L, 5.89 mmol) was dissolved in anhydrous CH_2Cl_2 (25 mL) and the mixture was cooled to and maintained at -78°C . Oxalyl chloride (249 μ L, 2.94 mmol) was added dropwise and the reaction was stirred for 15 min. **173** (700 mg, 2.26 mmol), dissolved in CH_2Cl_2 (20 mL), was then added dropwise and after 15 min stirring, Et_3N (1.5 mL, 11.3 mmol) was added dropwise. The reaction was then allowed to slowly warm to RT over a period of 2 h. The reaction was quenched with H_2O (20 mL) and organics extracted followed by washing with 2.0 M HCl (2 x 20 mL), NaHCO_3 (sat. aq. 20 mL), H_2O (20 mL) and brine (20 mL). Drying (MgSO_4), filtration and concentration *in vacuo* gave an off white solid. Flash chromatography (applied in CH_2Cl_2 ; eluted 50:1 to 5:1 $\text{CH}_2\text{Cl}_2/\text{EtOAc}$) gave the title compound as a white solid (543 mg, 1.76 mmol, 78%). Mpt: $128\text{--}130^\circ\text{C}$; $R_f = 0.31$ (5:1 $\text{CH}_2\text{Cl}_2/\text{EtOAc}$); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3399 (C-H stretch), 2936 (N-H stretch), 1677 (C=O stretch); ^1H NMR (500 MHz, CDCl_3): $\delta_{\text{H}} = 3.96$ (s, 3H, **9-H**), 3.97 (s, 3H, **10-H**), 4.88 (d, $J = 4.2$ Hz, 1H, **1-H**), 6.43 (s, 1H, **12-H**), 6.94 (d, $J = 8.2$ Hz, 1H, **5-H**), 7.57 (d, $J = 1.9$ Hz, 1H, **8-H**), 7.64 (d, $J = 2.7$ Hz, 1H, **16-H**), 7.70 (dd, $J = 8.2, 1.9$ Hz, 1H, **4-H**), 7.96 (d, $J = 2.7$ Hz, 1H, **15-H**); ^{13}C NMR (125 MHz, CDCl_3): $\delta_{\text{C}} = 47.3$ (**C-1**), 55.8 (**C-9**), 55.9 (**C-10**), 109.7 (**C-8**), 110.0 (**C-5**), 122.3 (**C-4**), 127.3 (**C-3**), 130.8 (**C-16**), 135.1 (**C-18**), 139.8 (**C-15**), 149.0 (**C-7**), 150.1 (**C-13**), 153.9 (**C-6**), 192.1 (**C-2**); LRMS m/z (Cl^+): 310 [$\text{M}(^{37}\text{Cl})+\text{H}$] $^+$, 308 [$\text{M}(^{35}\text{Cl})+\text{H}$] $^+$; HRMS m/z (Cl^+): Found 308.0814 [$\text{M}(^{35}\text{Cl})+\text{H}$] $^+$; $\text{C}_{14}\text{H}_{15}\text{ClN}_3\text{O}_3$ requires 308.0811.

2-[(3-Chloropyrazin-2-yl)amino]-1-(3,5-dimethylphenyl)ethanone (179)



DMSO (1.71 mL, 24.2 mmol) was dissolved in anhydrous CH₂Cl₂ (170 mL) and the mixture was cooled to and maintained at -78 °C. Oxalyl chloride (1.02 mL, 12.1 mmol) was added dropwise and the reaction was stirred for 20 min. **174** (2.58 g, 9.30 mmol), dissolved in CH₂Cl₂ (30 mL), was then added dropwise and after 20 min stirring, Et₃N (6.18 mL, 46.6 mmol) was added dropwise. The reaction was then allowed to slowly warm to RT over a period of 2½ h. The reaction was quenched with H₂O (50 mL) and organics extracted followed by washing with 2.0 M HCl (2 x 40 mL), NaHCO₃ (sat. aq. 40 mL), H₂O (40 mL) and brine (40 mL). Drying (MgSO₄), filtration and concentration *in vacuo* gave a yellow solid (2.31 g, 8.37 mmol, 90%) which was deemed clean enough to continue without any further purification. Mpt: Decomposed before melting; *R_f* = 0.76 (9:1 CH₂Cl₂/EtOAc); IR (ν_{max}/cm⁻¹, thin film): 3405 (aromatic C-H stretch), 2916 (N-H stretch), 1681 (C=O stretch), 1578 (N-H bend), 1497 (aromatic C=C stretch); ¹H NMR (500 MHz, CDCl₃): δ_H = 2.40 (s, 6H, **7-H**), 4.89 (d, *J* = 4.0 Hz, 1H, **1-H**), 6.43 (s, 1H, **8-H**), 7.27 (s, 1H, **6-H**), 7.65 (d, *J* = 3.0 Hz, 1H, **12-H**), 7.67 (s, 2H, **4-H**), 7.97 (d, *J* = 3.0 Hz, 1H, **11-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = 20.9 (**C-7**), 47.8 (**C-1**), 125.4 (**C-4**), 130.9 (**C-12**), 134.2 (**C-3**), 135.1 (**C-14**), 135.5 (**C-6**), 138.4 (**C-5**), 139.8 (**C-11**), 150.0 (**C-9**), 193.6 (**C-2**); LRMS *m/z* (ES⁻): 276 [M(³⁷Cl)-H]⁻, 274 [M(³⁵Cl)-H]⁻; HRMS *m/z* (ES⁻): Found 274.0762 [M(³⁵Cl)-H]⁻; C₁₄H₁₃ClN₃O requires 274.0747.

2-[(3-Chloropyrazin-2-yl)amino]-1-(3-thienyl)ethanone (180)

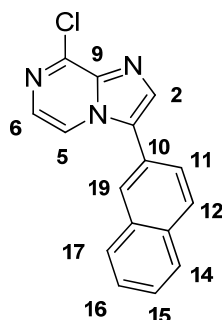


DMSO (128 µL, 1.81 mmol) was dissolved in anhydrous CH₂Cl₂ (9 mL) and the mixture was cooled to and maintained at -78 °C. Oxalyl chloride (77.0 µL, 0.906 mmol) was added dropwise and the reaction was stirred for 20 min. **175** (178 mg, 0.697 mmol), dissolved in CH₂Cl₂ (5 mL), was then added dropwise and after 20 min stirring, Et₃N (463 µL, 3.48 mmol) was added dropwise. The reaction was then allowed to slowly warm to RT over a period of 2½ h. The reaction was quenched with H₂O (20 mL) and organics extracted followed by washing with 2.0 M HCl (2 x 10 mL), NaHCO₃ (sat. aq. 10 mL), H₂O (10 mL) and brine (10 mL). Drying (MgSO₄), filtration and concentration *in vacuo* gave an orange sticky solid. Flash

chromatography (applied in CH₂Cl₂; eluted 50:1 to 20:1 CH₂Cl₂/EtOAc) gave the title compound as a yellow solid (135 mg, 0.531 mmol, 76%). Mpt: 130-134 °C; *R_f* = 0.85 (2:1 CH₂Cl₂/EtOAc); IR (ν_{max}/cm⁻¹, thin film): 3407 (aromatic C-H stretch), 3107 (C-H stretch), 2917 (N-H stretch), 1682 (C=O stretch), 1582 (N-H bend); ¹H NMR (600 MHz, CDCl₃): δ_H = 4.84 (d, *J* = 4.4 Hz, 1H, **1-H**), 6.33 (s, 1H, **9-H**), 7.41 (dd, *J* = 5.0, 2.9 Hz, 1H, **5-H**), 7.65 (dd, *J* = 5.0, 1.2 Hz, 1H, **4-H**), 7.66 (d, *J* = 2.8 Hz, 1H, **13-H**), 7.96 (d, *J* = 2.7 Hz, **12-H**), 8.26 (dd, *J* = 1.2, 2.9 Hz, 1H, **7-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 49.0 (**C-1**), 70.4 (**C-2**), 121.6 (**C-4**), 125.5 (**C-7**), 126.7 (**C-5**), 131.3 (**C-13**), 135.6 (**C-15**), 138.8 (**C-12**), 143.1 (**C-3**), 150.8 (**C-10**); LRMS *m/z* (Cl⁺): 256 [M(³⁷Cl)+H]⁺, 254 [M(³⁵Cl)+H]⁺; HRMS *m/z* (Cl⁺): Found 254.0147 [M(³⁵Cl)+H]⁺; C₁₀H₉ClN₃OS requires 254.0155.

6.1.2.2.9 Synthesis of 3-aryl-8-chloro-imidazo[1,2-a]pyrazine

8-Chloro-3-(2-naphthyl)imidazo[1,2-a]pyrazine (181)



Method A:

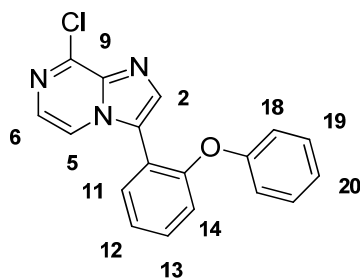
176 (903 mg, 3.03 mmol) was dissolved in anhydrous toluene (40 mL) and the mixture was cooled on ice. TFA (1.64 mL, 21.2 mmol) was added and the reaction was allowed to stir on ice for 30 min, followed by the addition of trifluoroacetic anhydride (2.95 mL, 21.2 mmol). The reaction mixture was then stirred on ice for a further 30 minutes and then at RT for 65 h. The reaction was then diluted with toluene (20 mL) and washed with NaHCO₃ solution (10% w/v, 3 x 20 mL) and brine (20 mL). The organics were dried (MgSO₄), filtered and concentrated *in vacuo* to give crude amber oil. Purification was carried out *via* flash chromatography (applied in CH₂Cl₂; eluted 80:1 to 10:1 CH₂Cl₂/EtOAc) to afford the title compound as an off white solid

(386 mg, 1.38 mmol, 45%). Mpt: 166 °C; R_f = 0.21 (10:1 CH₂Cl₂/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3102, 3052 (aromatic C-H stretches), 1333 (aromatic C=C stretch); ¹H NMR (600 MHz, CDCl₃): δ_{H} = 7.58-7.62 (m, 2H, **15,16-H**), 7.64 (dd, J = 8.6, 1.7 Hz, 1H, **11-H**), 7.73 (d, J = 4.6 Hz, 1H, **6-H**), 7.92-7.95 (m, 2H, **14,17-H**), 8.02 (s, 1H, **2-H**), 8.04-8.05 (m, 2H, **12,19-H**), 8.30 (d, J = 4.6 Hz, 1H, **5-H**); ¹³C NMR (125 MHz, CDCl₃): δ_{C} = 116.4 (**C-5**), 124.7 (**C-10**), 125.2 (**C-11**), 127.3 (**C-15**), 127.4 (**C-16**), 127.6 (**C-19**), 128.0 (**C-17**), 128.2 (**C-17**), 128.6 (**C-6**), 129.4 (**C-3**), 129.7 (**C-12**), 133.4 (**C-13**), 133.5 (**C-18**), 134.8 (**C-2**), 138.4 (**C-9**), 144.5 (**C-8**); LRMS m/z (ES⁺): 282 [M(³⁷Cl)+H]⁺, 280 [M(³⁵Cl)+H]⁺; HRMS m/z (ES⁺): Found 280.0646 [M(³⁵Cl)+H]⁺; C₁₆H₁₁ClN₃ requires 280.0642.

Method A (Attempted formation from 210):

210 (50.2 mg, 0.227 mmol), 2,3-dichloropyrazine (32.7 mg, 0.227 mmol) and NaHCO₃ (38.2 mg, 0.453 mmol) were suspended in ^tBuOH (1 mL) and the reaction mixture was stirred under reflux for 20 h. After this period neither, starting material nor product mass was observed by LCMS and so reaction was discarded.

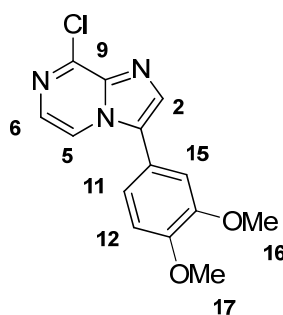
8-Chloro-3-(2-phenoxyphenyl)imidazo[1,2-a]pyrazine (**182**)



177 (1.33 g, 3.92 mmol) was dissolved in anhydrous toluene (50 mL) and the mixture was cooled on ice. TFA (2.11 mL, 24.7 mmol) was added and the reaction was allowed to stir on ice for 30 min, followed by the addition of trifluoroacetic anhydride (3.81 mL, 24.7 mmol). The reaction mixture was then stirred on ice for a further 30 min and then at RT for 68 h. The reaction was then diluted with toluene (50 mL) and washed with NaHCO₃ solution (10% w/v, 3 x 30 mL) and brine (40 mL). The organics were dried (MgSO₄), filtered and concentrated *in vacuo* to give a crude brown oil. Purification was carried out *via* flash chromatography (applied in CH₂Cl₂; eluted 50:1 to 5:1 CH₂Cl₂/EtOAc) to afford the title compound as a sticky yellow solid (1.26 g,

3.92 mmol, 100%). R_f = 0.25 (9:1 CH₂Cl₂/EtOAc); IR (ν_{\max} /cm⁻¹, thin film): 1460 (aromatic C=C stretch), 1231 (aromatic C-O stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 6.83–6.85 (m, 2H, **18-H**), 7.05–7.10 (m, 2H, **14,20-H**), 7.23–7.26 (m, 2H, **19-H**), 7.29 (td, J = 7.5, 1.2 Hz, 1H, **12-H**), 7.46–7.49 (m, 1H, **13-H**), 7.53 (dd, J = 7.5, 1.2 Hz, 1H, **11-H**), 7.70 (d, J = 4.2 Hz, 1H, **6-H**), 7.86 (s, 1H, **2-H**), 8.01 (d, J = 4.2 Hz, 1H, **5-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 118.3 (**C-5**), 118.5 (**C-18**), 118.8 (**C-22**), 124.2 (overlapping signals, **C-10,12,20**), 126.1 (**C-3**), 130.0 (**C-19**), 131.5 (**C-13**), 132.0 (**C-11**), 135.6 (**C-2**), 138.4 (**C-9**), 143.9 (**C-8**), 155.0 (**C-15**), 156.0 (**C-17**); LRMS m/z (EI⁺): 323 [M(³⁷Cl)]⁺, 321 [M(³⁵Cl)]⁺; HRMS m/z (EI⁺): Found: 321.0659 [M(³⁵Cl)]⁺; C₁₈H₁₂ClN₃O requires 321.0663.

8-Chloro-3-(3,4-dimethoxyphenyl)imidazo[1,2-a]pyrazine (**183**)



Method A:

178 (280 mg, 0.912 mmol) was dissolved in anhydrous toluene (20 mL) and the mixture was cooled on ice. TFA (490 μ L, 6.39 mmol) was added and the reaction was allowed to stir on ice for 30 min, followed by the addition of trifluoroacetic anhydride (887 μ L, 6.39 mmol). The reaction mixture was then stirred on ice for a further 30 min and then at RT for 68 h. The reaction was then diluted with toluene (50 mL) and washed with NaHCO₃ solution (10% w/v, 3 x 30 mL) and brine (40 mL). The organics were dried (MgSO₄), filtered and concentrated *in vacuo* to give a crude yellow solid. Purification was carried out *via* flash chromatography (applied in CH₂Cl₂; eluted 9:1 to 2:1 CH₂Cl₂/EtOAc) to afford the title compound as a white solid (46.8 mg, 0.162 mmol, 18%). Mpt: > 200 °C; R_f = 0.32 (2:1 CH₂Cl₂/EtOAc); IR (ν_{\max} /cm⁻¹, thin film): 2960, 2924 (aromatic C-H stretch), 1732 (aromatic C-C stretch); ¹H NMR (400 MHz, CDCl₃): δ_H = 3.93 (s, 3H, **16-H**), 3.96 (s, 3H, **17-H**), 6.99 (d, J = 2.0 Hz, 1H, **15-H**), 7.03 (d, J = 8.3 Hz, 1H, **12-H**), 7.10 (dd, J = 8.3, 2.0 Hz, 1H, **11-H**), 7.67 (d, J = 4.6 Hz, 1H, **6-H**), 7.84 (s, 1H, **2-H**), 8.15 (d, J

= 4.6 Hz, 1H, **5-H**); ^{13}C NMR (100 MHz, CDCl_3): δ_{C} = 56.1 (**C-16**), 56.2 (**C-17**), 111.4 (**C-15**), 111.8 (**C-12**), 116.3 (**C-5**), 119.8 (**C-10**), 121.0 (**C-11**), 128.2 (**C-6**), 129.2 (**C-3**), 134.2 (**C-2**), 138.0 (**C-9**), 144.3 (**C-8**), 149.8 (**C-14**), 150.1 (**C-13**); LRMS $m/z(\text{ES}^+)$: 290 $[\text{M}(^{35}\text{Cl})+\text{H}]^+$, 292 $[\text{M}(^{37}\text{Cl})+\text{H}]^+$; HRMS $m/z(\text{ES}^+)$: Found 290.0683 $[\text{M}(^{35}\text{Cl})+\text{H}]^+$; $\text{C}_{14}\text{H}_{13}\text{ClN}_3\text{O}_2$ requires 290.0696.

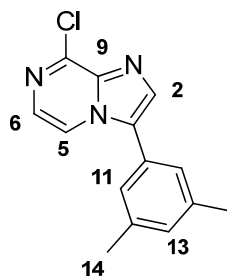
Method B:

178 (415 mg, 1.35 mmol) was dissolved in TFA (676 μL , 8.77 mmol) and the mixture was cooled on ice, under Ar. Trifluoroacetic anhydride (469 μL , 3.37 mmol) was added after 10 min and the reaction was stirred on ice for 15 min before stirring at RT for 21 h. The reaction was stopped, diluted with CH_2Cl_2 (50 mL) and washed with NaHCO_3 solution (10% w/v, 3 x 10 mL), H_2O (10 mL) and brine (10 mL). The organics were dried (MgSO_4), filtered and concentrated *in vacuo* to give a yellow solid. Purification was carried out *via* flash chromatography (applied in CH_2Cl_2 ; eluted 20:1 to 2:1 $\text{CH}_2\text{Cl}_2/\text{EtOAc}$) to afford the title compound as an off white solid (13.6 mg, 0.047 mmol, 3%).

Method C:

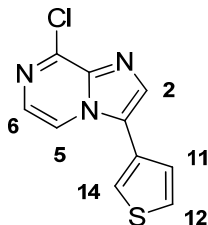
178 (542 mg, 1.76 mmol) was dissolved in anhydrous CH_2Cl_2 (20 mL) and the mixture was cooled on ice. TFA (950 μL , 12.3 mmol) was added and the reaction was allowed to stir on ice for 30 min, followed by the addition of trifluoroacetic anhydride (1.72 mL, 12.3 mmol). The reaction mixture was then stirred on ice for a further 30 min and then at RT for 68 h. The reaction was then diluted with CH_2Cl_2 (50 mL) and washed with NaHCO_3 solution (10% w/v, 3 x 10 mL) and brine (40 mL). The organics were dried (MgSO_4), filtered and concentrated *in vacuo* to give crude yellow solid. Purification was carried out *via* flash chromatography (applied in CH_2Cl_2 ; eluted 50:1 to 5:1 $\text{CH}_2\text{Cl}_2/\text{EtOAc}$) to afford the title compound as an off white solid (99.8 mg, 0.345 mmol, 20%).

8-Chloro-3-(3,5-dimethylphenyl)imidazo[1,2-a]pyrazine (184)



179 (2.29 g, 8.31 mmol) was dissolved in anhydrous toluene (90 mL) and the mixture was cooled on ice. TFA (4.48 mL, 58.1 mmol) was added and the reaction was allowed to stir on ice for 30 min, followed by the addition of trifluoroacetic anhydride (8.09 mL, 58.1 mmol). The reaction mixture was then stirred on ice for a further 30 min and then at RT for 68 h. The reaction was then diluted with toluene (50 mL) and washed with NaHCO₃ solution (10% w/v, 3 x 40 mL) and brine (1 x 40 mL). The organics were dried (MgSO₄), filtered and concentrated *in vacuo* to give crude orange sticky solid. Purification was carried out *via* flash chromatography (applied in CH₂Cl₂; eluted 40:1 CH₂Cl₂/EtOAc) to afford the title compound as a yellow solid (532 mg, 2.06 mmol, 25%). Mpt: 178-180 °C; *R_f* = 0.32 (9:1 CH₂Cl₂/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 2918 (C-H stretch), 1603, 1457 (aromatic C=C stretches), 1336; ¹H NMR (600 MHz, CDCl₃): δ_{H} = 2.41 (s, 6H, **14-H**), 7.14 (s, 1H, **13-H**), 7.16 (s, 2H, **11-H**), 7.69 (d, *J* = 4.6 Hz, 1H, **6-H**), 7.87 (s, 1H, **2-H**), 8.21 (d, *J* = 4.6 Hz, 1H, **5-H**); ¹³C NMR (150 MHz, CDCl₃): δ_{C} = 21.5 (**C-14**), 116.6 (**C-5**), 125.9 (**C-11**), 127.4 (**C-10**), 128.3 (**C-6**), 129.6 (**C-3**), 131.3 (**C-13**), 134.5 (**C-2**), 138.3 (**C-9**), 139.5 (**C-12**), 144.4 (**C-8**); LRMS *m/z* (EI⁺): 259 [M(³⁷Cl)]⁺, 257 [M(³⁵Cl)]⁺; HRMS *m/z* (CI⁺): Found: 257.0717 [M(³⁵Cl)]⁺; C₁₄H₁₂ClN₃ requires 257.0714.

8-Chloro-3-(3-thienyl)imidazo[1,2-a]pyrazine (185)

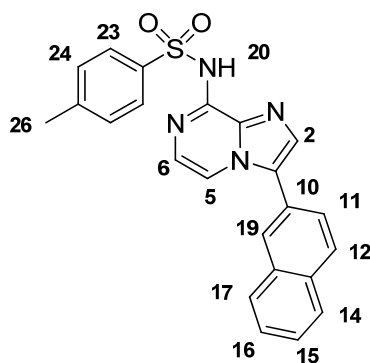


180 (345 mg, 1.36 mmol) was dissolved in anhydrous toluene (15 mL) and the mixture was cooled on ice. TFA (790 μ L, 10.3 mmol) was added and the reaction was allowed to stir on ice

for 30 minutes, followed by the addition of trifluoroacetic anhydride (1.43 mL, 10.3 mmol). The reaction mixture was then stirred on ice for a further 30 minutes and then at RT for 68 h. The reaction was then diluted with toluene (20 mL) and washed with aq. NaHCO₃ (10% w/v, 3 x 10 mL) and brine (1 x 10 mL). The organics were dried (MgSO₄), filtered and concentrated *in vacuo* to give crude orange oil. Purification was carried out *via* flash chromatography (applied in CH₂Cl₂; eluted 20:1 to 5:1 CH₂Cl₂/EtOAc) to afford the title compound as an off white solid (105 mg, 0.447 mmol, 33%). Mpt: 178-180 °C; *R*_f = 0.34 (5:1 CH₂Cl₂/EtOAc); IR (ν_{max}/cm⁻¹, thin film): 3098 (aromatic C-H stretch), 1462 (thiophene stretch), 1341 (aromatic C=C stretches); ¹H NMR (600 MHz, CDCl₃): δ_H = 7.34–7.36 (m, 1H, **12-H**), 7.58 (s, 1H, **14-H**), 7.59 (d, *J* = 0.7 Hz, 1H, **11-H**), 7.73 (d, *J* = 4.6 Hz, 1H, **6-H**), 7.91 (s, 1H, **2-H**), 8.19 (d, *J* = 4.6 Hz, 1H, **5-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 116.7 (**C-5**), 124.2 (**C-14**), 125.0 (**C-10**), 126.7 (**C-12**), 127.7 (**C-3**), 128.0 (**C-11**), 128.6 (**C-6**), 134.6 (**C-2**), 138.1 (**C-9**), 144.5 (**C-8**); LRMS *m/z* (CI⁺): 238 [M(³⁷Cl)+H]⁺, 236 [M(³⁵Cl)+H]⁺; HRMS *m/z* (CI⁺): Found 236.0057 [M(³⁵Cl)+H]⁺; C₁₀H₇ClN₃S requires 236.0049.

6.1.2.2.10 Synthesis of 4-methyl-N-(3-Arylimidazo[1,2-a]pyrazin-8-yl)benzenesulfonamides

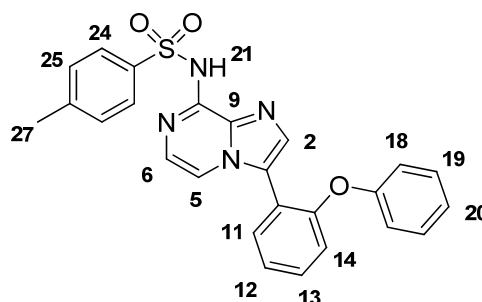
4-Methyl-N-(3-(2-naphthyl)imidazo[1,2-a]pyrazine-8-yl)benzenesulfonamide (4)



All glassware was evacuated and flushed with Ar prior to use. **181** (12.3 mg, 0.044 mmol), 4-toluene sulfonamide (9.10 mg, 0.053 mmol), K₂CO₃ (7.30 mg, 0.053 mmol), Pd(dba)₂ (0.120 mg, 1 mol%) and ^tBu-XPhos (0.600 mg, 5 mol%) were taken up in ^tBuOH (1 mL) and the reaction was stirred under reflux under Ar for 40 h. The reaction mixture was cooled to RT, diluted with MeOH and filtered through Celite (pre-washed with MeOH). Flash chromatography (applied in CH₂Cl₂; eluted 10:1 to 1:1 CH₂Cl₂/EtOAc) afforded the target compound as a white solid (7.50

mg, 0.018 mmol, 41%). Mpt: Decomposed before melting; R_f = 0.62 (1:1 CH₂Cl₂/EtOAc); IR (ν_{\max} /cm⁻¹, thin film): 3258, 3112 (aromatic C-H stretches), 2923 (N-H stretch), 2854 (C-H stretch), 1579 (N-H bend), 1381 (S=O asymmetric stretch), 1130 (S=O symmetric stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_H = 2.36 (s, 3H, **26-H**), 7.16 (bs, 1H, **6-H**), 7.39 (d, J = 8.2 Hz, 2H, **24-H**), 7.60-7.62 (m, 2H, **15,16-H**), 7.74 (d, J = 8.6 Hz, 1H, **11-H**), 7.87 (bd, J = 6.8 Hz, 1H, **5,23-H**), 7.91 (s, 1H, **2-H**), 8.00-8.04 (m, 2H, **14,17-H**), 8.11 (d, J = 8.6 Hz, **12-H**), 8.21 (s, 1H, **19-H**), 11.70 (bs, 1H, **7/20-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_C = 21.0 (**C-26**), 108.7 (**C-5**), 116.8 (**C-6**), 124.6 (**C-10**), 125.7 (**C-11**), 126.1 (**C-23**), 127.1 (overlapping signals, **C-15,16**), 127.4 (**C-19**), 128.1 (overlapping signals, **C-14,17**), 129.0 (**C-12**), 129.6 (**C-24**), 130.6 (**C-3**), 132.8 (overlapping signals, **C-13,18**), 133.3 (**C-2**), 135.6 (**C-9**), 140.1 (**C-22**), 142.6 (**C-25**), 144.5 (**C-8**); LRMS m/z (ES⁺): 415 [M+H]⁺, 260 [M-SO₂C₆H₄CH₃+H]⁺; HRMS m/z (ES⁺): Found 415.1230 [M+H]⁺; C₂₃H₁₉N₄O₂S requires 415.1229.

4-Methyl-N-(3-(2-phenoxyphenyl)imidazo[1,2-a]pyrazine-8-yl)benzenesulfonamide (**5**)



Method A:

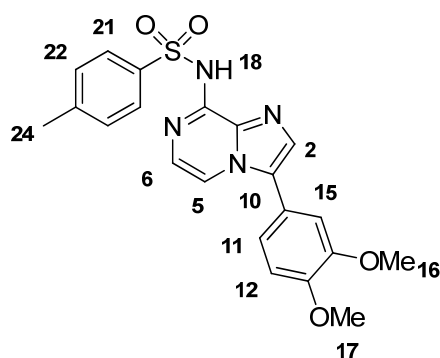
All glassware was evacuated and flushed with Ar prior to use. **182** (516 mg, 1.60 mmol), 4-toluene sulfonamide (330 mg, 1.93 mmol), K₂CO₃ (266 mg, 1.93 mmol), Pd(dba)₂ (5.20 mg, 1 mol%) and ^tBu-XPhos (26.0 mg, 5 mol%) were taken up in ^tBuOH (10 mL) and the reaction was stirred under reflux under Ar for 40 h. The reaction mixture was cooled to RT, diluted with MeOH and filtered through Celite (pre-washed with MeOH). Flash Chromatography was carried out (applied in CH₂Cl₂; eluted 10:1 to 1:1 CH₂Cl₂/EtOAc followed by 10% MeOH/CH₂Cl₂) to afford the title compound as yellow solid (188 mg, 0.412 mmol 26%). Mpt: > 200 °C; R_f = 0.18 (2:1 CH₂Cl₂/EtOAc); IR (ν_{\max} /cm⁻¹, thin film): 3243 (N-H stretch), 2917 (C-H stretch), 1588 (N-H bend), 1396 (S=O asymmetric stretch), 1233 (aromatic C-O stretch), 1139 (S=O symmetric

stretch); ^1H NMR (600 MHz, CDCl_3): δ_{H} = 2.37 (s, 3H, **27-H**), 6.85 (d, J = 7.8 Hz, 2H, **18-H**), 7.04 (d, J = 8.4 Hz, 2H, **6, 14H**), 7.07 (t, J = 7.2 Hz, 1H, **20H**), 7.23-7.27 (m, 5H, **12,19,25-H**), 7.43-7.46 (m, 3H, **5,11,13-H**), 7.65 (s, 1H, **2-H**), 7.94 (bs, 2H, **24-H**), 11.45 (bs, 1H, **7/21-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = 21.7 (**C-27**), 109.5 (**C-5**), 115.1 (**C-6**), 118.6 (**C-10**), 118.8 (**C-18**), 118.9 (**C-14**), 124.0 (**C-12**), 124.2 (**C-20**), 126.8 (**C-24**), 128.1 (**C-3**), 129.4 (**C-25**), 130.0 (**C-19**), 131.5 (**C-13**), 132.1 (**C-11**), 134.6 (**C-2**), 136.0 (**C-9**), 139.5 (**C-23**), 143.2 (**C-26**), 146.0 (**C-8**), 155.0 (**C-15**), 155.9 (**C-17**); LRMS m/z (ES^+): 457 [$\text{M}+\text{H}$] $^+$, 302 [$\text{M}-\text{C}_6\text{H}_5\text{-SO}_2$] $^+$. HRMS m/z (ES^-): Found: 455.1183 [$\text{M}-\text{H}$] $^-$; $\text{C}_{25}\text{H}_{19}\text{N}_4\text{O}_3\text{S}$ requires 455.1178.

Method B:

182 (756 mg, 2.35 mmol), 4-toluene sulfonamide (483 mg, 2.82 mmol) and Et_3N (654 μL , 4.70 mmol) were dissolved in anhydrous DMF (15 mL) and the reaction was stirred under reflux, under Ar for 16 h. The reaction was cooled to RT, quenched with H_2O (30 mL) and extracted with CH_2Cl_2 (3 x 40 mL). The combined organic extracts were washed with water (6 x 30 mL) and brine (30 mL), dried (MgSO_4), filtered and concentrated *in vacuo* to give a brown liquid. No product mass was found by LCMS and so no further work was carried out.

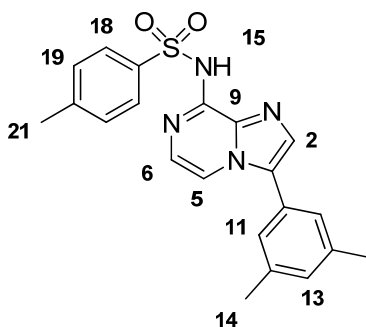
N-(3-(3,4-Dimethoxyphenyl)imidazo[1,2-*a*]pyrazine-8-yl)-4-methyl-benzenesulfonamide (**6**)



All glassware was evacuated and flushed with Ar prior to use. **183** (95.0 mg, 0.033 mmol), 4-toluene sulfonamide (67.5 mg, 0.039 mmol), K_2CO_3 (54.4 mg, 0.039 mmol), $\text{Pd}(\text{dba})_2$ (0.950 mg, 1 mol%) and $t\text{-Bu-XPhos}$ (4.75 mg, 5 mol%) were taken up in $t\text{-BuOH}$ (2 mL) and the reaction was stirred under reflux under Ar for 40 h. The reaction mixture was cooled to RT, diluted with

MeOH and filtered through Celite (pre-washed with MeOH). Flash Chromatography was carried out (applied in CH₂Cl₂; eluted 50:1 to 20:1 to 5:1 to 2:1 to 1:1 CH₂Cl₂/EtOAc) to afford the title compound as a yellow solid (42.3 mg, 0.010 mmol 30%). Mpt: >200 °C; *R*_f = 0.08 (1:1 CH₂Cl₂/EtOAc); IR (ν_{max}/cm⁻¹, thin film): 3244 (N-H stretch), 3129 (aromatic C-H stretch), 2838 (C-H stretch), 1582 (N-H bend), 1395 (S=O asymmetric stretch), 1254 (aromatic C-O stretch), 1134 (S=O symmetric stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 2.34 (s, 3H, **24-H**), 3.89 (s, 3H, **16-H**), 3.93 (s, 3H, **17-H**), 6.93 (d, *J* = 1.8 Hz, 1H, **15-H**), 6.98 (d, *J* = 8.3 Hz, 1H, **12-H**), 7.02 (dd, *J* = 8.3, 1.8 Hz, **11-H**), 7.13 (bs, 1H, **6-H**), 7.21 (d, *J* = 8.1 Hz, 2H, **22-H**), 7.41 (bs, 1H, **5-H**), 7.64 (s, 1H, **2-H**), 7.92 (d, *J* = 8.1 Hz, 2H, **21-H**), 11.53 (s, 1H, **18-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 21.5 (**C-24**), 56.1 (**C-16**), 56.2 (**C-17**), 108.0 (**C-5**), 111.5 (**C-15**), 111.7 (**C-12**), 116.1 (**C-6**), 119.5 (**C-10**), 121.2 (**C-11**), 126.6 (**C-21**), 129.4 (**C-22**), 131.4 (**C-3**), 133.1 (**C-2**), 135.6 (**C-9**), 139.3 (**C-20**), 143.2 (**C-23**), 145.8 (**C-8**), 149.6 (**C-14**), 150.1 (**C-13**); LRMS *m/z* (ES⁺): 447 [M+Na]⁺, 425 [M+H]⁺; HRMS *m/z* (ES⁻): Found 423.0940 [M+H]⁺; C₂₁H₂₁N₄O₄S requires 423.0949.

***N*-(3-(3,5-Dimethylphenyl)imidazo[1,2-*a*]pyrazine-8-yl)-4-methyl-benzenesulfonamide (7)**



Method A:

All glassware was evacuated and flushed with Ar prior to use. **184** (93.8 mg, 0.364 mmol), 4-toluene sulfonamide (74.8 mg, 0.437 mmol), K₂CO₃ (60.4 mg, 0.437 mmol), Pd(dba)₂ (2.10 mg, 1 mol%) and ^tBu-XPhos (7.70 mg, 5 mol%) were taken up in ^tBuOH (2 mL) and the reaction was stirred under reflux under Ar for 40 h. The reaction mixture was cooled to RT, diluted with MeOH and filtered through Celite (pre-washed with MeOH). Flash Chromatography was carried out (applied in CH₂Cl₂; eluted 9:1 to 1:1 CH₂Cl₂/EtOAc followed by 5% MeOH/CH₂Cl₂) to afford the title compound as a pale yellow solid (96.2 mg, 0.245 mmol, 67%). Mpt: >200 °C; *R*_f =

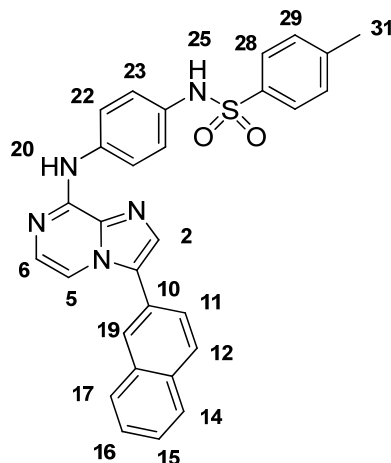
0.5 (CH₂Cl₂/10% MeOH); IR (ν_{max} /cm⁻¹, thin film): 3253 (aromatic C-H stretch), 1591 (N-H bend), 1399 (S=O asymmetric stretch), 1141 (S=O symmetric stretch); ¹H NMR (600 MHz, CD₂Cl₂): δ_{H} = 2.38 (s, 6H, **14-H**), 2.39 (s, 3H, **21-H**), 6.98 (bs, 1H, **6-H**), 7.11 (s, 2H, **11-H**), 7.13 (s, 1H, **13-H**), 7.30 (d, J = 8.0 Hz, 2H, **19-H**), 7.50 (bs, 1H, **5-H**), 7.64 (s, 1H, **2-H**), 7.90 (bd, J = 6.1 Hz, 2H, **18-H**), 11.33 (bs, 1H, **7/15-H**); ¹³C NMR (150 MHz, CD₂Cl₂): δ_{C} = 21.4 (**C-14**), 21.7 (**C-21**), 108.9 (**C-5**), 115.8 (**C-6**), 125.9 (**C-3**), 126.4 (**C-11**), 126.8 (**C-18**), 129.8 (**C-19**), 131.4 (**C-13**), 132.2 (**C-10**), 133.6 (**C-2**), 136.1 (**C-9**), 139.4 (**C-12**), 139.7 (**C-17**), 143.9 (**C-20**), 146.2 (**C-8**); LRMS m/z (ES⁺): 415 [M+Na]⁺, 393 [M+H]⁺; HRMS m/z (ES⁺): Found: 393.1366 [M+H]⁺; C₂₁H₂₁N₄O₂S requires 393.1385.

Method B:

184 (58.6 mg, 0.228 mmol), 4-toluene sulfonamide (46.7 mg, 0.273 mmol), K₂CO₃ (126 mg, 0.910 mmol) in MeCN (2 mL) were heated to 95 °C in a sealed tube. After 18 h, the vessel was allowed to slowly cool to RT followed by the careful release of the cap. The slurry was diluted with 0.1 M HCl (20 mL) and extracted with EtOAc (3 x 30 mL). The combined organics were washed with brine (20 mL), dried (MgSO₄), filtered and concentrated *in vacuo* to give crude white solid. Flash chromatography (applied in CH₂Cl₂; eluted 2:1 CH₂Cl₂/EtOAc followed by 5% MeOH/CH₂Cl₂) afforded the title compound as a pale yellow solid (14.4 mg, 0.037 mmol, 16%).

6.1.2.2.11 Synthesis of 4-methyl-N-(4-(3-Aryl-imidazo[1,2-a]pyrazin-8-ylamino)phenyl)benzene-sulfonamides

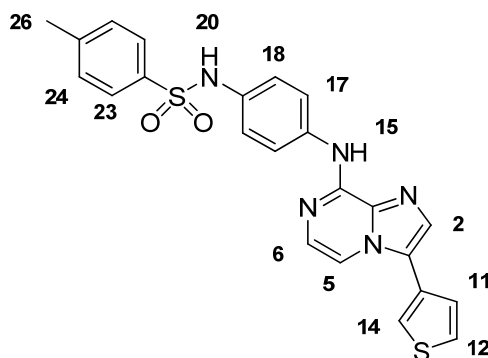
4-Methyl-N-(4-((3-(2-naphthyl)imidazo[1,2-a]pyrazine-8-yl)amino)phenyl)benzene-sulfonamide (12)



All glassware was evacuated and flushed with Ar prior to use. **181** (283 mg, 1.01 mmol), *N*-(4-aminophenyl)-4-methylbenzenesulfonamide (318 mg, 1.21 mmol), K₂CO₃ (167 mg, 1.21 mmol), Pd(dba)₂ (5.80 mg, 1 mol%) and ^tBu-XPhos (21.5 mg, 5 mol%) were taken up in ^tBuOH (6 mL) was added and the reaction was stirred under reflux under Ar for 46 h. The reaction mixture was cooled to RT, diluted with MeOH (100 mL) and filtered through Celite (pre-washed with MeOH). Flash chromatography (applied in CH₂Cl₂; eluted 100:1 to 50:1 to 8:1 CH₂Cl₂/EtOAc) was carried out to give the title compound as a yellow solid (181 mg, 0.355 mmol, 35%). Mpt: >200 °C; *R*_f = 0.12 (10:1 CH₂Cl₂/EtOAc); IR (ν_{max}/cm⁻¹, thin film): 3240 (N-H stretch), 3057 (aromatic C-H stretch), 1623 (aromatic C=C stretch), 1500 (N-H stretch), 1330 (S=O asymmetric stretch) 1154 (S=O symmetrical stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 2.37 (s, 3H, **31-H**), 7.08-7.10 (m, 2H, **23-H**), 7.17 (s, 1H, **25-H**), 7.21 (d, *J* = 8.3 Hz, 2H, **29-H**), 7.52 (d, *J* = 4.7 Hz, 1H, **6-H**), 7.57-7.58 (m, 2H, **15,16-H**), 7.64 (dd, *J* = 8.5, 1.6 Hz, 1H, **11-H**), 7.65 (d, *J* = 8.3 Hz, 2H, **28-H**), 7.73 (s, 1H, **2-H**), 7.79-7.80 (m, 3H, **5,22-H**), 7.91-7.92 (m, 2H, **14,17-H**), 8.00 (d, *J* = 8.5 Hz, 1H, **12-H**), 8.03 (s, 1H, **19-H**), 8.39 (bs, 1H, **20-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 21.6 (**C-31**), 109.4 (**C-5**), 120.3 (**C-22**), 123.7 (**C-23**), 125.2 (**C-10**), 125.4 (**C-11**), 127.1 (overlapping signals, **C-15,16**), 127.3 (overlapping signals, **C-19,28**), 127.9 (**C-14**), 128.1 (**C-17**), 128.9 (**C-3**), 129.0 (**C-6**), 129.3 (**C-12**), 129.7 (**C-29**), 130.4 (**C-2**), 131.2 (**C-24**), 133.1 (overlapping signals, **C-9,13**), 133.4 (**C-18**), 136.1 (**C-27**), 137.2 (**C-21**), 143.7 (**C-30**), 146.2 (C-

8); LRMS m/z (ES^-): 504 $[M-H]^-$; HRMS m/z (ES^-): Found 504.1503 $[M-H]^-$; $C_{29}H_{22}N_5O_2S$ requires 504.1494.

4-Methyl-N-(4-((3-(3-thienyl)imidazo[1,2-a]pyrazine-8-yl)amino)phenyl)benzenesulfonamide (13)



Method A:

All glassware was evacuated and flushed with Ar prior to use. **185** (35.0 mg, 0.149 mmol), *N*-(4-aminophenyl)-4-methylbenzenesulfonamide (47.8 mg, 0.178 mmol), K_2CO_3 (24.6 mg, 0.178 mmol), $Pd(dba)_2$ (0.900 mg, 1 mol%) and *t*Bu-XPhos (3.20 mg, 5 mol%) were taken up in *t*BuOH (2 mL) was added and the reaction was stirred under reflux under Ar for 46 h. The reaction mixture was cooled to RT, diluted with MeOH (100 mL) and filtered through Celite (pre-washed with MeOH). Flash chromatography (applied in pet. ether; eluted 3:2 pet. ether/EtOAc) was carried out to give a mixture of the product and starting sulfonamide. Reverse phase preparative HPLC (40 to 70% MeCN/ H_2O over 22 min) carried out to afford title compound as off white solid (12.4 mg, 0.027 mmol, 18%). Mpt: 108-110 °C; R_f = 0.62 (1:1 CH_2Cl_2 /EtOAc); IR (ν_{max}/cm^{-1} , thin film): 3112 (aromatic C-H stretch), 1624 (aromatic C=C stretch), 1542, 1506 (N-H bends), 1334 (S=O asymmetric stretch), 1159 (S=O symmetrical stretch); 1H NMR (600 MHz, CD_3OD): δ_H = 2.38 (s, 3H, **26-H**), 7.17 (d, J = 5.4 Hz, 1H, **6-H**), 7.28 (d, J = 9.0 Hz, 2H, **17-H**), 7.33 (d, J = 8.4 Hz, 2H, **24-H**), 7.48 (d, J = 9.0 Hz, 2H, **18-H**), 7.49, (dd, J = 5.4, 1.2 Hz, 1H, **11-H**), 7.72-7.73 (m, 3H, **12-H**, **23-H**), 7.92 (dd, J = 2.4, 1.2 Hz, 1H, **14-H**), 7.96 (bs, 1H, **2-H**), 8.02 (d, J = 5.4 Hz, 1H, **5-H**); 1H NMR (600 MHz, $CDCl_3$): δ_H = 2.38 (s, 3H, **26-H**), 6.69 (s, 1H, **20-H**), 7.07 (d, J = 8.8 Hz, 2H, **18-H**), 7.22 (d, J = 8.2 Hz, 2H, **24-H**), 7.34 (dd, J = 4.0, 2.0 Hz, 1H, **11-H**), 7.52 (d, J = 4.7 Hz, 1H, **6-H**), 7.54-7.56 (m, 2H, **12,14-H**), 7.63 (d, J = 8.2 Hz, 2H, **23-H**),

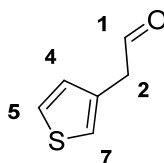
7.64 (s, 1H, **2-H**), 7.71 (d, $J = 4.7$ Hz, 1H, **5-H**), 7.79 (d, $J = 8.8$ Hz, 2H, **17-H**), 8.34 (bs, 1H, **15-H**); ^{13}C NMR (150 MHz, CD_3OD): $\delta_{\text{C}} = 21.4$ (**C-26**), 112.2 (**C-5**), 119.8 (**C-6**), 122.7 (**C-17**), 126.1 (**C-14**), 126.6 (**C-18**), 128.0 (**C-11**), 128.3 (**C-10**), 128.3 (**C-23**), 128.9 (**C-12**), 129.1 (**C-3**), 130.7 (**C-24**), 132.1 (**C-19**), 133.0 (**C-9**), 134.2 (**C-2**), 138.2 (**C-22**), 138.8 (**C-16**), 145.3 (**C-25**), 146.2 (**C-8**); ^{13}C NMR (150 MHz, CDCl_3): 21.7 (**C-26**), 109.7 (**C-5**), 120.3 (**C-17**), 123.6 (**C-12**), 123.9 (**C-18**), 124.5 (**C-10**), 126.9 (**C-11**), 127.4 (**C-14**), 127.5 (overlapping signals, **C-2**, **23**), 128.1 (**C-3**), 129.1 (**C-6**), 129.8 (**C-24**), 131.2 (**C-19**), 132.9 (**C-9**), 136.2 (**C-22**), 137.4 (**C-16**), 143.9 (**C-25**), 146.1 (**C-8**); LRMS m/z (ES^+): 484 $[\text{M}+\text{Na}]^+$, 462 $[\text{M}+\text{H}]^+$; HRMS m/z (ES^+): Found 462.1065 $[\text{M}+\text{H}]^+$; $\text{C}_{23}\text{H}_{20}\text{N}_5\text{S}_2\text{O}_2$ requires 462.1058.

Method B:

All glassware was evacuated and flushed with Ar prior to use. $\text{Pd}_2(\text{dba})_3$ (3.7 mg, 1 mol%), DavePhos (4.8 mg, 3 mol%) and NaO^tBu (54.2 mg, 0.564 mmol) were dissolved in anhydrous toluene (4 mL). **185** (94.9 mg, 0.403 mmol) and *N*-(4-aminophenyl)-4-methylbenzenesulfonamide (127 mg, 0.484 mmol) were added and the reaction was stirred under reflux, under Ar for 20 h. The reaction was cooled to RT and solvent removed, before the residue was taken up in CH_2Cl_2 (50 mL) and washed with NaHCO_3 (sat. aq. 30 mL), H_2O (30 mL) and brine (30 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Flash chromatography (pet. ether; 2:1 to 1:1 pet. ether/ EtOAc) followed by reverse phase preparative HPLC (35 to 55% $\text{MeCN}/\text{H}_2\text{O}$ over 10 min) afforded the title compound as a white solid (56.7 mg, 0.123 mmol, 30%).

6.1.2.2.12 Alternative Approach to 3-Aryl Imidazo[1,2-a]pyrazines

2-(3-Thienyl)acetaldehyde (212)



Method A:

2-(3-Thienyl)ethanol (0.450 g, 3.57 mmol) was dissolved in DMSO (4 mL). Et₃N (0.95 mL, 7.14 mmol) was added and the reaction mixture was stirred at RT for 5 min. Sulfur trioxide pyridine complex (1.14 g, 7.14 mmol) was dissolved in DMSO (4 mL) and was added dropwise the reaction mixture which was then stirred for 1 h. A colour change of colourless to yellow to orange was observed. The reaction was then cooled to 0 °C and quenched with H₂O (20 mL). The organics were extracted with CH₂Cl₂ (3 x 20 mL), which were then further washed with H₂O (5 x 20 mL), dried (Na₂SO₄), filtered and concentrated *in vacuo* to give an orange oil. Flash chromatography (applied in pet. ether; eluted 100% pet. ether to 50:1 to 10:1 to 5: 1 to 2: 1 to 1:1 pet. ether/CH₂Cl₂) afforded the title compound, with major impurities, as an orange oil. NMR was consistent with literature values.³⁰¹ R_f = 0.52 (CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃): δ_H = 3.73 (d, J = 2.3 Hz, 2H, **2-H**), 6.98 (dd, J = 5.0, 1.3 Hz, 1H, **4-H**), 7.14-7.15 (m, 1H, **7-H**), 7.35 (dd, J = 5.0, 3.0 Hz, 1H, **5-H**), 9.74 (t, J = 2.3 Hz, 1H, **1-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = 44.6 (**C-2**), 123.1 (**C-7**), 126.2 (**C-5**), 128.1 (**C-4**), 131.3 (**C-3**), 198.4 (**C-1**); m/z (Cl⁺): 83 [M-CH₂CHO]⁺.

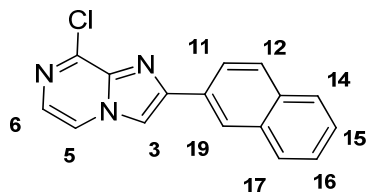
Method B:

DMSO (1.65 mL, 23.2 mmol) was dissolved in anhydrous CH₂Cl₂ (150 mL) and the reaction mixture was cooled to -78 °C. Oxalyl chloride (985 μ L, 11.6 mmol) was added dropwise and the mixture was allowed to stir for 15 min. 2-(3-thienyl)ethanol (1.14 g, 8.92 mmol), dissolved in CH₂Cl₂ (30 mL), was added dropwise to the mixture and stirred for a further 15 min. Et₃N (5.93 mL, 44.6 mmol) was then added dropwise (colour change of colourless to orange observed) and the mixture was then gently warmed to RT over a period of 2 h. The mixture was quenched with H₂O (80 mL) and the organic phase was extracted, followed by washing with 2.0 M HCl (2 x 80 mL), NaHCO₃ (sat. aq. 80 mL), H₂O (80 mL) and brine (80 mL). The organic extracts were dried (MgSO₄), filtered and solvent removed *in vacuo* to give a green oil (1.09 g). TLC and NMR revealed an extremely messy reaction mixture and so further work was abandoned.

6.1.2.3 Synthesis of 2-Aryl Imidazo[1,2-*a*]pyrazines

6.1.2.3.1 Synthesis of 2-aryl-8-chloro-imidazo[1,2-*a*]pyrazines

8-Chloro-2-(2-naphthyl)imidazo[1,2-*a*]pyrazine (214)



Method A:

2-(Bromoacetyl)naphthalene (3.14 g, 12.6 mmol), 2-amino-3-chloropyrazine (1.63 g, 12.6 mmol), NaHCO₃ (1.32 g, 16.7 mmol) and ^tBuOH (60 mL) were stirred under reflux for 40 h. The reaction mixture was cooled to RT and solvent removed *in vacuo*. The resulting orange solid was taken up in H₂O (100 mL) and extracted with CH₂Cl₂ (3x 150 mL). The combined organics were washed with H₂O (75 mL) and brine (75 mL), dried (MgSO₄), filtered and concentrated *in vacuo* to give crude orange solid. On addition of CH₂Cl₂ and MeOH (~1:1), the resulting insoluble material was filtered off to give title compound as an off white fluffy solid (1.05 g). Purification of the remaining filtrate *via* flash chromatography (applied in pet. ether; eluted 10% to 20% to 33% EtOAc) afforded the title compound as a pale orange/brown solid (338 mg, Total: 1.38 g, 4.95 mmol, 39%). Mpt: Decomposed before melting; *R_f* = 0.34 (1:1 pet. ether/ EtOAc); IR (ν_{max}/cm⁻¹, thin film): 3026, 2921 (aromatic C-H stretch), 1495 (aromatic C=C stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_H = 7.55-7.58 (m, 2H, **15,16-H**), 7.76 (d, *J* = 4.4 Hz, 1H, **6-H**), 7.95-7.97 (m, 1H, **17-H**), 8.04 (d, *J* = 8.6, 1H, **12-H**), 8.07-8.09 (m, 1H, **17-H**), 8.16 (dd, *J* = 8.6, 1.7 Hz, 1H, **11-H**), 8.64 (s, 1H, **19-H**), 8.67 (d, *J* = 4.4 Hz, H, **5-H**), 8.87 (s, 1H, **3-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 113.7 (**C-3**), 120.6 (**C-5**), 124.1 (**C-11**), 124.9 (**C-19**), 126.6 (**C-15**), 126.7 (**C-16**), 127.7 (overlapping signals, **C-6,17**), 128.4 (**C-14**), 128.6 (**C-12**), 129.9 (**C-10**), 133.1 (**C-18**), 133.2 (**C-13**), 137.5 (**C-9**), 141.2 (**C-8**), 146.4 (**C-2**); LRMS *m/z* (ES⁺): 282 [M(³⁷Cl)+H]⁺, 280 [M(³⁵Cl)+H]⁺; HRMS *m/z* (EI⁺): Found 279.0557 [M(³⁵Cl)]⁺; C₁₆H₁₀N₃Cl requires 279.0559; Anal. Calcd for C₁₆H₁₀N₃Cl: C, 68.70; H, 3.60; N, 15.02. Found C, 68.48; H, 3.51; N, 14.75%.

Method B:

2-(Bromoacetyl)naphthalene (50.0 mg, 0.201 mmol), 2-amino-3-chloropyrazine (26.0 mg, 0.201 mmol), NaHCO₃ (21.1 mg, 0.251 mmol) and solvent (1 mL) were stirred under reflux for 20 h. After this time the reaction was cooled to RT and a sample submitted for LCMS. The percentage of product by the UV trace and by ES⁺ TIC trace was recorded

Method C:

2-(Bromoacetyl)naphthalene (256 mg, 1.03 mmol), 2-amino-3-chloropyrazine (53.3 mg, 0.411 mmol), 1.0 M HCl (5 drops) and H₂O/THF (1:1 20 mL) were stirred at 80 °C for 40 h. The reaction mixture was then cooled to RT, diluted with H₂O (50 mL) and extracted CH₂Cl₂ (3 x 40 mL). The combined organic layers were washed with brine (40 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 10% to 20% to 33% EtOAc) afforded the title compound as an orange solid (19.2 mg, 0.069 mmol, 17%).

Method D (Microwave):

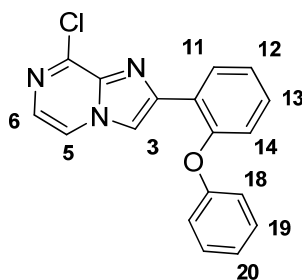
In a 5 mL microwave vial, 2-(bromoacetyl)naphthalene (50.0 mg, 0.201 mmol), 2-amino-3-chloropyrazine (26.0 mg, 0.201 mmol), NaHCO₃ (21.1 mg, 0.251 mmol) and solvent (2 mL) were stirred at 160 °C for 10 min, with a pressure limit of 20 bar. Removal of the reaction solvent *in vacuo*, followed by flash chromatography (applied in pet. ether; eluted 10% to 20% to 33% EtOAc) afforded the title compound as an orange solid.

Entry	Method	Base/Acid	Solvent	Temp/°C	Time/h	Yield/%
1	A	NaHCO ₃	EtOH	Reflux	6	20 ^a
2	A	Et ₃ N	Dioxane	Reflux	40	38
3	A	NaHCO ₃	EtOH	Reflux	16	16
4	A	NaHCO ₃	EtOH	Reflux	22	22
5	B	NaHCO ₃	MeOH	Reflux	20	2/0
6	B	NaHCO ₃	EtOH	Reflux	20	70/22 ^b

Entry	Method	Base/Acid	Solvent	Temp/°C	Time/h	Yield/%
7	B	NaHCO ₃	IPA	Reflux	20	100/45 ^b
8	B	NaHCO ₃	^t BuOH	Reflux	20	92/50 ^b
9	B	NaHCO ₃	DMF	Reflux	20	37/36 ^b
10	A	NaHCO ₃	^t BuOH	Reflux	40	39
11	A	NaHCO ₃	IPA	Reflux	40	26
12	A	KO ^t Bu	DMF	70	16	0
13	A	KO ^t Bu	NMP	100	16	0
14	A	Cs ₂ CO ₃	MeCN	70	16	8
15	C	HCl (cat.)	H ₂ O/THF	85	40	17
16	D	NaHCO ₃	^t BuOH	160	10 min	1
17	D	NaHCO ₃	MeCN	160	10 min	5
18	D	NaHCO ₃	DMF	160	10 min	4

Table 30: Variation in reaction conditions used for cyclisation to form 8-chloro-2-(2-naphthyl)imidazo[1,2-*a*]pyrazine, 214. ^a NaHCO₃ still present; ^b Not isolated yield: values are % peak are by ES+/UV from LCMS; Methods A: Basic condensation reaction; Method B: Solvent screening and analysing crude mixtures by LCMS; Method C: Acidic condensation; Method D: Microwave irradiation (20 bar pressure limit)

8-Chloro-2-(2-phenoxyphenyl)imidazo[1,2-*a*]pyrazine (215)



Method A:

2-Bromo-1-(2-phenoxyphenyl)ethanone (438 mg, 1.50 mmol), 2-amino-3-chloropyrazine (195 mg, 1.50 mmol), NaHCO₃ (158 mg, 1.88 mmol) and ^tBuOH (9 mL) were stirred under reflux for 48 h. The reaction was cooled to RT and solvent removed *in vacuo*. The sample was taken up in CH₂Cl₂ (75 mL) and washed with H₂O (3 x 50 mL). The combined aqueous extracts were further extracted with CH₂Cl₂ (4 x 50 mL), and the organic extracts were combined, washed brine (50 mL), dried (MgSO₄), filtered and solvent removed *in vacuo*. Flash chromatography (applied in pet. ether; eluted 25:1 to 10:1 to 5:1 to 3:1 pet. ether/EtOAc) afforded a pale yellow solid (156 mg, 0.485 mmol, 32%). Mpt: > 200 °C; *R*_f = 0.75 (1:1 pet. ether/ EtOAc); IR (*v*_{max}/cm⁻¹, thin film): 1071-1225 (aromatic C-O stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 6.97 (dd, *J* = 8.2, 1.5 Hz, 1H, **14-H**), 7.07 (d, *J* = 7.6 Hz, 2H, **18-H**), 7.16 (t, *J* = 7.4 Hz, 1H, **20-H**), 7.29-7.34 (m, 2H, **12,13-H**), 7.38 (dd, *J* = 8.6, 7.6 Hz, 2H, **19-H**), 7.65 (d, *J* = 4.5 Hz, 1H, **6-H**), 7.99 (d, *J* = 4.5 Hz, 1H, **5-H**), 8.31 (s, 1H, **3-H**), 8.59 (dd, *J* = 7.6, 1.9 Hz, 1H, **11-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 115.3 (**C-3**), 118.5 (**C-5**), 118.8 (**C-18**), 119.0 (**C-14**), 123.7 (**C-20**), 123.8 (**C-10**), 124.1 (**C-12**), 127.8 (**C-6**), 129.7 (**C-13**), 129.9 (**C-19**), 130.1 (**C-11**), 137.1 (**C-9**), 143.2 (**C-2**), 143.3 (**C-8**), 154.6 (**C-15**), 156.5 (**C-17**); LRMS *m/z* (ES⁺): 324 [M(³⁷Cl)+H]⁺, 322 [M(³⁵Cl)+H]⁺; HRMS *m/z* (CI⁺): Found 322.07554 [M(³⁵Cl)+H]⁺; C₁₈H₁₃ClN₃O requires 322.0747.

Method B:

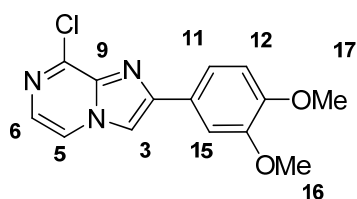
2-(Phenoxy)phenacyl bromide (52.0 mg, 0.179 mmol), 2-amino-3-chloropyrazine (27.8 mg, 0.214 mmol), NaHCO₃ (18.8 mg, 0.223 mmol) and MeOH (3 mL) were stirred under reflux for 24 h. The reaction was cooled to RT and solvent removed *in vacuo*. The sample was taken up in CH₂Cl₂ (50 mL) and washed with H₂O (3 x 10 mL). The combined aqueous extracts were further washed with CH₂Cl₂ (2 x 10 mL), and the organic extracts were combined, dried (MgSO₄), filtered and solvent removed *in vacuo* to give crude yellow solid (64.9 mg). No mass was present and so no further work was carried out.

Method C:

2-(Phenoxy)phenacyl bromide (70.0 mg, 0.241 mmol), 2-amino-3-chloropyrazine (31.2 mg, 0.241 mmol), NaHCO₃ (25.3 mg, 0.301 mmol) and EtOH (1 mL) were stirred under reflux for 20 h. The reaction was cooled to RT and solvent removed *in vacuo*. The sample was taken up in CH₂Cl₂ (25 mL) and washed with H₂O (3 x 10 mL). The combined aqueous extracts were further

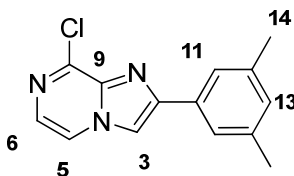
washed with CH₂Cl₂ (4 x 10 mL), and the organic extracts were combined, dried (Na₂SO₄), filtered and solvent removed *in vacuo* to give a crude yellow/orange solid (74.4 mg). Flash Chromatography (applied in CH₂Cl₂; eluted 50:1 to 20:1 CH₂Cl₂/Et₂O) afforded title compound as pale yellow oil (6.5 mg, 0.020 mmol, 8%).

8-Chloro-2-(3,4-dimethoxyphenyl)imidazo[1,2-a]pyrazine (216)



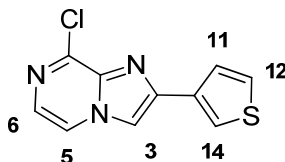
A mixture of **197** (229 mg, 0.885 mmol), 2-amino-3-chloropyrazine (115 mg, 0.885 mmol) and NaHCO₃ (93.0 mg, 1.11 mmol) in ^tBuOH (5 mL) were stirred under Ar, under reflux for 40 h. After this point, the reaction mixture was cooled to RT and solvent removed *in vacuo*. The resulting orange solid was dissolved in CH₂Cl₂ (75 mL) and washed with H₂O (3 x 30 mL). The aqueous layers were extracted further with CH₂Cl₂ (2 x 40 mL) before the combined organics were washed with brine (50 mL), dried (MgSO₄), filtered and solvent removed *in vacuo* to give a light brown solid. Flash chromatography (applied in pet. ether; eluted 3:1 pet. ether/EtOAc) was carried out to afford the title compound as a pale yellow solid (155 mg, 0.536 mmol, 61%). Mpt: Decomposed before melting; *R*_f = 0.11 (1:1 pet. ether/EtOAc); IR (ν_{max}/cm⁻¹, thin film): 3142, 2933 (aromatic C-H stretches), 2833 (C-H stretch), 1498 (aromatic C=C stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 3.93 (s, 3H, **16-H**), 4.00 (s, 3H, **17-H**), 6.93 (d, *J* = 8.3 Hz, 1H, **12-H**), 7.48 (dd, *J* = 8.3, 2.1 Hz, 1H, **11-H**), 7.59 (d, *J* = 2.1 Hz, 1H, **15-H**), 7.65 (d, *J* = 4.5, 1H, **6-H**), 7.96 (s, 1H, **3-H**), 8.02 (d, *J* = 4.5 Hz, 1H, **5-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 56.0 (**C-16**), 56.1 (**C-17**), 109.6 (**C-15**), 110.6 (**C-3**), 111.2 (**C-12**), 118.3 (**C-5**), 119.3 (**C-11**), 125.2 (**C-10**), 128.0 (**C-6**), 138.0 (**C-9**), 143.1 (**C-8**), 148.3 (**C-2**), 149.3 (**C-13**), 149.9 (**C-14**); LRMS *m/z* (ES⁺): 292 [**263**⁷Cl+H]⁺, 290 [M(³⁵Cl)+H]⁺; HRMS *m/z* (ES⁺): Found 290.0687 [M(³⁵Cl)+H]⁺; C₁₄H₁₃ClN₃O₂ requires 290.0696.

8-Chloro-2-(3,5-dimethylphenyl)imidazo[1,2-a]pyrazine (217)



A mixture of **198** (530 mg, 2.33 mmol), 2-amino-3-chloropyrazine (302 mg, 2.33 mmol) and NaHCO₃ (245 mg, 2.92 mmol) in *t*-BuOH (11 mL) were stirred under Ar, under reflux for 40 h. After this point, the reaction mixture was cooled to RT and solvent removed *in vacuo*. The resulting orange solid was dissolved in CH₂Cl₂ (100 mL) and washed with H₂O (3 x 40 mL). The aqueous layers were extracted further with CH₂Cl₂ (2 x 50 mL) before the combined organics were washed with brine (75 mL), dried (MgSO₄), filtered and solvent removed *in vacuo* to give crude orange solid. Flash chromatography (1st: applied in pet. ether; eluted 9:1 to 5:1 to 2:1 pet. ether/EtOAc; 2nd: applied in CH₂Cl₂; eluted 9:1 CH₂Cl₂/EtOAc) was carried out to afford the title compound as a pale yellow solid (245 mg, 0.951 mmol, 41%). Mpt: 158-162 °C; *R*_f = 0.68 (2:1 CH₂Cl₂/EtOAc); IR (ν_{max}/cm⁻¹, thin film): 2920 (C-H stretch), 1365 (aromatic C=C stretch); ¹H NMR (500 MHz, CDCl₃): δ_H = 2.38 (s, 6H, **14-H**), 7.05 (s, 1H, **13-H**), 7.62 (s, 2H, **11-H**), 7.66 (d, *J* = 4.5 Hz, 1H, **6-H**), 7.99 (s, 1H, **3-H**), 8.02 (d, *J* = 4.5 Hz, 1H, **5-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = 21.3 (**C-14**), 111.3 (**C-3**), 118.4 (**C-5**), 124.5 (**C-11**), 128.0 (**C-6**), 131.1 (**C-13**), 132.1 (**C-10**), 138.2 (**C-9**), 138.6 (**C-12**), 143.4 (**C-8**), 148.6 (**C-2**); LRMS *m/z* (EI⁺): 257 [M(³⁵Cl)]⁺, 259 [M(³⁷Cl)]⁺; HRMS *m/z* (EI⁺): Found 257.0718 [M(³⁵Cl)]⁺; C₁₄H₁₂ClN₃ requires 257.0714.

8-Chloro-2-(3-thienyl)imidazo[1,2-a]pyrazine (218)



Method A:

2-Bromo-1-(3-thienyl)-1-ethanone (293 mg, 1.43 mmol), 2-amino-3-chloropyrazine (185 mg, 1.43 mmol), NaHCO₃ (150 mg, 1.79 mmol) and *t*-BuOH (6 mL) were stirred under reflux for 41 h.

The reaction was cooled to RT and solvent removed *in vacuo*. The sample was taken up in H₂O (70 mL) and extracted with CH₂Cl₂ (2 x 50 mL). The combined organics were washed with H₂O (2 x 30 mL) and brine (30 mL) before they were dried (MgSO₄), filtered and concentrated *in vacuo* to give crude brown/orange solid. Flash chromatography (applied in pet. ether; eluted 2:1 pet. ether/EtOAc) afforded a pale yellow solid (109 mg, 0.464 mmol, 32%). Mpt: decomposed before melting; *R*_f = 0.33 (1:1 pet. ether/ EtOAc); IR (ν_{max} /cm⁻¹, thin film): 1240-1480 (thiophene stretch); ¹H NMR (600 MHz, CDCl₃): δ_{H} = 7.44 (dd, *J* = 5.0, 3.0 Hz, 1H, **12-H**), 7.58 (dd, *J* = 5.0, 1.3 Hz, 1H, **11-H**), 7.69 (d, *J* = 4.4 Hz, 1H, **6-H**), 7.93 (s, 1H, **3-H**), 7.96 (dd, *J* = 3.0, 1.3 Hz, 1H **14-H**), 8.04 (d, *J* = 4.4 Hz, 1H, **5-H**); ¹³C NMR (150 MHz, CDCl₃): δ_{C} = 111.1 (**C-3**), 118.4 (**C-5**), 123.6 (**C-14**), 126.0 (**C-12**), 126.6 (**C-11**), 128.1 (**C-6**), 134.1 (**C-10**), 138.0 (**C-9**), 143.3 (**C-8**), 144.3 (**C-2**); LRMS *m/z* (ES⁺): 238 [M(³⁷Cl)+H]⁺, 236 [M(³⁵Cl)+H]⁺; HRMS *m/z* (ES⁺): Found: 236.0056 [M(³⁵Cl)+H]⁺; C₁₀H₇ClN₃S requires 236.0049.

Method B:

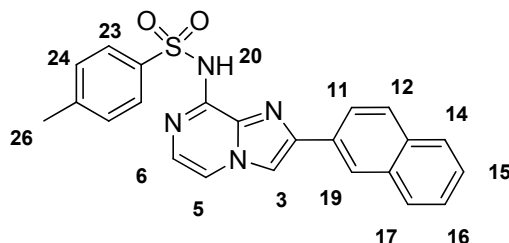
2-Bromo-1-(3-thienyl)-1-ethanone (50.0 mg, 0.244 mmol), 2-amino-3-chloropyrazine (37.9 mg, 0.293 mmol), NaHCO₃ (25.6 mg, 0.305 mmol) and MeOH (3 mL) were stirred under reflux for 24 h. The reaction was cooled to RT and solvent removed *in vacuo*. The sample was taken up in CHCl₃ (50 mL) and washed with H₂O (3 x 10 mL). The combined aqueous extracts were further washed with CHCl₃ (2 x 10 mL), and the organic extracts were combined, dried (MgSO₄), filtered and solvent removed *in vacuo* to give crude yellow solid (80.8 mg). No desired product mass was observed by MS and so work abandoned.

Method C:

2-Bromo-1-(3-thienyl)-1-ethanone (50.0 mg, 0.244 mmol), 2-amino-3-chloropyrazine (31.6 mg, 0.244 mmol), NaHCO₃ (25.6 mg, 0.305 mmol) and EtOH (1 mL) were stirred under reflux for 20 h. The reaction was cooled to RT and solvent removed *in vacuo*. The sample was taken up in CH₂Cl₂ (25 mL) and washed with H₂O (3 x 10 mL). The combined aqueous extracts were further washed with CH₂Cl₂ (4 x 10 mL), and the organic extracts were combined, dried (MgSO₄), filtered and solvent removed *in vacuo* to give crude yellow/orange solid (61.7 mg). Flash chromatography (applied in pet. ether; eluted 10:1 to 5:1 to 2:1 pet. ether/EtOAc) afforded the title compound as a pale yellow solid. (7.7 mg, 0.033 mmol, 13%).

6.1.2.3.2 Synthesis of 4-methyl-N-(2-Arylimidazo[1,2-a]pyrazin-8-yl)benzenesulfonamides

4-Methyl-N-[2-(2-naphthyl)imidazo[1,2-a]pyrazine-8-yl]benzenesulfonamide (8)



Method A:

All glassware was evacuated and flushed with Ar prior to use. **214** (50.0 mg, 0.178 mmol), 4-toluene sulfonamide (36.9 mg, 0.215 mmol), K_2CO_3 (29.7 mg, 0.215 mmol), $Pd(dba)_2$ (1.03 mg, 1 mol%) and $^tBu-XPhos$ (3.80 mg, 5 mol%) were taken up in tBuOH (3 mL) and the reaction was stirred under reflux for 24 h. The reaction mixture was cooled to RT, diluted with MeOH (100 mL) and filtered through Celite (pre-washed with MeOH). Flash chromatography (applied in CH_2Cl_2 ; eluted 5% to 10% to 20% EtOAc; or applied in pet. ether; eluted 20% to 33% to 50% to 66% EtOAc) afforded the target compound as a white solid (28.0 mg, 0.068 mmol, 38%). Mpt: $>200\text{ }^\circ C$; $R_f = 0.32$ (1:1 CH_2Cl_2 / EtOAc); IR (ν_{max}/cm^{-1} , thin film): 3253 (N-H stretch), 1361 (S=O asymmetric stretch), 1135 (S=O symmetric stretch), 1116; 1H NMR (600 MHz, $(CD_3)_2SO$): $\delta_H = 2.37$ (s, 3H, **26-H**), 7.16 (bd, $J = 5.2$ Hz, 1H, **6-H**), 7.39 (d, $J = 8.1$ Hz, 2H, **24-H**), 7.51-7.54 (m, 2H, **15,16-H**), 7.86 (bd, $J = 5.2$ Hz, 1H, **5-H**), 7.89 (d, $J = 8.1$ Hz, 2H, **23-H**), 7.92 (d, $J = 7.7$ Hz, 1H, **14-H**), 7.98 (d, $J = 8.6$ Hz, 1H, **12-H**), 8.01-8.05 (m, 2H, **11,17-H**), 8.52 (s, 1H, **19-H**), 8.59 (s, 1H, **3-H**), 11.69 (s, 1H, **20-H**); ^{13}C NMR (150 MHz, $(CD_3)_2SO$): $\delta_C = 21.0$ (**C-26**), 111.0 (**C-5**), 115.3 (**C-3**), 116.8 (**C-6**), 123.8 (**C-11**), 124.2 (**C-19**), 126.2 (**C-23**), 126.3 (**C-15**), 126.6 (**C-16**), 127.7 (**C-14**), 128.3 (**C-17**), 128.4 (**C-12**), 129.5 (**C-24**), 130.0 (**C-10**), 132.8 (**C-13**), 133.2 (**C-18**), 135.6 (**C-9**), 140.0 (**C-22**), 142.7 (**C-25**), 144.5 (**C-8**), 145.3 (**C-2**); LRMS m/z (ES^+): 415 $[M+H]^+$, (ES^-): 413 $[M-H]^-$, HRMS m/z (ES^+): Found 415.1219 $[M+H]^+$; $C_{23}H_{19}N_4O_2S$ requires 415.1229.

Method B (Microwave):

In a 2 mL microwave vial, catalyst, ligand and base were dissolved in solvent. **214** and 4-toluene sulfonamide (1.2 eq) were added and the reaction mixture was stirred at the stated temperature and for the given time with a 20 bar pressure limit. The solvent was removed *in vacuo* and the

crude mixture was purified *via* flash chromatography (applied in CH₂Cl₂; eluted 5% to 10% to 20% EtOAc; or applied in pet. ether; eluted 20% to 33% to 50% to 66% EtOAc) afforded the title compound.

Method C:

214, 4-toluene sulfonamide (2 eq) and base (in the case of NaH, the base was pre-activated by stirring in anhydrous hexanes and drying) were dissolved in solvent and stirred at the stated temperature for the given time. The reaction was cooled to RT and quenched with brine (in the case of NaH: with NH₄Cl (Sat.Aq)) and extracted EtOAc (3x). The combined organic layers were washed with H₂O (5x) and brine, dried (MgSO₄), filtered and solvent removed. Flash chromatography (applied in CH₂Cl₂; eluted 5% to 10% to 20% EtOAc; or applied in pet. ether; eluted 20% to 33% to 50% to 66% EtOAc) afforded the title compound.

Method D (Microwave):

In a 2 mL microwave vial, **214**, 4-toluene sulfonamide (1.2 eq) and base were added and the reaction mixture was stirred at the stated temperature and for the given time, with a 20 bar pressure limit. The solvent was removed *in vacuo* and the crude mixture was purified *via* flash chromatography (applied in CH₂Cl₂; eluted 5% to 10% to 20% EtOAc; or applied in pet. ether; eluted 20% to 33% to 50% to 66% EtOAc) afforded the title compound.

Method E:

NaH was washed by stirring in anhydrous hexanes, syringing out the solvent and drying. Under Ar, the reaction solvent was added to the flask, followed by 4-toluene sulfonamide (2 eq) in solvent. The contents were then stirred at RT for 20 min before **227** in solvent was added dropwise, and the reaction was stirred at the stated temperature for the given time. The reaction was cooled to RT and quenched with NH₄Cl (Sat.Aq) and extracted EtOAc (3x). The combined organic layers were washed with H₂O (5x) and brine, dried (MgSO₄), filtered and solvent removed. Flash chromatography (applied in pet. ether; eluted 20% to 33% to 50% to 66% EtOAc) afforded the title compound.

Method F (Microwave):

In a 2 mL microwave vial, **227**, 4-toluene sulfonamide (2 eq) and base were added and the reaction mixture was stirred at the stated temperature and for the given time with a 20 bar

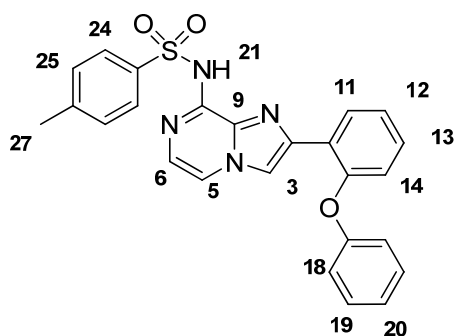
pressure limit. The solvent was removed *in vacuo* and the crude mixture was purified *via* flash chromatography (pet. ether; 20% to 33% to 50% to 66% EtOAc) to afford the title compound.

Entry	Method	Catalyst	Ligand	Base	Solvent	Temp/°C	Time/h	Yield/%
1	A	Pd(dba) ₂ (1 mol%)	Q-Phos (2 mol%)	NaO ^t Bu (1.5 eq)	toluene	80	22	0
2	A	Pd(dba) ₂ (1 mol%)	Q-Phos (2 mol%)	NaO ^t Bu (1.5 eq)	THF	50	72	0
3	A	Pd(dba) ₂ (1 mol%)	^t Bu-XPhos (5 mol%)	K ₂ CO ₃ (1.2 eq)	THF	Reflux	24	0
4	A	Pd(dba) ₂ (1 mol%)	^t Bu-XPhos (5 mol%)	K ₂ CO ₃ (1.2 eq)	^t BuOH	Reflux	24	38
5	B	Pd(dba) ₂ (1 mol%)	^t Bu-XPhos (5 mol%)	K ₂ CO ₃ (1.2 eq)	^t BuOH	120	30 min	11
6	A	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4 eq)	^t BuOH	Reflux	24	5
7	A	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4 eq)	dioxane	Reflux	24	66
8	A	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4 eq)	dioxane	105 ^a	24	17
9	B	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4 eq)	dioxane	130	30 min	1
10	A	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4 eq)	toluene	Reflux	16	12
11	A	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.4 eq)	toluene	Reflux	24	67
12	A	Pd ₂ (dba) ₃ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4 eq)	dioxane	Reflux	24	81
13	A	Pd ₂ (dba) ₃ (1 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.4 eq)	toluene	Reflux	24	93
14	B	Pd ₂ (dba) ₃ (1 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.4 eq)	toluene	160	10 min	46
15	A	Pd(OAc) ₂ (0.5 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.2 eq)	toluene	Reflux	21	64
16	A	Pd(OAc) ₂ (0.5 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.2 eq)	toluene	120 ^a	21	28
17	B	Pd(OAc) ₂ (0.5 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.2 eq)	toluene	95	30	0
18	B	Pd(PPh ₃) ₄ (5 mol%)	-	NaO ^t Bu (1.4 eq)	toluene	160	10 min	8

Entry	Method	Catalyst	Ligand	Base	Solvent	Temp/°C	Time/h	Yield/%
19	B	Pd(dppf)Cl ₂ (5 mol%)		NaO ^t Bu (1.4 eq)	toluene	160	10 min	38
20	A	CuI (15 mol%)	Diamine ^b (30 mol%)	K ₂ CO ₃ (2 eq)	DMF	100	72	3
21	C	-	-	DIPEA (5 eq)	NMP	140	24	0
22	C ^c	-	-	DIPEA (3.8 eq)	dioxane	100	21	0
23	C	-	-	NaH (2 eq)	DMSO	RT	21	0
24	C	-	-	NaH (2 eq)	DMSO	100	24	18
25	C	-	-	NaH (2 eq)	DMF	100	24	22
26	D	-	-	DIPEA (1.5 eq)	MeCN	160	10	0
27	E	-	-	NaH (2 eq)	DMSO	RT	20	18
28	E	-	-	NaH (2 eq)	DMSO	100	20	62
29	E	-	-	NaH (2 eq)	DMF	100	20	72
30	F	-	-	DIPEA (2 eq)	MeCN	160	10	0

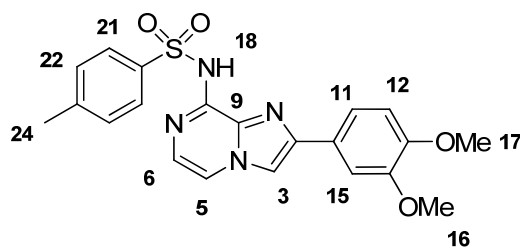
Table 31: Reaction optimisation for the formation of 8. ^a Reaction carried out in sealed tube; ^b *N,N'*-dimethylcyclohexane-1,2-diamine; ^c 1.4 eq of 4-toluene sulfonamide used

4-Methyl-N-[2-(2-phenoxyphenyl)imidazo[1,2-a]pyrazine-8-yl]benzenesulfonamide (9)



All glassware was evacuated and flushed with Ar prior to use. **215** (130 mg, 0.405 mmol), 4-toluene sulfonamide (83.1 mg, 0.486 mmol), K₂CO₃ (67.1 mg, 0.127 mmol), Pd(dba)₂ (1.30 mg, 1 mol%) and ^tBu-XPhos (6.50 mg, 5 mol%) were taken up in ^tBuOH (3 mL) and the reaction was stirred under reflux for 48 h. The reaction mixture was cooled to RT, diluted with MeOH (100 mL) and filtered through Celite (pre-washed with MeOH). Flash chromatography was carried out (applied in CH₂Cl₂; eluted 100% CH₂Cl₂ to 40:1 to 30:1 to 10:1 to 1:1 CH₂Cl₂/EtOAc) to afford the title compound as a yellow solid (23.9 mg, 0.052 mmol 13%). Mpt: >200 °C; *R*_f = 0.18 (9:1 CH₂Cl₂/EtOAc); IR (ν_{max}/cm⁻¹, thin film): 3245 (N-H stretch), 1586 (N-H bend), 1393 (S=O asymmetric stretch), 1227 (aromatic C-O stretch), 1115 (S=O symmetric stretch); ¹H NMR (600 MHz, CD₂Cl₂): δ_H = 2.43 (s, 3H, **27-H**), 6.91 (bs, 1H, **6-H**), 6.96 (d, *J* = 8.1 Hz, 1H, **14-H**), 7.08 (dd, *J* = 8.8, 0.7 Hz, 2H, **18-H**), 7.17 (t, *J* = 7.1 Hz, 1H, **20-H**), 7.27 (t, *J* = 7.6 Hz, 1H, **12-H**), 7.31-7.37 (m, 4H, **5,13,25-H**), 7.38-7.41 (m, 2H, **19-H**), 7.91 (bd, *J* = 5.7 Hz, 2H, **24-H**), 8.12 (s, 1H, **3-H**), 8.48 (dd, *J* = 7.6, 1.7 Hz 1H, **11-H**), 11.41 (s, 1H, **21-H**); ¹³C NMR (150 MHz, CD₂Cl₂): δ_C = 21.3 (**C-27**), 110.2 (**C-5**), 115.0 (**C-6**), 117.7 (**C-3**), 118.8 (**C-18**), 118.9 (**C-14**), 123.7 (**C-20**), 123.8 (**C-10**), 123.9 (**C-12**), 126.2 (**C-24**), 129.1 (**C-11**), 129.5 (**C-15,25**), 130.0 (**C-19**), 134.7 (**C-9**), 139.4 (**C-23**), 142.1 (**C-2**), 143.5 (**C-26**), 145.5 (**C-8**), 154.3 (**C-15**), 156.5 (**C-17**); LRMS *m/z* (ES⁺): 457 [M+H]⁺, HRMS *m/z* (ES⁻): Found 455.1167 [M-H]⁻; C₂₅H₁₉N₄O₃S requires 455.1178.

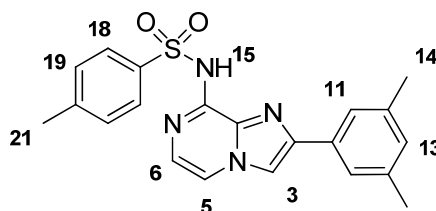
***N*-[2-(3,4-Dimethoxyphenyl)imidazo[1,2-*a*]pyrazine-8-yl]-4-methyl-benzenesulfonamide (10)**



All glassware was evacuated and flushed with Ar prior to use. **216** (200 mg, 0.691 mmol), 4-toluene sulfonamide (142 mg, 0.829 mmol), K₂CO₃ (115 mg, 0.829 mmol), Pd(dba)₂ (2.00 mg, 1 mol%) and ^tBu-XPhos (10.0 mg, 5 mol%) were taken up in ^tBuOH (4 mL) and the reaction was stirred under reflux for 48 h. The reaction mixture was cooled to RT, diluted with MeOH (100 mL) and filtered through Celite (pre-washed with MeOH). Flash chromatography was carried out (applied in CH₂Cl₂; eluted 100% CH₂Cl₂ to 50:1 to 10:1 to 2:1 to 1:2 to 1:9 CH₂Cl₂/EtOAc

followed by CH₂Cl₂/10% MeOH) gave the title compound as a yellow sticky solid (5.2 mg, 0.012 mmol, 2%). Mpt: >200 °C; *R_f* = 0.26 (1:1 CH₂Cl₂/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3274, 3138 (aromatic C-H stretches), 1584 (N-H bend), 1389 (S=O asymmetric stretch), 1113 (S=O symmetric stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_{H} = 2.36 (s, 3H, **24-H**), 3.78 (s, 3H, **16-H**), 3.83 (s, 3H, **17-H**), 7.02 (d, *J* = 8.2 Hz, 1H, **12-H**), 7.13 (bd, *J* = 5.2 Hz, 1H, **6-H**), 7.37 (d, *J* = 8.2 Hz, 1H, **22-H**), 7.45 (bs, 1H, **11-H**), 7.47 (bs, 1H, **15-H**), 7.81 (d, *J* = 5.2 Hz, 1H, **5-H**), 7.87 (d, *J* = 8.0 Hz, 2H, **21-H**), 8.41 (s, 1H, **3-H**), 11.63 (s, 1H, **18-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_{C} = 21.0 (**C-24**), 55.5 (**C-16**), 55.6 (**C-17**), 108.9 (**C-15**), 111.0 (**C-5**), 111.9 (**C-12**), 114.3 (**C-3**), 116.6 (**C-6**), 118.2 (**C-11**), 125.3 (**C-10**), 126.1 (**C-21**), 129.5 (**C-22**), 135.0 (**C-9**), 139.8 (**C-20**), 142.7 (**C-23**), 144.4 (**C-8**), 145.6 (**C-2**), 149.0 (**C-14**), 149.1 (**C-13**); LRMS *m/z* (ES⁺): 423 [M+H]⁺; HRMS *m/z* (ES⁺): Found 423.1115 [M+H]⁺; C₂₁H₂₁N₄O₄S requires 423.1127.

***N*-[2-(3,5-Dimethylphenyl)imidazo[1,2-*a*]pyrazine-8-yl]-4-methyl benzenesulfonamide (**11**)**



Method A:

A mixture of **217** (100 mg, 0.388 mmol), 4-toluene sulfonamide (79.8 mg, 0.466 mmol), Pd(dppf)Cl₂ (6.30 mg, 2 mol%) and Cs₂CO₃ (152 mg, 0.466 mmol) in anhydrous toluene (3 mL) were stirred under reflux, under Ar. After 21 h, the reaction was cooled to RT, diluted with toluene (20 mL) and washed with H₂O (10 mL) and brine (10 mL). Further extraction of the aqueous layers was carried out using CH₂Cl₂ (3 x 10 mL). The combined organics were dried (MgSO₄), filtered and solvent removed *in vacuo*. Flash chromatography (applied in CH₂Cl₂; eluted 19:1 CH₂Cl₂/EtOAc) afforded the title compound as a yellow solid (43.4 mg, 0.111 mmol, 29%). Mpt: >200 °C; *R_f* = 0.37 (2:1 CH₂Cl₂/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 2928 (C-H stretch), 1595 (N-H bend), 1118 (S=O symmetric stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_{H} = 2.31 (s, 6H, **14-H**), 2.36 (s, 3H, **21-H**), 6.97 (s, 1H, **13-H**), 7.13 (t, *J* = 5.4 Hz, 1H, **6-H**), 7.38 (d, *J* = 8.1 Hz, 2H, **19-H**), 7.54 (s, 2H, **11-H**), 7.81 (d, *J* = 5.4 Hz, 1H, **5-H**), 7.87 (d, *J* = 8.1 Hz, 2H,

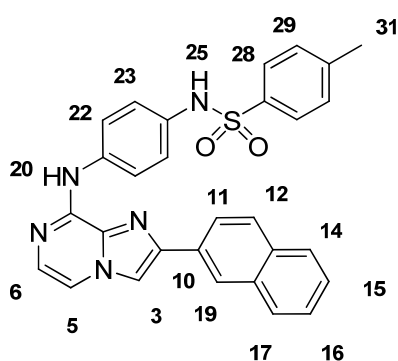
18-H), 8.42 (s, 1H, **3-H**), 11.63 (bd, $J = 5.4$ Hz, 1H, **7/15-H**); ^{13}C NMR (150 MHz, $(\text{CD}_3)_2\text{SO}$): $\delta_{\text{C}} = 21.0$ (overlapping signals, **C-14,21**), 111.0 (**C-5**), 114.8 (**C-3**), 116.7 (**C-6**), 123.3 (**C-11**), 126.1 (**C-18**), 129.5 (**C-19**), 129.8 (**C-13**), 132.4 (**C-10**), 135.2 (**C-9**), 137.9 (**C-12**), 139.9 (**C-17**), 142.6 (**C-20**), 144.5 (**C-8**), 145.5 (**C-2**); LRMS m/z (ES^+): 393 $[\text{M}+\text{H}]^+$, HRMS m/z (ES^-): Found 391.1236 $[\text{M}-\text{H}]^-$; $\text{C}_{21}\text{H}_{19}\text{N}_4\text{O}_2$ requires 391.1229.

Method B:

All glassware was evacuated and flushed with Ar prior to use. **217** (21.5 mg, 0.0835 mmol), 4-toluene sulfonamide (17.2 mg, 0.100 mmol), K_2CO_3 (13.8 mg, 0.100 mmol), $\text{Pd}(\text{dba})_2$ (0.20 mg, 1 mol%) and $^t\text{Bu-XPhos}$ (1.10 mg, 5 mol%) were taken up in $^t\text{BuOH}$ (1 mL) and the reaction was stirred under reflux for 48 h. The reaction mixture was cooled to RT, diluted with MeOH (100 mL) and filtered through Celite (pre-washed with MeOH). Flash chromatography was carried out (applied in CH_2Cl_2 ; eluted 100% CH_2Cl_2 to 50:1 to 30:1 $\text{CH}_2\text{Cl}_2/\text{EtOAc}$), but contamination of the pure product was evident by NMR with not enough sample to re-purify.

6.1.2.3.3 Synthesis of Synthesis of 4-methyl-N-(4-(2-Aryl-imidazo[1,2-a]pyrazin-8-ylamino)-phenyl)benzenesulfonamides

4-Methyl-N-[4-[[2-(2-naphthyl)imidazo[1,2-a]pyrazine-8-yl]amino]phenyl]benzenesulfonamide (14)



Method A:

All glassware was evacuated and flushed with Ar prior to use. **214** (50.0 mg, 0.178 mmol), *N*-(4-aminophenyl)-4-methylbenzenesulfonamide (56.3 mg, 0.215 mmol), K_2CO_3 (29.7 mg, 0.215 mmol), $\text{Pd}(\text{dba})_2$ (1.03 mg, 1 mol%) and $^t\text{Bu-XPhos}$ (3.80 mg, 5 mol%) were taken up in $^t\text{BuOH}$

(3 mL) and the reaction was stirred under reflux for 24 h. The reaction mixture was cooled to RT, diluted with MeOH (100 mL) and filtered through Celite (pre-washed with MeOH). Flash chromatography (applied in toluene; eluted 3:1 toluene/EtOAc) gave the title compound as a light brown solid (12.4 mg, 0.025 mmol, 14%). Mpt: decomposed before melting; R_f = 0.50 (1:1 pet. ether/EtOAc); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3126 (N-H stretch), 2923, 2853 (C-H stretches), 1507 (N-H bend), 1325 (S=O asymmetric stretch), 1143 (S=O symmetric stretch); ^1H NMR (600 MHz, CD_3OD): δ_{H} = 2.42 (s, 3H, **31-H**), 7.20 (d, J = 5.2 Hz, 1H, **6-H**), 7.29 (d, J = 8.9 Hz, 2H, **22-H**), 7.36 (d, J = 8.2 Hz, 2H, **29-H**), 7.52-7.56 (m, 2H, **15,16-H**), 7.58 (d, J = 8.9 Hz, 2H, **23-H**), 7.75 (d, J = 8.2 Hz, 2H, **28-H**), 7.92 (d, J = 7.1 Hz, 1H, **17-H**), 7.96-7.99 (m, 3H, **5,12,14-H**), 8.13 (dd, J = 8.5, 1.7 Hz, 1H, **11-H**), 8.53 (s, 1H, **3-H**), 8.56 (s, 1H, **19-H**); ^{13}C NMR (150 MHz, CD_3OD): δ_{C} = 21.4 (**C-31**), 113.9 (**C-5**), 115.9 (**C-3**), 115.9 (**C-6**), 122.9 (**C-22**), 124.9 (**C-11**), 125.8 (**C-23**), 126.1 (**C-19**), 127.6/127.7 (**C-15,16**), 128.4 (**C-28**), 128.9 (**C-17**), 129.3 (**C-14**), 129.7 (**C-12**), 130.7 (**C-29**), 131.0 (**C-10**), 133.5 (**C-9, 24**), 135.0 (**C-13,18**), 137.7 (**C-21**), 138.2 (**C-27**), 145.2 (**C-30**), 146.1 (**C-8**), 148.1 (**C-2**); LRMS m/z (ES⁺): 506 $[\text{M}+\text{H}]^+$, (ES⁻): 504 $[\text{M}-\text{H}]^-$; HRMS m/z (ES⁺): Found 506.1651 $[\text{M}+\text{H}]^+$; $\text{C}_{29}\text{H}_{24}\text{N}_5\text{O}_2\text{S}$ requires 506.1651.

Method B (Microwave):

In a 2 mL microwave vial, catalyst, ligand and base were dissolved in solvent. **214** and *N*-(4-aminophenyl)-4-methylbenzenesulfonamide (1.2 eq) were added and the reaction mixture was stirred at the stated temperature and for the given time with a 20 bar pressure limit. The solvent was removed *in vacuo* and the crude mixture was purified *via* flash chromatography (applied in toluene; eluted 3:1 toluene/EtOAc) to afford the title compound.

Method C:

214, *N*-(4-aminophenyl)-4-methylbenzenesulfonamide (2 eq) and base (in the case of NaH, the base was pre-activated by stirring in anhydrous hexanes and drying) were dissolved in solvent and stirred at the stated temperature for the given time. The reaction was cooled to RT and quenched with brine (in the case of NaH: with NH_4Cl (Sat.Aq)) and extracted EtOAc (3x). The combined organic layers were washed with H_2O (5x) and brine, dried (MgSO_4), filtered and solvent removed *in vacuo*. No further work was carried out.

Method D (Microwave):

In a 2 mL microwave vial, **214**, *N*-(4-aminophenyl)-4-methylbenzenesulfonamide (1.2 eq) and base were added and the reaction mixture was stirred at the stated temperature and for the given time with a 20 bar pressure limit. No further work was carried out.

Method E:

NaH was washed by stirring in anhydrous hexanes, syringing out the solvent and drying. Under Ar, the reaction solvent was added to the flask, followed by *N*-(4-aminophenyl)-4-methylbenzenesulfonamide (2 eq) in solvent. The contents were then stirred at RT for 20 min before **227** in solvent was added dropwise, and the reaction was stirred at the stated temperature for the given time. The reaction was cooled to RT and quenched with NH₄Cl (Sat.Aq) and extracted EtOAc (3x). The combined organic layers were washed with H₂O (5x) and brine, dried (MgSO₄), filtered and solvent removed *in vacuo*. No further work was carried out.

Method F (Microwave):

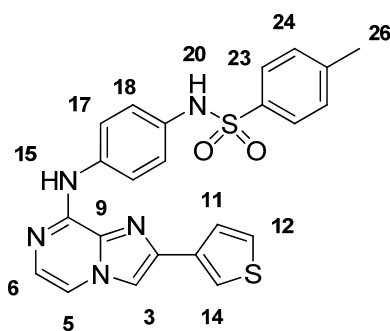
In a 2 mL microwave vial, **227**, *N*-(4-aminophenyl)-4-methylbenzenesulfonamide (2 eq) and base were added and the reaction mixture was stirred at the stated temperature and for the given time with a 20 bar pressure limit. No further work was carried out.

Entry	Method	Catalyst	Ligand	Base	Solvent	Temp/°C	Time/h	Yield/%
1	A	Pd(dba) ₂ (1 mol%)	^t Bu-XPhos (5 mol%)	K ₂ CO ₃ (1.2 eq)	^t BuOH	Reflux	24	14
2	A	Pd(dba) ₂ (1 mol%)	^t Bu-XPhos (5 mol%)	K ₂ CO ₃ (1.4 eq)	^t BuOH	Reflux	40	0
3	A	Pd(dba) ₂ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4)	dioxane	Reflux	40	6
4	A	Pd ₂ (dba) ₃ (1 mol%)	DavePhos (3 mol%)	Cs ₂ CO ₃ (1.4)	dioxane	Reflux	24	Trace
5	A	Pd ₂ (dba) ₃ (1 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.4 eq)	toluene	Reflux	24	22
6	B	Pd ₂ (dba) ₃ (1 mol%)	DavePhos (3 mol%)	NaO ^t Bu (1.4 eq)	toluene	160	10 min	47
7	B	Pd(PPh ₃) ₄ (5 mol%)	-	NaO ^t Bu (1.4 eq)	toluene	160	10 min	23

8	B	Pd(dppf)Cl ₂ (5 mol%)		-	NaO ^t Bu (1.4 eq)	toluene	160	10 min	4
9	C	-	-	-	Et ₃ N (1.2 eq)	DMF	Reflux	40	0
10	C ^a	-	-	-	DIPEA (3.8 eq)	dioxane	100	20	0
11	C	-	-	-	DIPEA (5 eq)	NMP	140	20	0
12	C	-	-	-	DIPEA (2.5 eq)	^t BuOH	120	16	0
13	C	-	-	-	NaH (2 eq)	DMSO	100	24	0
14	C	-	-	-	NaH (2 eq)	DMF	100	24	0
15	D	-	-	-	DIPEA (1.5 eq)	MeCN	160	10 min	0
16	E	-	-	-	NaH (2 eq)	DMSO	RT	18	0
17	E	-	-	-	NaH (2 eq)	DMSO	100	18	0
18	F	-	-	-	DIPEA (2 eq)	MeCN	160	10 min	0

Table 32: Variation in reaction conditions for formation of 14. ^a 1.4 eq of *N*-(4-aminophenyl)-4-methylbenzenesulfonamide used

4-Methyl-N-[4-[[2-(3-thienyl)imidazo[1,2-a]pyrazine-8-yl]amino]phenyl]benzenesulfonamide (15)

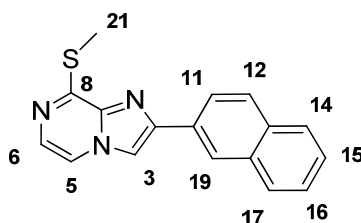


All glassware was evacuated and flushed with Ar prior to use. **218** (128 mg, 0.540 mmol), *N*-(4-aminophenyl)-4-methylbenzenesulfonamide (171 mg, 0.650 mmol), K₂CO₃ (90.1 mg, 0.652 mmol), Pd(dba)₂ (3.12 mg, 1 mol%) and ^tBu-XPhos (11.5 mg, 5 mol%) were taken up in ^tBuOH

(5 mL) and the reaction was stirred under reflux for 40 h. The reaction mixture was cooled to RT, diluted with MeOH (100 mL) and filtered through Celite (pre-washed with MeOH). Flash chromatography (applied in pet. ether; eluted 1:1 pet. ether/EtOAc) was carried to give the title compound as a yellow solid (19.2 mg, 0.042 mmol, 8%). Mpt: 158-164 °C; R_f = 0.57 (1:1 CH₂Cl₂/Et₂O); IR (ν_{\max} /cm⁻¹, thin film): 3116 (N-H and C-H stretches), 1671, 1508 (N-H bend), 1328 (S=O asymmetric stretch), 1140 (S=O symmetric stretch); ¹H NMR (600 MHz, CD₃OD): δ_H = 2.41 (s, 3H, **26-H**), 7.17 (bd, J = 4.5 Hz, 1H, **6-H**), 7.29 (d, J = 8.8 Hz, 2H, **17-H**), 7.35 (d, J = 8.2 Hz, 2H, **24-H**), 7.53 (d, J = 8.8, 2H, **18-H**), 7.57 (dd, J = 4.9, 2.9 Hz, 1H, **12-H**), 7.66 (dd, J = 4.9, 1.1 Hz, 1H, **11-H**), 7.75 (d, J = 8.2, 2 H, **23-H**), 7.96 (d, J = 5.4 Hz, 1H, **5-H**), 7.98 (dd, J = 2.9, 1.1 Hz, 1H, **14-H**), 8.30 (s, 1H, **3-H**); ¹³C NMR (150 MHz, CD₃OD): δ_C = 20.0 (**C-26**), 112.6 (**C-5**), 114.3 (**C-3**), 118.8 (**C-6**), 121.4 (**C-17**), 122.3 (**C-14**), 124.8 (**C-18**), 125.5 (**C-11**), 126.5 (**C-12**), 127.0 (**C-23**), 129.3, (**C-24**), 131.4 (**C-16**), 134.0 (**C-9,10**), 136.8 (overlapping signals, **C-19,22**), 143.2 (**C-2**), 143.8 (**C-25**), 144.4 (**C-8**); LRMS m/z (ES⁺) 462 [M+H]⁺; HRMS m/z (ES⁺): Found 462.1047 [M+H]⁺; C₂₃H₂₀N₅O₂S₂ requires 462.1058.

6.1.2.3.4 Alternative Compounds used during Buchwald-Hartwig Optimisation

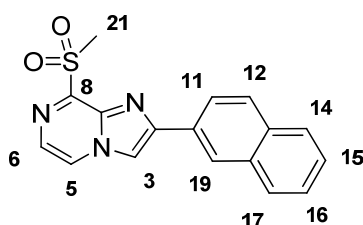
8-(Methylthio)-2-(naphthalen-2-yl)imidazo[1,2-a]pyrazine (226)



214 (1.12 g, 4.01 mmol) was dissolved in anhydrous DMSO (16 mL). NaSMe (337 mg, 4.81 mmol) was added portionwise and the reaction was stirred at 100 °C for 16 h. The mixture was then cooled to RT, diluted with brine (50 mL) and extracted with CH₂Cl₂ (100 mL). The organic layer was washed with H₂O (5 x 30 mL) and brine (30 mL), dried (MgSO₄), filtered and solvent removed *in vacuo*. Flash chromatography (applied in CH₂Cl₂; eluted 0% to 1% to 2% EtOAc) afforded the title compound as an off white/yellow solid (989 mg, 3.40 mmol, 85%). Mpt: 168 °C; R_f = 0.47 (5% EtOAc/CH₂Cl₂); IR (ν_{\max} /cm⁻¹, thin film): 3055 (aromatic C-H stretch), 1470, 1432 (aromatic C=C stretches), 1368 (aromatic C-S stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 2.71 (s, 3H, **21-H**), 7.47-7.52 (m, 2H, **15,16-H**), 7.72 (d, J = 4.5 Hz, 1H, **6-H**), 7.82 (d, J = 4.5

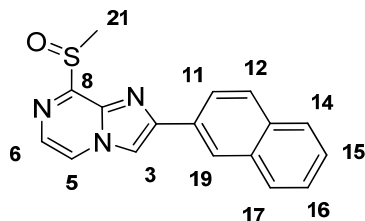
Hz, 1H, **5-H**), 7.85 (d, $J = 7.4$ Hz, 1H, **17-H**), 7.90 (d, $J = 8.5$ Hz, 1H, **12-H**), 7.94 (d, $J = 7.4$ Hz, 1H, **14-H**), 7.98 (s, 1H, **3-H**), 8.04 (dd, $J = 8.5, 1.6$ Hz, 1H, **11-H**), 8.55 (s, 1H, **19-H**); ^{13}C NMR (150 MHz, CDCl_3): $\delta_{\text{C}} = 12.3$ (**C-21**), 110.3 (**C-3**), 115.1 (**C-5**), 124.3 (**C-11**), 125.6 (**C-19**), 126.4 (**C-15**), 126.5 (**C-16**), 127.9 (**C-17**), 128.5 (**C-12**), 128.6 (**C-14**), 129.0 (**C-6**), 130.2 (**C-10**), 133.5 (overlapping signals, **C-13,18**), 138.9 (**C-9**), 146.4 (**C-2**), 154.4 (**C-8**); LRMS m/z (ES^+): 292 $[\text{M}+\text{H}]^+$; HRMS m/z (ES^+): Found 292.0909 $[\text{M}+\text{H}]^+$; $\text{C}_{17}\text{H}_{14}\text{N}_3\text{S}$ requires 292.0908; Anal. Calcd. for $\text{C}_{17}\text{H}_{13}\text{N}_3\text{S}$: C, 70.08; H, 4.50; N, 14.42. Found C, 69.85; H, 4.28; N, 14.42%.

8-(Methylsulfonyl)-2-(naphthalen-2-yl)imidazo[1,2-a]pyrazine (227)



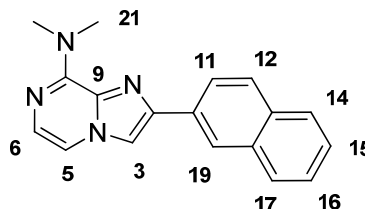
226 (1.77 g, 6.07 mmol) was dissolved in anhydrous CH_2Cl_2 (50 mL) and the mixture was cooled on ice. *m*CPBA (5.23 g, 30.3 mmol) was added in one portion and the reaction continued to stir at RT for 5 h. The reaction was partitioned with NaHCO_3 (40 mL) and extracted with CH_2Cl_2 (3 x 30 mL); the combined organic extracts were then washed with brine (30 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Flash chromatography (applied in CH_2Cl_2 ; eluted 2% to 5% EtOAc) afforded the title compound as a yellow solid (1.10 g, 3.40 mmol, 56%). Mpt: >200 $^{\circ}\text{C}$; $R_f = 0.22$ (10% EtOAc/ CH_2Cl_2); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3121, 3010 (aromatic C-H stretch), 1312 (S=O asymmetric stretch), 1138 (S=O symmetric stretch); ^1H NMR (600 MHz, CDCl_3): $\delta_{\text{H}} = 3.84$ (s, 3H, **21-H**), 7.51-7.54 (m, 2H, **15,16-H**), 7.86-7.87 (m, 1H, **14-H**), 7.92 (d, $J = 8.5$ Hz, 1H, **12-H**), 7.95-7.96 (m, 1H, **17-H**), 8.02 (d, $J = 4.3$ Hz, 1H, **6-H**), 8.08 (dd, $J = 8.5, 1.4$ Hz, 1H, **11-H**), 8.26 (s, 1H, **3-H**), 8.32 (d, $J = 4.3$ Hz, 1H, **5-H**), 8.59 (s, 1H, **19-H**); ^{13}C NMR (150 MHz, CDCl_3): $\delta_{\text{C}} = 41.7$ (**C-21**), 110.9 (**C-3**), 122.1 (**C-5**), 124.3 (**C-11**), 126.7 (**C-19**), 126.8 (**C-15**), 127.0 (**C-16**), 127.9 (**C-14**), 128.1 (**C-6**), 128.7 (**C-17**), 128.8 (**C-12**), 129.1 (**C-10**), 133.5 (**C-13**), 134.0 (**C-18**), 136.3 (**C-9**), 148.6 (**C-8**), 149.8 (**C-2**); LRMS m/z (EI^+): 323 $[\text{M}]^+$; HRMS m/z (EI^+): Found 323.0727 $[\text{M}]^+$; $\text{C}_{17}\text{H}_{13}\text{N}_3\text{O}_2\text{S}$ requires 323.0723.

8-(Methylsulfinyl)-2-(naphthalen-2-yl)imidazo[1,2-a]pyrazine (228)



Mpt: Decomposed before melting; $R_f = 0.05$ (10% EtOAc/CH₂Cl₂); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3111 (aromatic C-H stretch), 1360 (aromatic C=C and C-N stretches), 1069 (S=O stretch); ¹H NMR (600 MHz, CDCl₃): $\delta_{\text{H}} = 3.28$ (s, 3H, **21-H**), 7.52-7.55 (m, 2H, **15,16-H**), 7.85-7.88 (m, 1H, **14-H**), 7.93-7.95 (m, 2H, **12,17-H**), 8.03 (dd, $J = 8.5, 1.6$ Hz, 1H, **11-H**), 8.11 (d, $J = 4.4$ Hz, 1H, **6-H**), 8.19 (bs, 1H, **5-H**), 8.19 (bs, 1H, **3-H**), 8.51 (s, 1H, **19-H**); ¹³C NMR (150 MHz, CDCl₃): $\delta_{\text{C}} = 39.8$ (C-**21**), 110.6 (C-**3**), 120.0 (C-**5**), 124.1 (C-**11**), 126.1 (C-**19**), 126.8 (C-**15**), 126.9 (C-**16**), 128.0 (C-**14**), 128.6 (C-**17**), 128.9 (C-**12**), 129.4 (C-**10**), 129.8 (C-**6**), 133.4 (C-**13**), 133.6 (C-**18**), 136.6 (C-**9**), 148.5 (C-**2**), 155.8 (C-**8**); LRMS m/z (EI⁺): 307 [M]⁺, 291 [M-O]⁺, 245 [M-S(O)Me]⁺.

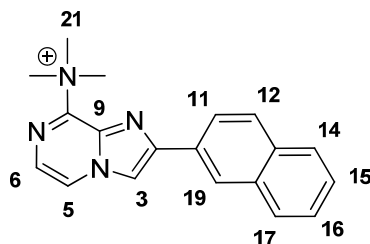
***N,N*-Dimethyl-2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-amine (230)**



214 (50 mg, 0.179 mmol) was dissolved in anhydrous DMSO (3 mL) in a sealed tube. 4.2 N Me₃N/EtOH (100 μ L, 0.358 mmol) was added and the mixture was stirred at 60 °C for 16 h. The solvent was removed and purification *via* flash chromatography (applied in CH₂Cl₂; eluted 24:1 CH₂Cl₂/EtOAc) afforded the title compound as an off white solid (38.0 mg, 0.132 mmol, 73%). $R_f = 0.31$ (5:1 CH₂Cl₂/EtOAc); ¹H NMR (600 MHz, (CD₃)₂SO): $\delta_{\text{H}} = 3.56$ (s, 6H, **21-H**), 7.33 (d, $J = 4.4$ Hz, 1H, **6-H**), 7.49-7.55 (m, 2H, **15,16-H**), 7.84 (d, $J = 4.4$ Hz, 1H, **5-H**), 7.92 (d, $J = 7.9$ Hz, 1H, **14-H**), 7.99 (d, $J = 8.3$ Hz, 2H, **12,17-H**), 8.10 (dd, $J = 8.5, 1.6$ Hz, 1H, **11-H**), 8.49 (bs, 2H, **3,19-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): $\delta_{\text{C}} = 39.3$ (C-**21**), 110.6 (C-**5**), 111.5 (C-**3**), 123.7 (C-**19**), 124.0 (C-**11**), 126.0 (C-**15**), 126.5 (C-**16**), 127.7 (C-**14**), 128.0 (C-**6**), 128.1 (C-**12**),

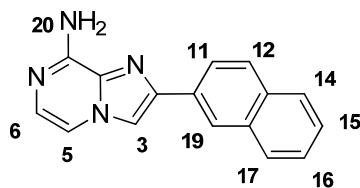
128.3 (C-17), 130.9 (C-10), 132.7 (C-13), 133.2 (C-18), 133.7 (C-9), 142.2 (C-2), 149.4 (C-8); LRMS m/z (ES^+): 289 $[M+H]^+$; HRMS m/z (ES^+): Found 289.1446 $[M+H]^+$; $C_{18}H_{17}N_4$ requires 289.1453.

***N,N,N*-Trimethyl-2-(naphthalen-2-yl)imidazo[1,2-*a*]pyrazin-8-aminium (229)**



214 (50 mg, 0.179 mmol) was dissolved in anhydrous DMF (3 mL) in a sealed tube. 4.2 N $Me_3N/EtOH$ (100 μL , 0.358 mmol) was added and the mixture was stirred at RT for 16 h. The solvent was removed and purification *via* flash chromatography (applied in CH_2Cl_2 ; eluted 1:1 $CH_2Cl_2/EtOAc$ to 100% MeOH) afforded the title compounds as a sticky pale brown solid (5.20 mg, 0.017 mmol, 10%). R_f = 0.25 (1:1 $CH_2Cl_2/MeOH$); 1H NMR (600 MHz, $(CD_3)_2SO$): δ_H = 3.94 (s, 6H, **21-H**), 7.58-7.60 (m, 2H, **15,16-H**), 7.98-8.00 (m, 2H, **6,17-H**), 8.07-8.10 (m, 2H, **12,14-H**), 8.25 (dd, J = 8.5, 1.6 Hz, 1H, **11-H**), 8.72 (s, 1H, **19-H**), 9.00 (d, J = 4.3 Hz, 1H, **5-H**), 9.06 (s, 1H, **3-H**); ^{13}C NMR (150 MHz, $(CD_3)_2SO$): δ_C = 53.8 (**C-21**), 113.5 (**C-3**), 123.9 (**C-5**) 124.2 (**C-11**), 125.5 (overlapping signals, **C-6,19**), 126.9 (overlapping signals, **C-15,16**), 127.9 (**C-17**), 128.4 (**C-14**), 128.8 (**C-12**), 129.4 (**C-10**), 132.4 (**C-9**), 133.1 (**C-13**), 133.3 (**C-18**), 144.7 (**C-8**), 146.5 (**C-2**); LRMS m/z (ES^+): 303 $[M]^+$; HRMS m/z (ES^+): Found 303.1603 $[M]^+$; $C_{19}H_{19}N_4$ requires 303.1610.

***2*-(Naphthalen-2-yl)imidazo[1,2-*a*]pyrazin-8-amine (231)**



Method A:

In a sealed tube, **227** (21.0 mg, 0.065 mmol) was dissolved in 2.0 M NH₃/IPA (2 mL), and the mixture was heated at 100 °C for 16 h. Removal of solvent *in vacuo* followed by flash chromatography (applied in CH₂Cl₂; eluted 1% to 10% MeOH) afforded the title compound as a sticky yellow solid (3.40 mg, 0.013 mmol, 20%). Mpt: >200 °C; *R_f* = 0.49 (20:1 CH₂Cl₂/MeOH); IR (ν_{max}/cm⁻¹, thin film): 3314, 2956-3055 (N-H and aromatic C-H stretches), 1620 (aromatic C=C stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 6.10 (bs, 2H, **20-H**), 7.29 (d, *J* = 4.7 Hz, 1H, **6-H**), 7.47-7.52 (m, 2H, **15,16-H**), 7.54 (d, *J* = 4.7 Hz, 1H, **5-H**), 7.85 (d, *J* = 8.4 Hz, 1H, **17-H**), 7.90 (d, *J* = 1.0 Hz, 2H, **12,14-H**), 7.92 (s, 1H, **3-H**), 8.00 (d, *J* = 8.5 Hz, 1H, **11-H**), 8.44 (s, 1H, **19-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 111.4 (**C-3**), 112.8 (**C-5**), 123.8 (**C-11**), 125.2 (**C-19**), 126.6 (**C-15**), 126.7 (**C-16**), 127.8 (signals overlapping, **C-6,17**), 128.4 (**C-12**), 128.8 (**C-14**), 129.4 (**C-10**), 133.5 (signals overlapping, **C-9,13,18**), 146.1(**C-8**), 148.4 (**C-2**); LRMS *m/z* (EI⁺): 260 [M]⁺; HRMS *m/z* (CI⁺): Found 261.1141 [M+H]⁺; C₁₆H₁₃N₄ requires 261.1140.

Method B:

In a sealed tube **214**, and any further reagents were taken up in solvent and the reaction was heated at the stated temperature for the given time. The reaction mixture was allowed to cool to RT and the top was carefully unscrewed to relieve the pressure. The contents were transferred to a round bottom flask and the solvent removed *in vacuo*. The compound was purified using flash chromatography (applied in CH₂Cl₂; eluted 1:1 CH₂Cl₂, followed by MeOH flush).

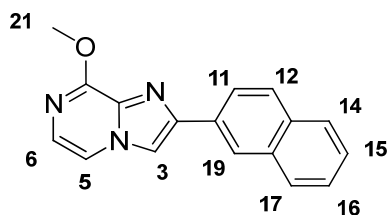
Method C:

All glassware was flushed with Ar prior to use. **214** (100 mg, 0.358 mmol) was dissolved in dry THF (5 mL). Lithium amide (8.21 mg, 0.358 mmol) was added and the reaction was stirred under reflux for 22 h. The mixture was left to cool to RT and solvent was removed *in vacuo*. Brine (20 mL) was added, and was extracted with CHCl₃ (3 x 30 mL), combined, dried (MgSO₄) and solvent removed. NMR indicated that no reaction had occurred and so no further work was carried out.

Entry	Method	Catalyst	Ligand	Base	Solvent	Temp/°C	Time/h	Yield/%
1	A	-	-	-	2 M NH ₃ /IPA	RT	16	0
2	A	-	-	-	2 M NH ₃ /IPA	100	16	20
3	A	-	-	-	7 M NH ₃ /MeOH	80	16	27
4	B	-	-	-	2 M NH ₃ /EtOH	120	24	20
5	B				NH ₄ OH (sat. aq.)	90	16	7
6	B			NH ₄ OH (sat. aq.)	IPA	90	16	Trace
7	B			NH ₄ OAc	2 M NH ₃ /IPA	90	72	Trace
8	B	Pd(dba) ₂ (1 mol%)	DavePhos (1.2 mol%)	LHMDS (3 eq)	dioxane	100	48	0
9	B	Pd(dba) ₂ (0.5 mol%)	^t Bu-XPhos (1.2 mol%)	NHMDS (1.2 eq)	THF	65	16	0
10	C	-	-	LiNH ₂ (1 eq)	THF	Reflux	16	0

Table 33: Variation in reaction conditions for formation of **231**

8-Methoxy-2-(naphthalen-2-yl)imidazo[1,2-a]pyrazine (232)



227 (19.8 mg, 0.061 mmol) and 7.0 M NH₃/MeOH (2 mL) were stirred in a sealed tube at 80 °C for 16 h. Removal of the solvent *in vacuo* followed by flash chromatography (applied in CH₂Cl₂; eluted 0.5% MeOH) afforded the title compound as a white solid (16.5 mg, 0.06 mmol, 98%). Mpt: 184 °C; *R_f* = 0.76 (5% MeOH/CH₂Cl₂); IR (ν_{max}/cm⁻¹, thin film): 3138, 3054 (aromatic C-H stretches), 2949 (C-H stretch), 1525, 1504 (aromatic C=C and C=N stretches), 1325 (aromatic C-O stretch), 1130 (C-O stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 4.21 (s, 3H, **21-H**), 7.40 (d, *J* = 4.6 Hz, 1H, **6-H**), 7.47-7.51 (m, 2H, **15,16-H**), 7.74 (d, *J* = 4.6 Hz, 1H, **5-H**), 7.84-7.85 (m, 1H,

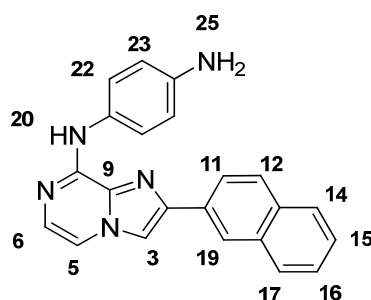
17-H), 7.89-7.90 (m, 1H, **12-H)**, 7.92-7.93 (m, 1H, **14-H)**, 8.00, (s, 1H, **3-H)**, 8.03 (dd, $J = 8.5$, 1.5 Hz, 1H, **11-H)**, 8.60 (s, 1H, **19-H)**; ^{13}C NMR (150 MHz, CDCl_3): $\delta_{\text{C}} = 54.4$ (**C-21**), 111.0 (**C-3**), 114.4 (**C-5**), 124.2 (**C-11**), 125.2 (**C-19**), 126.4 (**C-15**), 126.5 (**C-16**), 126.7 (**C-6**), 127.8 (**C-17**), 128.5 (**C-12**), 128.5 (**C-14**), 130.1 (**C-10**), 133.5 (**C-13**), 133.7 (**C-18**), 133.9 (**C-9**), 149.0 (**C-2**), 154.8 (**C-8**); LRMS m/z (ES^+): 276 $[\text{M}+\text{H}]^+$; HRMS m/z (ES^+): Found 276.1144 $[\text{M}+\text{H}]^+$; $\text{C}_{17}\text{H}_{14}\text{N}_3\text{O}$ requires 276.1137.

6.1.3 Synthesis of 2nd Generation Compounds

6.1.3.1 Imidazo[1,2-*a*]pyrazine Compounds

6.1.3.1.1 Variations in Position 8

*N*¹-(2-(Naphthalen-2-yl)imidazo[1,2-*a*]pyrazin-8-yl)benzene-1,4-diamine (233)



Method A:

All glassware was dried and purged with Ar prior to use. $\text{Pd}_2(\text{dba})_3$ (9.17 mg, 1 mol%), DavePhos (11.8 mg, 3 mol%) and NaO^tBu (135 mg, 1.402 mmol) were dissolved in anhydrous toluene (10 mL). **214** (280 mg, 1.00 mmol) and 1,4-diaminobenzene (130 mg, 1.20 mmol) were added and the reaction was stirred under reflux, under Ar for 16 h. The reaction was cooled to RT and solvent removed *in vacuo*, before the residue was taken up in CH_2Cl_2 (100 mL) and washed with H_2O (3 x 50 mL) and brine (30 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Flash Chromatography (applied in CH_2Cl_2 ; eluted 15:1 to 5:1 to 1:1) afforded the title compound as a sticky brown solid (95.0 mg, 0.271 mmol, 27%). $R_f = 0.22$ (1:1 $\text{CH}_2\text{Cl}_2/\text{EtOAc}$); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3335, 3051 (C-H and N-H stretches), 1622, 1541, 1507 (aromatic C=C and C=N stretches); ^1H NMR (600 MHz, CDCl_3): $\delta_{\text{H}} = 3.71$ (bs, 2H, **25-H**), 6.76 (ap.d, $J = 8.7$ Hz, 2H, **23-H**), 7.42 (d, $J = 4.6$ Hz, 1H, **6-H**), 7.48-7.53 (m, 3H, **5,15,16-H**), 7.61 (d, $J = 9.5$ Hz,

2H, **22-H**), 7.86 (d, $J = 7.5$ Hz, 1H, **14-H**), 7.90 (s, 1H, **3-H**), 7.92-7.94 (m, 3H, **12,17,20-H**), 8.02 (dd, $J = 8.5, 1.7$ Hz, 1H, **11-H**), 8.48 (s, 1H, **19-H**); ^{13}C NMR (150 MHz, CDCl_3): $\delta_{\text{C}} = 110.8$ (**C-5**), 111.0 (**C-3**), 115.8 (**C-23**), 122.4 (**C-22**), 124.1 (**C-11**), 124.9 (**C-19**), 126.3 (**C-15,16**), 127.9 (**C-14**), 128.4 (overlapping signals, **C-12,17**), 128.7 (**C-6**), 130.6 (overlapping signals, **C-10,21**), 133.4 (**C-13**), 133.7 (**C-9**), 133.8 (**C-18**), 142.7 (**C-24**), 144.7 (**C-2**), 146.8 (**C-8**); LRMS m/z (ES^+): 352 $[\text{M}+\text{H}]^+$, 338, 181; HRMS m/z (ES^+): Found 352.1563 $[\text{M}+\text{H}]^+$; $\text{C}_{22}\text{H}_{18}\text{N}_5$ requires 352.1562.

Method B (Microwave):

In a 2 mL microwave vial, catalyst (1 mol%), ligand (3 mol%) and base (1.4 eq) were dissolved in anhydrous solvent. **214** and 1,4-diaminobenzene (1.2 eq) were added and the reaction mixture was stirred at 160 °C for 10 min with a 20 bar pressure limit. The solvent was removed *in vacuo* and the crude mixture was purified *via* flash chromatography (applied in CH_2Cl_2 ; eluted 10% to 20% to 50% EtOAc) to afford the title compound.

Method C (Microwave):

In a 2 mL microwave vial, **214**, 1,4-diaminobenzene (1.2 eq) and base (1.5 eq) were dissolved in stated solvent and the reaction mixture was stirred at 160 °C for 10 min with a 20 bar pressure limit. The solvent was removed *in vacuo* and the crude mixture was purified *via* flash chromatography (applied in CH_2Cl_2 ; eluted 10% to 20% to 50% EtOAc) to afford the title compound.

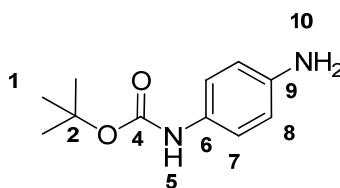
Method D (Microwave):

In a 2 mL microwave vial, **227**, 1,4-diaminobenzene (2.0 eq) and base (2.0 eq) were dissolved in stated solvent and the reaction mixture was stirred at 160 °C for 10 min with a 20 bar pressure limit. The solvent was removed *in vacuo* and the crude mixture was purified *via* flash chromatography (applied in CH_2Cl_2 ; eluted 10% to 20% to 50% EtOAc) to afford the title compound.

Exp	Method	Catalyst	Ligand	Base	Solvent	Temp/°C	Time/h	Yield/%
1	A	Pd(dba) ₂	DavePhos	Cs ₂ CO ₃	dioxane	Reflux	40	17
2	A	Pd ₂ (dba) ₃	DavePhos	NaO ^t Bu	toluene	Reflux	16	27
3	B	Pd ₂ (dba) ₃	DavePhos	NaO ^t Bu	toluene	160	10	32
4	B	Pd(PPh ₃) ₄ ^a	-	NaO ^t Bu	toluene	160	10	0
5	B	Pd(dppf)Cl ₂ ^a	-	NaO ^t Bu	toluene	160	10	20
6	C	-	-	DIPEA	MeCN	160	10	0
7	D	-	-	DIPEA	MeCN	160	10	0

Table 34: Different reaction conditions used for the formation of 233/212. ^a 5 mol% used

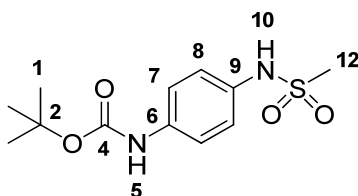
***tert*-Butyl 4-aminophenylcarbamate (235)²⁶⁰**



Benzene-1,4-diamine (6.49 g, 60 mmol) was dissolved in THF/DMF (3:1, 80 mL). K₂CO₃ (3.04 g, 22.0 mmol) in H₂O (10 mL) was added followed by Boc₂O (4.37 g, 20.0 mmol) portionwise. The reaction was stirred at RT for 5 h before pouring into cold H₂O (100 mL) and extracting with CHCl₃ (4 x 70 mL). The combined organic extracts were washed with Brine (70 mL), dried (MgSO₄), filtered and solvent removed to give crude brown/red oil. Flash chromatography (applied in CH₂Cl₂; eluted 1% to 2% to 5% acetone) afforded the title compound as a yellow solid (3.91 g, 18.8 mmol, 94%) with the NMR consistent with literature values.³⁰² Mpt: 94-96°C [Lit.³⁰³ 114-116°C]; *R_f* = 0.6 (20% acetone/CH₂Cl₂); IR (ν_{max}/cm⁻¹, thin film): 3365 (N-H stretch), 2970 (C-H stretch), 1738 (C=O stretch), 1693 (N-H bend), 1513, 1233; ¹H NMR (600 MHz, CDCl₃): δ_H = 1.49 (s, 9H, **1-H**), 3.52 (bs, 2H, **10-H**), 6.35 (bs, 1H, **5-H**), 6.60-6.63 (m, 2H, **8-H**), 7.12 (bs, 2H, **7-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 28.5 (**C-1**), 80.1 (**C-2**), 115.7 (**C-8**), 121.0 (**C-7**), 129.8 (**C-6**), 142.5 (**C-9**), 153.4 (**C-4**); LRMS *m/z* (EI⁺): 208 [M]⁺, 152 [M-^tBu]⁺,

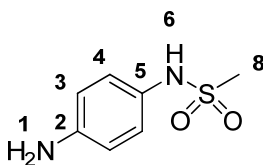
108 [M-Boc]⁺; HRMS m/z (EI⁺): Found 208.1210 [M]⁺; C₁₁H₁₆N₂O₂ requires 208.1206; Anal. Calcd. for C₁₁H₁₆N₂O₂: C, 63.44; H, 7.74; N, 13.45. Found C, 63.13; H, 7.81; N, 13.29%.

'Butyl 4-(methanesulfonamido)phenylcarbamate (236)



235 (500 mg, 2.40 mmol) was dissolved in anhydrous CH₂Cl₂ (20 mL), Et₃N (186 μL, 2.40 mmol) added and the yellow solution was cooled on ice. MeSO₂Cl (670 μL, 4.80 mmol) was added dropwise and the reaction was left to stir at RT for 16 h (a precipitate formed after 30 min of stirring). The mixture was diluted with H₂O (20 mL) and organics extracted (3 x 30 mL). The combined extracts were washed with H₂O (20 mL) and brine (20 mL), dried (MgSO₄), filtered and solvent removed *in vacuo*. Flash chromatography (applied in CH₂Cl₂; eluted 0% to 1% to 2% to 5% acetone) afforded the title compound as an off white solid (173 mg, 0.604 mmol, 25%), with NMR consistent with literature values.³⁰⁴ Mpt: 190 °C [Lit.³⁰⁴ 194-196 °C]; *R_f* = 0.39 (10% acetone/CH₂Cl₂); IR (ν_{max}/cm⁻¹, thin film): 3338 (N-H stretch), 3178 (aromatic C-H stretch), 2978 (C-H stretch), 1698 (C=O stretch), 1527 (N-H bend), 1321 (S=O asymmetric stretch), 1147 (S=O symmetric stretch); ¹H NMR (600 MHz, CD₃OD): δ_H = 1.51 (s, 9H, **1-H**), 2.90 (s, 3H, **12-H**), 7.15-7.18 (m, 2H, **8-H**), 7.37 (d, *J* = 8.5 Hz, 2H, **7-H**); ¹³C NMR (150 MHz, CD₃OD): δ_C = 28.7 (**C-1**), 38.8 (**C-12**), 80.9 (**C-2**), 120.6 (**C-7**), 123.5 (**C-8**), 133.9 (**C-9**), 138.0 (**C-6**), 155.3 (**C-4**); LRMS m/z (ES⁻): 285 [M-H]⁻, 255, 148; HRMS m/z (ES⁻): Found 285.0900 [M-H]⁻; C₁₂H₁₇N₂O₄S requires 285.0909; Anal. Calcd. for C₁₂H₁₈N₂O₄S: C, 50.33; H, 6.34; N, 9.78. Found C, 50.17; H, 6.30; N, 9.50%.

***N*-(4-Aminophenyl)methanesulfonamide 237**



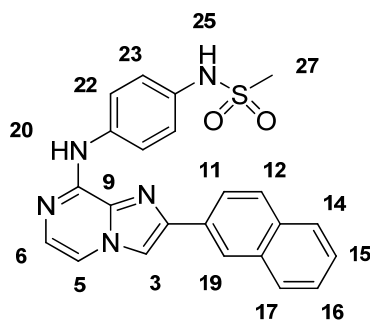
Method A:

Benzene-1,4-diamine (3.14 g, 29.0 mmol), was dissolved in anhydrous CH₂Cl₂ (100 mL), Et₃N (1.62 mL, 11.61 mmol) added and the mixtures cooled on ice. MeSO₂Cl (0.449 mL, 5.81 mmol) was added dropwise and the reaction was stirred at RT for 16 h. The mixture was then diluted with CH₂Cl₂ (50 mL) and washed NaHCO₃ (sat. aq. 50 mL). The aqueous layer was then extracted with CH₂Cl₂ (4 x 50 mL) as well as EtOAc (4 x 50 mL). Each of the organic extracts were washed with brine (50 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in CH₂Cl₂; eluted 1% to 2% to 5% MeOH) afforded the title compound as a white solid (844 mg, 4.54 mmol, 78%) with NMR consistent with literature values.³⁰⁵ Mpt: 89 °C [Lit.³⁰⁵ 116-117 °C]; *R_f* = 0.53 (10% MeOH/CH₂Cl₂); IR (ν_{max} /cm⁻¹, thin film): 3459, 3375, 3330 (C-H and N-H stretches), 1625, 1518 (N-H bend), 1309 (S=O asymmetric stretch), 1152 (S=O symmetric stretch); ¹H NMR (600 MHz, CD₃OD): δ_{H} = 2.87 (s, 3H, **8-H**), 6.71-6.73 (m, 2H, **3-H**), 7.02-7.05 (m, 2H, **4-H**); ¹³C NMR (150 MHz, CD₃OD): δ_{C} = 36.8 (**C-8**), 115.4 (**C-3**), 124.4 (**C-4**), 127.5 (**C-5**), 145.8 (**C-2**); LRMS *m/z* (CI⁺): 187 [M+H]⁺, 107; HRMS *m/z* (CI⁺): Found 187.0539 [M+H]⁺; C₇H₁₁N₂O₂S requires 187.0541.

Method B:

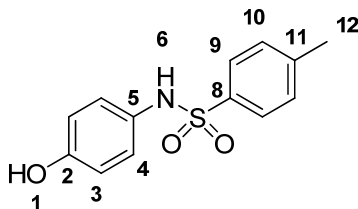
236 (164 mg, 0.582 mmol) was dissolved in CH₂Cl₂/TFA (1:1, 10 mL) and stirred at RT for 16 h. Removal of the solvent *in vacuo*, *via* trituration with MeOH, gave the crude product which upon purification by flash chromatography (applied in CH₂Cl₂; eluted 0% to 20% acetone) afforded the title compound as a brown oil/solid (123 mg, 0.409 mmol, 70%).

N-(4-(2-(naphthalen-2-yl)imidazo[1,2-*a*]pyrazin-8-ylamino)phenyl)methanesulfonamid (**238**)



All glassware was dried and purged with Ar prior to use. $\text{Pd}_2(\text{dba})_3$ (1.64 mg, 1 mol%), DavePhos (2.11 mg, 3 mol%) and NaO^tBu (24.1 mg, 0.250 mmol) were dissolved in anhydrous toluene. **214** (50.0 mg, 0.179 mmol) and **237** (39.9 mg, 0.215 mmol) were added and the reaction was stirred under reflux, under Ar for 20 h. The reaction was cooled to RT and solvent removed *in vacuo*, before the residue was taken up in CH_2Cl_2 (50 mL) and washed with NaHCO_3 (30 mL), H_2O (30 mL) and brine (30 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Flash chromatography (applied in toluene; eluted 2:1 toluene/EtOAc) afforded the title compound as an off white solid (5.2 mg, 0.012 mmol, 7%). Mpt: Decomposed before melting; R_f = 0.32 (1:1 toluene/EtOAc); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3248, 3056, 2926, 2854 (aromatic C-H and N-H stretch), 1624 (N-H bend), 1543 (aromatic C=C symmetric and C=N stretches), 1508 (aromatic C=C asymmetric stretch), 1326 (S=O asymmetric stretch), 1152 (S=O symmetric stretch); ^1H NMR (600 MHz, CDCl_3): δ_{H} = 3.01 (s, 3H, **27-H**), 6.39 (s, 1H, **25-H**), 7.27-7.29 (m, 2H, **23-H**), 7.47 (d, J = 4.6 Hz, 1H, **6-H**), 7.49-7.54 (m, 2H, **15,16-H**), 7.60 (d, J = 4.6 Hz, 1H, **5-H**), 7.87 (d, J = 7.8 Hz, 1H, **14-H**), 7.92-7.95 (m, 5H, **3,12,17,22-H**), 8.02 (dd, J = 8.5, 1.6 Hz, 1H, **11-H**), 8.18 (s, 1H, **20-H**), 8.48 (s, 1H, **19-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = 39.3 (**C-27**), 111.2 (**C-3**), 111.9 (**C-5**), 128.2 (**C-6**), 120.8 (**C-22**), 123.3 (**C-23**), 124.1 (**C-11**), 125.0 (**C-19**), 126.4 (**C-15**), 126.6 (**C-16**), 127.9 (**C-14**), 128.2 (**C-17**), 128.8 (**C-12**), 130.4 (**C-10**), 131.1 (**C-24**), 133.4 (**C-13**), 133.7 (overlapping signals, **C-9,18**), 137.7 (**C-21**), 145.1 (**C-2**), 146.1 (**C-8**); LRMS m/z (ES^+): 430 $[\text{M}+\text{H}]^+$; HRMS m/z (ES^+): Found 430.1324 $[\text{M}+\text{H}]^+$; $\text{C}_{23}\text{H}_{30}\text{N}_5\text{O}_2\text{S}$ requires 430.1338.

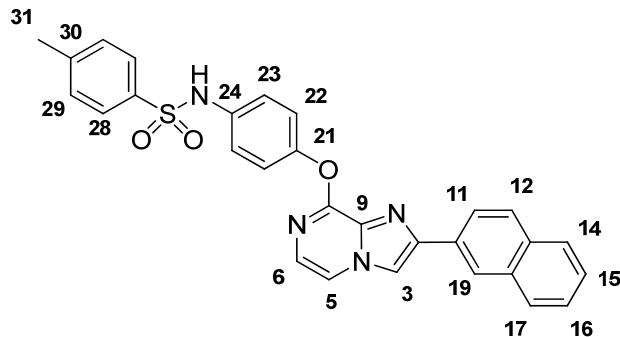
N-(4-Hydroxyphenyl)-4-methylbenzenesulfonamide (**240**)¹⁸⁰



A solution of 4-aminophenol (500 mg, 4.58 mmol) in anhydrous pyridine (9 mL) was cooled on ice and 4-methylbenzene-1-sulfonyl chloride (1.05 g, 5.50 mmol) was added portionwise. The resulting red solution was stirred under Ar at RT for 18 h, before diluting with Et_2O (100 mL) and washing with H_2O (50 mL), 5% HCl (50 mL), H_2O (50 mL) and brine (30 mL); drying

(MgSO₄) filtering and concentrating *in vacuo*. Flash chromatography (applied in toluene; eluted 5:1 to 4:1 toluene/Et₂O) afforded the title compound as a pale yellow solid (934 mg, 3.55 mmol, 78%) with the NMR consistent with literature values.¹⁸⁰ Mpt: 134-136 °C [Lit.¹⁸⁰ 144-146 °C]; *R_f* = 0.33 (2:1 toluene/Et₂O); IR (ν_{max} /cm⁻¹, thin film): 3445 (O-H stretch), 3234 (aromatic C-H stretch), 1509 (N-H bend), 1263 (S=O asymmetric stretch), 1153 (S=O symmetric stretch), 1090 (C-OH stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_{H} = 2.38 (s, 3H, **12-H**), 6.57-6.60 (m, 2H, **3-H**), 6.81-6.83 (m, 2H, **4-H**), 7.31 (d, *J* = 8.1 Hz, 2H, **10-H**), 7.53 (d, *J* = 8.1 Hz, 2H, **9-H**), 9.29 (s, 1H, **1-H**), 9.66 (bs, 1H, **6-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_{C} = 21.0 (**C-12**), 115.5 (**C-3**), 123.9 (**C-4**), 126.8 (**C-9**), 129.5 (**C-10**), 136.7 (**C-8**), 142.8 (**C-11**), 154.8 (**C-2**) 128.6 (**C-5**); LRMS *m/z* (ES⁻): 262 [M-H]⁻, 308 [M+Formic Acid]⁺; HRMS *m/z* (ES⁻): Found 262.0545 [M-H]⁻; C₁₃H₁₂NO₃S requires 262.0538; Anal. Calcd. for C₁₃H₁₃NO₃S: C, 59.30; H, 4.98; N, 5.32. Found C, 59.01; H, 4.73; N, 5.26%.

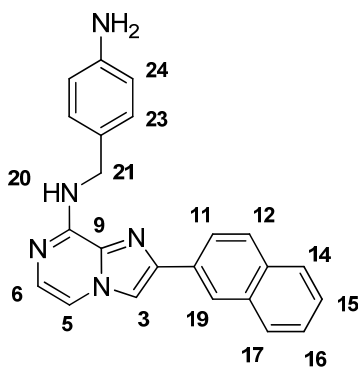
4-Methyl-N-(4-(2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-yloxy)phenyl)benzenesulfonamide (241)



NaH was washed by stirring NaH (60% in mineral oil; 12.4 mg, 0.310 mmol) in anhydrous hexanes (3 mL) for 20 min, removing the solvent using a syringe and drying the contents under high vacuum. DMF (0.5 mL) was added followed by **240** (81.4 mg, 0.310 mmol) in DMF (1 mL) and the mixture was stirred at RT for 20 min. **227** (50.0 mg, 0.155 mmol) in DMF (1.5 mL) was added and the resulting deep red solution was heated at 100 °C under Ar for 16 h. The mixture was then diluted with EtOAc (50 mL) and washed with NH₄Cl (sat. aq. 20 mL) and H₂O (5 x 20 mL). The combined aqueous layers were then re-extracted with EtOAc (2 x 30 mL), followed by washing the combined organics with brine (30 mL), drying (MgSO₄), filtering and concentrating *in vacuo*. The crude material was purified *via* flash chromatography (applied in

toluene; eluted 25% EtOAc/toluene) to give the title compound as an off white-pink solid (36.4 mg, 0.072 mmol, 47%). Mpt: 128 °C; R_f = 0.21 (2:1 toluene/EtOAc); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3568, 3049 (aromatic C-H stretch), 1488 (N-H bend), 1330 (S=O asymmetric stretch), 1153 (S=O symmetric stretch and aromatic C-O stretch); ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{H} = 2.39 (s, 3H, **31-H**), 7.15-7.17 (m, 2H, **23-H**), 7.21-7.13 (m, 2H, **22-H**), 7.33 (d, J = 4.6 Hz, 1H, **6-H**), 7.39 (d, J = 8.2 Hz, 2H, **29-H**), 7.52-7.56 (m, 2H, **15,16-H**), 7.70 (d, J = 8.2 Hz, 2H, **28-H**), 7.94 (d, J = 7.6 Hz, 1H, **17-H**), 8.01-8.04 (m, 2H, **12,14-H**), 8.15 (dd, J = 8.5, 1.6 Hz, 1H, **11-H**), 8.32 (d, J = 4.6 Hz, 1H, **5-H**), 8.61 (s, 1H, **19-H**), 8.74 (s, 1H, **3-H**), 10.34 (s, 1H, **25-H**); ^{13}C NMR (150 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{C} = 21.0 (**C-31**), 112.9 (**C-3**), 116.9 (**C-5**), 121.3 (**C-23**), 122.7 (**C-22**), 124.0 (**C-11**), 124.5 (**C-19**), 125.5 (**C-6**), 126.4 (**C-15**), 126.6 (**C-16**), 126.8 (**C-28**), 127.7 (**C-17**), 128.3 (**C-14**), 128.5 (**C-12**), 129.9 (**C-29**), 130.4 (**C-10**), 132.8 (**C-9**), 132.9 (**C-13**), 133.2 (**C-18**), 135.0 (**C-24**), 136.7 (**C-27**), 143.4 (**C-30**), 145.1 (**C-2**), 148.7 (**C-21**), 153.1 (**C-8**); LRMS m/z (ES $^-$): 505 $[\text{M-H}]^-$; HRMS m/z (ES $^-$): Found 505.1323 $[\text{M+H}]^+$; $\text{C}_{29}\text{H}_{23}\text{N}_4\text{O}_3\text{S}$ requires 505.1334.

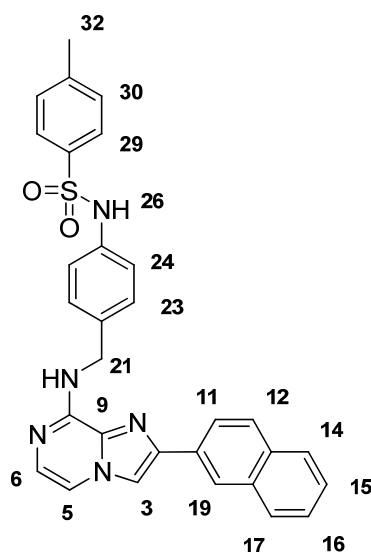
***N*-(4-Aminobenzyl)-2-(naphthalen-2-yl)imidazo[1,2-*a*]pyrazin-8-amine (243)**



All glassware was dried and purged with Ar prior to use. $\text{Pd}_2(\text{dba})_3$ (1.64 mg, 1 mol%), DavePhos (2.11 mg, 3 mol%) and NaO^tBu (24.1 mg, 0.250 mmol) were dissolved in anhydrous toluene (2 mL). **214** (50.0 mg, 0.179 mmol) and 4-(aminomethyl)aniline (24.3 μL , 0.215 mmol) were added and the reaction was stirred under reflux, under Ar for 20 h. The reaction was cooled to RT and solvent removed *in vacuo*, before the residue was taken up in CH_2Cl_2 (50 mL) and washed with NaHCO_3 (sat. aq. 30 mL), H_2O (30 mL) and brine (30 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 4:1 to 3:1 to 2:1 to 1:1 to 1:3 pet. ether/EtOAc) afforded the title compound as a light yellow oil (20.1 mg, 0.055

mmol, 31%). $R_f = 0.52$ (3:1 EtOAc/pet. ether); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3326 (aromatic C-H stretch), 1619 (N-H bend), 1544, 1519 (aromatic C=C and C=N stretches); ^1H NMR (600 MHz, CD_3OD): $\delta_{\text{H}} = 4.58$ (s, 2H, **21-H**), 6.71-6.74 (m, 2H, **24-H**), 7.19 (d, $J = 8.4$ Hz, 2H, **23-H**), 7.25 (d, $J = 4.7$ Hz, 1H, **6-H**), 7.44-7.49 (m, 2H, **15,16-H**), 7.65 (d, $J = 4.7$ Hz, 1H, **5-H**), 7.82-7.84 (m, 1H, **14-H**), 7.85-7.88 (m, 2H, **12,17-H**), 7.94 (dd, $J = 8.5, 1.6$ Hz, 1H, **11-H**), 8.16 (s, 1H, **3-H**), 8.34 (s, 1H, **19-H**); ^{13}C NMR (150 MHz, CD_3OD): $\delta_{\text{C}} = 45.5$ (**C-21**), 111.5 (**C-5**), 113.1 (**C-3**), 116.7 (**C-24**), 124.9 (**C-11**), 125.5 (**C-19**), 127.2 (**C-15**), 127.5 (**C-16**), 128.7 (**C-14**), 129.1 (**C-6**), 129.2 (**C-17**), 129.2 (**C-22**), 129.5 (**C-12**), 129.9 (**C-23**), 131.7 (**C-10**), 134.6 (overlapping signals, **C-9,13**), 135.0 (**C-18**), 145.4 (**C-2**), 148.1 (**C-25**), 149.8 (**C-8**); LRMS m/z (ES^+): 366 $[\text{M}+\text{H}]^+$, 273 $[\text{M}-\text{aniline}]^+$, 261 $[\text{M}-\text{CH}_2-\text{aniline}]^+$; HRMS m/z (ES^+): Found 366.1716 $[\text{M}+\text{H}]^+$; $\text{C}_{23}\text{H}_{20}\text{N}_5$ requires 366.1719.

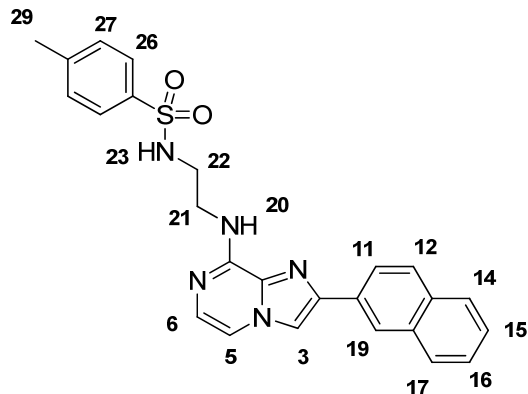
4-Methyl-N-(4-((2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-ylamino)methyl)phenyl)benzene-sulfonamide (244)



243 (18.0 mg, 0.049 mmol) was dissolved in anhydrous pyridine (1 mL) and the mixture was cooled on ice. 4-Methylbenzene-1-sulfonyl chloride (11.3 mg, 0.059 mmol) was added and the deep yellow/orange solution was stirred under Ar at RT for 16 h. The solvent was then removed *in vacuo* and the crude material was purified *via* flash chromatography (applied in CH_2Cl_2 ; eluted 10% to 20% EtOAc) to afford the title compound as pale green sticky solid (8.20 mg, 0.016 mmol, 32%). $R_f = 0.44$ (20% EtOAc/ CH_2Cl_2); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3240, 3050 (aromatic C-

H stretches), 2923, 2823 (C-H and N-H stretches), 1544 (N-H bend), 1509 (aromatic C=C and C=N stretches), 1332 (S=O asymmetric stretch), 1136 (S=O symmetric stretch); ^1H NMR (600 MHz, CDCl_3): δ_{H} = 2.47 (s, 3H, **32-H**), 4.76 (bs, 2H, **21-H**), 6.49 (bs, 1H, **20-H**), 6.73 (bs, 1H, **26-H**), 7.03 (d, J = 8.5 Hz, 2H, **24-H**), 7.22 (d, J = 8.2 Hz, 2H, **30-H**), 7.29 (d, J = 8.5 Hz, 2H, **23-H**), 7.35 (d, J = 4.5 Hz, 1H, **6-H**), 7.46-7.51 (m, 3H, **5,15,16-H**), 7.64 (d, J = 8.2 Hz, 2H, **29-H**), 7.83-7.85 (m, 1H, **14-H**), 7.87-7.90 (m, 3H, **3,12,17-H**), 7.95 (dd, J = 8.6, 1.2 Hz, 1H, **11-H**), 8.40 (s, 1H, **19-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = 21.7 (**C-32**), 44.2 (**C-21**), 110.3 (**C-5**), 111.0 (**C-3**), 122.0 (**C-24**), 124.0 (**C-11**), 124.8 (**C-19**), 126.3 (**C-15**), 126.6 (**C-16**), 127.4 (**C-29**), 127.9 (Overlapping signals, **C-12,17**), 128.7 (**C-6**), 129.0 (**C-23**), 129.8 (**C-30**), 130.5 (**C-10**), 133.3 (**C-13**), 133.6 (**C-9**), 133.7 (**C-18**), 135.6 (**C-22**), 135.8 (**C-25**), 136.2 (**C-28**), 144.0 (**C-31**), 144.6 (**C-2**), 148.8 (**C-8**); LRMS m/z (ES^-): 518 $[\text{M-H}]^-$; HRMS m/z (ES^-): Found 518.1658 $[\text{M-H}]^-$; $\text{C}_{30}\text{H}_{24}\text{N}_5\text{O}_2\text{S}$ requires 518.1651.

4-Methyl-N-(2-(2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-ylamino)ethyl)benzenesulfonamide (247)



Method A:

To a solution of **245** (65.9 mg, 0.310 mmol) in anhydrous DMF (2 mL) was added DIPEA (53.9 μL , 0.310 mmol) followed by **227** (50.0 mg, 0.155 mmol) and the reaction mixture was stirred at 100 $^{\circ}\text{C}$ for 16 h. Cooling to RT and removal of the solvent was followed by flash chromatography (applied in toluene; eluted 2:1 toluene/EtOAc), which yielded the title compound as a white solid (15 mg, 0.033 mmol, 21%). Mpt: 158-160 $^{\circ}\text{C}$; R_f = 0.26 (3:1 EtOAc/pet. ether); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3412 (N-H stretch), 3029, 2924 (aromatic C-H

stretches), 2853 (C-H stretch), 1621, 1538 (N-H bends), 1327 (S=O asymmetric stretch), 1158 (S=O symmetric stretch); ^1H NMR (600 MHz, CDCl_3): δ_{H} = 2.24 (s, 3H, **29-H**), 3.32 (bd, J = 4.7 Hz, 2H, **22-H**), 3.70-3.73 (m, 2H, **21-H**), 6.32 (bs, 1H, **20-H**), 6.55 (bs, 1H, **23-H**), 7.10 (d, J = 8.1 Hz, 2H, **27-H**), 7.29 (d, J = 4.6 Hz, 1H, **6-H**), 7.45 (d, J = 4.6 Hz, 1H, **5-H**), 7.48-7.52 (m, 2H, **15,16-H**), 7.63 (d, J = 8.1 Hz, 2H, **26-H**), 7.85-7.86 (m, 2H, **3,14-H**), 7.88-7.91 (m, 2H, **12,17-H**), 7.94 (d, J = 8.4 Hz, 1H, **11-H**), 8.37 (s, 1H, **19-H**); ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{H} = 2.30 (s, 3H, **29-H**), 3.03 (q, J = 6.3 Hz, 2H, **22-H**), 3.53 (q, J = 6.3 Hz, 2H, **21-H**), 7.23-7.24 (m, 3H, **6,27-H**), 7.45 (t, J = 5.9 Hz, 1H, **20-H**), 7.50-7.56 (m, 2H, **15,16-H**), 7.63 (d, J = 8.2 Hz, 2H, **26-H**), 7.67 (t, J = 5.5 Hz, 1H, **23-H**), 7.77 (d, J = 4.6 Hz, 1H, **5-H**), 7.94 (d, J = 7.9 Hz, 1H, **14-H**), 7.96 (d, J = 7.9 Hz, 1H, **17-H**), 8.00 (d, J = 8.6 Hz, 1H, **12-H**), 8.11 (dd, J = 8.6, 1.6 Hz, 1H, **11-H**), 8.46 (s, 1H, **3-H**), 8.52 (s, 1H, **19-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = 21.6 (**C-29**), 40.7 (**C-21**), 45.0 (**C-22**), 110.6 (**C-5**), 111.0 (**C-3**), 124.0 (**C-11**), 124.8 (**C-19**), 126.4 (**C-15**), 126.6 (**C-16**), 127.0 (**C-26**), 127.9 (**C-6,14**), 128.4 (**C-17**), 128.7 (**C-12**), 129.5 (**C-27**), 130.4 (**C-10**), 133.2 (**C-9**), 133.4 (**C-13**), 133.7 (**C-18**), 137.0 (**C-25**), 143.3 (**C-28**), 144.9 (**C-2**), 149.5 (**C-8**); ^{13}C NMR (150 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{C} = 21.0 (**C-29**), 40.1 (**C-21**), 41.6 (**C-22**), 110.3 (**C-5**), 112.0 (**C-3**), 123.8 (**C-19**), 124.1 (**C-11**), 126.1 (**C-15**), 126.6 (**C-16**), 126.6 (**C-26**), 127.7 (**C-14**), 128.0 (**C-17**), 128.1 (**C-6**), 128.3 (**C-12**), 129.4 (**C-27**), 131.0 (**C-10**), 132.6 (**C-13**), 132.9 (**C-9**), 133.2 (**C-18**), 137.3 (**C-25**), 142.4 (**C-28**), 143.0 (**C-2**), 148.4 (**C-8**); LRMS m/z (ES^+): 458 $[\text{M}+\text{H}]^+$; HRMS m/z (EI^+): Found 458.1656 $[\text{M}+\text{H}]^+$; $\text{C}_{25}\text{H}_{24}\text{N}_5\text{O}_2\text{S}$ requires 458.1651.

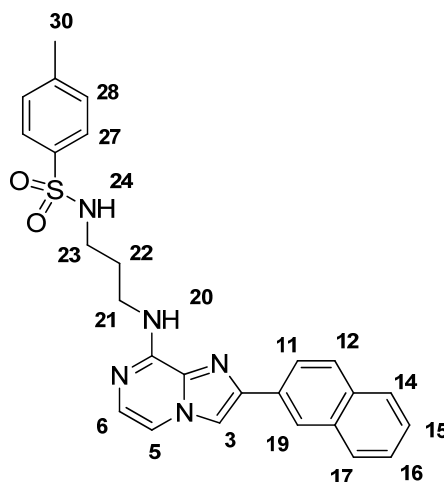
Method B:

All glassware was dried and purged with Ar prior to use. $\text{Pd}_2(\text{dba})_3$ (1.64 mg, 1 mol%), DavePhos (2.11 mg, 3 mol%) and NaO^tBu (24.1 mg, 0.250 mmol) were dissolved in anhydrous toluene (2 mL). **214** (50.0 mg, 0.179 mmol) and **245** (45.7 mg, 0.215 mmol) were added and the reaction was stirred under reflux, under Ar for 16 h. The reaction was cooled to RT and purification by flash chromatography (toluene; 10% to 20% to 50% EtOAc) afforded the title compound as an off-white solid (6.20 mg, 0.014 mmol, 8%).

Method C:

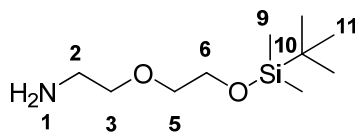
214 (50.0 mg, 0.179 mmol), **245** (57.2 mg, 0.268 mmol) and DIPEA (78.1 μL , 0.447 mmol) were stirred in $^n\text{BuOH}$ at 80 $^\circ\text{C}$ for 16 h. The reaction was cooled to RT and purification by flash chromatography (toluene; 10% to 20% to 50% EtOAc) afforded the title compound as an off-white solid (4.90 mg, 0.011 mmol, 6%).

4-Methyl-N-(3-(2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-ylamino)propyl)benzene-sulfonamide (248)



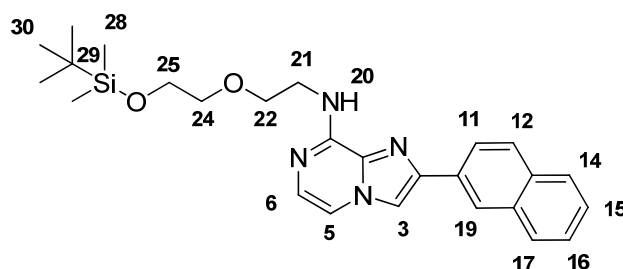
To a solution of **247** (73.1 mg, 0.322 mmol) in anhydrous DMF (2 mL) was added DIPEA (56.1 μ L, 0.322 mmol) followed by **227** (52.0 mg, 0.161 mmol) and the reaction mixture was stirred at 100 $^{\circ}$ C for 16 h. Cooling to RT and removal of the solvent was followed by flash chromatography (applied in toluene; eluted 2:1 toluene/EtOAc), which yielded the title compound as a white solid (24.9 mg, 0.053 mmol, 33%). Mpt: Decomposed before melting; R_f = 0.26 (3:1 EtOAc/pet. ether); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3023, 2971 (aromatic C-H and N-H stretches), 1740, 1548 (N-H bends), 1370 (S=O asymmetric stretch), 1217 (S=O symmetric stretch); ^1H NMR (600 MHz, CDCl_3): δ_{H} = 1.79-1.81 (m, 2H, **22-H**), 2.35 (s, 3H, **30-H**), 3.00 (bd, J = 5.4 Hz, 2H, **23-H**), 3.68 (q, J = 5.8 Hz, 2H, **21-H**), 6.27 (bs, 1H, **20-H**), 7.02 (bs, 1H, **24-H**), 7.21 (d, J = 8.2 Hz, 2H, **28-H**), 7.33 (d, J = 4.7 Hz, 1H, **6-H**), 7.43 (d, J = 4.6 Hz, 1H, **5-H**), 7.46-7.51 (m, 2H, **15,16-H**), 7.72 (d, J = 8.2 Hz, 2H, **27-H**), 7.84-7.85 (m, 1H, **14-H**), 7.86 (s, 1H, **3-H**), 7.88-7.90 (m, 2H, **12,17-H**), 7.95 (dd, J = 8.5, 1.6 Hz, 1H, **11-H**), 8.38 (s, 1H, **19-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = 21.6 (**C-30**), 30.3 (**C-22**), 38.3 (**C-21**), 39.7 (**C-23**), 110.2 (**C-5**), 111.1 (**C-3**), 124.0 (**C-11**), 124.7 (**C-19**), 126.3 (**C-15**), 126.6 (**C-16**), 127.1 (**C-27**), 127.9 (**C-14**), 128.1 (**C-6**), 128.4 (**C-17**), 128.7 (**C-12**), 129.7 (**C-28**), 130.5 (**C-10**), 133.3 (**C-9,13**), 133.7 (**C-18**), 137.6 (**C-26**), 143.1 (**C-29**), 144.8 (**C-2**), 149.7 (**C-8**); LRMS m/z (ES^+): 472 [$\text{M}+\text{H}$] $^+$; HRMS m/z (EI^+): Found 471.1726 [M] $^+$; $\text{C}_{26}\text{H}_{25}\text{N}_5\text{O}_2\text{S}$ requires 471.1724.

2-(2-(*tert*-Butyldimethylsilyloxy)ethoxy)ethanamine (**250**)¹⁸¹



To 2-(2-aminoethoxy)ethanol (0.500 mL, 5.02 mmol) dissolved in anhydrous pyridine (5 mL) was added imidazole (527 mg, 7.75 mmol) and *tert*-butylchlorodimethylsilane (756 mg, 5.02 mmol) and the reaction was stirred at RT under Ar for 16 h. The solvent was removed *in vacuo* and the residue was taken up in CH₂Cl₂ (70 mL) and washed NaHCO₃ (sat. aq. 2 x 40 mL), H₂O (40 mL) and brine (40 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in CH₂Cl₂; eluted 1% to 2% to 5% MeOH) afforded the title compound as a colourless oil (830 mg, 3.79 mmol, 76%) with the NMR consistent with literature values.¹⁸¹ R_f = 0.37 (10% MeOH/CH₂Cl₂); IR (ν_{\max} /cm⁻¹, thin film): 3367, 2929, 2856 (N-H and C-H stretches), 1253 (Si-C stretch), 1100 (Si-O and C-O stretches); ¹H NMR (600 MHz, CDCl₃): δ_H = 0.06 (s, 6H, **9-H**), 0.89 (s, 9H, **11-H**), 1.79 (s, 2H, **1-H**), 2.86 (t, J = 5.3 Hz, 2H, **2-H**), 3.51 (t, J = 5.3 Hz, **3-H**), 3.53 (t, J = 5.3 Hz, 2H, **5-H**), 3.76 (t, J = 5.3 Hz, 2H, **6-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = -5.1 (**C-9**), 18.6 (**C-10**), 26.0 (**C-11**), 41.9 (**C-2**), 62.8 (**C-6**), 72.5 (**C-5**), 73.3 (**C-3**); LRMS m/z (ES): 242 [M+Na]⁺, 220 [M+H]⁺; HRMS m/z (ES⁺): Found 220.1737 [M+H]⁺; C₁₀H₂₆NO₂Si requires 220.1733.

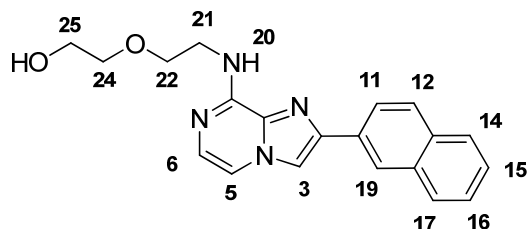
N-(2-(2-(*tert*-Butyldimethylsilyloxy)ethoxy)ethyl)-2-(naphthalen-2-yl)imidazo[1,2-*a*]pyrazin-8-amine (**251**)



All glassware was dried and purged with Ar prior to use. Pd₂(dba)₃ (1.64 mg, 1 mol%), DavePhos (2.11 mg, 3 mol%) and Cs₂CO₃ (81.6 mg, 0.250 mmol) were dissolved in anhydrous dioxane (2 mL). **214** (50.0 mg, 0.179 mmol) and **250** (47.0 mg, 0.215 mmol) were added and the

reaction was stirred under reflux, under Ar for 16 h. The reaction was cooled to RT and solvent removed, before the residue was taken up in CH₂Cl₂ (50 mL) and washed with H₂O (3 x 30 mL) and brine (30 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in toluene; eluted 10% EtOAc) afforded the title compound as a yellow oil (26.3 mg, 0.057 mmol, 31%). *R_f* = 0.17 (20% EtOAc/toluene); IR (ν_{max} /cm⁻¹, thin film): 3062 (N-H and aromatic C-H stretches), 2953, 2927, 2856 (C-H stretches), 1631, 1461 (aromatic C=C and C=N stretches), 1254 (Si-C stretch), 1104 (Si-O and C-O stretches); ¹H NMR (600 MHz, CDCl₃): δ_{H} = 0.10 (s, 6H, **28-H**), 1.91 (s, 9H, **30-H**), 3.62 (t, *J* = 5.3 Hz, 2H, **22-H**), 3.79-3.80 (m, 2H, **24-H**), 3.82-3.84 (m, 4H, **21,25-H**), 6.49 (bs, 1H, **21-H**), 7.21 (d, *J* = 4.7 Hz, 1H, **6-H**), 7.40 (d, *J* = 4.7 Hz, 1H, **5-H**), 7.46-7.51 (m, 2H, **15,16-H**), 7.82 (s, 1H, **3-H**), 7.84 (bd, *J* = 7.9 Hz, 1H, **14-H**), 7.88-7.92 (m, 2H, **12,17-H**), 7.99 (dd, *J* = 8.5, 1.7 Hz, 1H, **11-H**), 8.43 (s, 1H, **19-H**); ¹³C NMR (150 MHz, CDCl₃): δ_{C} = -5.1 (**C-28**), 18.4 (**C-29**), 26.1 (**C-30**), 40.7 (**C-25**), 62.7 (**C-21**), 69.9 (**C-24**), 72.7 (**C-22**), 109.9 (**C-5**), 110.8 (**C-3**), 124.2 (**C-11**), 124.7 (**C-19**), 126.1 (**C-15**), 126.5 (**C-16**), 127.9 (**C-14**), 128.4 (**C-17**), 128.5 (**C-6**), 128.6 (**C-12**), 130.9 (**C-10**), 133.3 (**C-13**), 133.7 (**C-9**), 133.8 (**C-18**), 144.5 (**C-2**), 149.2 (**C-8**); LRMS *m/z* (ES⁺): 439 (Not correct mass).

2-(2-(2-(Naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-ylamino)ethoxy)ethanol (252)

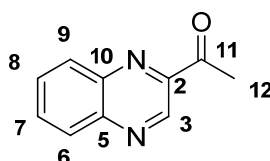


251 (26.0 mg, 0.056 mmol) was taken up in THF (2 mL) and the mixture was cooled on ice. TBAF (1.0 M solution in THF, containing 5 wt% H₂O; 112 μ L, 0.112 mmol) was added and the reaction was stirred at RT for 22 h. Quenching with H₂O (10 mL) and extracting with EtOAc (3 x 20 mL) was followed by washing the combined organics with brine (20 mL), drying (MgSO₄), filtering and concentrating *in vacuo*. Flash chromatography (1st: applied in pet. ether; eluted 50% to 90% to 95% EtOAc; 2nd: applied in CH₂Cl₂; eluted 1% to 2% MeOH) afforded the title compound as a colourless oil (10.1 mg, 0.029 mmol, 52%). *R_f* = 0.08 (9:1 EtOAc/pet. ether) or 0.76 (10% MeOH/CH₂Cl₂); IR (ν_{max} /cm⁻¹, thin film): 3347 (O-H stretch), 3134, 3055 (N-H and aromatic C-H stretches), 2925, 2869 (C-H stretches), 1620, 1548 (aromatic C=C and C=N

stretches), 1125 (C-O stretch); ^1H NMR (600 MHz, CDCl_3): $\delta_{\text{H}} = 3.50$ (bs, 1H, **26-H**), 3.68 (t, $J = 4.4$ Hz, 2H, **24-H**), 3.80-3.81 (m, 4H, **22,25-H**), 3.84-3.86 (m, 2H, **21-H**), 6.70 (bs, 1H, **20-H**), 7.31 (d, $J = 4.7$ Hz, 1H, **6-H**), 7.40 (d, $J = 4.7$ Hz, 1H, **5-H**), 7.46-7.51 (m, 2H, **15,16-H**), 7.81 (s, 1H, **3-H**), 7.84 (d, $J = 7.6$ Hz, **14-H**), 7.88-7.90 (m, 2H, **12,17-H**), 7.95 (dd, $J = 8.5, 1.5$ Hz, 1H, **11-H**), 8.38 (s, 1H, **19-H**); ^{13}C NMR (150 MHz, CDCl_3): $\delta_{\text{C}} = 40.9$ (**C-21**), 61.8 (**C-25**), 70.0 (**C-22**), 72.8 (**C-24**), 110.0 (**C-5**), 110.9 (**C-3**), 124.1 (**C-11**), 124.8 (**C-19**), 126.2 (**C-15**), 126.5 (**C-16**), 127.9 (**C-14**), 128.4 (**C-17**), 128.6 (overlapping signals, **C-6,12**), 130.5 (**C-10**), 133.3 (**C-13**), 133.6 (**C-9**), 133.7 (**C-18**), 144.5 (**C-2**), 149.1 (**C-8**); LRMS m/z (ES^+): 371 $[\text{M}+\text{Na}]^+$, 349 $[\text{M}+\text{H}]^+$, 287 $[\text{M}-(\text{OCH}_2\text{CH}_2\text{OH})]^+$; HRMS m/z (ES^+): Found 349.1653 $[\text{M}+\text{H}]^+$; $\text{C}_{20}\text{H}_{21}\text{N}_4\text{O}_2$ requires 349.2665.

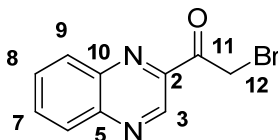
6.1.3.1.2 Variations with aromatic Group in Position 2

1-(Quinoxalin-2-yl)ethanone (254)¹⁸³



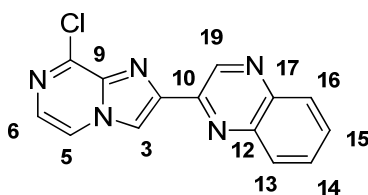
Quinoxaline (1.19 g, 9.11 mmol), pyruvic acid (1.90 mL, 27.3 mmol), AgNO_3 (0.124 g, 0.729 mmol), $\text{N}_2\text{H}_8\text{S}_2\text{O}_8$ (3.12 g, 13.7 mmol) and H_2SO_4 (0.490 mL, 9.11 mmol) were stirred in $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ (1:1, 150 mL) at 40 °C for 2½ h. The solution was then basified *via* the addition of NaOH and the organics extracted (3 x 75 mL) and washed brine (50 mL), dried (MgSO_4), filtered and solvent removed. Flash chromatography (applied in pet. ether; eluted 3:1 pet. ether/EtOAc) afforded the title compound as a yellow solid (618 mg, 3.91 mmol, 43%) with the NMR consistent with literature values.³⁰⁶ Mpt: 70-74 °C [Lit.³⁰⁶ 76.5-77.5 °C]; $R_f = 0.24$ (3:1 pet. ether/EtOAc); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 1689 (C=O stretch), 1357 (aromatic C=C and C-N stretches); ^1H NMR (500 MHz, CDCl_3): $\delta_{\text{H}} = 2.84$ (s, 3H, **12-H**), 7.82-7.90 (m, 2H, **7,8-H**), 8.14-8.21 (m, 2H, **6,9-H**), 9.47 (s, 1H, **3-H**); ^{13}C NMR (125 MHz, CDCl_3): $\delta_{\text{C}} = 25.6$ (**C-12**), 129.5 (**C-6**), 130.5 (**C-8**), 130.8 (**C-9**), 132.3 (**C-7**), 141.1 (**C-10**), 143.1 (**C-3**), 143.9 (**C-5**), 146.6 (**C-2**), 199.8 (**C-11**); LRMS m/z (EI^+): 172 $[\text{M}]^+$, 130 $[\text{M}-\text{Ac}]^+$, 86; HRMS m/z (EI^+): Found 172.0637 $[\text{M}]^+$; $\text{C}_{10}\text{H}_8\text{N}_2\text{O}$ requires 172.0631.

2-Bromo-1-(quinoxalin-2-yl)ethanone (255)



Pyridinium tribromide (2.94 g, 9.18 mmol) was added to a stirred solution of **254** (580 mg, 3.67 mmol) in $\text{CHCl}_3/\text{EtOH}$ (1:1, 60 mL) and the mixture was heated at 50 °C for 16 h. Removal of the solvent *in vacuo* was followed by addition of H_2O (100 mL) and extraction with EtOAc (3 x 60 mL). The combined organic extracts were further washed with H_2O (50 mL) and brine (50 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Flash chromatography (CH_2Cl_2) afforded the title compound as a brown solid (656 mg, 2.61 mmol, 71%) with the NMR consistent with literature values.³⁰⁷ Mpt: Decomposed before melting [Lit.³⁰⁷ 113-117 °C]; R_f = 0.26 (CH_2Cl_2); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 1708 (C=O stretch), 1392 (aromatic C=C and C-N stretches), 762 (C-Br stretch); ^1H NMR (600 MHz, CDCl_3): δ_{H} = 4.96 (s, 2H, **12-H**), 7.88-7.90 (m, 1H, **8-H**), 7.93-7.96 (m, 1H, **7-H**), 8.20-8.21 (m, 2H, **6,9-H**), 9.53 (s, 1H, **3-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = 31.3 (**C-12**), 129.7 (**C-6**), 130.6 (**C-9**), 131.3 (**C-8**), 133.0 (**C-7**), 141.0 (**C-10**), 143.4 (**C-3**), 144.3 (**C-5**), 144.7 (**C-2**), 192.4 (**C-11**); LRMS m/z (EI^+): 252 [$\text{M}^{(81}\text{Br})^+$], 250 [$\text{M}^{(79}\text{Br})^+$], 142, 115, 113; HRMS m/z (EI^+): Found 249.9740 [$\text{M}^{(79}\text{Br})^+$]; $\text{C}_{10}\text{H}_7\text{BrN}_2\text{O}$ requires 249.9736; Anal. Calcd. for $\text{C}_{10}\text{H}_7\text{BrN}_2\text{O}$: C, 47.84; H, 2.81; N, 11.16. Found C, 47.70; H, 2.68; N, 10.86%.

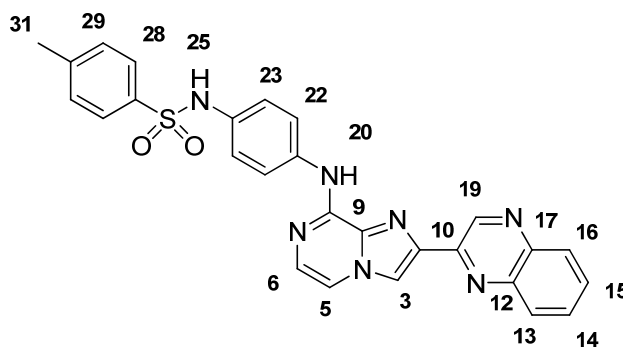
2-(8-Chloroimidazo[1,2-a]pyrazin-2-yl)quinoxaline (256)



255 (648 mg, 2.58 mmol), 2-amino-3-chloropyrazine (334 mg, 2.58 mmol) and NaHCO_3 (271 mg, 3.23 mmol) in $t\text{BuOH}$ (15 mL) were stirred under reflux for 40 h. The solvent was removed *in vacuo* and the resulting residual was taken up in CH_2Cl_2 (100 mL) and washed H_2O (2 x 40 mL) and brine (40 mL), dried (MgSO_4), filtered and solvent removed *in vacuo*. Flash chromatography (applied in pet. ether; eluted 3:1 to 1:1 to 1:3 pet. ether/EtOAc) afforded the title

compound as an orange solid (342 mg, 1.21 mmol, 47%). Mpt: >200 °C; R_f = 0.11 (1:1 pet. ether/EtOAc); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 2924 (aromatic C-H stretch), 1675, 1495, 1354 (aromatic C=C and C-N stretches), 1200; ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{H} = 7.84 (d, J = 4.5 Hz, 1H, **6-H**), 7.89-7.95 (m, 2H, **14,15-H**), 8.15-8.17 (m, 2H, **13,16-H**), 8.73 (d, J = 4.5 Hz, 1H, **5-H**), 9.08 (s, 1H, **3-H**), 9.71 (s, 1H, **19-H**); ^{13}C NMR (150 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{C} = 116.9 (**C-3**), 121.0 (**C-5**), 128.3 (**C-6**), 129.0 (**C-13**), 129.1 (**C-16**), 130.5 (**C-15**), 131.1 (**C-14**), 137.7 (**C-9**), 141.4 (**C-12**), 141.8 (**C-17**), 142.2 (**C-8**), 143.4 (**C-19**), 143.9 (**C-10**), 146.4 (**C-2**); LRMS m/z (CI): 282 $[\text{M}(^{35}\text{Cl})+\text{H}]^+$, 284 $[\text{M}(^{37}\text{Cl})+\text{H}]^+$; HRMS m/z (CI): Found 282.0552 $[\text{M}(^{35}\text{Cl})+\text{H}]^+$; $\text{C}_{14}\text{H}_9\text{ClN}_5$ requires 282.0547.

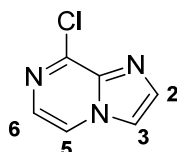
4-Methyl-N-(4-(2-(quinoxalin-2-yl)imidazo[1,2-a]pyrazin-8-ylamino)phenyl)benzenesulfonamide (257)



All glassware was dried and purged with Ar prior to use. $\text{Pd}(\text{dba})_2$ (2.04 mg, 1 mol%), DavePhos (4.19 mg, 3 mol%) and Cs_2CO_3 (162 mg, 0.497 mmol) were stirred in dissolved in 1,4-dioxane (5 mL). **256** (100 mg, 0.355 mmol) and *N*-(4-aminophenyl)-4-methylbenzenesulfonamide (112 mg, 0.426 mmol) were added and the reaction was stirred under reflux for 40 h. The reaction was cooled to RT and solvent removed *in vacuo*. The resulting residue was taken up in CH_2Cl_2 (60 mL) and washed with NaHCO_3 (sat. aq. 40 mL), H_2O (30 mL) and brine (30 mL), dried (MgSO_4), filtered and solvent removed *in vacuo*. Flash chromatography (applied in toluene; eluted 0% to 50% EtOAc) afforded the title compound as a light yellow solid (12.3 mg, 0.024 mmol, 7%). Mpt: Decomposed before melting; R_f = 0.49 (1:1 $\text{CH}_2\text{Cl}_2/\text{EtOAc}$); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3135 (aromatic C-H stretch), 3061 (C-H stretch), 1495 (N-H bend), 1291 (S=O asymmetric stretch), 1201 (S=O symmetric stretch); ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{H} = 2.34 (s, 3H, **31-H**), 7.05 (d, J = 8.9 Hz, 2H, **23-H**), 7.35 (d, J = 8.3 Hz, 2H, **29-H**), 7.44 (d, J = 4.6 Hz,

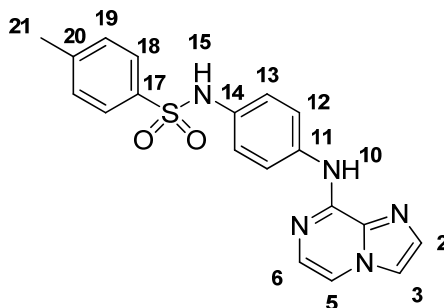
1H, **6-H**), 7.64 (d, $J = 8.3$ Hz, 2H, **28-H**), 7.84-7.86 (m, 1H, **15-H**), 7.88-7.90 (m, 3H, **14,22-H**), 8.03 (d, $J = 4.6$ Hz, 1H, **5-H**), 8.10-8.14 (m, 2H, **13,16-H**), 8.80 (s, 1H, **3-H**), 9.61 (s, 1H, **20-H**), 9.78 (s, 1H, **19-H**), 10.06 (bs, 1H, **25-H**); ^{13}C NMR (150 MHz, $(\text{CD}_3)_2\text{SO}$): $\delta_{\text{C}} = 21.0$ (**C-31**), 112.5 (**C-5**), 115.9 (**C-3**), 121.1 (**C-23**), 121.2 (**C-22**), 126.8 (**C-28**), 128.2 (**C-6**), 128.8 (**C-13**), 129.1 (**C-16**), 129.7 (**C-29**), 129.9 (**C-15**), 130.9 (**C-14**), 132.1 (**C-24**), 133.4 (**C-9**), 136.6 (**C-21**), 136.7 (**C-27**), 141.2 (**C-10**), 141.4 (**C-17**), 141.5 (**C-12**), 143.1 (**C-30**), 143.7 (**C-19**), 146.1 (**C-8**), 147.0 (**C-2**) ; LRMS m/z (ES^+): 530 $[\text{M}+\text{Na}]^+$, 508 $[\text{M}+\text{H}]^+$; HRMS m/z (ES^+): Found 508.1565 $[\text{M}+\text{H}]^+$; $\text{C}_{27}\text{H}_{22}\text{N}_7\text{O}_2\text{S}$ requires 508.1556.

8-Chloroimidazo[1,2-a]pyrazine (259)



2-Amino-3-chloropyrazine (200 mg, 1.54 mmol) and NaHCO_3 (162 mg, 1.93 mmol) were suspended in $t\text{BuOH}$. Chloroacetaldehyde (50% w/v in H_2O ; 200 μL , 1.54 mmol) was added and the reaction mixture was stirred under reflux for 40 h. The reaction was then cooled to RT and solvent removed *in vacuo*. The residual material was taken up in CH_2Cl_2 (100 mL) washed H_2O (40 mL) and brine (40 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 3:1 to 2:1 to 1:3 pet. ether/EtOAc) afforded the title compound as an off white solid (118 mg, 0.773 mmol, 50%). Mpt: Decomposed before melting; $R_f = 0.17$ (2:1 EtOAc/pet. ether); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3144, 3105 (aromatic C-H stretches), 1432, 1330 (aromatic C=C and C=N stretches), 905, 800, 738 (aromatic C-Cl stretch); ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$): $\delta_{\text{H}} = 7.73$ (d, $J = 4.5$ Hz, 1H, **6-H**), 7.87 (d, $J = 0.9$ Hz, 1H, **2-H**), 8.28 (d, $J = 0.9$ Hz, 1H, **3-H**), 8.66 (d, $J = 4.5$ Hz, 1H, **5-H**); ^{13}C NMR (150 MHz, $(\text{CD}_3)_2\text{SO}$): $\delta_{\text{C}} = 117.3$ (**C-3**), 120.8 (**C-5**), 127.3 (**C-6**), 135.5 (**C-2**), 137.1 (**C-9**), 141.7 (**C-8**); LRMS m/z (CI^+): 154 $[\text{M}(^{35}\text{Cl})+\text{H}]^+$, 156 $[\text{M}(^{37}\text{Cl})+\text{H}]^+$; HRMS m/z (CI^+): Found 154.0166 $[\text{M}(^{35}\text{Cl})+\text{H}]^+$; $\text{C}_6\text{H}_5\text{ClN}_3$ requires 154.0172.

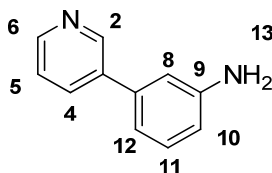
N-(4-(Imidazo[1,2-*a*]pyrazin-8-ylamino)phenyl)-4-methylbenzenesulfonamide (**260**)



All glassware was dried and purged with Ar prior to use. $\text{Pd}_2(\text{dba})_3$ (2.97 mg, 1 mol%), DavePhos (3.83 mg, 3 mol%) and NaO^tBu (43.8 mg, 0.456 mmol) were dissolved in anhydrous toluene (3 mL). **259** (49.8 mg, 0.324 mmol) and *N*-(4-aminophenyl)-4-methylbenzenesulfonamide (106 mg, 0.389 mmol) were added and the reaction was stirred under reflux, under Ar for 16 h. The reaction was cooled to RT and solvent removed *in vacuo*, before the residue was taken up in CH_2Cl_2 (50 mL) and washed with H_2O (3 x 30 mL) and brine (30 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 2:1 pet. ether/EtOAc) afforded the title compound as a brown solid (69.7 mg, 0.184 mmol, 56%). Mpt: Decomposed before melting; R_f = 0.30 (3:1 EtOAc/pet. ether); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3338, 3045 (aromatic C-H stretches), 1537 (N-H bend), 1505 (aromatic C=C and C=N stretches), (1326) S=O asymmetric stretch, 1152 (S=O symmetric stretch); ^1H NMR (600 MHz, CDCl_3): δ_{H} = 2.37 (s, 3H, **21-H**), 6.88 (s, 1H, **15-H**), 7.06 (d, J = 8.8 Hz, 2H, **13-H**), 7.21 (d, J = 8.3 Hz, 2H, **19-H**), 7.45 (d, J = 4.6 Hz, 1H, **6-H**), 7.56-7.57 (m, 3H, **2,3,5-H**), 7.63 (d, J = 8.3 Hz, 2H, **18-H**), 7.76 (d, J = 8.8 Hz, 2H, **12-H**), 8.19 (s, 1H, **10-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = 21.7 (**C-21**), 111.9 (**C-5**), 115.2 (**C-3**), 120.3 (**C-12**), 123.9 (**C-13**), 127.4 (**C-18**), 128.4 (**C-6**), 129.7 (**C-19**), 131.1 (**C-14**), 131.9 (**C-2**), 133.2 (**C-9**), 136.2 (**C-17**), 137.4 (**C-11**), 143.9 (**C-20**), 146.0 (**C-8**); LRMS m/z (ES^+): 380 $[\text{M}+\text{H}]^+$, 402 $[\text{M}+\text{Na}]^+$; HRMS m/z (ES^+): Found 380.1168 $[\text{M}+\text{H}]^+$; $\text{C}_{19}\text{H}_{18}\text{N}_5\text{O}_2\text{S}$ requires 380.1181.

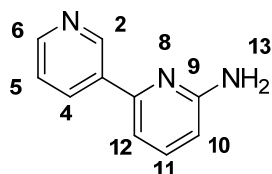
6.1.3.2 Maybridge Analogues

3-(Pyridin-3-yl)aniline (262)



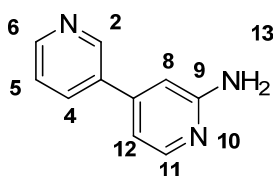
Pyridin-3-ylboronic acid (621 mg, 5.05 mmol) and 2-bromoaniline (500 μ L, 4.59 mmol) were dissolved in toluene/EtOH (1:1; 30 mL). Na_2CO_3 (25 mL, aq. 10% w/v) was added, the vessel was purged with Ar, $\text{Pd}(\text{PPh}_3)_4$ (292 mg, 5 mol%) was added and the mixture was stirred at 80 $^\circ\text{C}$ for 16 h. The reaction was then cooled to RT, diluted with H_2O (50 mL) and extracted with EtOAc (3 x 75 mL). The combined organics were then washed with H_2O (50 mL) and brine (2 x 30 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Purification *via* flash chromatography (applied in CH_2Cl_2 ; eluted 5% to 10% to 20% to 40% to 50% EtOAc) afforded the title compound as a yellow/brown solid (470 mg, 2.76 mmol, 60%), with NMR values consistent with literature.³⁰⁸ Mpt: 54 $^\circ\text{C}$ [Lit.³⁰⁸ 74.4-75.8 $^\circ\text{C}$]; R_f = 0.17 (1:1 CH_2Cl_2 /EtOAc); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3440, 3296 (aromatic C-H stretches), 3184, 3055 (N-H stretches), 1602, 1476, 1408 (aromatic C=C stretches); ^1H NMR (600 MHz, CDCl_3): δ_{H} = 3.63 (bs, 2H, **13-H**), 6.72 (ddd, J = 7.9, 2.2, 0.5 Hz, 1H, **10-H**), 6.87 (t, J = 2.0 Hz, 1H, **8-H**), 6.95-6.96 (m, 1H, **12-H**), 7.25 (t, J = 7.9 Hz, 1H, **11-H**), 7.22 (dd, J = 7.9, 4.9 Hz, 1H, **5-H**), 7.83 (dt, J = 7.9, 1.9 Hz, 1H, **4-H**), 8.57 (bd, J = 4.1 Hz, 1H, **6-H**), 8.81 (bs, 1H, **2-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = 113.7 (**C-8**), 114.9 (**C-10**), 117.8 (**C-12**), 123.6 (**C-5**), 130.2 (**C-11**), 134.5 (**C-4**), 137.0 (**C-3**), 139.1 (**C-7**), 147.2 (**C-9**), 148.3 (**C-2**), 148.6 (**C-6**); LRMS m/z (EI^+): 170 [M] $^+$; HRMS m/z (EI^+): Found 170.0841 [M] $^+$; $\text{C}_{11}\text{H}_{10}\text{N}_2$ requires 170.0839.

2,3'-Bipyridin-6-amine (267)



Pyridin-3-ylboronic acid (52.3 mg, 0.428 mmol) and 2-amino-6-chloropyridine (50.0 mg, 0.389 mmol) were dissolved in toluene/EtOH (1:1; 3 mL). Na_2CO_3 (2.5 mL, aq. 10% w/v) was added, the vessel was purged with Ar, $\text{Pd}(\text{PPh}_3)_4$ (22.5 mg, 5 mol%) was added and the mixture was stirred at 80 °C for 16 h. The reaction was then cooled to RT, diluted with H_2O (30 mL) and extracted with EtOAc (3 x 20 mL). The combined organics were then washed with H_2O (20 mL) and brine (2 x 20 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Purification *via* flash chromatography (applied in toluene; eluted 1:1 to 1:2 to 1:5 to 1:9 toluene/EtOAc) afforded the title compound as an off white solid (7.20 mg, 0.042 mmol, 11%) with the NMR consistent with literature values.³⁰⁹ Mpt: 92-94 °C [Lit.³¹⁰ 108 °C]; R_f = 0.19 (9:1 EtOAc/toluene); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3310 (aromatic C-H stretch), 3165 (N-H stretch), 1636, 1567 (aromatic C=C stretches), 1467, 1434; ^1H NMR (600 MHz, CDCl_3): δ_{H} = 4.56 (bs, 2H, **13-H**), 6.52 (d, J = 8.2 Hz, 1H, **10-H**), 7.11 (d, J = 7.4 Hz, 1H, **12-H**), 7.37 (dd, J = 7.9, 4.8 Hz, 1H, **5-H**), 7.54 (t, J = 7.8 Hz, 1H, **11-H**), 8.26 (dt, J = 7.9, 1.8 Hz, 1H, **4-H**), 8.61, (bd, J = 3.2 Hz, 1H, **6-H**), 9.13 (s, 1H, **2-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = 108.0 (**C-10**), 111.1 (**C-12**), 123.5 (**C-5**), 134.3 (**C-4**), 135.2 (**C-3**), 138.7 (**C-11**), 148.4 (**C-2**), 149.7 (**C-6**), 153.4 (**C-7**), 158.6 (**C-9**); LRMS m/z (EI^+): 171 [M] $^+$; HRMS m/z (EI^+): Found 171.0784 [M] $^+$; $\text{C}_{10}\text{H}_9\text{N}_3$ requires 171.0791.

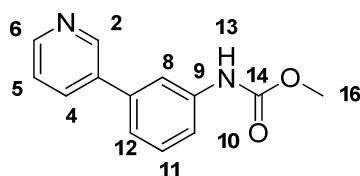
3,4'-Bipyridin-2'-amine (268)



Pyridin-3-ylboronic acid (52.3 mg, 0.428 mmol) and 2-amino-4-chloropyridine (50.0 mg, 0.389 mmol) were dissolved in toluene/EtOH (1:1; 3 mL). Na_2CO_3 (2.5 mL, aq. 10% w/v) was added, the vessel was purged with Ar, $\text{Pd}(\text{PPh}_3)_4$ (22.5 mg, 5 mol%) was added and the mixture was stirred at 80 °C for 16 h. The reaction was then cooled to RT, diluted with H_2O (30 mL) and extracted with EtOAc (3 x 20 mL). The combined organics were then washed with H_2O (20 mL) and brine (2 x 20 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Purification *via* flash chromatography (applied in toluene: eluted 1:1 to 1:2 to 1:5 to 1:9 toluene/EtOAc followed by CH_2Cl_2 ; 2% to 5% MeOH) afforded the title compound as an off white solid (9.70 mg, 0.057 mmol, 15%) with the NMR consistent with literature values.³¹¹ Mpt: 102-106 °C; R_f = 0.38 (10%

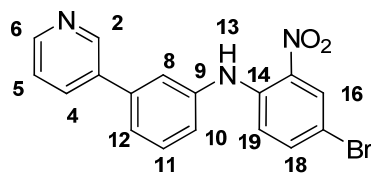
MeOH/CH₂Cl₂); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3431, 3293 (aromatic C-H stretches), 3171 (N-H stretch), 1628, 1537, 1408 (aromatic C=C and C=N stretches); ¹H NMR (600 MHz, CDCl₃): δ_{H} = 4.84 (bs, 2H, **13-H**), 6.73 (s, 1H, **8-H**), 6.88 (dd, J = 5.5, 1.5 Hz, 1H, **12-H**), 7.39-7.42 (m, 1H, **5-H**), 7.88 (dt, J = 7.9, 2.0 Hz, 1H, **4-H**), 8.14 (d, J = 5.5 Hz, 1H, **11-H**), 8.67, (dd, J = 4.8, 1.5 Hz, 1H, **6-H**), 8.84 (d, J = 2.0 Hz, 1H, **2-H**); ¹³C NMR (150 MHz, CDCl₃): δ_{C} = 106.7 (**C-8**), 112.5 (**C-12**), 123.8 (**C-5**), 134.3 (**C-3**), 134.4 (**C-4**), 147.8 (**C-7**), 147.9 (**C-11**), 148.2 (**C-2**), 150.2 (**C-6**), 158.7 (**C-9**); LRMS m/z (EI⁺): 171 [M]⁺; HRMS m/z (EI⁺): Found 171.0797 [M]⁺; C₁₀H₉N₃ requires 171.0791.

Methyl 3-(pyridin-3-yl)phenylcarbamate (269)



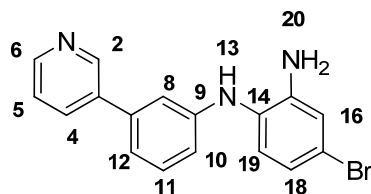
To a solution of **262** (27.0 mg, 0.159 mmol) dissolved in anhydrous CH₂Cl₂ (6 mL) was added Et₃N (35.4 μ L, 0.254 mmol). The mixture was purged, methyl chloroformate (18.4 μ L, 0.238 mmol) was added dropwise and the reaction was stirred at RT under Ar for 16 h. The mixture was diluted with CH₂Cl₂ (30 mL) and washed with H₂O (2 x 20 mL) and brine (20 mL), dried (MgSO₄), filtered and solvent removed *in vacuo*. Purification *via* flash chromatography (applied in pet. ether; eluted 1:1 pet. ether/EtOAc) afforded the title compound as a white solid (13.2 mg, 0.058 mmol, 37%). Mpt: 155 °C; R_f = 0.27 (2:1 EtOAc/pet. ether); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 2951 (N-H stretch), 1736 (C=O stretch); ¹H NMR (600 MHz, CDCl₃): δ_{H} = 3.80 (s, 3H, **16-H**), 7.22 (bs, 1H, **13-H**), 7.28 (dd, J = 8.6, 1.4 Hz, 1H, **12-H**), 7.38 (dd, J = 7.9, 4.8 Hz, 1H, **5-H**), 7.40-7.45 (m, 2H, **10,11-H**), 7.68 (bs, 1H, **8-H**), 7.90 (dt, J = 7.9, 1.9 Hz, 1H, **4-H**), 8.61 (bd, J = 3.9 Hz, 1H, **6-H**), 8.86 (bs, 1H, **2-H**); ¹³C NMR (150 MHz, CDCl₃): δ_{C} = 52.6 (**C-16**), 117.3 (**C-8**), 118.4 (**C-10**), 122.2 (**C-12**), 123.8 (**C-5**), 130.0 (**C-11**), 134.9 (**C-4**), 136.6 (**C-3**), 138.7 (**C-7**), 139.0 (**C-9**), 148.1 (**C-2**), 148.5 (**C-6**), 154.2 (**C-14**); LRMS m/z (CI⁺): 229 [M+H]⁺; HRMS m/z (CI⁺): Found 229.0971 [M+H]⁺; C₁₃H₁₃N₂O₂ requires 229.0977.

4-Bromo-2-nitro-N-(3-(pyridin-3-yl)phenyl)aniline (**270**)¹⁸⁸



To a solution of **262** (114 mg, 0.671 mmol) in anhydrous NMP (1 mL), was added anhydrous Et₃N (93.5 μ L, 0.671 mmol) and 4-bromo-1-fluoro-2-nitrobenzene (82.7 μ L, 0.671 mmol) and the mixture was stirred at 120 °C for 20 h. After this time, the reaction was cooled to RT, poured into cold H₂O (50 mL) and extracted EtOAc (3 x 30 mL). TLC of the aqueous layer showed signs of product and so the pH was adjusted *via* the addition of NaHCO₃ followed by extraction with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (30 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 10% to 20% to 33% EtOAc) afforded the title compound as a deep orange oil (87.3 mg, 0.236 mmol, 35%). R_f = 0.29 (1:1 pet. ether/EtOAc); ¹H NMR (600 MHz, CDCl₃): δ_H = 7.19 (d, J = 9.2 Hz, 1H, **19-H**), 7.30-7.32 (m, 1H, **10-H**), 7.39 (dd, J = 7.7, 4.7 Hz, 1H, **5-H**), 7.45-7.48 (m, 3H, **8,12,18-H**), 7.54 (t, J = 8.3 Hz, 1H, **11-H**), 7.87 (dt, J = 7.7, 2.0 Hz, 1H, **4-H**), 8.37 (d, J = 2.3 Hz, 1H, **16-H**), 8.63 (dd, J = 4.7, 1.5 Hz, 1H, **6-H**), 8.84 (d, J = 2.0 Hz, 1H, **2-H**), 9.52 (s, 1H, **13-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 109.1 (C-17), 117.9 (C-19), 123.1 (C-8), 123.8 (C-5), 124.0 (C-10), 124.9 (C-12), 129.0 (C-16), 130.8 (C-11), 133.8 (C-15), 134.5 (C-4), 135.7 (C-3), 138.7 (C-18), 139.2 (C-9), 140.0 (C-7), 141.9 (C-14), 148.3 (C-2), 149.2 (C-6); LRMS m/z (ES⁺): 370 [M(⁷⁹Br)+H]⁺, 372 [M(⁸¹Br)+H]⁺; HRMS m/z (ES⁺): Found 370.0191 [M(⁷⁹Br)+H]⁺; C₁₇H₁₃BrN₃O₂ requires 370.0191.

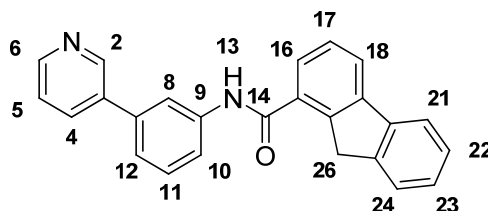
4-Bromo-N¹-(3-(pyridin-3-yl)phenyl)benzene-1,2-diamine (**271**)



To a solution of **270** (85.0 mg, 0.230 mmol) in anhydrous MeOH (2 mL) was added 10% Pd/C (8.5 mg, 10% wt/wt). The vessel was evacuated and purged with Ar (3x), and under static

vacuum a balloon of H₂ was added. The reaction was stirred at RT under H₂ atmosphere for 18 h, after which time the balloon was removed and the vessel evacuated and purged Ar (3x). The contents were filtered through Celite (pre-washed with MeOH), the filtrate concentrated *in vacuo* and flash chromatography (applied in toluene; eluted 1:1 toluene/EtOAc) afforded the title compound as brown oil (21.8 mg, 0.064 mmol, 28%). *R_f* = 0.27 (1:1 toluene/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3358 (aromatic C-H stretches), 3034 (N-H stretch), 1603, 1587, 1494 (aromatic C=C and C-N stretches); ¹H NMR (600 MHz, CDCl₃): δ_{H} = 3.63 (bs, 2H, **20-H**), 5.28 (bs, 1H, **13-H**), 6.76 (dd, *J* = 7.9, 1.9 Hz, 1H, **10-H**), 6.85-6.88 (m, 2H, **8,18-H**), 6.96 (d, *J* = 2.2 Hz, 1H, **16-H**), 7.01 (d, *J* = 8.3 Hz, 1H, **19-H**), 7.03 (d, *J* = 7.9 Hz, 1H, **12-H**), 7.31 (t, *J* = 7.9 Hz, 1H, **11-H**), 7.37 (dd, *J* = 7.9, 4.9 Hz, 1H, **5-H**), 7.86 (dt, *J* = 7.9, 1.9 Hz, 1H, **4-H**), 8.57 (dd, *J* = 4.9, 1.3 Hz, 1H, **6-H**), 8.80 (d, *J* = 1.9 Hz, 1H, **2-H**); ¹³C NMR (150 MHz, CDCl₃): δ_{C} = 113.6 (**C-8**), 115.1 (**C-10**), 118.5 (**C-12**), 118.8 (**C-16**), 119.2 (**C-17**), 121.9 (**C-18**), 123.8 (**C-5**), 126.8 (**C-14**), 126.9 (**C-19**), 130.3 (**C-11**), 135.1 (**C-3**), 135.1 (**C-7**), 143.8 (**C-15**), 145.8 (**C-9**), 147.8 (**C-2**), 148.0 (**C-6**); LRMS *m/z* (ES⁺): 340 [M(⁷⁹Br)+H]⁺, 342 [M(⁸¹Br)]⁺; HRMS *m/z* (ES⁺): Found 340.0449 [M(⁷⁹Br)+H]⁺; C₁₇H₁₅BrN₃ requires 340.0449.

N-(3-(Pyridin-3-yl)phenyl)-9*H*-fluorene-1-carboxamide (**274**)

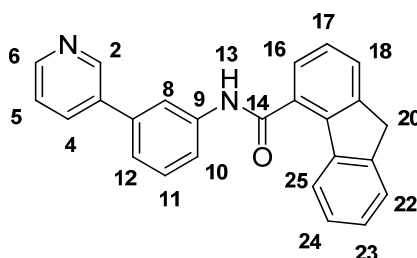


Formation of the Acid Chloride (**273**) *in situ*: 9*H*-fluorene-1-carboxylic acid (75.0 mg, 0.357 mmol) and thionyl chloride (500 μ L) were stirred under reflux for 16 h. The reaction was concentrated *in vacuo* and used as crude for next step.

To a solution of **262** (60.7 mg, 0.357 mmol) in anhydrous CH₂Cl₂ (1 mL) was added pyridine (86.3 μ L, 1.07 mmol) followed by the dropwise addition of **273** (81.6 mg, 0.357 mmol) in anhydrous CH₂Cl₂ (1.5 mL). The reaction mixture was stirred at RT for 5 h, followed by diluting with CH₂Cl₂ (50 mL) and washing with NaHCO₃ (sat. aq. 2 x 30 mL) and brine (30 mL), drying (MgSO₄), filtering and concentrating *in vacuo*. Flash chromatography (applied in pet. ether; eluted 1:1 pet. ether/EtOAc) afforded the title compound as a yellow solid (80.3 mg, 0.222 mmol,

62%) with the NMR values similar to those previously reported.¹⁹⁰ Mpt: 185-186 °C; R_f = 0.23 (1:2 pet. ether/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3237 (N-H stretch), 3037 (aromatic C-H stretch), 1648 (C=O stretch), 1589 (N-H bend), 1540, 1395 (aromatic C=C and C=N stretches); ¹H NMR (600 MHz, (CD₃)₂SO): δ_{H} = 4.22 (s, 2H, **26-H**), 7.36 (t, J = 7.4 Hz, 1H, **23-H**), 7.42 (t, J = 7.4 Hz, 1H, **22-H**), 7.48 (d, J = 7.8 Hz, 1H, **12-H**), 7.51-7.54 (m, 2H, **5,11-H**), 7.59 (t, J = 7.6 Hz, 1H, **17-H**), 7.64 (d, J = 7.4 Hz, 1H, **24-H**), 7.78 (d, J = 7.6 Hz, 1H, **16-H**), 7.88 (d, J = 7.9 Hz, 1H, **10-H**), 7.98 (d, J = 7.4 Hz, 1H, **21-H**), 8.06 (dt, J = 7.9, 1.9 Hz, 1H, **4-H**), 8.13 (d, J = 7.6 Hz, 1H, **18-H**), 8.19 (s, 1H, **8-H**), 8.60 (dd, J = 4.7, 1.4 Hz, 1H, **6-H**), 8.89 (d, J = 1.9 Hz, 1H, **2-H**), 10.50 (s, 1H, **13-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_{C} = 36.9 (**C-26**), 118.4 (**C-8**), 119.7 (**C-10**), 120.3 (**C-21**), 122.2 (**C-12**), 122.6 (**C-18**), 124.0 (**C-5**), 125.2 (**C-24**), 125.9 (**C-16**), 126.8 (**C-22**), 127.2 (**C-17**), 127.4 (**C-23**), 129.6 (**C-11**), 132.7 (**C-15**), 134.2 (**C-4**), 135.6 (**C-3**), 137.6 (**C-7**), 140.0 (**C-9**), 140.1 (**C-20**), 142.2 (**C-19**), 142.3 (**C-27**), 143.3 (**C-25**), 147.6 (**C-2**), 148.7 (**C-6**), 166.6 (**C-14**); LRMS m/z (ES⁺): 363 [M+H]⁺; HRMS m/z (ES⁺): Found 363.1498 [M+H]⁺; C₂₅H₁₉N₂O requires 363.1497.

***N*-(3-(Pyridin-3-yl)phenyl)-9H-fluorene-4-carboxamide (277)**

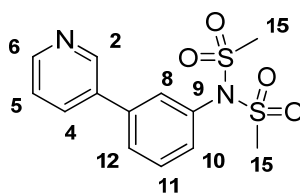


Formation of the Acid Chloride (**276**) *in situ*: 9H-fluorene-4-carboxylic acid (75.0 mg, 0.357 mmol) and thionyl chloride (500 μ L) were stirred under reflux for 16 h. The reaction was concentrated *in vacuo* and used as crude for next step.

To a solution of **262** (60.7 mg, 0.357 mmol) in anhydrous CH₂Cl₂ (1 mL) was added pyridine (86.3 μ L, 1.071 mmol) followed by the dropwise addition of **273** (81.6 mg, 0.357 mmol) in anhydrous CH₂Cl₂ (1.5 mL). The reaction mixture was stirred at RT for 5 h, followed by diluting with CH₂Cl₂ (50 mL) and washing with NaHCO₃ (sat. aq. 2 x 30 mL) and brine, drying (MgSO₄), filtering and concentrating *in vacuo*. Flash chromatography (applied in toluene; eluted 2:1 toluene/EtOAc) afforded the title compound as a yellow solid (81.6 mg, 0.225 mmol, 63%). Mpt:

98-100 °C; R_f = 0.32 (1:1 toluene/EtOAc); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3035 (aromatic C-H and N-H stretches), 1654 (C=O stretch), 1591 (N-H bend), 1539, 1397 (aromatic C=C and C=N stretches); ^1H NMR (600 MHz, CD_3OD): δ_{H} = 3.98 (s, 2H, **20-H**), 7.28 (t, J = 7.3 Hz, 1H, **24-H**), 7.31 (td, J = 7.3, 1.0 Hz, 1H, **23-H**), 7.42 (t, J = 7.5 Hz, 1H, **17-H**), 7.50-7.57 (m, 4H, **5,10,11,16-H**), 7.60 (d, J = 7.3 Hz, 1H, **22-H**), 7.72 (d, J = 7.5 Hz, 1H, **18-H**), 7.85-7.87 (m, 2H, **12,25-H**), 8.10 (t, J = 1.8 Hz, 1H, **8-H**), 8.15 (dt, J = 8.0, 2.0 Hz, 1H, **4-H**), 8.54 (dd, J = 4.9, 1.4 Hz, 1H, **6-H**), 8.85 (d, J = 2.0 Hz, 1H, **2-H**); ^{13}C NMR (150 MHz, CD_3OD): δ_{C} = 36.1 (**C-20**), 118.3 (**C-8**), 119.7 (**C-12**), 122.1 (**C-25**), 122.7 (**C-10**), 124.0 (**C-5**), 124.6 (**C-22**), 125.2 (**C-16**), 126.0 (**C-17**), 126.2 (**C-18**), 126.3 (**C-24**), 126.8 (**C-23**), 129.5 (**C-11**), 131.3 (**C-15**), 135.1 (**C-4**), 136.8 (**C-3**), 137.7 (**C-27**), 137.9 (**C-7**), 139.4 (**C-9**), 139.5 (**C-26**), 143.6 (**C-21**), 144.3 (**C-19**), 146.8 (**C-2**), 147.4 (**C-6**), 169.7 (**C-14**); LRMS m/z (ES^+): 363 $[\text{M}+\text{H}]^+$, 404 $[\text{M}+\text{MeCN}]^+$; HRMS m/z (ES^+): Found 363.1483 $[\text{M}+\text{H}]^+$; $\text{C}_{25}\text{H}_{19}\text{N}_2\text{O}$ requires 363.1497.

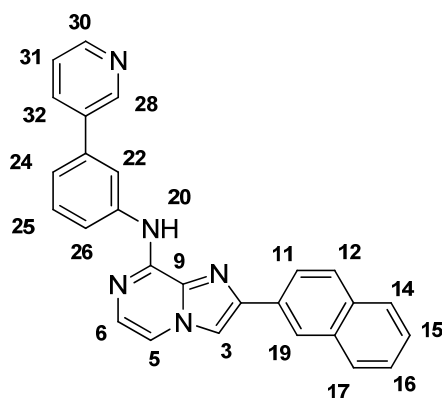
***N*-(Methylsulfonyl)-*N*-(3-(pyridin-3-yl)phenyl)methanesulfonamide (279)**



To a solution of **262** (50.7 mg, 0.298 mmol) in anhydrous CH_2Cl_2 (3 mL) was added Et_3N (83.1 μL , 0.596 mmol) and the mixture was cooled on ice. Methanesulfonyl chloride (27.7 μL , 0.358 mmol) was added and the reaction was stirred at RT for 16 h. The solidified reaction was diluted with CH_2Cl_2 (50 mL) and washed with NaHCO_3 (sat. aq. 30 mL), H_2O (30 mL) and brine (30 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Flash chromatography (applied in toluene; eluted 1:1 toluene/EtOAc) afforded the title compound as a white solid (80.1 mg, 0.246 mmol, 82%). Mpt: 174-175 °C; R_f = 0.33 (2:1 EtOAc/toluene); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 2996, 2918 (C-H stretch), 1334 (S=O asymmetrical stretch), 1153 (S=O symmetrical stretch); ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{H} = 3.59 (s, 6H, **15-H**), 7.52-7.54 (m, 1H, **5-H**), 7.57-7.59 (m, 1H, **10-H**), 7.63 (t, J = 7.8 Hz, 1H, **11-H**), 7.88-7.91 (m, 2H, **8,12-H**), 8.17 (dt, J = 7.9, 2.1 Hz, 1H, **4-H**), 8.62 (dd, J = 4.8, 1.5 Hz, 1H, **6-H**), 8.98 (d, J = 2.1 Hz, 1H, **2-H**); ^{13}C NMR (150 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{C} = 43.2 (**C-15**), 123.9 (**C-5**), 128.6 (**C-12**), 129.3 (**C-8**), 130.3 (**C-11**), 130.6 (**C-10**), 134.1 (**C-3**), 134.5 (**C-4**), 134.8 (**C-9**), 138.6 (**C-7**), 147.9 (**C-2**), 149.1 (**C-6**); LRMS m/z

(EI⁺): 326 [M]⁺, 248 [M-(SO₂Me)]⁺; HRMS m/z (EI⁺): Found 326.0395 [M]⁺; C₁₃H₁₄N₂O₄S₂ requires 326.0390; Anal. Calcd. for C₁₃H₁₄N₂O₄S₂: C, 47.84; H, 4.32; N, 8.58. Found C, 47.77; H, 4.31; N, 8.44%.

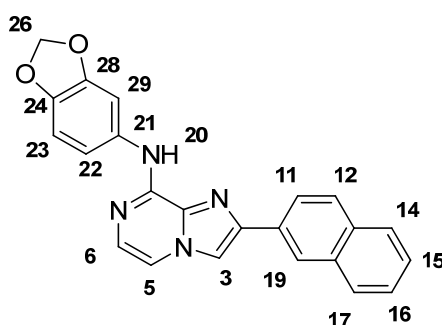
2-(Naphthalen-2-yl)-N-(3-(pyridin-3-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine (280)



All glassware was dried and purged with Ar prior to use. Pd₂(dba)₃ (1.28 mg, 1 mol%), DavePhos (1.65 mg, 3 mol%) and NaO^tBu (18.8 mg, 0.195 mmol) were dissolved in anhydrous toluene (2 mL). **214** (39.0 mg, 0.140 mmol) and **262** (28.5 mg, 0.168 mmol) were added and the reaction was stirred under reflux, under Ar for 16 h. The reaction was cooled to RT, and solvent removed *in vacuo*. The crude material was taken up in CH₂Cl₂ (50 mL) and washed H₂O (30 mL) and NaHCO₃ (sat. aq. 30 mL). The combined aqueous layers were then re-extracted with CH₂Cl₂ (2 x 30 mL) followed by washing the combined organics with brine (30 mL), drying (MgSO₄), filtering and concentrating *in vacuo*. Purification by flash chromatography (applied in toluene; eluted 20% to 33% EtOAc) afforded the title compound as a sticky brown solid (18.9 mg, 0.046 mmol, 33%). *R_f* = 0.26 (1:1 toluene/EtOAc); IR (ν_{max}/cm⁻¹, thin film): 2922 (N-H and aromatic C-H stretches), 1514 (aromatic C=C stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 7.32 (d, *J* = 7.8 Hz, 1H, **24-H**), 7.42 (dd, *J* = 7.8, 4.8 Hz, 1H, **31-H**), 7.49-7.53 (m, 4H, **6,15,16,25-H**), 7.61 (d, *J* = 4.6 Hz, 1H, **5-H**), 7.87 (d, *J* = 7.8 Hz, 1H, **14-H**), 7.92 (dd, *J* = 8.2, 1.6 Hz, 1H, **26-H**), 7.93-7.95 (m, 3H, **3,12,17-H**), 7.98 (dt, *J* = 7.8, 1.9 Hz, 1H, **32-H**), 8.03 (dd, *J* = 8.5, 1.6 Hz, 1H, **11-H**), 8.28 (bt, *J* = 1.6 Hz, 1H, **22-H**), 8.32 (bs, 1H, **20-H**), 8.49 (s, 1H, **19-H**), 8.62 (dd, *J* = 4.8, 1.4 Hz, 1H, **30-H**), 8.94 (d, *J* = 1.9 Hz, 1H, **28-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 111.2 (**C-3**), 111.8 (**C-5**), 118.3 (**C-22**), 119.3 (**C-26**), 121.8 (**C-24**), 123.8 (**C-31**), 124.1 (**C-11**), 125.0 (**C-19**), 126.4 (**C-15**), 126.6 (**C-16**), 127.9 (**C-14**), 128.4 (**C-17**), 128.5 (**C-6**), 128.7 (**C-12**),

129.9 (C-25), 130.4 (C-10), 133.4 (C-13), 133.7 (C-18), 133.8 (C-9), 134.9 (C-32), 136.8 (C-27), 138.7 (C-23), 140.2 (C-21), 145.0 (C-2), 146.2 (C-8), 148.4 (C-28), 148.4 (C-30); LRMS m/z (ES^+): 414 $[M+H]^+$; HRMS m/z (ES^+): Found 414.1706 $[M+H]^+$; $C_{27}H_{20}N_5$ requires 414.1719.

***N*-(Benzo[d][1,3]dioxol-5-yl)-2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-amine (281)**



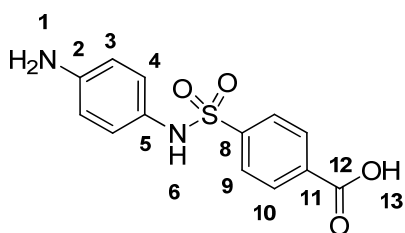
All glassware was dried and purged with Ar prior to use. $Pd_2(dba)_3$ (1.64 mg, 1 mol%), DavePhos (2.11 mg, 3 mol%) and NaO^tBu (24.1 mg, 0.250 mmol) were dissolved in anhydrous toluene (2 mL). **214** (50.0 mg, 0.179 mmol) and benzo[d][1,3]dioxol-5-amine, **264** (29.4 mg, 0.215 mmol) were added and the reaction was stirred under reflux, under Ar for 16 h. The reaction was cooled to RT and solvent removed *in vacuo*, before the residue was taken up in CH_2Cl_2 (50 mL) and washed with H_2O (3 x 30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (1st: applied in pet. ether; eluted 3:1 to 1:1 to 1:3 pet. ether/EtOAc; 2nd: applied in toluene; eluted 15:1 toluene/EtOAc) afforded the title compound as a brown solid (11.7 mg, 0.031 mmol, 17%). Mpt: Decomposed before melting; R_f = 0.38 (10% EtOAc/pet. ether); IR (ν_{max}/cm^{-1} , thin film): 3392 (aromatic C-H stretch), 1889, 1625, 1551, 1496, 1484 (aromatic C=C and C=N stretches); 1H NMR (600 MHz, $CDCl_3$): δ_H = 5.99 (s, 2H, **26-H**), 6.84 (d, J = 8.3 Hz, 1H, **23-H**), 7.16 (bd, J = 7.5 Hz, 1H, **22-H**), 7.44 (bd, J = 4.4 Hz, 1H, **6-H**), 7.48-7.53 (m, 2H, **15,16-H**), 7.55 (d, J = 4.4 Hz, 1H, **5-H**), 7.63 (d, J = 1.7 Hz, 1H, **29-H**), 7.86 (d, J = 7.9 Hz, 1H, **14-H**), 7.92-7.95 (m, 3H, **3,12,17-H**), 8.02 (dd, J = 8.5, 1.6 Hz, 2H, **11,20-H**), 8.48 (s, 1H, **19-H**); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C = 101.3 (C-26), 103.0 (C-29), 108.4 (C-23), 111.1 (C-3), 113.3 (C-5), 124.1 (C-11), 124.9 (C-19), 126.3 (C-15), 126.6 (C-16), 127.9 (C-14), 128.5 (overlapping signals, C-6,17), 128.7 (C-12), 130.4 (C-10), 133.4 (overlapping signals, C-9,13), 133.5 (C-21), 133.8 (C-18), 143.7 (C-24), 144.0 (C-2), 146.3 (C-

8), 147.9 (C-28); LRMS m/z (ES^+): 381 $[M+H]^+$; HRMS m/z (EI^+): Found 380.1273 $[M]^+$; $C_{23}H_{16}N_4O_2$ requires 380.1268.

6.1.4 Synthesis PEGylated Imidazo[1,2-*a*]pyrazines

6.1.4.1 Synthesis of the Carbamate Linker

4-(*N*-(4-aminophenyl)sulfamoyl)benzoate (310)



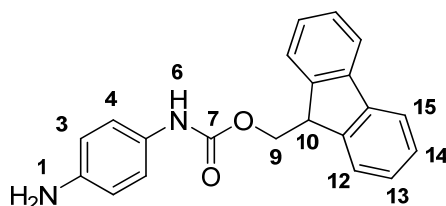
Method A:

Benzene-1,4,-diamine (4.11 g, 38.0 mmol) was taken up in anhydrous CH_2Cl_2 (150 mL), Et_3N (5.30 mL, 38.0 mmol) was added and the mixture was cooled to 0 °C. 4-(Chlorosulfonyl)benzoic acid (1.68 g, 7.60 mmol) was added and the reaction was stirred at RT for 16 h. Removal of the reaction solvent *in vacuo*, followed by purification *via* flash chromatography (applied in CH_2Cl_2 ; eluted 1% to 2% to 5% to 10% to 20% MeOH) afforded the title compound as a partial triethylamine salt, as dark brown/black solid (2.74 g, 6.79 mmol, 92%). Mpt: >200°C; R_f = 0.20 (10% MeOH/ CH_2Cl_2); IR (ν_{max}/cm^{-1} , thin film): 3329, 3256 (N-H and O-H stretches), 2979 (C-H stretch), 1603 (C=O stretch), 1409 (N-H bend), 1400, 1331 (S=O asymmetric stretch), 1164 (S=O symmetric stretch); 1H NMR (600 MHz, $(CD_3)_2SO$): δ_H = 1.14 (t, J = 7.2 Hz, 3H, Et_3N : CH_3), 2.94-2.96 (m, 2H, Et_3N : CH_2), 3.39 (bs, 3H, **1,13-H**), 6.35-6.38 (m, 2H, **3-H**), 6.64-6.66 (m, 2H, **4-H**), 7.65 (d, J = 8.2Hz, 2H, **9-H**), 8.01 (d, J = 8.2 Hz, 2H, **10-H**), 9.55 (bs, 1H, **6-H**); ^{13}C NMR (150 MHz, $(CD_3)_2SO$): δ_C = 9.01 (C-14), 45.3 (C-15), 114.0 (C-3), 124.9 (C-4), 125.0 (C-5), 126.6 (C-9), 129.6 (C-10), 139.0 (C-11), 141.7 (C-8), 146.7 (C-2), 168.2 (C-12); LRMS m/z (ES^+): 342 $[M+K]^+$, 316 $[M+Na]^+$, 195 $[M-Benzoic\ Acid + Na]^+$, 161 $[M-131]^+$; (ES^-): 337 $[M+Formic\ Acid]^-$, 291 $[M-H]^-$; HRMS m/z (ES^-): Found 291.0426 $[M-H]^-$; $C_{13}H_{11}N_2O_4S$ requires 291.0440; Anal. Calcd. For $C_{13}H_{11}N_2O_4S$: C, 57.99; H, 6.92; N, 10.68. Found C, 48.90; H, 5.08; N, 8.86%.

Method B:

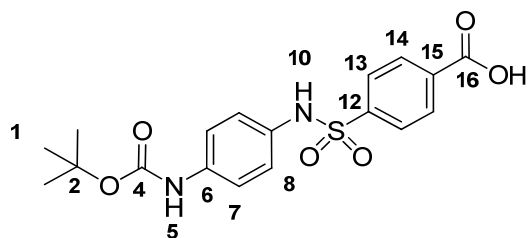
312 (41.4 mg, 0.106 mmol) was taken up in anhydrous CH₂Cl₂ (5 mL) and TFA (5 mL) was added to the mixture which was then stirred at RT for 5 h. Removal of solvent *in vacuo* followed by flash chromatography (applied in CH₂Cl₂; eluted 10% to 20% MeOH) afforded the title compound as a colourless oil (11.2 mg, 0.038 mmol, 36%).

(9H-Fluoren-9-yl)methyl 4-aminophenylcarbamate (311)



Benzene-1,4-diamine (4.00 g, 37.0 mmol) was taken up in anhydrous CH₂Cl₂ (200 mL) and the vessel was purged with Ar. Et₃N (1.72 mL, 12.3 mmol) was added followed by Fmoc-OSu (4.16 g, 12.3 mmol) and the reaction was stirred under Ar for 16 h. The resulting pink suspension was diluted with CH₂Cl₂ (100 mL) and washed with H₂O (100 mL). Extraction with CH₂Cl₂ (3 x 75 mL) was followed by combining the organic layers and washing with brine (75 mL), drying (MgSO₄), filtering and concentrating *in vacuo*. Flash chromatography (applied in pet. ether; eluted 5:1 to 1:1 pet. ether/EtOAc) afforded the title compound as a yellow solid (3.23 g, 9.77 mmol, 79%) with NMR values similar to those reported for the HCl salt.³¹² Mpt: 178-180 °C; *R_f* = 0.33 (1:1 pet. ether/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3306 (aromatic C-H stretch), 1682 (C=O stretch), 1528 (N-H bend), 1231 (COOR stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_{H} = 4.27 (t, *J* = 6.7 Hz, 1H, **10-H**), 4.39 (bd, *J* = 6.0 Hz, 2H, **9-H**), 4.80 (s, 2H, **1-H**), 6.48 (bd, *J* = 6.7 Hz, 2H, **3-H**), 7.08 (bd, *J* = 6.7 Hz, 2H, **4-H**), 7.35 (ap.t, *J* = 7.5 Hz, 2H, **13-H**), 7.42 (ap.t, *J* = 7.5 Hz, 2H, **14-H**), 7.74 (bd, *J* = 5.6 Hz, 2H, **12-H**), 7.91 (d, *J* = 7.5 Hz, 2H, **15-H**), 9.22 (bs, 1H, **6-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_{C} = 46.7 (**C-10**), 62.5 (**C-9**), 114.0 (**C-3**), 120.2 (**C-15**), 120.4 (**C-4**), 125.2 (**C-12**), 127.1 (**C-13**), 127.3 (**C-5**), 127.7 (**C-14**), 129.0 (**C-2**), 140.8 (**C-16**), 143.9 (**C-11**), 153.4 (**C-7**); LRMS *m/z* (EI⁺): 330 [*M*]⁺; HRMS *m/z* (EI⁺): Found 330.1356 [*M*]⁺; C₂₁H₁₈N₂O₂ requires 330.1363; Anal. Calcd. for C₂₁H₁₈N₂O₂: C, 76.34; H, 5.49; N, 8.48. Found C, 76.27; H, 5.53; N, 8.47%.

4-(N-(4-(tert-Butoxycarbonylamino)phenyl)sulfamoyl)benzoic acid (312)



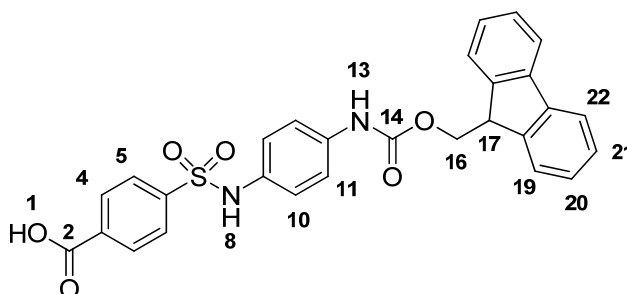
Method A:

235 (1.74 g, 8.38 mmol) was taken up in acetone, 4-(chlorosulfonyl)benzoic acid (616 mg, 2.79 mmol) was added and the mixture was stirred at RT for 16 h. The solvent was then removed *in vacuo*, the crude material was treated with NaHCO₃ (sat. aq. 100 mL), and the resulting precipitate was filtered. 2 M HCl (75 mL) was slowly added to the filtrate and the resulting material was filtered and dried to give a white solid (230 mg, 0.786 mmol, 28%).

Method B:

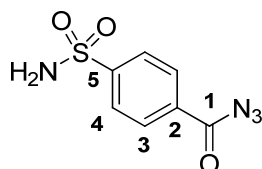
235 (2.68 g, 12.9 mmol) was taken up in acetone, 4-(chlorosulfonyl)benzoic acid (2.84 g, 12.9 mmol) was added and the mixture was stirred at RT for 16 h. The solvent was removed and the crude material was purified *via* flash chromatography (applied in toluene; eluted 10% to 20% to 30% to 40% to 50% to 80% to 100% EtOAc then 5% MeOH/EtOAc) to give the title compound as a light brown solid (1.72 g, 4.40 mmol, 34%). Mpt: >200°C; *R_f* = 0.28 (9:1 Et₂O/toluene); IR (ν_{max} /cm⁻¹, thin film): 3367 (N-H stretch), 3271 (O-H stretch), 2844 (C-H stretch), 1670, 1679 (C=O stretches), 1530 (N-H bend), 1338 (S=O asymmetric stretch), 1290 (COOR stretch), 1157 (S=O symmetric stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_{H} = 1.43 (s, 9H, **1-H**), 6.92 (d, *J* = 8.8 Hz, 2H, **8-H**), 7.28 (d, *J* = 8.8 Hz, 2H, **7-H**), 7.78 (d, *J* = 8.5 Hz, 2H, **13-H**), 8.04 (d, *J* = 8.5 Hz, 2H, **14-H**), 9.29 (bs, 1H, **5-H**), 10.15 (s, 1H, **9-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_{C} = 28.1 (**C-1**), 79.1 (**C-2**), 118.7 (**C-7**), 122.4 (**C-8**), 127.1 (**C-13**), 130.1 (**C-14**), 131.0 (**C-6**), 134.4 (**C-9**), 136.7 (**C-15**), 143.0 (**C-12**), 152.7 (**C-4**), 166.2 (**C-16**); LRMS *m/z* (ES⁻): 391 [M-H]⁻ HRMS *m/z* (ES⁻): Found 391.0979 [M-H]⁻; C₁₈H₁₉N₂O₆S requires 391.0964.

4-(N-(4-(((9H-Fluoren-9-yl)methoxy)carbonylamino)phenyl)sulfamoyl)benzoic acid (313)



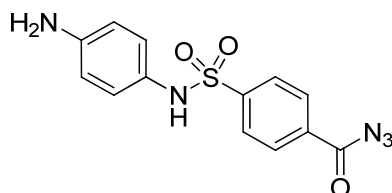
311 (3.23 g, 9.77 mmol) was taken up in acetone (100 mL). 4-(Chlorosulfonyl)benzoic acid (2.16 g, 9.77 mmol) was added and the mixture was stirred at RT for 16 h. The solvent was removed *in vacuo* and the crude material was purified *via* flash chromatography (applied in CH₂Cl₂; eluted 0.5% to 1% to 5% to 10% to 20% MeOH) afforded the title compound as a pale brown solid (936 mg, 1.82 mmol, 19%). Mpt: >200 °C; *R_f* = 0.22 (10% MeOH/CH₂Cl₂); IR (ν_{max} /cm⁻¹, thin film): 3351 (N-H stretch), 3274 (aromatic C-H stretch), 1705, 1683 (C=O stretches), 1521 (N-H bend), 1333 (S=O asymmetric stretch), 1220 (COOR stretch), 1161 (S=O symmetric stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_{H} = 4.28 (t, *J* = 6.5 Hz, 1H, **17-H**), 4.44 (bs, 2H, **16-H**), 6.95 (bs, 2H, **10-H**), 7.33 (t, *J* = 7.5 Hz, 4H, **11,20-H**), 7.41 (t, *J* = 7.5 Hz, **21-H**), 7.72 (bd, *J* = 7.1 Hz, 2H, **19-H**), 7.79 (d, *J* = 8.5 Hz, 2H, **5-H**), 7.89 (d, *J* = 7.5 Hz, 2H, **22-H**), 8.06 (d, *J* = 8.5 Hz, 2H, **4-H**), 9.67 (bs, 1H, **13-H**), 10.19 (bs, 1H, **8-H**), 13.46 (bs, 1H, **1-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_{C} = 46.6 (**C-17**), 65.6 (**C-16**), 119.0 (**C-11**), 120.2 (**C-22**), 122.3 (**C-10**), 125.1 (**C-19**), 127.0 (**C-5**), 127.2 (**C-20**), 127.7 (**C-21**), 130.1 (**C-4**), 131.5 (**C-9,12**), 134.4 (**C-3**), 140.8 (**C-23**), 143.1 (**C-6**), 143.8 (**C-18**), 153.4 (**C-14**), 166.2 (**C-2**); LRMS *m/z* (ES⁻): 513 [M-H]⁻; HRMS *m/z* (ES⁻): Found 513.1142 [M+H]⁺; C₂₈H₂₁N₂O₆S requires 513.1120; Anal. Calcd. for C₂₈H₂₂N₂O₆S: C, 65.36; H, 4.31; N, 5.44. Found C, 65.17; H, 4.33; N, 5.35%.

4-Sulfamoylbenzoyl azide (315)



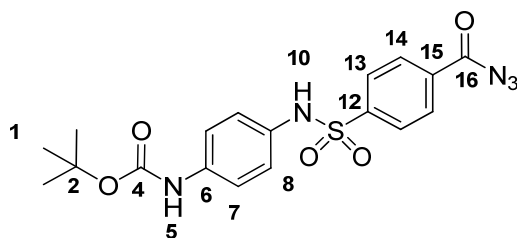
4-Sulfamoylbenzoic acid (1.00 g, 4.97 mmol), PPh₃ (2.61 g, 9.94 mmol) and NaN₃ (0.388 g, 5.96 mmol) were suspended in anhydrous acetone (10 mL). To this milky white suspension was added trichloroacetonitrile (0.997 mL, 9.94 mmol) dropwise and the reaction was left to stir at RT for 18 h. The solvent was removed *in vacuo* (no heat) and the resulting dark yellow slurry was diluted with CH₂Cl₂ (100 mL) and washed with H₂O (60 mL) and brine (50 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 3:1 to 2:1 to 1:1 pet. ether/EtOAc) afforded the title compound as a white solid (907 mg, 4.01 mmol, 81%). Mpt: 118 °C; *R_f* = 0.23 (1:1 pet. ether/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3362 (N-H stretch), 3258 (aromatic C-H stretch), 2137 (N=N=N stretch), 1687 (C=O stretch), 1339 (S=O asymmetric stretch), 1238, 1155 (S=O symmetrical stretch); ¹H NMR (600 MHz, CD₃OD): δ_{H} = 8.01-8.02 (m, 2H, **4-H**), 8.17-8.18 (m, 2H, **3-H**); ¹³C NMR (150 MHz, CD₃OD): δ_{C} = 127.6 (**C-4**), 131.0 (**C-3**), 134.9 (**C-2**), 150.2 (**C-5**), 172.8 (**C-1**); LRMS/HRMS *m/z* (ES⁺): no product mass present; Anal. Calcd. for C₇H₆N₄O₃S: C, 37.17; H, 2.67; N, 24.77. Found C, 37.27; H, 2.49; N, 24.40%.

4-(N-(4-Aminophenyl)sulfamoyl)benzoyl azide (316)



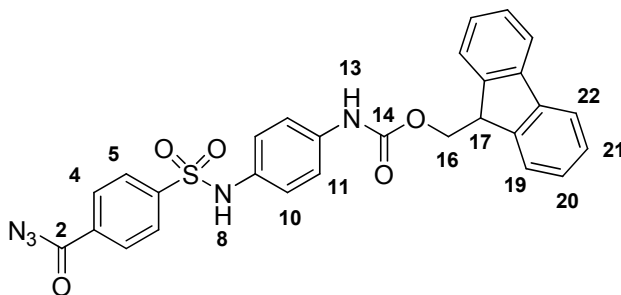
310 (640 mg, 2.19 mmol), PPh₃ (1.15 g, 4.38 mmol) and NaN₃ (171 mg, 2.63 mmol) were suspended in anhydrous acetone (5 mL). Trichloroacetonitrile (440 μ L, 4.38 mmol) was added dropwise and the reaction was left to stir at RT for 18 h. The solvent was removed *in vacuo* (no heat) and the resulting dark brown slurry was diluted with CH₂Cl₂ (75 mL) and washed with H₂O (50 mL) and brine (50 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. The reaction profile by TLC was deemed too messy to continue and so no further work was carried out.

***tert*-Butyl 4-(4-(azidocarbonyl)phenylsulfonamido)phenylcarbamate (317)**



312 (1.66 g, 4.22 mmol), PPh_3 (2.22 g, 8.44 mmol) and NaN_3 (329 mg, 5.07 mmol), were suspended in anhydrous acetone (15 mL). Trichloroacetonitrile (847 μL , 8.44 mmol) was added dropwise and the clear brown/green solution was stirred at RT (after a few minutes a white precipitate was evident). After stirring for 16 h the reaction was concentrated *in vacuo* (no heat), diluted with CH_2Cl_2 (100 mL) and washed with H_2O (60 mL) and brine (60 mL), dried (MgSO_4), filtered and solvent removed. Flash chromatography (applied in pet. ether; eluted 5:1 to 4:1 to 3:1 to 2:1) afforded the title compound as an off white solid (835 mg, 2.00 mmol, 48%). Mpt: 165 $^\circ\text{C}$; R_f = 0.6 (1:1 pet. ether/EtOAc); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3349, 3267 (aromatic C-H and N-H stretches), 2141 ($-\text{N}=\text{N}^+=\text{N}^-$ stretch), 1699, 1686 (C=O stretches), 1519 (N-H bend), 1301 (S=O asymmetric stretch), 1245 (COOR stretch), 1158 (S=O stretch); ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{H} = 1.43 (s, 9H, **1-H**), 6.92 (ap.d, J = 8.9 Hz, 2H, **8-H**), 7.29 (bd, J = 8.5 Hz, 2H, **7-H**), 7.83 (d, J = 6.9 Hz, 2H, **13-H**), 8.08 (d, J = 6.7 Hz, 2H, **14-H**), 9.31 (bs, 1H, **5-H**), 10.20 (s, 1H, **10-H**); ^{13}C NMR (150 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{C} = 28.1 (**C-1**), 79.1 (**C-2**), 118.7 (**C-7**), 122.6 (**C-8**), 127.5 (**C-13**), 129.9 (**C-14**), 130.8 (**C-6**), 133.5 (**C-15**), 136.9 (**C-9**), 144.4 (**C-12**), 152.7 (**C-4**), 171.0 (**C-16**); LRMS and HRMS m/z (EI^+): no product mass.

(9H-Fluoren-9-yl)methyl 4-(4-(azidocarbonyl)phenylsulfonamido)phenylcarbamate (318)

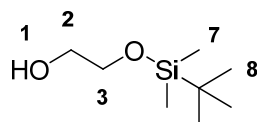


313 (200 mg, 0.389 mmol), PPh₃ (204 mg, 0.778 mmol) and NaN₃ (30.4 mg, 0.467 mmol) were suspended in anhydrous acetone (1 mL). Trichloroacetonitrile (78.0 μ L, 0.788 mmol) was added dropwise and the reaction was left to stir at RT for 18 h. The solvent was removed *in vacuo* and the resulting dark yellow slurry was diluted with CH₂Cl₂ (50 mL) and washed with H₂O (40 mL) and brine (40 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 3:1 to 1:1 pet. ether/EtOAc) afforded the title compound as a white solid (164 mg, 0.305 mmol, 78%). Mpt: 150-152 °C; *R_f* = 0.61 (1:1 pet. ether/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3265 (aromatic C-H stretch), 2189 (-N=N⁺=N⁻ stretch), 1707, 1686 (C=O stretches), 1529 (N-H bend), 1256 (S=O asymmetric stretch), 1220 (C-O stretch), 1160 (S=O symmetric stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_{H} = 4.28 (t, *J* = 6.5 Hz, 1H, **17-H**) 4.44 (bs, 2H, **16-H**), 6.94 (bd, *J* = 6.4 Hz, 2H, **10-H**), 7.33 (t, *J* = 7.3 Hz, 4H, **11,21-H**), 7.42 (t, *J* = 7.3 Hz, 2H, **20-H**), 7.72 (d, *J* = 7.3 Hz, 2H, **22-H**), 7.83-7.85 (m, 2H, **5-H**), 7.90 (d, *J* = 7.3 Hz, 2H, **19-H**), 8.09 (d, *J* = 8.5 Hz, 2H, **4-H**), 9.68 (bs, 1H, **13-H**), 10.25 (s, 1H, **8-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_{C} = 46.6 (**C-17**), 65.6 (**C-16**), 120.2 (**C-19**), 122.4 (**C-10**), 125.1 (**C-22**), 127.2 (overlapping signals, **C-11,21**), 127.4 (**C-5**), 127.7 (**C-20**), 129.9 (**C-4**), 131.5 (overlapping signals, **C-9,12**), 133.6 (**C-3**), 140.8 (**C-18**), 143.8 (**C-23**), 144.4 (**C-6**), 153.4 (**C-14**), 171.0 (**C-2**); LRMS *m/z* (ES⁺): 534, 443.

6.1.4.2 Synthesis of PEGylated Free Hydroxyl Imidazo[1,2-*a*]pyrazines

6.1.4.2.1 PEGylated-Benzenesulfonamide-bound Imidazo[1,2-*a*]pyrazines

2-(*tert*-Butyl(dimethyl)silyl)oxyethanol (**338**)



Method A:

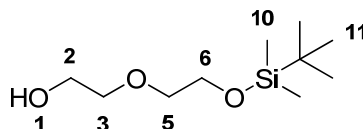
Anhydrous CH₂Cl₂ (100 mL) was added to a dry round bottom flask which was then evacuated and back-flushed with Ar. Ethylene glycol (2.00 mL, 35.9 mmol) and imidazole (7.32 g, 108 mmol) were added and the mixture was cooled to 0 °C whilst stirring. *tert*-Butyl(dimethyl)silyl chloride (6.49 g, 43.0 mmol) was added and the reaction was then stirred at 0 °C for 40 min.

After this time, the reaction was quenched by the addition of H₂O (250 mL) and the organic layer separated. The remaining aqueous layer was then further extracted with CH₂Cl₂ (2 x 100 mL) before the combined organics were washed with brine (200 mL) and dried (MgSO₄). Concentrating *in vacuo* gave a crude oil which was purified *via* flash chromatography (applied in Pet ether; *in vacuo* 30:1 to 19:1 to 5:1 pet. ether/EtOAc) to give the title compound as a colourless oil (2.16 g, 12.3 mmol, 34%) with the NMR consistent with literature values.³¹³ *R*_f = 0.60 (5:1 pet. ether/EtOAc); *v*_{max}/cm⁻¹ (thin film): 3370 (O-H stretch), 2955, 2858 (C-H stretches); ¹H NMR (500 MHz, CDCl₃): δ_H = 0.08 (s, 6H, **7-H**), 0.91 (s, 9H, **8-H**), 1.98 (bs, 1H, **1-H**), 3.63-3.65 (m, 2H, **2-H**), 3.70-3.72 (m, 2H, **3-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = -5.3 (**C-7**), 18.4 (**C-6**), 26.0 (**C-8**), 63.8 (**C-2**), 64.1 (**C-3**); LRMS *m/z* (CI⁺): 177 [M+H]⁺; HRMS (CI⁺): Found 177.1303 [M+H]⁺; C₈H₂₁O₂Si requires 177.1311.

Method B:²⁶⁴

Ethylene glycol (3.00 mL, 53.8 mmol), Et₃N (11.2 mL, 80.7 mmol) and DMAP (65.7 mg, 1 mol%) were dissolved in anhydrous CH₂Cl₂ (300 mL) and cooled to 0 °C. *tert*-Butyl(dimethyl)silyl chloride (9.74 g, 64.6 mmol) was added to the reaction mixture in 1 mL portions over a period of 1 h. The reaction was then allowed to stir at RT for 16 h before diluting with H₂O (150 mL) and extracting the organic phase. This was then further washed with NaHCO₃ (sat. aq. 75 mL), NH₄Cl (sat. aq. 75 mL), H₂O (75 mL) and brine (75 mL), before drying (MgSO₄). The solvent was removed *in vacuo* to give crude pale yellow oil, which upon purification using flash chromatography (applied in CH₂Cl₂: eluted 30:1 to 5:1 CH₂Cl₂/EtOAc) afforded the title compound as a colourless oil (5.74 g, 32.6 mmol, 61%).

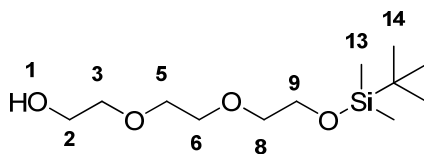
2-(2-(*tert*-Butyldimethylsilyloxy)ethoxy)ethanol (339)



Diethylene glycol (1.31 mL, 13.8 mmol), Et₃N (2.88 mL, 20.7 mmol) and DMAP (16.8 mg, 1 mol%) was dissolved in anhydrous CH₂Cl₂ (100 mL) and cooled on ice. *tert*-Butyl(dimethyl)silyl chloride (2.50 g, 16.5 mmol) was added portionwise and the reaction was stirred at RT for 16 h. The reaction was then washed with H₂O (60 mL), NaHCO₃ (sat. aq. 60

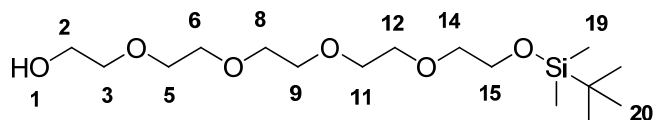
mL), H₂O (60 mL) and brine (60 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 25:1 to 19:1 to 5:1 to 2:1 pet. ether/EtOAc) afforded the title compound as a colourless oil (1.49 g, 6.75 mmol, 50%) with the NMR consistent with literature values.³¹⁴ R_f = 0.13 (5:1 pet. ether/EtOAc); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3343 (O-H stretch), 2954, 2929, 2885, 2858 (C-H stretch), 1253 (Si-C stretch), 1058 (C-O and Si-O stretches), 832 (Si-C stretch); ¹H NMR (600 MHz, CDCl₃): δ_{H} = 0.08 (s, 6H, **10-H**), 0.90 (s, 9H, **11-H**), 2.12 (bs, 1H, **1-H**), 3.59-3.53 (m, 4H, **3,5-H**), 3.71-3.73 (m, 2H, **2-H**), 3.78 (t, J = 4.9 Hz, 2H, **6-H**); ¹³C NMR (150 MHz, CDCl₃): δ_{C} = -5.4 (**C-10**), 18.5 (**C-9**), 26.0 (**C-11**), 62.0 (**C-2**), 63.0 (**C-6**), 72.5 (**C-3**), 72.7 (**C-5**); LRMS m/z (CI⁺): 220 [M]⁺, 107 [M- Si(CH₃)₂(CH₃)₃]⁺; HRMS m/z (CI⁺): Found 220.1502 [M]⁺; C₁₀H₂₄O₃Si requires 220.1495.

2,2,3,3-Tetramethyl-4,7,10-trioxa-3-siladodecan-12-ol (340)



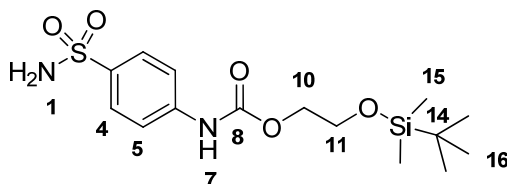
Triethylene glycol (1.89 mL, 13.9 mmol), Et₃N (2.90 mL, 20.8 mmol) and DMAP (16.9 mg, 1 mol%) was dissolved in anhydrous CH₂Cl₂ (100 mL) and cooled on ice. *tert*-Butyl(dimethyl)silyl chloride (2.51 g, 16.6 mmol) was added portionwise and the reaction was stirred at RT for 16 h. The reaction was then washed with H₂O (60 mL), NaHCO₃ (sat. aq. 60 mL), H₂O (60 mL) and brine (60 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 19:1 to 9:1 to 4:1 to 1:1 pet. ether/EtOAc) afforded the title compound as a colourless oil (1.70 g, 6.46 mmol, 47%) with NMR consistent with literature values.³¹⁵ R_f = 0.12 (2:1 pet. ether/EtOAc); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3455 (O-H stretch), 2952, 2929, 2858 (C-H stretch), 1252 (Si-C stretch), 1100 (C-O and Si-O stretches), 832 (Si-C stretch); ¹H NMR (600 MHz, CDCl₃): δ_{H} = 0.07 (s, 6H, **13-H**), 0.89 (s, 9H, **14-H**), 2.43 (bs, 1H, **1-H**), 3.57 (t, J = 5.3 Hz, 2H, **8-H**), 3.61-3.62 (m, 2H, **3-H**), 3.67 (s, 4H, **5,6-H**), 3.73 (bs, 2H, **2-H**), 3.77 (t, J = 5.3 Hz, 2H, **9-H**); ¹³C NMR (150 MHz, CDCl₃): δ_{C} = -5.2 (**C-13**), 18.5 (**C-12**), 26.0 (**C-14**), 61.9 (**C-2**), 62.8 (**C-9**), 70.6 (**C-5**), 70.9 (**C-6**), 72.6 (**C-3**), 72.8 (**C-8**); LRMS m/z (CI⁺): 265 [M+H]⁺, 207 [M-*t*Bu]⁺; HRMS m/z (CI⁺): Found 265.1841 [M+H]⁺; C₁₂H₂₉O₄Si requires 265.1835.

2,2,3,3-Tetramethyl-4,7,10,13,16-pentaoxa-3-silaooctadecan-18-ol (341)



Penta(ethylene) glycol (1.78 mL, 8.39 mmol), Et₃N (1.75 mL, 12.6 mmol) and DMAP (10.3 mg, 0.084 mmol) were dissolved in anhydrous CH₂Cl₂ (90 mL) and cooled to 0 °C. *tert*-Butyl(dimethyl)silyl chloride (1.52 g, 10.07 mmol), dissolved in anhydrous CH₂Cl₂ (10 mL) was added dropwise and the reaction was stirred at RT for 16 h. The mixture was diluted with H₂O (60 mL) and the organic phase was extracted followed by washing with saturated NaHCO₃ (sat. aq. 60 mL), NH₄Cl (sat. aq. 60 mL), H₂O (60 mL) and brine (60 mL), before drying (MgSO₄). The solvent was removed *in vacuo* to give crude colourless oil, which upon purification using flash chromatography (1st: applied in pet. ether; eluted 19:1 to 10:1 to 5:1 pet. ether/EtOAc; 2nd: applied in CH₂Cl₂; eluted 1:1 CH₂Cl₂/EtOAc) afforded the title compound as a colourless oil (567 mg, 1.61 mmol, 19%) with the NMR consistent with literature values.³¹⁶ *R*_f = 0.19 (5:1 EtOAc/pet. ether); IR (ν_{max}/cm⁻¹, thin film): 3444 (O-H stretch), 2927, 2857 (C-H stretch), 1251 (Si-C stretch), 1098 (C-O and Si-O stretches); ¹H NMR (500 MHz, CDCl₃): δ_H = 0.06 (s, 6H, **19-H**), 0.89 (s, 9H, **20-H**), 2.18 (bs, 1-H, **1-H**), 3.55 (t, *J* = 5.5 Hz, 2H, **14-H**), 3.60-3.61 (m, 2H, **2-H**), 3.62-3.68 (m, 12H, **5,6,8,9,11,12-H**), 3.71-3.72 (m, 2H, **3-H**), 3.76 (t, *J* = 5.5 Hz, 2H, **15-H**); ¹³C NMR (125 MHz, CDCl₃): δ_C = -5.2 (**C-19**), 18.4 (**C-18**), 26.0 (**C-20**), 61.8 (**C-3**), 62.8 (**C-15**), 70.4-70.8 (**C-5,6,8,9,11,12**), 72.3 (**C-2**), 72.7 (**C-14**); LRMS *m/z* (ES⁺): 375 [M+Na]⁺; HRMS *m/z* (ES⁺): Found 375.2170 [M+Na]⁺; C₁₆H₃₆O₆NaSi requires 375.2179.

2-(*tert*-Butyldimethylsilyloxy)ethyl 4-sulfamoylphenylcarbamate (319)



Method A:

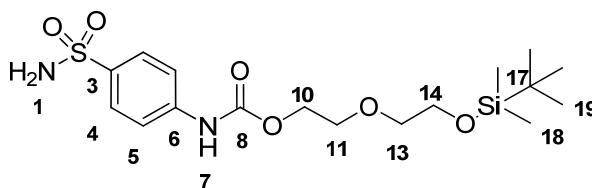
To a suspension of 4-sulfamoylbenzoic acid (2.00 g, 9.94 mmol) in anhydrous toluene (20 mL) was added Et₃N (1.62 mL, 11.6 mmol). Diphenylphosphoryl azide (2.50 mL, 11.6 mmol) in

anhydrous toluene (5 mL) was added dropwise to the reaction mixture and stirred at RT for 30 min followed by heating at 90 °C for 30 min. **338** (2.10 g, 11.9 mmol) in anhydrous DMF (5 mL) was then added and the reaction was stirred at 100 °C for 4 h. The reaction was cooled to RT and solvent removed *in vacuo*. The crude material was taken up in EtOAc (100 mL) and washed with NaHCO₃ (sat. aq. 50 mL), H₂O (4 x 40 mL) and brine (40 mL); dried (MgSO₄), filtered and concentrated *in vacuo* to give an orange/brown sticky solid. Flash chromatography (applied in pet. ether; eluted 5:1 to 1:1 pet. ether/EtOAc) afforded the title compound as a pale yellow solid (394 mg, 1.05 mmol, 11%).

Method B:

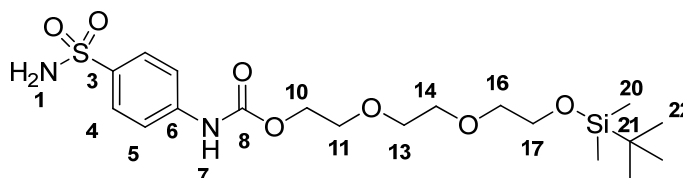
338 (114 mg, 0.646 mmol) was dissolved in anhydrous toluene (20 mL) and **315** (175 mg, 0.775 mmol) was added and the reaction was stirred under reflux for 16 h. The solvent was removed *in vacuo* and purification *via* flash chromatography (applied in toluene; eluted 5:1 to 3:1 to 2:1 to 1:1 toluene/EtOAc) afforded the title compound as a white solid (121 mg, 0.323 mmol, 50%). Mpt: 136-140 °C; *R_f* = 0.49 (2:1 EtOAc/pet. ether); IR (ν_{max} /cm⁻¹, thin film): 3325, 3236 (C-H stretch), 2950, 2928, 2857 (C-H and N-H stretch), 1719 (C=O stretch), 1598, 1540 (N-H bends), 1334 (S=O asymmetric stretch), 1242 (Si-C stretch), 1156 (S=O symmetric stretch), 1063 (Si-O stretch); ¹H NMR (600 MHz, CD₃OD): δ_{H} = 0.10 (s, 6H, **15-H**), 0.90 (s, 9H, **16-H**), 3.87-3.89 (m, 2H, **11-H**), 4.23-4.24 (m, 2H, **10-H**), 7.61 (d, *J* = 8.8 Hz, 2H, **5-H**), 7.79-7.81 (m, 2H, **4-H**); ¹³C NMR (150 MHz, CD₃OD): δ_{C} = -5.3 (**C-15**), 19.1 (**C-14**), 26.3 (**C-16**), 62.7 (**C-11**), 67.4 (**C-10**), 119.0 (**C-5**), 128.2 (**C-4**), 138.5 (**C-3**), 144.1 (**C-6**), 155.5 (**C-8**); LRMS *m/z* (ES⁻): 373 [M-H]⁻; HRMS *m/z* (ES⁻): Found 373.1253 [M-H]⁻; C₁₅H₂₅N₂O₅SSi requires 373.1274; Anal. Calcd. for C₁₅H₂₆N₂O₅SSi: C, 48.10; H, 7.00; N, 7.48. Found C, 48.36; H, 7.03; N, 7.40%.

2-(2-(*tert*-Butyldimethylsilyloxy)ethoxy)ethyl 4-sulfamoylphenylcarbamate (**320**)



339 (129 mg, 0.588 mmol) was dissolved in anhydrous toluene (15 mL) and **315** (160 mg, 0.706 mmol) was added and the reaction was stirred under reflux for 16 h. The solvent was removed *in vacuo* and purification *via* flash chromatography (applied in pet. ether; eluted 5:1 to 3:1 to 2:1 to 1:1 pet. ether/EtOAc) afforded the title compound as a white solid (92.0 mg, 0.220 mmol, 37%). Mpt: 75 °C; R_f = 0.11 (2:1 pet. ether/EtOAc); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3336, 3282 (N-H and aromatic C-H stretches), 2929, 2856 (C-H stretch), 1729 (C=O stretch), 1597, 1540 (N-H bends), 1341 (S=O asymmetric stretch), 1235 (Si-C stretch), 1158 (S=O symmetric stretch), 1065 (C-O and Si-O stretches); ^1H NMR (600 MHz, CD_3OD): δ_{H} = 0.07 (s, 6H, **17-H**), 0.89 (s, 9H, **19-H**), 3.58 (t, J = 5.0 Hz, 2H, **14-H**), 3.76 (t, J = 4.7 Hz, 2H, **11-H**), 3.78 (t, J = 5.7 Hz, 2H, **13-H**), 4.30 (t, J = 4.7 Hz, **10-H**), 7.61 (d, J = 8.8 Hz, 2H, **5-H**), 7.80 (d, J = 8.8 Hz, 2H, **4-H**); ^{13}C NMR (150 MHz, CD_3OD): δ_{C} = -5.2 (**C-17**), 19.2 (**C-18**), 26.4 (**C-19**), 64.0 (**C-14**), 65.4 (**C-10**), 70.5 (**C-11**), 73.7 (**C-13**), 118.9 (**C-5**), 128.2 (**C-4**), 138.5 (**C-3**), 144.1 (**C-6**), 155.4 (**C-8**); LRMS m/z (ES^+): 441 $[\text{M}+\text{Na}]^+$; HRMS m/z (ES^+): Found 441.1484 $[\text{M}+\text{Na}]^+$; $\text{C}_{17}\text{H}_{30}\text{N}_2\text{O}_6\text{NaSSi}$ requires 441.1492; Anal. Calcd. for $\text{C}_{17}\text{H}_{30}\text{N}_2\text{O}_6\text{SSi}$: C, 48.78; H, 7.22; N, 6.69. Found C, 48.68; H, 7.01; N, 6.40%.

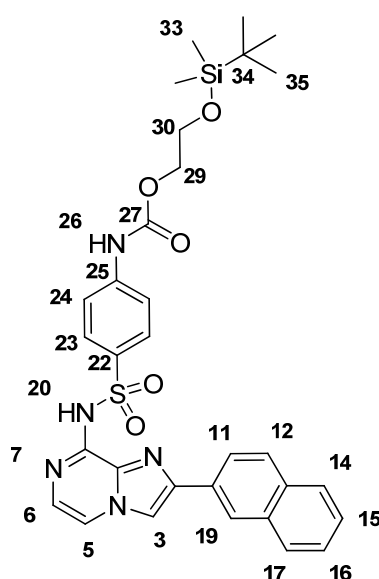
2,2,3,3-Tetramethyl-4,7,10-trioxa-3-siladodecan-12-yl-4-sulfamoylphenylcarbamate (321)



340 (148 mg, 0.560 mmol) was dissolved in anhydrous toluene (14 mL) and **315** (152 mg, 0.672 mmol) was added and the reaction was stirred under reflux for 16 h. The solvent was removed *in vacuo* and purification *via* flash chromatography (applied in CH_2Cl_2 ; eluted 2% MeOH) afforded the title compound as a white solid (119 mg, 0.256 mmol, 46%). Mpt: 76 °C; R_f = 0.53 (10% MeOH/ CH_2Cl_2); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3303 (N-H and aromatic C-H stretch), 2951, 2927, 2901, 2855 (C-H stretches), 1718 (C=O stretch), 1596, 1543 (N-H bends), 1332 (S=O asymmetric stretch), 1241 (Si-C stretch), 1155 (S=O symmetric stretch), 1100 (C-O and Si-O stretches); ^1H NMR (600 MHz, CD_3OD): δ_{H} = 0.07 (s, 6H, **20-H**), 0.90 (s, 9H, **22-H**), 3.54-3.56 (m, 2H, **16-H**), 3.65-3.68 (m, 4H, **13,14-H**), 3.75-3.77 (m, 4H, **11,17-H**), 4.29-4.30 (m, 2H, **10-H**), 7.61 (d, J = 8.9 Hz, 2H, **5-H**), 7.79-7.81 (m, 2H, **4-H**); ^{13}C NMR (150 MHz, CD_3OD): δ_{C} =

5.2 (C-20), 19.2 (C-21), 26.4 (C-22), 63.9 (C-17), 65.4 (C-10), 70.4 (C-11), 71.6 (C-13), 71.7 (C-14), 73.8 (C-16), 118.9 (C-5), 128.3 (C-4), 138.5 (C-3), 144.1 (C-6), 155.4 (C-8); LRMS m/z (ES^+): 485 $[M+Na]^+$, 463 $[M+H]^+$; HRMS m/z (ES^+): Found 485.1746 $[M+Na]^+$; $C_{19}H_{34}N_2O_7NaSSi$ requires 485.1754; Anal. Calcd. for $C_{19}H_{34}N_2O_7SSi$: C, 49.33; H, 7.41; N, 6.06. Found C, 49.53; H, 7.50; N, 5.82%.

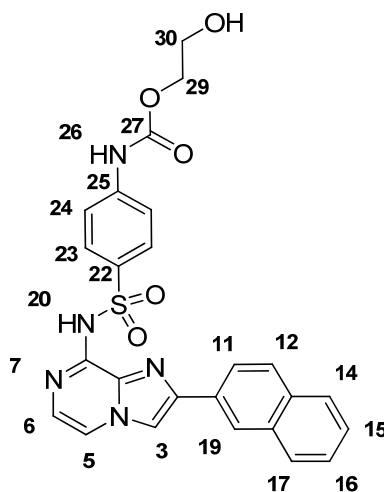
2-(tert-Butyldimethylsilyloxy)ethyl-4-(N-(2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-yl)-sulfamoyl)phenylcarbamate (342)



All glassware was dried and purged with Ar prior to use. $Pd_2(dba)_3$ (1.64 mg, 1 mol%), DavePhos (2.11 mg, 3 mol%) and NaO^tBu (24.1 mg, 0.250 mmol) were dissolved in anhydrous toluene (2 mL). **214** (50.0 mg, 0.179 mmol) and **319** (80.3 mg, 0.215 mmol) were added and the reaction was stirred under reflux, under Ar for 16 h. The reaction was cooled to RT and solvent removed *in vacuo*, before the residue was taken up in CH_2Cl_2 (50 mL) and washed with H_2O (3 x 30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 2:1 to 1:1 to 1:2 pet. ether/EtOAc) afforded the title compound as a pale yellow solid (44.5 mg, 0.072 mmol, 40%). Mpt: $>200\ ^\circ C$; R_f = 0.43 (1:3 pet. ether/EtOAc); IR (ν_{max}/cm^{-1} , thin film): 3275 (aromatic C-H stretch), 2952, 2927, 2880, 2854 (C-H and N-H stretches), 1736 (C=O stretch), 1595, 1535 (N-H bends), 1395 (S=O asymmetric stretch), 1219 (Si-C stretch), 1134 (S=O symmetric stretch), 1065 (C-O and Si-O stretches); 1H NMR (600 MHz, $(CD_3)_2SO$): δ_H = 0.04 (s, 6H, **33-H**), 0.83 (s, 9H, **35-H**), 3.81 (t, J = 4.7 Hz, 2H,

30-H), 4.16 (t, $J = 4.7$ Hz, 2H, **29-H**), 7.16 (t, $J = 5.5$ Hz, 1H, **6-H**), 7.50-7.55 (m, 2H, **15,16-H**), 7.65 (d, $J = 8.8$ Hz, 2H, **24-H**), 7.86 (d, $J = 5.5$ Hz, 1H, **5-H**), 7.92 (d, $J = 8.8$ Hz, 3H, **14,23-H**), 7.98 (d, $J = 8.6$ Hz, 1H, **12-H**), 8.02-8.05 (m, 2H, **11,17-H**), 8.52 (s, 1H, **19-H**), 8.59 (s, 1H, **3-H**), 10.13 (s, 1H, **26-H**), 11.63 (bd, $J = 3.3$ Hz, 1H, **7/20-H**); ^{13}C NMR (150 MHz, $(\text{CD}_3)_2\text{SO}$): $\delta_{\text{C}} = -5.3$ (**C-33**), 18.0 (**C-34**), 25.8 (**C-35**), 61.2 (**C-30**), 66.0 (**C-29**), 110.9 (**C-5**), 115.1, (**C-3**), 116.6 (**C-6**) 117.7 (**C-24**), 123.8 (**C-11**), 124.2 (**C-19**), 126.3 (**C-15**), 126.6 (**C-16**), 127.4 (**C-23**), 127.8 (**C-14**), 128.3 (**C-12**), 128.4 (**C-17**), 130.0 (**C-10**), 132.8 (**C-13**), 133.2 (**C-18**), 135.6 (**C-9**), 135.9 (**C-22**), 142.9 (**C-25**), 145.2 (**C-2**), 145.5 (**C-8**), 153.2 (**C-27**); LRMS m/z (ES^+): 618 $[\text{M}+\text{H}]^+$, 438 $[\text{M}-(\text{CO}_2\text{CH}_2\text{CH}_2\text{OTBDMS})+\text{Na}]^+$; HRMS m/z (ES^+): Found 618.2219 $[\text{M}+\text{H}]^+$; $\text{C}_{31}\text{H}_{36}\text{N}_5\text{O}_5\text{SSi}$ requires 618.2206; Anal. Calcd. for $\text{C}_{31}\text{H}_{35}\text{N}_5\text{O}_5\text{SSi}$: C, 60.27; H, 5.71; N, 11.34. Found C, 60.08; H, 5.50; N, 11.19%.

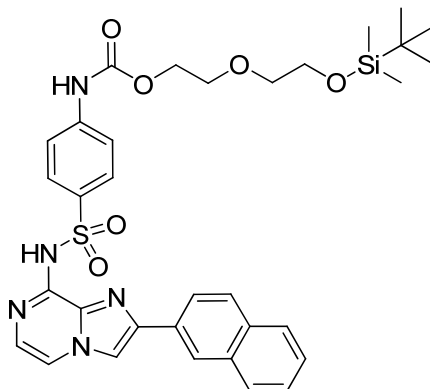
2-Hydroxyethyl-4-(N-(2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-yl)sulfamoyl)phenyl-carbamate (343)



342 (39.6 mg, 0.064 mmol) was taken up in THF (2 mL) and the mixture was cooled on ice. TBAF (1.0 M solution in THF, containing 5 wt% H_2O ; 128 μL , 0.128 mmol) was added and the reaction was stirred at RT for 6 h. Quenching with H_2O (20 mL) and extracting with EtOAc (3 x 20 mL) was followed by washing the combined organics with brine (20 mL), drying (MgSO_4), filtering and concentrating *in vacuo*. Flash chromatography (applied in CH_2Cl_2 ; eluted 2% MeOH) afforded the title compound as an off white (9.90 mg, 0.020 mmol, 31%). Mpt: Decomposed before melting; $R_f = 0.36$ (10% MeOH/ CH_2Cl_2); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3252 (O-

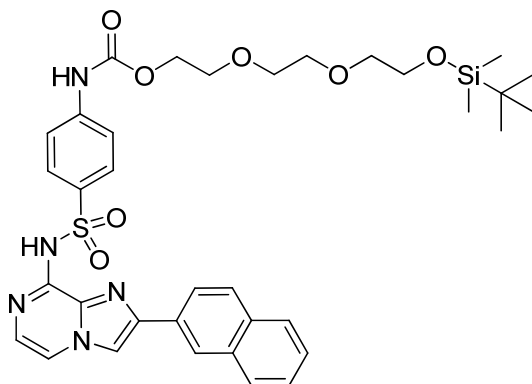
H and aromatic C-H stretch), 2921 (N-H and C-H stretches), 1592 (C=O stretch), 1123 (S=O symmetric stretch), 1114 (C-O stretch); ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{H} = 3.62 (q, J = 5.1 Hz, 2H, **30-H**), 4.11 (t, J = 5.1 Hz, 2H, **29-H**), 4.84 (t, J = 5.1 Hz, 1H, **31-H**), 7.14 (bs, 1H, **6-H**), 7.50-7.55 (m, 2H, **15,16-H**), 7.63 (bs, 2H, **24-H**), 7.84 (bs, 1H, **5-H**), 7.91-7.93 (m, 3H, **14,23-H**), 7.98 (d, J = 8.6 Hz, 1H, **12-H**), 8.02-8.05 (m, 2H, **11,17-H**), 8.52 (s, 1H, **19-H**), 8.57 (bs, 1H, **3-H**), 10.15 (bs, 1H, **26-H**), 11.63 (bs, 1H, **7/20-H**); ^{13}C NMR (150 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{C} = 59.2 (**C-30**), 66.4 (**C-29**), 110.9 (**C-5**), 115.1 (**C-3**), 116.6 (**C-6**), 117.5 (**C-24**), 123.8 (**C-11**), 124.1 (**C-19**), 126.6 (overlapping signals, **C-15,16**), 127.4 (**C-14**), 127.7 (**C-23**), 128.2 (**C-12**), 128.4 (**C-17**), 130.8 (**C-10**), 132.8 (**C-13**), 133.2 (**C-18**), 135.6 (**C-9**), 135.9 (**C-22**), 142.9 (**C-25**), 145.2 (**C-2**), 145.5 (**C-8**), 153.5 (**C-27**); LRMS m/z (ES^+): 504 $[\text{M}+\text{H}]^+$, 382; HRMS m/z (ES^+): Found 504.1345 $[\text{M}+\text{H}]^+$; $\text{C}_{25}\text{H}_{22}\text{N}_5\text{O}_5\text{S}$ requires 504.1342.

2-(2-(tert-Butyldimethylsilyloxy)ethoxy)ethyl-4-(N-(2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-yl)sulfamoyl)phenylcarbamate (344)



All glassware was dried and purged with Ar prior to use. $\text{Pd}_2(\text{dba})_3$ (1.64 mg, 1 mol%), DavePhos (2.11 mg, 3 mol%) and NaO^tBu (24.1 mg, 0.250 mmol) were dissolved in anhydrous toluene (2 mL). **214** (50.0 mg, 0.179 mmol) and **320** (89.7 mg, 0.215 mmol) were added and the reaction was stirred under reflux, under Ar for 16 h. The reaction was cooled to RT and solvent removed, before the residue was taken up in CH_2Cl_2 (50 mL) and washed with H_2O (3 x 30 mL) and brine (30 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. LCMS showed trace signs of product. No further work was carried out at this stage.

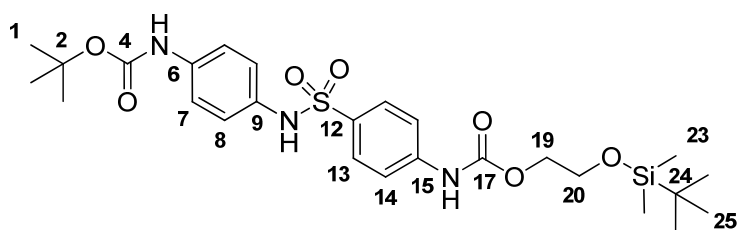
2,2,3,3-Tetramethyl-4,7,10-trioxa-3-siladodecan-12-yl-4-(N-(2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-yl)sulfamoyl)phenylcarbamate (345)



All glassware was dried and purged with Ar prior to use. Pd₂(dba)₃ (1.64 mg, 1 mol%), DavePhos (2.11 mg, 3 mol%) and NaO^tBu (24.1 mg, 0.250 mmol) were dissolved in anhydrous toluene (2 mL). **214** (50.0 mg, 0.179 mmol) and **321** (99.2 mg, 0.215 mmol) were added and the reaction was stirred under reflux, under Ar for 16 h. The reaction was cooled to RT and solvent removed, before the residue was taken up in CH₂Cl₂ (50 mL) and washed with H₂O (3 x 30 mL) and brine (30 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. LCMS showed trace signs of product. No further work was carried out at this stage.

6.1.4.2.2 PEGylated-N-(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2-a]pyrazines

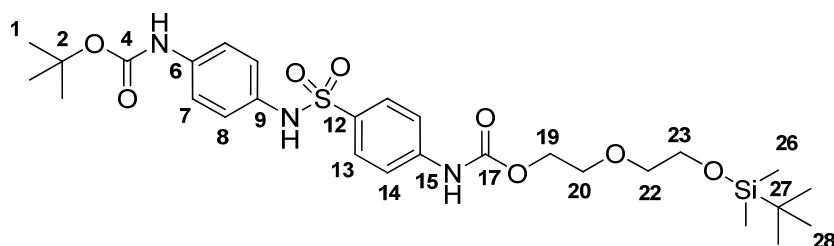
2-(tert-Butyldimethylsilyloxy)ethyl-4-(N-(tert-butyl-4-aminophenylcarbamate)sulfamoyl)-phenylcarbamate (329)



338 (83.1 mg, 0.472 mmol) was dissolved in anhydrous toluene (12 mL) and **317** (236 mg, 0.567 mmol) was added and the reaction was stirred under reflux for 16 h. The solvent was removed *in vacuo* and purification *via* flash chromatography (applied in toluene; eluted 20% Et₂O) afforded the title compound as an off white solid (233 mg, 0.412 mmol, 87%). Mpt: 164 °C; *R_f* = 0.49 (1:1

toluene/Et₂O); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3321, 3221 (N-H and aromatic C-H stretches), 2955, 2930 (C-H stretch), 1714, 1683 (C=O stretches), 1532 (N-H bend), 1241 (COOR and Si-C stretches), 1338 (S=O asymmetric stretch), 1160 (S=O symmetric stretch), 1058 (C-O and Si-O stretches); ¹H NMR (600 MHz, CD₃OD): δ_{H} = 0.09 (s, 6H, **23-H**), 0.89 (s, 9H, **25-H**), 1.49 (s, 9H, **1-H**), 3.86 (t, J = 4.8 Hz, 2H, **20-H**), 4.22 (t, J = 4.8 Hz, 2H, **19-H**), 6.94-6.96 (m, 2H, **8-H**), 7.24 (d, J = 8.5 Hz, 2H, **7-H**), 7.54 (d, J = 8.9 Hz, 2H, **14-H**), 7.57-7.59 (m, 2H, **13-H**); ¹³C NMR (150 MHz, CD₃OD): δ_{C} = -5.3 (**C-23**), 19.1 (**C-24**), 16.3 (**C-25**), 18.7 (**C-1**), 62.7 (**C-20**), 67.5 (**C-19**), 80.8 (**C-2**), 118.9 (**C-14**), 120.3 (**C-7**), 124.2 (**C-8**), 129.9 (**C-13**), 133.4 (**C-9**), 134.1 (**C-12**), 138.0 (**C-6**), 144.7 (**C-15**), 155.3 (**C-17**), 155.5 (**C-4**); LRMS m/z (ES^+): 588 [$\text{M}+\text{Na}$]⁺, 510 [$\text{M-Boc}+\text{Formic Acid}$]⁺; HRMS m/z (ES^+): Found 588.2164 [$\text{M}+\text{Na}$]⁺; C₂₆H₃₉N₃O₇NaSSi requires 588.2176; Anal. Calcd. for C₂₆H₃₉N₃O₇SSi: C, 55.20; H, 6.95; N, 7.43. Found C, 54.94; H, 6.77; N, 7.22%.

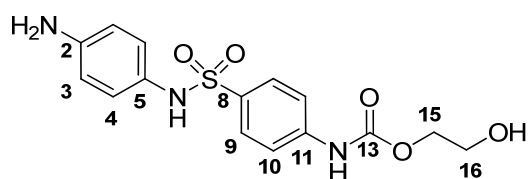
2-(tert-Butyldimethylsilyloxy)ethoxy)ethyl-4-(N-(tert-butyl-4-aminophenylcarbamate)-sulfamoyl)phenylcarbamate (330)



339 (116 mg, 0.526 mmol) was dissolved in anhydrous toluene (13 mL) and **317** (263 mg, 0.630 mmol) was added and the reaction was stirred under reflux for 16 h. The solvent was removed *in vacuo* and purification *via* flash chromatography (applied in toluene; eluted 9% EtOAc/1% Et₃N to 50% EtOAc) afforded the title compound as an off white solid (208 mg, 0.341 mmol, 65%). R_f = 0.65 (1:1 toluene/EtOAc); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3277, 2931 (C-H and N-H stretches), 1697 (C=O stretch), 1596, 1523 (N-H bends), 1328 (S=O asymmetric stretch), 1218 (Si-C stretch), 1155 (S=O symmetric stretch), 1093, 1059 (C-O and Si-O stretches); ¹H NMR (600 MHz, CD₃OD): δ_{H} = 0.05 (s, 6H, **26-H**), 0.88 (s, 9H, **28-H**), 1.49 (s, 9H, **1-H**), 3.57 (t, J = 5.0 Hz, 2H, **22-H**), 3.74 (dt, J = 4.7, 3.3 Hz, 2H, **20-H**), 3.76 (t, J = 5.0 Hz, 2H, **23-H**), 4.28 (t, J = 4.7 Hz, 2H, **19-H**), 6.94-6.97 (m, 2H, **8-H**), 7.24 (d, J = 8.7 Hz, 2H, **7-H**), 7.52 (d, J = 8.9 Hz, 2H, **14-H**), 7.58-7.60 (m, 2H, **13-H**); ¹³C NMR (150 MHz, CD₃OD): δ_{C} = -5.2 (**C-26**), 19.2 (**C-27**), 26.4 (**C-**

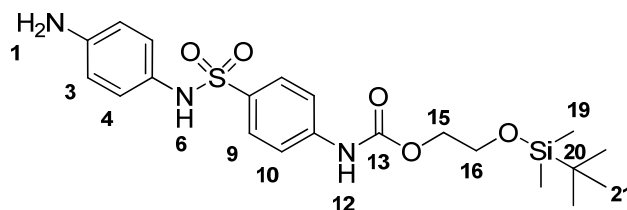
28), 28.7 (**C-1**), 64.0 (**C-23**), 65.4 (**C-23**), 70.5 (**C-20**), 73.7 (**C-22**), 80.9 (**C-2**), 118.8 (**C-14**), 120.3 (**C-7**), 124.2 (**C-8**), 129.4 (**C-13**), 133.4 (**C-9**), 134.1 (**C-12**), 138.0 (**C-6**), 144.7 (**C-15**), 155.2 (**C-4**), 155.3 (**C-17**); LRMS m/z (ES^+): 632 $[M+H]^+$, 554 $[M-^tBu]^+$; HRMS m/z (ES^+): Found 632.2422 $[M+H]^+$; $C_{28}H_{43}N_3O_8NaSSi$ requires 632.2438.

2-Hydroxyethyl-4-(N-(4-aminophenyl)sulfamoyl)phenylcarbamate (326)



329 (240 mg, 0.425 mmol) was dissolved in 7.5 N HCl/EtOAc (20 mL) and the reaction was stirred at RT for 30 min. The organic layer was extracted after diluting with H_2O (30 mL). The aqueous layer was then neutralised *via* the addition of NaOH and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 1:1 to 1:4 to 1:9 pet. ether/EtOAc) afforded the title compound as a pale brown solid (96.4 mg, 0.275 mmol, 65%). Mpt: Decomposed before melting; R_f = 0.15 (1:9 pet. ether/EtOAc); IR (ν_{max}/cm^{-1} , thin film): 3233 (O-H, aromatic C-H stretches), 1717 (C=O stretch), 1593, 1535, 1510 (N-H bends), 1312 (S=O asymmetric stretch), 1220 (COOR stretch), 1150 (S=O symmetric stretch), 1050 (C-O stretch); 1H NMR (600 MHz, CD_3OD): δ_H = 3.77 (t, J = 4.8 Hz, 2H, **16-H**), 4.21 (t, J = 4.8 Hz, 2H, **15-H**), 6.55-6.57 (m, 2H, **3-H**), 6.75-6.77 (m, 2H, **4-H**), 7.50-7.54 (m, 4H, **9,10-H**); ^{13}C NMR (150 MHz, CD_3OD): δ_C = 61.3 (**C-16**), 67.6 (**C-15**), 116.7 (**C-3**), 118.7 (**C-10**), 126.5 (**C-4**), 128.8 (**C-5**), 129.5 (**C-9**), 134.2 (**C-8**), 144.5 (**C-11**), 147.0 (**C-2**), 155.4 (**C-13**); LRMS m/z (ES^+): 374 $[M+Na]^+$, 352 $[M+H]^+$, 331 $[M-(CH_2CH_2OH)+Na]^+$, 302 $[M-(O_2CH_2CH_2OH)+Na]^+$; HRMS m/z (ES^+): Found 374.0772 $[M+Na]^+$; $C_{15}H_{17}N_3O_5NaS$ requires 374.0787.

2-(tert-Butyldimethylsilyloxy)ethyl-4-(N-(4-aminophenyl)sulfamoyl)phenylcarbamate (346)



Method A:

326 (88.5 mg, 0.252 mmol), Et₃N (52.7 μ L, 0.378 mmol) and DMAP (0.283 mg, 1 mol%) were dissolved in anhydrous CH₂Cl₂ (10 mL). *tert*-Butylchlorodimethylsilane (45.6 mg, 0.302 mmol) was added portionwise and the reaction was stirred at RT for 18 h. The mixture was diluted with CH₂Cl₂ (50 mL) and washed with H₂O (30 mL), NaHCO₃ (sat. aq. 30 mL), NH₄Cl (sat. aq. 30 mL) and brine (30 mL), dried (MgSO₄), filtered and solvent removed *in vacuo*. Flash chromatography (applied in pet. ether; eluted 1:1 to 1:9 pet. ether/EtOAc) afforded the title compound as an off white sticky solid (6.30 mg, 0.018 mmol, 7%). *R*_f = 0.69 (9:1 EtOAc/pet. ether); IR (ν_{max} /cm⁻¹, thin film): 3287 (N-H and aromatic C-H stretches), 2954, 2929, 2857 (C-H stretches), 1715 (C=O stretch), 1595, 1532, 1512 (N-H bends), 1314 (S=O asymmetric stretch), 1222 (COOR and Si-C stretches), 1156 (S=O symmetric stretch), 1096, 1065 (C-O and Si-O stretches); ¹H NMR (600 MHz, CD₃OD): δ_{H} = 0.08 (s, 6H, **19-H**), 0.89 (s, 9H, **21-H**), 2.13 (bs, ~2H, **1-H**), 3.85 (bt, *J* = 4.8 Hz, 2H, **16-H**), 4.25 (bt, *J* = 4.8 Hz, 2H, **15-H**), 6.18 (bs, 1H, **6-H**), 6.55 (bs, 2H, **3-H**), 8.81 (bd, *J* = 7.7 Hz, 2H, **4-H**), 6.90 (bs, 1H, **12-H**), 7.43 (d, *J* = 8.4 Hz, 2H, **10-H**), 7.60 (d, *J* = 8.3 Hz, 2H, **9-H**); ¹³C NMR (150 MHz, CD₃OD): δ_{C} = -5.2 (**C-19**), 18.5 (**C-20**), 26.0 (**C-21**), 61.5 (**C-16**), 67.1 (**C-15**), 115.7 (**C-3**), 117.9 (**C-10**), 126.4 (**C-4**), 128.9 (**C-9**), 132.6 (**C-5**), 133.2 (**C-8**), 142.1 (**C-11**), 145.4 (**C-2**), 153.2 (**C-13**); LRMS *m/z* (EI⁺): 465 [M]⁺; HRMS *m/z* (EI⁺): Found 465.1752 [M]⁺. C₂₁H₃₁N₃O₅SSi requires 465.1748.

Method B:

326 (76.6 mg, 0.218 mmol) was dissolved in anhydrous DMF (1.5 mL), imidazole (29.7 mg, 0.436 mmol) was added and the mixture was stirred at RT for 15 min. *tert*-Butylchlorodimethylsilane (49.3 mg, 0.327 mmol) in DMF (0.5 mL) was added and the reaction was stirred at RT for 16 h. Removal of the solvent *in vacuo* followed by flash chromatography

(applied in pet. ether; eluted 1:1 to 1:9 pet. ether/EtOAc) afforded a trace quantity of the title compound as an off white sticky solid.

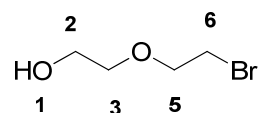
Method C:

To a suspension of **310** (690 mg, 2.36 mmol) in anhydrous toluene (3.15 mL) was added Et₃N (385 μ L, 2.76 mmol). Diphenylphosphoryl azide (596 μ L, 2.76 mmol) in anhydrous toluene (2.76 mL) was added dropwise to the reaction mixture and stirred at RT for 2 h followed by heating at 90 °C for 90 min. **338** (499 mg, 2.84 mmol) in anhydrous DMF (1.4 mL) was then added and the reaction was stirred at 90 °C for 16 h, before the reaction was cooled to RT and solvent removed *in vacuo*. The crude material was taken up in EtOAc (75 mL) and washed with NaHCO₃ (sat. aq. 50 mL), H₂O (3 x 50 mL) and brine (50 mL); dried (MgSO₄), filtered and concentrated *in vacuo*. LCMS and NMR showed no product mass and so no further work carried out.

6.1.4.3 Synthesis of PEGylated Maleimide Linked Imidazo[1,2-a]pyrazines

6.1.4.3.1 PEGylated-Benzenesulfonamide-bound Imidazo[1,2-a]pyrazines

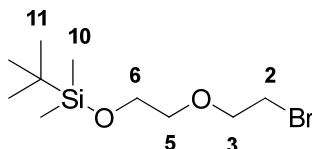
2-(2-Bromoethoxy)ethanol (**347**)²⁶⁷



Diethylene glycol (2.00 mL, 0.021 mol) was dissolved in anhydrous CH₂Cl₂ (75 mL) and the mixture was cooled on ice. Thienyl bromide (0.816 mL, 0.010 mol) was then added dropwise and stirred at 0 °C for 30 min. The reaction was then stirred at RT under Ar for 16 h before quenching with H₂O (150 mL) and NaHCO₃ (sat. aq. 75 mL). The organic layer was extracted, washed with H₂O (3 x 100 mL) and brine (1 x 75 mL), dried (MgSO₄) and concentrated *in vacuo* to give a light yellow oil. Flash chromatography (applied in pet. ether; eluted 1:1 pet. ether/EtOAc) afforded the title compound as colourless oil (787 mg, 5.15 mmol, 24%) with NMR consistent with literature values.²⁶⁷ *R*_f = 0.33 (1:1 pet. ether/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3397 (O-H stretch), 2871 (C-H stretch), 1117, 1059 (C-O stretches); ¹H NMR (600 MHz, CDCl₃): δ_{H} = 1.93 (bs, 1H, **1-H**), 3.49 (t, *J* = 6.0 Hz, 2H, **6-H**), 3.62-3.63 (m, 2H, **3-H**), 3.75-3.76 (m, 2H, **2-H**), 3.82 (t, *J* = 6.0 Hz, 2H, **5-H**); ¹³C NMR (150 MHz, CDCl₃): δ_{C} = 30.7 (**C-6**), 61.8

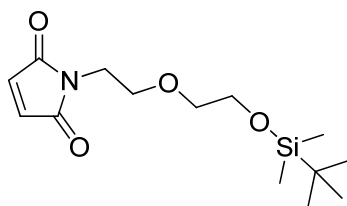
(C-2), 71.0 (C-5), 72.3 (C-3); LRMS m/z (CI): 169 $[M(^{79}\text{Br})]^+$, 171 $[M(^{81}\text{Br})]^+$, 151 $[M(^{79}\text{Br})\text{-OH}]^+$, 153 $[M(^{81}\text{Br})\text{-OH}]^+$, 107 $[M(^{79}\text{Br})\text{-OCH}_2\text{CH}_2\text{OH}]^+$, 109 $[M(^{81}\text{Br})\text{-OCH}_2\text{CH}_2\text{OH}]^+$; HRMS m/z (CI): Found 168.9869 $[M(^{79}\text{Br})]^+$; $\text{C}_4\text{H}_{10}\text{O}_2\text{Br}$ requires 168.9864.

(2-(2-Bromoethoxy)ethoxy)(tert-butyl)dimethylsilane (348)²⁶⁹



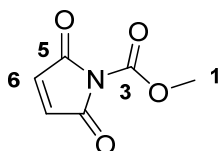
347 (351 mg, 2.30 mmol) was dissolved in anhydrous DMF (3 mL). *Tert*-butyldimethylsilyl chloride (381 mg, 3.52 mmol) was added in one portion followed by DIPEA (1.00 mL, 5.74 mmol) and the reaction mixture was allowed to stir at RT for 1 h. After diluting with EtOAc (50 mL), the reaction was washed with H_2O (20 mL), NH_4Cl (sat. aq. 20 mL) and brine (15 mL), followed by drying (MgSO_4) and concentrating *in vacuo* to give a red/orange oil. Flash chromatography (applied in pet. ether; eluted 9:1 pet. ether/EtOAc) gave the title compound as a colourless oil (473 mg, 1.67 mmol, 73%) with NMR consistent with literature values.²⁶⁹ R_f = 0.55 (9:1 pet. ether/EtOAc); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 2929, 2857 (C-H stretch), 1255 (Si-C stretch), 1105 (C-O and Si-O stretches), 834, 778 (Si-C stretches); ^1H NMR (600 MHz, CDCl_3): δ_{H} = 0.07 (s, 6H, **10-H**), 0.89 (s, 9H, **11-H**), 3.46 (t, J = 6.4 Hz, 2H, **2-H**), 3.59 (t, J = 5.2 Hz, 2H, **5-H**), 3.77 (t, J = 5.2 Hz, 2H, **6-H**), 3.82 (t, J = 6.4 Hz, 2H, **3-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = -5.2 (C-10), 18.5 (C-9), 26.0 (C-11), 30.6 (C-2), 62.9 (C-6), 71.4 (C-3), 72.7 (C-5); LRMS m/z (ES^+): 306 $[M(^{79}\text{Br})+\text{Na}]^+$, 308 $[M(^{81}\text{Br})+\text{Na}]^+$, 283 $[M(^{79}\text{Br})+\text{H}]^+$, 285 $[M(^{81}\text{Br})+\text{H}]^+$, 242 $[M\text{-Br}+\text{K}]^+$; HRMS m/z (ES^+): Found 283.0782 $[M(^{79}\text{Br})+\text{H}]^+$; $\text{C}_{10}\text{H}_{24}\text{O}_2\text{SiBr}$ requires 283.0729.

Attempted formation of 1-(2-(2-(*tert*-butyldimethylsilyloxy)ethoxy)ethyl)-1*H*-pyrrole-2,5-dione (349)



NaH (60% dispersion in mineral oil; 60.5 mg, 1.51 mmol) was stirred in anhydrous hexanes (10 mL) for 20 min. Removal of the hexanes *via* syringe and drying under vacuum gave the activated base. Anhydrous THF (2 mL) was added and cooled on ice. Maleimide (147 mg, 1.51 mmol) in dry THF (2 mL) was added dropwise and the resulting off white milky solution was stirred for 10 min. **348** (473 mg, 1.51 mmol) in dry THF (3 mL) was added dropwise and the solution was stirred at RT for 5 h. TLC indicated no reaction and so it was heated to 50 °C for 16 h. No reaction was observed and so the reaction was abandoned.

Methyl maleimido-1-carboxylate (**352**)²⁷¹



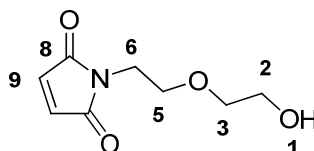
Method A:

To a cooled (0 °C) solution of maleimide (2.00 g, 20.6 mmol) in EtOAc (80 mL) was added a solution of *N*-methyl morpholine (2.50 mL, 22.7 mmol) in EtOAc (10 mL) over a period of 10 min. Methyl chloroformate (1.60 mL, 20.6 mmol) in EtOAc (5 mL) was then added dropwise and the solution turned cloudy with visible precipitate. The reaction mixture was stirred at RT for 1 h before diluting with EtOAc (100 mL) and washing with NaHCO₃ (sat. aq. 50 mL), H₂O (50 mL) and brine (50 mL). Drying (MgSO₄), filtering and removal of solvent *in vacuo* gave the crude material which upon purification *via* flash chromatography (applied in pet. ether; eluted 25% to 50% EtOAc) afforded the title compound as a white solid (742 mg, 4.79 mmol, 23%), with NMR consistent with literature values.²⁷¹ Mpt: 48-50 °C [Lit.²⁷¹ 60-61 °C]; *R_f* = 0.48 (1:1 pet. ether/EtOAc); IR (ν_{max}/cm⁻¹, thin film): 3102 (C-H stretch), 1795, 1754, 1712 (C=O and C=C stretches), 1441 (C-H Scissor), 1333, 1263; ¹H NMR (600 MHz, CDCl₃): δ_H = 3.97 (s, 3H, **1-H**), 6.84 (s, 2H, **6-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 54.5 (**C-1**), 135.4 (**C-6**), 148.2 (**C-3**), 165.8 (**C-5**); LRMS *m/z* (EI⁺): 155 [M]⁺; HRMS *m/z* (EI⁺): Found 155.0208 [M]⁺; C₆H₅O₄N requires 155.0213; Anal. Calcd. for C₆H₅O₄N: C, 46.46; H, 3.25; N, 9.03. Found C, 46.22; H, 3.26; N, 8.84%.

Method B:

To a stirred solution of maleimide (3.00 g, 30.9 mmol) in anhydrous THF (250 mL) was added methyl chloroformate (2.39 mL, 30.9 mmol) followed by *N*-methyl morpholine (3.40 mL, 30.9 mmol). The resulting cloudy pink solution was stirred at RT for 72 h, after which the reaction mixture was diluted with CH₂Cl₂ (300 mL) and washed H₂O (3 x 100 mL) and brine (100 mL). Drying (MgSO₄), filtering and concentrating *in vacuo* gave a crude brown sticky solid. Flash chromatography (applied in pet. ether; eluted 2:1 to 3:2 to 1:1 pet. ether/EtOAc) afforded the title compound as a white solid (1.53 g, 9.84 mmol, 32%).

2-(2-Maleimidoethoxy)ethanol (350)



Method A:

347 (311 mg, 2.03 mmol) and maleimide (197 mg, 2.03 mmol) were dissolved in anhydrous DMF (5 mL). K₂CO₃ (140 mg, 1.01 mmol) was added and the colourless reaction mixture was stirred at 50 °C under Ar. After 3½ h, the resulting red solution was cooled to RT, and LCMS indicated no product was present, even though no maleimide was present. The reaction was abandoned at this stage.

*Method B:*²⁷¹

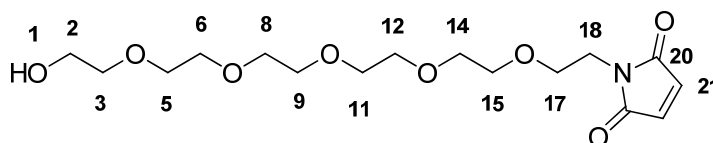
2-(2-Aminoethoxy)ethanol (463 µL, 4.64 mmol) was dissolved in NaHCO₃ (sat. aq. 20 mL) and stirred at 0 °C for 10 min. **352** (720 mg, 4.64 mmol) was added in one portion and the reaction was allowed to warm slowly to RT and stir for a further 30 min. The reaction was then extracted with CHCl₃ (3 x 40 mL), the combined organics washed with brine (20 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 2:1 to 1:5 pet. ether/EtOAc) afforded the title compound as a colourless gum (464 mg, 2.51 mmol, 54%) with NMR consistent with literature values.²⁷¹ *R*_f = 0.31 (5:1 pet. ether/EtOAc); IR (ν_{max}/cm⁻¹, thin film): 3456 (O-H stretch), 2874 (C-H stretch), 1686 (C=O stretch), 1404, 1118 (C-O

stretch); ^1H NMR (600 MHz, CDCl_3): $\delta_{\text{H}} = 2.01$ (bs, 1H, **1-H**), 3.56 (t, $J = 4.5$ Hz, 2H, **3-H**), 3.64 (t, $J = 5.5$ Hz, 2H, **5-H**), 3.68 (t, $J = 4.5$ Hz, 2H, **2-H**), 3.74 (t, $J = 5.5$ Hz, 2H, **6-H**), 6.72 (s, 2H, **9-H**); ^{13}C NMR (150 MHz, CDCl_3): $\delta_{\text{C}} = 37.5$ (**C-6**), 61.9 (**C-2**), 68.4 (**C-5**), 72.2 (**C-3**), 134.3 (**C-9**), 171.0 (**C-8**); LRMS m/z (CI^+): 186 $[\text{M}+\text{H}]^+$, 168 $[\text{M}-\text{OH}]^+$; HRMS m/z (CI^+): Found 186.0761 $[\text{M}-\text{OH}]^+$; $\text{C}_8\text{H}_{12}\text{O}_4\text{N}$ requires 186.0766.

Method C:

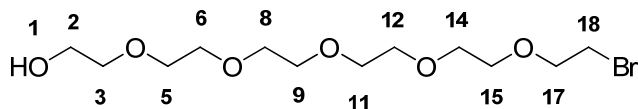
352 (1.53 g, 9.84 mmol) was dissolved in anhydrous CH_2Cl_2 (100 mL). 2-(2-Aminoethoxy)ethanol (0.981 mL, 9.84 mmol) was added and the reaction stirred at RT for 30 min. The resulting cloudy white solution was diluted with CH_2Cl_2 (100 mL) and washed with NaHCO_3 (sat. aq. 3 x 75 mL) and brine (75 mL); dried (MgSO_4), filtered and concentrated *in vacuo*. Flash chromatography (pet. ether; 1:1 to 1:3 to 1:5 pet. ether/EtOAc) afforded the title compound as a colourless oil (395 mg, 2.14 mmol, 22%).

1-(17-Hydroxy-3,6,9,12,15-pentaoxaheptadecyl)-maleimide (**355**)



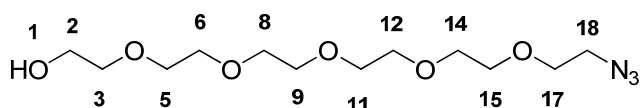
354 (545 mg, 1.94 mmol) was dissolved in NaHCO_3 (sat. aq. 10 mL) and the mixture was cooled on ice and stirred for 10 min. **352** (260 mg, 1.67 mmol) was added in one portion and the reaction was stirred on ice for 30 min. After this time, the reaction was extracted with CHCl_3 (3 x 30 mL) and the combined organics washed with brine (30 mL), dried (MgSO_4), filtered and solvent removed *in vacuo*. Flash chromatography (applied in CH_2Cl_2 ; eluted 5% MeOH) afforded the title compound as a pale yellow oil (391 mg, 1.08 mmol, 65%). $R_f = 0.29$ (5% MeOH/ CH_2Cl_2); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3460 (O-H stretch), 2868 (C-H stretch), 1703 (C=O stretch), 1097 (C-O stretch); ^1H NMR (600 MHz, CDCl_3): $\delta_{\text{H}} = 3.58$ -3.68 (m, 20H, **3,5,6,8,9,11,12,14,15,17-H**), 3.71-3.73 (m, 4H, **2,18-H**), 6.70 (s, 2H, **21-H**); ^{13}C NMR (150 MHz, CDCl_3): $\delta_{\text{C}} = 37.2$ (**C-18**), 61.8 (**C-2**), 67.6 (**C-3**), 70.1-70.7 (**C-5,6,8,9,11,12,14,15**), 72.7 (**C-17**), 134.3 (**C-21**), 170.8 (**C-20**); LRMS m/z (CI^+): 362 $[\text{M}+\text{H}]^+$, 318 $[\text{M}-\text{CH}_2\text{CH}_2\text{OH}]^+$, 115; HRMS m/z (CI^+): Found 362.1823 $[\text{M}+\text{H}]^+$; $\text{C}_{16}\text{H}_{28}\text{NO}_8$ requires 362.1815.

17-Bromo-3,6,9,12,15-pentaoxaheptadecan-1-ol (357)



Hexaethylene glycol (1.80 mL, 7.08 mmol) was dissolved in anhydrous CH_2Cl_2 (25 mL) and the mixture was cooled on ice. Thienyl bromide (0.274 mL, 3.5 mmol) was then added dropwise and stirred at 0 °C for 30 min. The reaction was then stirred at RT under Ar for 16 h before quenching with H_2O (50 mL) and NaHCO_3 (sat. aq. 25 mL). The organic layer was extracted, washed with H_2O (3 x 20 mL) and brine (20 mL), dried (MgSO_4) and concentrated *in vacuo* to give a light yellow oil. Flash chromatography (applied in CH_2Cl_2 ; eluted 5% MeOH/ CH_2Cl_2) afforded the title compound as colourless oil (510 mg, 51.5 mmol, 20%) with NMR consistent with literature values.³¹⁷ R_f = 0.38 (5% MeOH/ CH_2Cl_2); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3469 (O-H stretch), 2869 (C-H stretch), 1102 (C-O stretch); ^1H NMR (600 MHz, CDCl_3): δ_{H} = 1.98 (bs, 1H, **1-H**), 3.48 (t, J = 6.3 Hz, 2H, **18-H**), 3.61 (t, J = 4.5 Hz, 2H, **3-H**), 3.66 (bs, 16H, **5,6,8,9,11,12,14,15-H**), 3.72 (t, J = 4.5 Hz, 2H, **2-H**), 3.81 (t, J = 6.3 Hz, 2H, **17-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = 30.5 (**C-18**), 61.8 (**C-2**), 70.3 (**C-15**), 70.6-70.7 (**C-5, 6,8,9,11,12**), 71.3 (**C-17**), 72.8 (**C-3**); LRMS m/z (ES⁺): 345 [$\text{M}(^{79}\text{Br})+\text{H}$]⁺, 347 [$\text{M}(^{81}\text{Br})+\text{H}$]⁺.

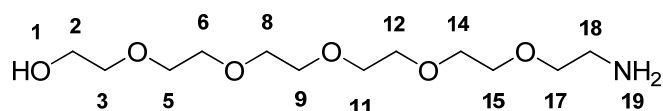
17-Azido-3,6,9,12,15-pentaoxaheptadecan-1-ol (358)



357 (1.80 g, 5.20 mmol) was dissolved in anhydrous DMF (22 mL). NaN_3 (0.675 g, 10.4 mmol) was added and the reaction was stirred at 70 °C for 3½ h. After this time, the mixture was cooled to RT and the solvent removed. Diluting with CH_2Cl_2 (100 mL), washing with brine (50 mL), drying (MgSO_4), filtering and concentrating *in vacuo* gave the title compound as pale yellow oil (1.47 g, 4.79 mmol, 92%) with NMR consistent with literature values.³¹⁸ R_f = 0.38 (5% MeOH/ CH_2Cl_2); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3472 (O-H stretch), 2869 (C-H stretch), 2098 ($-\text{N}=\text{N}^+=\text{N}^-$ stretch), 1096 (C-O stretch); ^1H NMR (600 MHz, CDCl_3): δ_{H} = 2.00 (s, 1H, **1-H**), 3.39 (t, J = 5.0 Hz, 2H, **18-H**), 3.60-3.62 (m, 2H, **3-H**), 3.65-3.68 (m, 18H, **5,6,8,9,11,12,14,15,16,17-H**), 3.72 (bd, J = 3.3 Hz, 2H, **2-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = 50.8 (**C-18**), 61.8 (**C-2**),

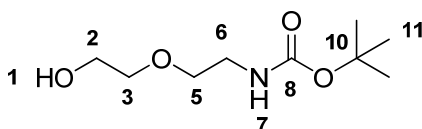
70.2-70.8 (C-5,6,8,9,11,12,14,15,16,17), 72.7 (C-3); LRMS m/z (ES^+): 330 $[M+H]^+$, 302 $[M-N_2+Na]^+$; HRMS m/z (ES^+): Found 330.1641 $[M+H]^+$; $C_{12}H_{25}N_3O_6Na$ requires 330.1641.

17-Amino-3,6,9,12,15-pentaoxaheptadecan-1-ol (354)



358 (1.47 g, 4.49 mmol) was dissolved in anhydrous MeOH (25 mL). 10% Pd/C (150 mg, 10% w/w) was added, the vessel was evacuated and purged with Ar (3x), and under static vacuum a balloon of H_2 was added. The reaction was stirred at RT for 5 h, after which time the balloon was removed and the vessel evacuated and purged with Ar (3x). The contents were filtered through Celite (pre-washed with MeOH) and the filtrate concentrated *in vacuo*. Flash chromatography (applied in CH_2Cl_2 ; eluted 5% to 20% MeOH to 2% Et_3N /20% MeOH) afforded the title compound as a yellow oil (545 mg, 1.93 mmol, 43%) with NMR consistent with literature values.³¹⁸ R_f = 0.10 (20% MeOH/ CH_2Cl_2); IR (ν_{max}/cm^{-1} , thin film): 3376 (O-H stretch), 2868 (C-H and N-H stretches), 1099 (C-O stretch); 1H NMR (600 MHz, $CDCl_3$): δ_H = 2.01 (bs, 3H, **1,19-H**), 2.87-2.89 (m, 2H, **18-H**), 3.52-3.56 (m, 2H, **17-H**), 3.58-3.60 (m, 2H, **3-H**), 3.64-3.68 (m, 16H, **5,6,8,9,11,12,14,15-H**), 3.71-3.72 (m, 2H, **2-H**); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C = 41.6 (C-18), 61.5 (C-2), 70.1-70.7 (C-5,6,8,9,11,12,14,15), 72.9-73.2 (C-3,17); LRMS m/z (CI^+): 282 $[M+H]^+$, 167; HRMS m/z (EI^+): Found 282.1923 $[M+H]^+$; $C_{12}H_{28}NO_6$ requires 282.1917.

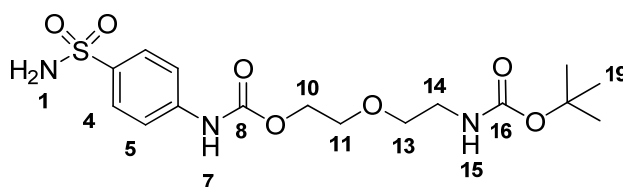
tert-Butyl 2-(2-hydroxyethoxy)ethylcarbamate (359)²⁷³



To a solution of 2-(2-aminoethoxy)ethanol (2.00 mL, 19.9 mmol) in $CHCl_3$ (20 mL) cooled on ice, was added $(Boc)_2O$ (4.35 g, 19.9 mmol) in $CHCl_3$ (20 mL). The reaction was stirred at RT for 90 min, followed by diluting with H_2O (50 mL) and then extracting with $CHCl_3$ (3 x 60 mL).

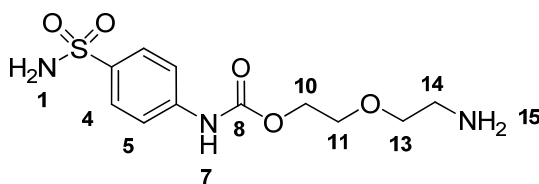
The combined organics were dried (MgSO₄), filtered, concentrated *in vacuo*, and then purified *via* flash chromatography (applied in CH₂Cl₂; eluted 2% to 5% to 10% MeOH) to afford the title compound as a colourless oil (3.93 g, 19.2 mmol, 96%) with NMR consistent with literature values.²⁷³ R_f = 0.83 (10% MeOH/CH₂Cl₂); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3353 (O-H stretch), 2976, 2931, 2871 (C-H and N-H stretches), 1687 (C=O stretch), 1520 (N-H bend), 1366 (C-H bend), 1169, 1123 (C-O stretches); ¹H NMR (500 MHz, CDCl₃): δ_{H} = 1.43 (s, 9H, **11-H**), 2.16 (bs, 1H, **1-H**), 3.32 (t, J = 5.1 Hz, 2H, **6-H**), 3.53-3.57 (m, 4H, **3,5-H**), 3.72-3.74 (m, 2H, **2-H**), 4.98 (bs, 1H, **7-H**); ¹³C NMR (125 MHz, CDCl₃): δ_{C} = 28.5 (**C-11**), 40.5 (**C-6**), 61.8 (**C-2**), 70.4 (**C-5**), 72.3 (**C-3**), 79.5 (**C-10**), 156.2 (**C-8**); LRMS m/z (CI⁺): 206 [M+H]⁺, 150 [M-^tBu]⁺; HRMS m/z (CI⁺): Found 206.1398 [M+H]⁺; C₉H₂₀NO₄ requires 206.1392.

2-(2-(tert-butyl carbamate)ethoxy)ethyl 4-sulfamoylphenylcarbamate (323)



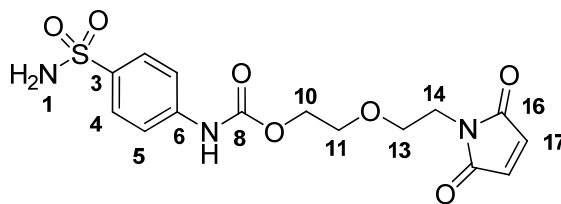
359 (993 mg, 4.39 mmol) was dissolved in anhydrous toluene (92 mL). **315** (751 mg, 3.66 mmol) was added and the reaction was stirred under reflux for 16 h. The reaction was cooled to RT, solvent removed and purification *via* flash chromatography (pet. ether; 2:1 to 1:1 to 1:2 pet. ether/EtOAc) afforded the title compound as a white solid (1.13 g, 2.81 mmol, 77%). Mpt: 94-95 °C; R_f = 0.35 (1:3 pet. ether/EtOAc); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3368, 3332 (aromatic C-H stretch), 3201, 3101, 2976, 2919 (C-H, N-H stretches), 1703, 1676 (C=O stretches), 1522 (N-H bend), 1333 (S=O asymmetric stretch), 1243 (COOR stretch), 1156 (S=O symmetric stretch), 1073 (C-O stretch); ¹H NMR (600 MHz, CD₃OD): δ_{H} = 1.41 (s, 9H, **19-H**), 3.22 (t, J = 5.7 Hz, 2H, **14-H**), 3.53 (t, J = 5.7 Hz, 2H, **13-H**), 3.71 (t, J = 4.7 Hz, 2H, **11-H**), 4.29 (t, J = 4.7 Hz, 2H, **10-H**), 7.60 (d, J = 8.8 Hz, 2H, **5-H**), 7.81 (ap.d, J = 8.8 Hz, 2H, **4-H**); ¹³C NMR (150 MHz, CD₃OD): δ_{C} = 28.8 (**C-19**), 41.3 (**C-14**), 65.4 (**C-10**), 70.2 (**C-11**), 71.0 (**C-13**), 80.2 (**C-18**), 119.0 (**C-5**), 128.3 (**C-4**), 138.7 (**C-3**), 144.1 (**C-6**), 155.4 (**C-8**), 158.2 (**C-16**); LRMS m/z (ES⁺): 426 [M+Na]⁺, 370 [M-^tBu+Na]⁺; HRMS m/z (ES⁺): Found 426.1306 [M+Na]⁺; C₁₆H₂₅N₃O₇NaS requires 426.1311; Anal. Calcd. for C₁₆H₂₅N₃O₇S: C, 47.63; H, 6.25; N, 10.42. Found C, 47.78; H, 6.46; N, 10.20%.

2-(2-Aminoethoxy)ethyl 4-sulfamoylphenylcarbamate (360)



323 (507 mg, 1.26 mmol) was dissolved in anhydrous CH_2Cl_2 (20 mL), TFA (20 mL) added and the reaction was stirred at RT for 18 h. The solvent was removed *via* co-evaporation with toluene and the crude material was purified by flash chromatography (applied in CH_2Cl_2 ; eluted 2% to 5% to 10% to 20% MeOH) to afford the title compound as a white solid (531 mg, 1.00 mmol, 79%). Mpt: 138-142 °C; R_f = 0.29 (20% MeOH/ CH_2Cl_2); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3311 (C-H stretch), 3194, 3083 (C-H and N-H stretches), 1678 (C=O stretch), 1542 (N-H bend), 1339 (S=O asymmetric stretch), 1160 (S=O symmetric stretch), 1123, 1060 (C-O stretch); ^1H NMR (600 MHz, CD_3OD): δ_{H} = 2.95 (t, J = 5.2 Hz, 2H, **14-H**), 3.54 (t, J = 5.2 Hz, 2H, **13-H**), 3.75 (t, J = 4.6 Hz, 2H, **11-H**), 4.33 (t, J = 4.6 Hz, 2H, **10-H**), 7.61 (d, J = 8.8 Hz, 2H, **5-H**), 7.78-7.82 (m, 2H, **4-H**); ^{13}C NMR (150 MHz, CD_3OD): δ_{C} = 41.4 (**C-14**), 65.3 (**C-10**), 70.4 (**C-11**), 70.8 (**C-13**), 115.3 (TFA), 117.2 (TFA), 119.0 (**C-5**), 119.2 (TFA), 121.1 (TFA), 128.3 (**C-4**), 138.5 (**C-3**), 144.1 (**C-6**), 155.5 (**C-8**), 163.1 (TFA); LRMS m/z (ES^+): 304 $[\text{M}+\text{H}]^+$; HRMS m/z (ES^+): Found 304.0953 $[\text{M}+\text{H}]^+$; $\text{C}_{11}\text{H}_{18}\text{N}_3\text{O}_5\text{S}$ requires 304.0967.

2-(2-(Maleimido)ethoxy)ethyl-4-sulfamoylphenylcarbamate (322)



Method A:

To a suspension of 4-sulfamoylbenzoic acid (93.7 mg, 0.466 mmol) in anhydrous toluene (0.655 mL) was added Et_3N (76.0 μL , 0.545 mmol). Diphenylphosphoryl azide (117 μL , 0.545 mmol) in anhydrous toluene (0.545 mL) was added dropwise to the reaction mixture and stirred at RT for 2 h followed by heating at 90 °C for 90 min. **350** (103 mg, 0.559 mmol) in anhydrous DMF (280

μL) was then added and the reaction was stirred at 90 °C for 16 h. The mixture was cooled to RT and solvent removed *in vacuo*. The crude material was taken up in EtOAc (50 mL) and washed with NaHCO_3 (sat. aq. 30 mL), H_2O (3 x 30 mL) and brine (30 mL); dried (MgSO_4), filtered and concentrated *in vacuo*. LCMS showed no product mass and so no further work carried out.

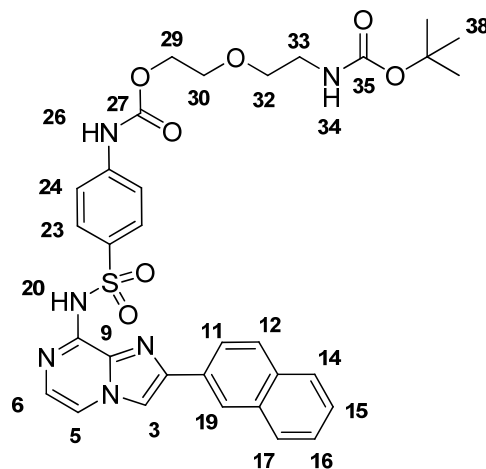
Method B:

350 (105 mg, 0.568 mmol) was taken up in anhydrous toluene (15 mL), **315** (154 mg, 0.782 mmol) was added and the reaction was stirred under reflux for 20 h. Removal of the solvent *in vacuo* and subsequent TLC and NMR analysis revealed no product formation. No further work was carried out.

Method C:

360 (415 mg, 0.664 mmol) was dissolved in NaHCO_3 (sat. aq. 40 mL) and **352** (206 mg, 1.33 mmol) was added in one portion. The reaction was stirred at RT for 30 min followed by diluting with EtOAc (75 mL), neutralising *via* addition of HCl (1.0 M) and extracting the organics (3 x 50 mL). The combined organics were washed with brine (60 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Purification by flash chromatography (applied in CH_2Cl_2 ; eluted 2% to 5% MeOH/ CH_2Cl_2) afforded the title compound as a white solid (244 mg, 0.637 mmol, 96%). Mpt: 118 °C; R_f = 0.31 (5% MeOH/ CH_2Cl_2); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3348 (N-H stretch), 3233 (aromatic C-H stretches), 2958, 2871 (C-H stretches), 1706 (C=O stretch), 1596, 1538 (N-H bends), 1336 (S=O asymmetric stretch), 1157 (S=O symmetric stretch), 1072 (C-O stretch); ^1H NMR (600 MHz, CD_3OD): δ_{H} = 3.64-3.66 (m, 2H, **13-H**), 3.68-3.71 (m, 4H, **11,14-H**), 4.23-4.25 (m, 2H, **10-H**), 6.75 (s, 2H, **17-H**), 7.29 (d, J = 8.8 Hz, 2H, **5-H**), 7.80-7.82 (m, 2H, **4-H**); ^{13}C NMR (150 MHz, CD_3OD): δ_{C} = 38.1 (**C-14**), 65.2 (**C-10**), 68.8 (**C-13**), 69.9 (**C-11**), 119.0 (**C-5**), 128.3 (**C-4**), 135.4 (**C-17**), 138.5 (**C-3**), 144.1 (**C-6**), 155.3 (**C-8**), 172.4 (**C-16**); LRMS m/z (ES^-): 428 [$\text{M}+\text{Formic Acid}$] $^+$, 382 [$\text{M}-\text{H}$] $^-$; HRMS m/z (ES^-): Found 382.0711 [$\text{M}-\text{H}$] $^-$; $\text{C}_{15}\text{H}_{16}\text{N}_3\text{O}_7\text{S}$ requires 382.0709.

2-(tert-butylcarbamate-ethoxy)ethyl-4-(N-(2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-yl)-sulfamoyl)phenylcarbamate (361)



Method A:

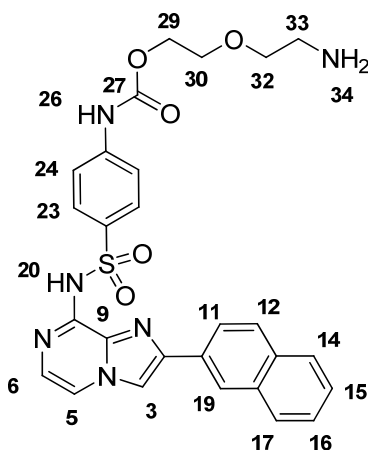
All glassware was dried and purged with Ar prior to use. Pd₂(dba)₃ (1.64 mg, 1 mol%), DavePhos (2.11 mg, 3 mol%) and NaO^tBu (24.1 mg, 0.250 mmol) were dissolved in anhydrous toluene (2 mL). **214** (50.0 mg, 0.179 mmol) and **323** (86.5 mg, 0.215 mmol) were added and the reaction was stirred under reflux, under Ar for 20 h. The reaction was cooled to RT and solvent removed, before the residue was taken up in CH₂Cl₂ (50 mL) and washed with H₂O (3 x 30 mL) and brine (30 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 3:1 to 2:1 to 1:1 to 1:3 to 1:5 pet. ether/EtOAc) afforded the title compound as a pale yellow solid (8.50 mg, 0.013 mmol, 7%).

Method B:

NaH was washed by stirring NaH (60% in Mineral Oil; 71.0 mg, 1.77 mmol) in anhydrous hexanes (25 mL) for 20 min, removing the solvent using a syringe and drying the contents under high vacuum. DMF (5 mL) was added followed by **323** (715 mg, 1.77 mmol) in DMF (8 mL) and the mixture was stirred at RT for 20 min. **227** (287 mg, 0.887 mmol) in DMF (17 mL) was added and the resulting dark brown solution was heated at 100 °C under Ar for 16 h. The solvent was then removed and the residue was diluted with EtOAc (100 mL) and washed with NH₄Cl (aq. Sat 50 mL) and H₂O (3 x 40 mL). The combined aqueous layers were then re-extracted with EtOAc (2x), followed by washing the combined organics with brine, drying (MgSO₄), filtering and concentrating *in vacuo*. Flash chromatography (applied in pet. ether; eluted 1:1 to 1:2 to 1:3

to 1:4 to 1:5 pet. ether/EtOAc) afforded the title compound as a light yellow solid (328 mg, 0.507 mmol, 57%). Mpt: >200 °C; R_f = 0.21 (9:1 EtOAc/pet. ether); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3230 (C-H stretch), 2972 (N-H stretch), 1697 (C=O stretch), 1584, 1530 (N-H bend), 1393 (S=O asymmetric stretch), 1221, 1114 (S=O symmetric stretch). 1064 (C-O stretch); ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{H} = 1.34 (s, 9H, **38-H**), 3.06 (q, J = 5.5 Hz, 2H, **33-H**), 3.47 (t, J = 5.5 Hz, 2H, **32-H**), 3.62 (t, J = 4.3 Hz, 2H, **30-H**), 4.20 (t, J = 4.3 Hz, 2H, **29-H**), 6.80 (t, J = 5.5 Hz, 1H, **34-H**), 7.16 (bs, 1H, **6-H**), 7.50-7.55 (m, 2H, **15,16-H**), 7.65 (d, J = 8.8 Hz, 2H, **24-H**), 7.86 (bd, J = 4.6 Hz, 1H, **5-H**), 7.92 (d, J = 7.4 Hz, 3H, **14,23-H**), 7.98 (d, J = 8.6 Hz, 1H, **12-H**), 8.02-8.05 (m, 2H, **11,17-H**), 8.52 (s, 1H, **19-H**), 8.59 (s, 1H, **3-H**), 10.22 (s, 1H, **26-H**), 11.63 (bs, 1H, **7/20-H**); ^{13}C NMR (150 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{C} = 28.3 (**C-38**), 40.0 (**C-33**), 63.9 (**C-29**), 68.2 (**C-30**), 69.1 (**C-32**), 77.7 (**C-37**), 110.9 (**C-5**), 115.3 (**C-3**), 116.8 (**C-6**), 117.6 (**C-24**), 123.8 (**C-11**), 124.2 (**C-19**), 126.3 (**C-15**), 126.6 (**C-16**), 127.4 (**C-23**), 127.7 (**C-14**), 128.3 (**C-12**), 128.4 (**C-17**), 130.0 (**C-10**), 132.8 (**C-13**), 133.2 (**C-18**), 135.6 (**C-9**), 135.8 (**C-22**), 142.9 (**C-25**), 145.2 (**C-2**), 153.3 (**C-27**), 155.6 (**C-35**) 145.5 (**C-8**); LRMS m/z (EI^+): 647 $[\text{M}+\text{H}]^+$, 591 $[\text{M}-t\text{Bu}]^+$; (ES^-): 691 $[\text{M}+\text{Formic Acid}]^+$, 645 $[\text{M}-\text{H}]^-$; HRMS m/z (ES^-): Found 645.2140 $[\text{M}-\text{H}]^-$; $\text{C}_{32}\text{H}_{33}\text{N}_6\text{O}_7\text{S}$ requires 645.2131.

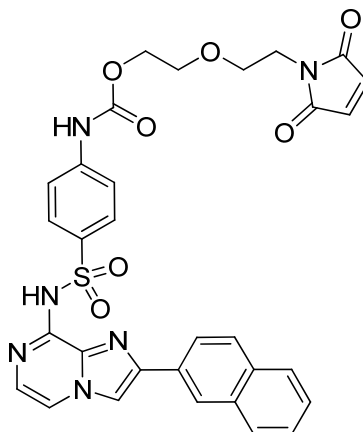
2-(2-Aminoethoxy)ethyl-4-(N-(2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-yl)sulfamoyl)phenylcarbamate (362)



361 (318 mg, 0.492 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL) and cooled on ice. TFA (15 mL) was added and the reaction was stirred at RT for 3 h. Removal of the solvent, *via* trituration with toluene, followed by flash chromatography (applied in CH_2Cl_2 ; eluted 5% to 10%

to 20% MeOH/CH₂Cl₂) afforded the title compound as an off white solid (390 mg, 0.389 mmol, 79%). Mpt: >200 °C; *R*_f = 0.26 (20% MeOH/CH₂Cl₂); IR (ν_{max}/cm⁻¹, thin film): 1726 (C=O stretch), 1675 (N-H bend), 1351 (S=O asymmetric stretch), 1200, 1120 (S=O symmetric stretch), 1071 (C-O stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_H = 2.99 (t, *J* = 5.3 Hz, 2H, **33-H**), 3.62 (t, *J* = 5.3 Hz, 2H, **32-H**), 3.69 (t, *J* = 4.5 Hz, 2H, **30-H**), 4.24 (t, *J* = 4.5 Hz, 2H, **29-H**), 7.14 (d, *J* = 5.0 Hz, 1H, **6-H**), 7.49-7.54 (m, 2H, **15,16-H**), 7.58 (d, *J* = 8.5 Hz, 2H, **24-H**), 7.80 (d, *J* = 5.0 Hz, 1H, **5-H**), 7.89-7.92 (m, 3H, **14,23-H**), 7.97 (d, *J* = 8.6 Hz, 1H, **12-H**), 8.01 (d, *J* = 7.9 Hz, 1H, **17-H**), 8.05 (dd, *J* = 8.6, 1.4 Hz, 1H, **11-H**), 8.49 (s, 1H, **3-H**), 8.52 (s, 1H, **19-H**), 10.09 (bs, 1H, **20-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_C = 38.6 (**C-33**), 63.6 (**C-29**), 66.7 (**C-32**), 68.5 (**C-30**), 110.7 (**C-5**) 116.3 (**C-3**), 117.4 (**C-24**), 118.3 (**C-6**), 123.9 (**C-11**), 124.0 (**C-19**) 126.1 (**C-15**), 126.5 (**C-16**) 127.7 (overlapping signals, **C-14,23**), 128.2 (**C-17**), 128.3 (**C-12**), 130.6 (**C-10**), 132.7 (**C-13**), 133.6 (**C-18**), 136.2 (**C-9**), 137.4 (**C-22**), 142.0 (**C-25**), 144.2 (**C-2**), 149.7 (**C-8**), 153.5 (**C-27**); LRMS *m/z* (ES⁺): 547 [M+H]⁺; HRMS *m/z* (ES⁺): Found 547.1757 [M+H]⁺; C₂₇H₂₇N₆O₅S requires 547.1764.

Attempted formation of 2-(2-(maleimido)ethoxy)ethyl 4-(N-(2-(naphthalen-2-yl)imidazo-[1,2-*a*]pyrazin-8-yl)sulfamoyl)phenylcarbamate (297)



Method A:

All glassware was dried and purged with Ar prior to use. Pd₂(dba)₃ (1.64 mg, 1 mol%), DavePhos (2.11 mg, 3 mol%) and Cs₂CO₃ (81.6 mg, 0.250 mmol) were dissolved in anhydrous dioxane (2 mL). **214** (50.0 mg, 0.179 mmol) and **322** (82.3 mg, 0.215 mmol) were added and the

reaction was stirred under reflux, under Ar for 16 h. The reaction was cooled to RT and solvent removed. LCMS showed no product mass and so no further work was carried out.

Method B:

NaH was washed by stirring NaH (60% in Mineral Oil; 5.20 mg, 0.130 mmol) in anhydrous hexanes (5 mL) for 20 min, removing the solvent using a syringe and drying the contents under high vacuum. DMF (0.5 mL) was added followed by **322** (50.0 mg, 0.065 mmol) in DMF (0.5 mL) and the mixture was stirred at RT for 20 min. **227** (21.1 mg, 0.130 mmol) in DMF (1 mL) was added and the resulting solution was stirred at 100 °C under Ar for 16 h. The solvent was then removed and the residue was diluted with EtOAc (50 mL) and washed with NH₄Cl (sat. aq. 30 mL) and H₂O (3 x 30 mL). The combined aqueous layers were then re-extracted with EtOAc (2 x 50 mL), followed by washing the combined organics with brine (40 mL), drying (MgSO₄), filtering and concentrating *in vacuo*. LCMS showed no product mass and so no further work was carried out.

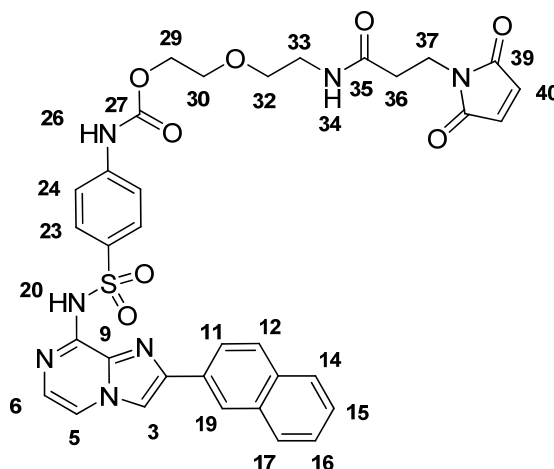
Method C:

362 (6.50 mg, 0.010 mmol) was dissolved in NaHCO₃ (sat. aq.; 1 mL) and **352** (3.10 mg, 0.020 mmol) was added in one portion. The reaction was stirred at RT for 2 h followed by diluting with H₂O (10 mL) and extracting with EtOAc (3 x 10 mL). The combined organics were washed with brine (10 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. LCMS showed signs of product, but the TLC reaction profile was extremely messy and so no further work was carried out. Crude LRMS *m/z* (ES⁺): 627 [M+H]⁺; Crude HRMS *m/z* (ES⁺): Found 627.1671; C₃₁H₂₇N₆O₇S requires 627.1662.

Method D:

362 (9.20 mg, 0.014 mmol) was dissolved in DMF (2 mL), Et₃N (3.88 µL, 0.028 mmol) and **352** (4.30 mg, 0.028 mmol) were added and the reaction was stirred at RT for 16 h. Removal of the solvent followed by LCMS indicated no reaction product evident and so no further work was carried out.

2-(2-(3-(Maleimido)propanamido)ethoxy)ethyl-4-(N-(2-(naphthalen-2-yl)imidazo[1,2-a]-pyrazin-8-yl)sulfamoyl)phenylcarbamate (363)

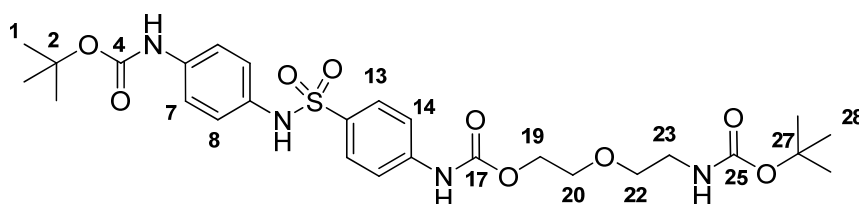


3-Maleimidopropanoic acid (62.7 mg, 0.371 mmol) and HBTU (211 mg, 0.556 mmol) were dissolved in anhydrous DMF (15 mL) and the mixture was purged with Ar. DIPEA (197 μ L, 1.11 mmol) was added and a colourless to orange colour change was observed after stirring at RT for 20 min. **362** (367 mg, 0.556 mmol) in anhydrous DMF (5 mL) was then added and the reaction was stirred at RT for 16 h. Removal of the solvent *in vacuo* was followed by diluting with EtOAc (50 mL) and washing with H₂O (4 x 30 mL) and brine (30 mL), drying (MgSO₄), filtering and concentrating *in vacuo*. Flash chromatography (applied in CH₂Cl₂; eluted 2% to 3% to 4% MeOH/CH₂Cl₂) afforded the title compound as an off white solid (146 mg, 0.210 mmol, 57%). Mpt: >200 °C; *R_f* = 0.43 (10% MeOH/CH₂Cl₂); IR (ν_{max} /cm⁻¹, thin film): 3254 (aromatic C-H stretch), 2925 (C-H and N-H stretches), 1706 (C=O stretch), 1589, 1527 (N-H bends), 1405 (S=O asymmetric stretch), 1224, 1140 (C-O and S=O symmetric stretch); ¹H NMR (600 MHz, CD₃CN): δ_{H} = 2.38 (t, *J* = 7.1 Hz, 2H, **36-H**), 3.27 (q, *J* = 5.6 Hz, 2H, **33-H**), 3.48 (t, *J* = 5.6 Hz, 2H, **32-H**), 3.64-3.67 (m, 4H, **20,37-H**), 4.24-4.26 (m, 2H, **29-H**), 6.58 (bs, 1H, **34-H**), 6.72 (s, 2H, **40-H**), 7.06 (bd, *J* = 5.4 Hz, 1H, **6-H**), 7.51-7.56 (m, 2H, **15,16-H**), 7.62-7.65 (m, 3H, **5,24-H**), 7.91-7.93 (m, 3H, **14,23-H**), 7.95-7.98 (m, 2H, **12,17-H**), 8.03 (dd, *J* = 8.5, 1.6 Hz, 1H, **11-H**), 8.20 (s, 1H, **3-H**), 8.31 (bs, 1H, **20-H**), 8.49 (s, 1H, **19-H**); ¹³C NMR (150 MHz, CD₃CN): δ_{C} = 33.8 (C-37), 33.9 (C-36), 38.4 (C-33), 64.0 (C-29), 68.3 (C-30), 68.9 (C-32), 110.3 (C-5), 114.5 (C-3), 115.8 (C-6), 117.6 (C-24), 123.4 (C-11), 124.2 (C-19), 126.1 (C-15), 126.2 (C-16), 127.1 (C-23), 127.3 (C-14), 127.9 (C-17), 128.2 (C-12), 129.4 (C-10), 132.9 (C-13), 133.2 (C-18), 133.9 (C-40), 135.4 (C-9), 135.8 (C-22), 145.2 (C-2), 142.5 (C-25), 144.7 (C-8), 153.1 (C-

27), 169.8 (**C-35**), 170.5 (**C-39**); LRMS m/z (ES^+): 698 $[M+H]^+$; Found 698.2010 $[M+H]^+$; $C_{34}H_{32}N_7O_8S$ requires 698.2033.

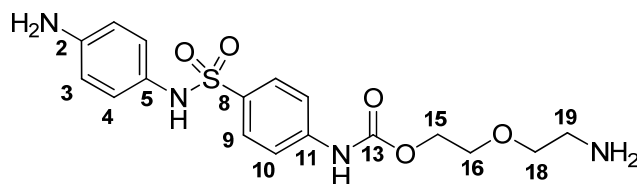
6.1.4.3.2 PEGylated-N-(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2-a]pyrazines

2-(2-Boc-aminoethoxy)ethyl-4-(N-(4-Boc-aminophenyl)sulfamoyl)phenylcarbamate (331)



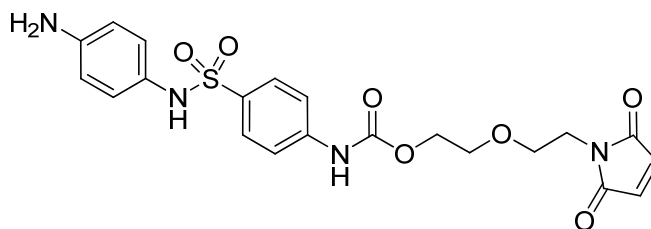
359 (76.9 mg, 0.375 mmol) was dissolved in anhydrous toluene (10 mL) and **317** (156 mg, 0.375 mmol) was added and the reaction was stirred under reflux for 16 h. The solvent was removed *in vacuo* and purification *via* flash chromatography (applied in pet. ether; eluted 25% to 50% EtOAc) afforded the title compound as pale yellow solid (165 mg, 0.278 mmol, 74%). Mpt: Decomposed at 88 °C before melting; R_f = 0.50 (1:3 pet. ether/EtOAc); IR (ν_{max}/cm^{-1} , thin film): 3352 (N-H and aromatic C-H stretches), 2976 (C-H stretch), 1684 (C=O stretch), 1524 (N-H bend), 1392 (S=O asymmetric stretch), 1247 (COOR stretch), 1154 (S=O symmetric stretch), 1069 (C-O stretch); 1H NMR (600 MHz, CD_3OD): δ_H = 1.39 (s, 9H, **28-H**), 1.49 (s, 9H, **1-H**), 3.21 (t, J = 5.6 Hz, 2H, **23-H**), 3.52 (t, J = 5.6 Hz, 2H, **22-H**), 3.70 (t, J = 4.7 Hz, 2H, **20-H**), 4.27 (t, J = 4.7 Hz, 2H, **19-H**), 6.94-6.97 (m, 2H, **8-H**), 7.24 (d, J = 8.7 Hz, 2H, **7-H**), 7.53 (d, J = 8.8 Hz, 2H, **14-H**), 7.58-7.60 (m, 2H, **13-H**); ^{13}C NMR (150 MHz, CD_3OD): δ_C = 28.7 (**C-28**), 28.7 (**C-1**), 41.2 (**C-23**), 65.4 (**C-19**), 70.1 (**C-20**), 71.0 (**C-21**), 80.1 (**C-27**), 80.3 (**C-2**), 118.8 (**C-14**), 120.3 (**C-7**), 124.1 (**C-8**), 129.4 (**C-13**), 133.4 (**C-9**), 134.1 (**C-12**), 137.9 (**C-6**), 144.6 (**C-15**), 155.3 (**C-4**), 155.3 (**C-17**), 158.4 (**C-25**); LRMS m/z (ES^+): 617 $[M+Na]^+$, 439 $[M-(2xBoc)+Formic\ Acid]^+$; HRMS m/z (ES^+): Found 617.2267 $[M+Na]^+$; $C_{27}H_{38}N_4O_9NaS$ requires 617.2257.

2-(2-Aminoethoxy)ethyl-4-(N-(4-aminophenyl)sulfamoyl)phenylcarbamate (364)



331 (160 mg, 0.269 mmol) was dissolved in CH₂Cl₂ (10 mL), TFA (10 mL) was added and the reaction was stirred at RT for 18 h. The solvent was removed *via* trituration with toluene, and the residual was diluted with H₂O (50 mL) and extracted EtOAc (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in CH₂Cl₂; eluted 5% to 10% to 20% MeOH) afforded the title compound as a sticky red solid (45.1 mg, 0.115 mmol, 43%). *R_f* = 0.06 (10% MeOH/CH₂Cl₂); IR (ν_{max} /cm⁻¹, thin film): 3249, 3104, 2924 (N-H and C-H stretches), 1704 (C=O stretch), 1594, 1512 (N-H bend), 1316 (S=O asymmetrical stretch), 1220 (COOR stretch), 1154 (S=O symmetrical stretch), 1069 (C-O stretch); ¹H NMR (600 MHz, CD₃OD): δ_{H} = 3.06 (t, *J* = 5.1 Hz, 2H, **19-H**), 3.70 (t, *J* = 5.1 Hz, 2H, **18-H**), 3.77 (t, *J* = 4.7 Hz, 2H, **16-H**), 4.34 (t, *J* = 4.7 Hz, 2H, **15-H**), 6.55-6.57 (m, 2H, **3-H**), 6.75-6.78 (m, 2H, **4-H**), 7.50-7.56 (m, 4H, **9,10-H**); ¹³C NMR (150 MHz, CD₃OD): δ_{C} = 40.9 (**C-19**), 65.0 (**C-15**), 68.7 (**C-18**), 70.5 (**C-16**), 116.7 (**C-3**), 118.7 (**C-10**), 126.4 (**C-4**), 128.8 (**C-5**), 129.5 (**C-9**), 134.4 (**C-8**), 144.4 (**C-11**), 147.0 (**C-2**); LRMS *m/z* (ES⁻): no product mass present.

Attempted formation of 2-(2-(maleimido)ethyl-4-(N-(4-aminophenyl)sulfamoyl)phenylcarbamate (327)



Method A:

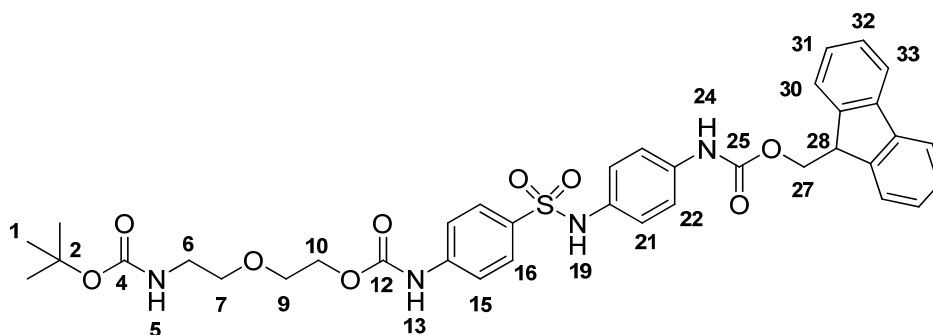
To a suspension of **310** (136 mg, 0.466 mmol) in anhydrous toluene (0.655 mL) was added Et₃N (76 μ L, 0.545 mmol). Diphenylphosphoryl azide (117 μ L, 0.545 mmol) in anhydrous toluene

(0.545 mL) was added dropwise to the reaction mixture and stirred at RT for 2 h followed by heating at 90 °C for 90 min. **350** (103 mg, 0.559 mmol) in anhydrous DMF (280 μ L) was then added and the reaction temperature was stirred at 90 °C for 16 h. The reaction was cooled to RT and solvent removed *in vacuo*. The crude material was taken up in EtOAc (75 mL) and washed with NaHCO₃ (sat. aq. 50 mL), H₂O (3 x 50 mL) and brine (50 mL); dried (MgSO₄), filtered and concentrated *in vacuo*. LCMS showed no product mass and so no further work carried out.

Method B:

364 (32.0 mg, 0.081 mmol) was dissolved in NaHCO₃ (sat. aq. 3 mL) and **352** (25.0 mg, 0.162 mmol) was added in one portion. The reaction was stirred at RT for 1 h, with TLC indicating no **352** remaining. LCMS showed no signs of product and so no further work was carried out.

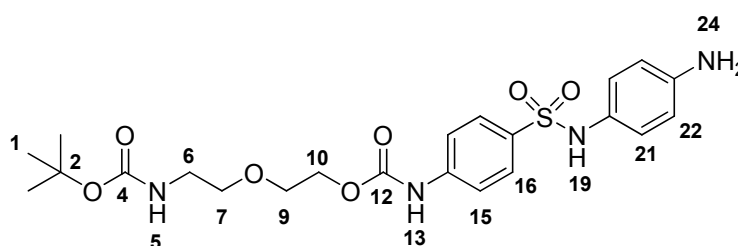
2-(2-Boc-aminoethoxy)ethyl-4-(N-(4-Fmoc-aminophenyl)sulfamoyl)phenylcarbamate (333)



359 (44.4 mg, 0.216 mmol) was dissolved in anhydrous toluene (10 mL) and **318** (140 mg, 0.260 mmol) was added and the reaction was stirred under reflux for 2 h. The solvent was removed *in vacuo* and purification *via* flash chromatography (applied in pet. ether; eluted 2:1 to 1:1 to 1:2 pet. ether/EtOAc) afforded the title compound as a white solid (30.9 mg, 0.043 mmol, 20%). Mpt: 162-164 °C; R_f = 0.11 (1:1 pet. ether/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3322 (N-H stretch), 3239 (aromatic C-H stretch), 2973 (C-H stretch), 1700 (C=O stretch), 1522 (N-H bend), 1240 (S=O asymmetric stretch), 1154 (S=O symmetric stretch), 1067, 1054 (C-O stretches); ¹H NMR (600 MHz, (CD₃)₂SO): δ_{H} = 1.34 (s, 9H, **1-H**), 3.06 (q, J = 5.9 Hz, 2H, **6-H**), 3.38-3.40 (m, 2H, **7-H**), 3.61 (t, J = 4.3 Hz, 2H, **9-H**), 4.19 (t, J = 4.3 Hz, 2H, **10-H**), 4.27 (t, J = 6.5 Hz, 1H, **28-H**), 4.43 (bs, 2H, **27-H**), 6.80 (t, J = 5.6 Hz, 1H, **5-H**), 6.94 (bs, 2H, **21-H**), 7.32-7.34 (m, 4H, **22,32-H**), 7.41 (t, J = 7.5 Hz, 2H, **31-H**), 7.56 (d, J = 8.9 Hz, 2H, **15-H**), 7.60 (d, J = 8.9 Hz, 2H, **16-H**),

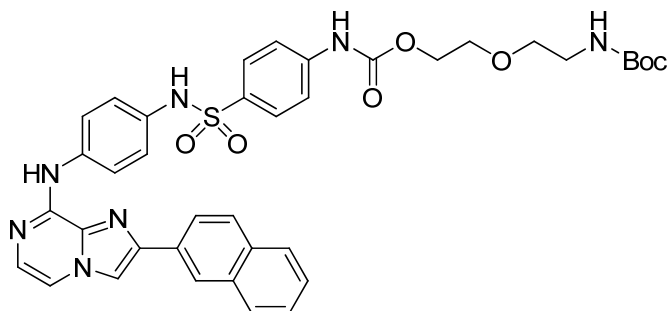
7.72 (bd, $J = 6.7$ Hz, 2H, **33-H**), 7.89 (d, $J = 7.5$ Hz, 2H, **30-H**), 9.64 (bs, 1H, **24-H**), 9.90 (s, 1H, **13-H**), 10.20 (s, 1H, **19-H**); ^{13}C NMR (150 MHz, $(\text{CD}_3)_2\text{SO}$): $\delta_{\text{C}} = 28.2$ (**C-1**), 39.1 (**C-6**), 46.6 (**C-28**), 63.9 (**C-10**), 65.5 (**C-27**), 68.2 (**C-9**), 69.2 (**C-7**), 77.7 (**C-2**), 117.7 (**C-15**), 120.2 (**C-30**), 121.7 (**C-21**), 125.1 (**C-33**), 127.2 (**C-22,32**), 127.7 (**C-31**), 128.0 (**C-16**), 131.5 (overlapping signals, **C-20,23**), 132.8 (**C-17**), 140.8 (**C-29**), 142.2 (**C-14**), 143.8 (**C-34**), 153.3 (**C-12**), 153.4 (**C-25**), 155.6 (**C-4**); LRMS m/z (ES^+): 739 $[\text{M}+\text{Na}]^+$, 180 [aromatic Fmoc Fragment] $^+$; HRMS m/z (ES^+): Found 739.2414 $[\text{M}+\text{Na}]^+$; $\text{C}_{37}\text{H}_{40}\text{N}_4\text{O}_9\text{NaS}$ requires 739.2402.

2-(2-Boc-aminoethoxy)ethyl-4-(N-(4-aminophenyl)sulfamoyl)phenylcarbamate (365)



333 (30.9 mg, 0.043 mmol) was dissolved in piperidine/ CH_2Cl_2 (40% v/v; 10 mL) and the reaction was stirred at RT for 30 min. The solvent was removed and flash chromatography (applied in CH_2Cl_2 ; eluted 3:1 $\text{CH}_2\text{Cl}_2/\text{EtOAc}$) afforded the title compound as a yellow oil (6.70 mg, 0.014 mmol, 33%). $R_f = 0.09$ (3:1 EtOAc/pet. ether); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3363 (N-H and aromatic C-H stretches), 2976 (C-H stretch), 1689 (C=O stretch), 1513 (N-H bend), 1314 (S=O asymmetric stretch), 1224 (COOR stretch), 1157 (S=O symmetric stretch), 1067 (C-O stretch); ^1H NMR (600 MHz, CD_3OD): $\delta_{\text{H}} = 1.41$ (s, 9H, **1-H**), 3.21-3.23 (m, 2H, **6-H**), 3.52 (t, $J = 5.5$ Hz, 2H, **7-H**), 3.70 (t, $J = 4.6$ Hz, 2H, **9-H**), 4.28 (t, $J = 4.6$ Hz, 2H, **10-H**), 6.55-6.57 (m, 2H, **22-H**), 6.75-6.77 (m, 2H, **21-H**), 7.50-7.55 (m, 4H, **15,16-H**); ^{13}C NMR (150 MHz, CD_3OD): $\delta_{\text{C}} = 28.7$ (**C-1**), 41.2 (**C-6**), 62.2 (**C-10**), 65.4 (**C-9**), 70.1 (**C-7**), 80.1 (**C-2**), 116.7 (**C-22**), 118.7 (**C-15**), 126.4 (**C-21**), 128.8 (**C-20**), 129.5 (**C-16**), 134.2 (**C-17**), 144.5 (**C-14**), 147.0 (**C-23**), 155.3 (**C-12**), 158.5 (**C-4**); LRMS m/z (ES^-): 493 $[\text{M}-\text{H}]^-$, 288 $[\text{M}-(\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{NHBoc})]^-$; HRMS m/z (ES^-): Found 493.1766 $[\text{M}+\text{H}]^+$; $\text{C}_{22}\text{H}_{29}\text{N}_4\text{O}_7\text{S}$ requires 493.1757;

2-(2-Boc-aminoethoxy)ethyl-4-(N-(4-aminophenyl(2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-yl)sulfamoyl)phenylcarbamate (366)

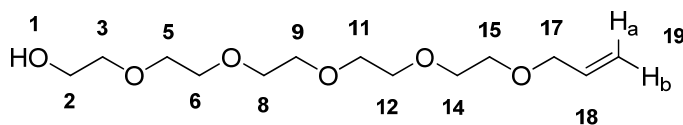


All glassware was dried and purged with Ar prior to use. $\text{Pd}_2(\text{dba})_3$ (0.105 mg, 1 mol%), DavePhos (0.135 mg, 3 mol%) and NaO^tBu (1.54 mg, 0.016 mmol) were dissolved in anhydrous toluene (2 mL). **214** (3.20 mg, 0.011 mmol) and **365** (6.76 mg, 0.014 mmol) were added and the reaction was stirred under reflux, under Ar for 16 h. LCMS showed desired product, but no further work was carried out at this stage. Crude LRMS m/z (ES^+): 738 $[\text{M}+\text{H}]^+$, 682 $[\text{M}-^t\text{Bu}]^+$, 638 $[\text{M}-\text{Boc}]^+$.

6.1.4.4 Synthesis of PEGylated Allyl and Alkyne Linked Imidazo[1,2-a]pyrazines

6.1.4.4.1 PEGylated-Benzenesulfonamide-bound Imidazo[1,2-a]pyrazines

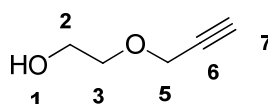
3,6,9,12,15-Pentaoxaoctadec-17-en-1-ol (374)



NaH (56.7 mg, (94.5 mg of 60% dispersion in mineral oil), 2.36 mmol) was washed by stirring in dry hexanes (10 mL) for 20 min. The hexanes were removed *via* syringe and the flask was dried under vacuum. After 15 min, anhydrous THF (2 mL) was added and the mixture was cooled to 0 °C. Penta(ethylene) glycol (1 mL, 4.73 mmol), dissolved in anhydrous THF (3 mL) was added dropwise to the solution, which was then allowed to stir for 15 min before allyl bromide (136 μL , 1.58 mmol) was added dropwise. The resulting solution was stirred at 0 °C for 90 min, before removal of the solvent *in vacuo*. Addition of CH_2Cl_2 (100 mL) was followed by washing with H_2O (50 mL) and brine (50 mL), drying (MgSO_4) and concentrating *in vacuo* to give a colourless

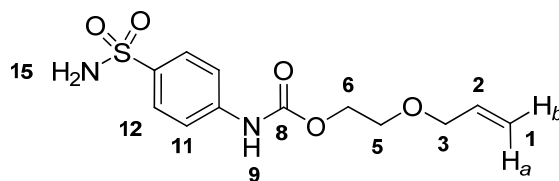
oil. Flash chromatography (applied in CH₂Cl₂; eluted 1:1 CH₂Cl₂/EtOAc to 45:50:5 CH₂Cl₂/EtOAc/MeOH) afforded the title compound as a light yellow oil (420 mg, 1.51 mmol, 96%) with NMR consistent with literature values.³¹⁹ R_f = 0.12 (1:5 CH₂Cl₂/EtOAc); IR (ν_{\max} /cm⁻¹, thin film): 3448 (O-H stretch), 2868 (C-H stretch), 1094 (C-O stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 2.45 (bs, 1H, **1-H**), 3.59-3.61 (m, 4H, **2,15-H**), 3.65-3.68 (m, 14H, **5,6,8,9,11,12,14-H**), 3.71-3.73 (m, 2H, **3-H**), 4.02 (d, J = 5.7 Hz, 2H, **17-H**), 5.17 (dd, J = 10.5, 1.3 Hz, 1H, **19-H_b**), 5.27 (dd, J = 17.2, 1.3 Hz, 1H, **19-H_a**), 5.88-5.93 (m, 1H, **18-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 61.8 (**C-3**), 69.5 (**C-15**), 70.4 (**C-14**), 70.4-70.7 (**C-5-12**), 72.4 (**C-17**), 72.7 (**C-2**), 117.3 (**C-19**), 134.8 (**C-18**); LRMS m/z (ES⁺): 302.

2-(Prop-2-ynyloxy)ethanol (**375**)²⁷⁴



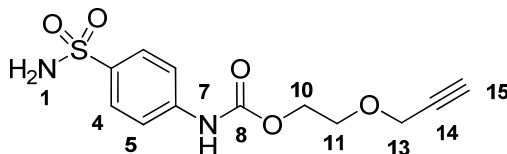
Ethylene glycol (5.00 mL, 0.089 mol) was cooled on ice and NaH (60% in mineral oil; 0.894 g, 0.022 mol) was added slowly to form a viscous white paste. Propargyl bromide (80% solution in toluene ; 2.49 mL, 0.022 mol) was added dropwise and the reaction mixture was stirred at 45 °C for 3h. The reaction was carefully quenched with H₂O (100 mL) and extracted with CHCl₃ (3 x 50 mL) and CH₂Cl₂ (5 x 50 mL). The combined organic extracts were dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 10% to 50% EtOAc/pet. ether) afforded the title compound as a light yellow oil (1.03 g, 0.013 mmol, 47%) with NMR consistent with literature values.²⁷⁴ R_f = 0.39 (1:1 pet. ether/EtOAc); IR (ν_{\max} /cm⁻¹, thin film): 3390 (O-H stretch), 3286 (\equiv C-H stretch), 2936 (C-H stretch), 2116 (C \equiv C stretch), 1354, 1105, 1065, 1027 (C-O stretches); ¹H NMR (600 MHz, CDCl₃): δ_H = 2.06 (bs, 1H, **1-H**), 2.46 (J = 2.4 Hz, 1H, **7-H**), 2.65 (t, J = 4.5 Hz, 2H, **3-H**), 3.77 (t, J = 4.5 Hz, 2H, **2-H**), 4.20 (d, J = 2.3 Hz, 2H, **5-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 58.5 (**C-5**), 61.8 (**C-2**), 71.3 (**C-3**), 74.9 (**C-7**), 79.6 (**C-6**); LRMS m/z (CI⁺): 101 [M+H]⁺; HRMS m/z (CI⁺): Found 101.0609 [M-H]⁻; C₅H₉O₂ requires 101.0603.

2-(Allyloxy)ethyl 4-sulfamoylphenylcarbamate (324)



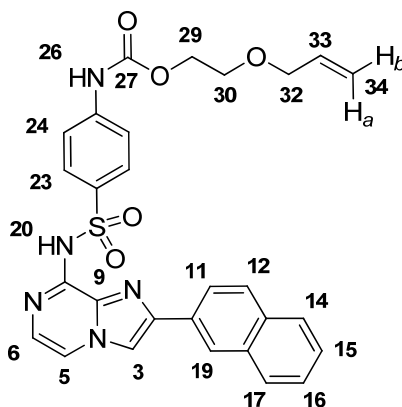
To a suspension of 4-sulfamoylbenzoic acid (2.01 g, 10.0 mmol) in anhydrous toluene (15 mL) was added Et₃N (1.63 mL, 11.7 mmol). Diphenylphosphoryl azide (2.52 mL, 11.7 mmol) in anhydrous toluene (10 mL) was added dropwise to the reaction mixture and stirred at RT for 30 min followed by heating at 90 °C for 30 min. 2-(allyloxy)ethanol (1.28 mL, 12.0 mmol) in anhydrous DMF (6 mL) was then added and the reaction was stirred at 90 °C for 16 h. The reaction was cooled to RT and solvent removed *in vacuo*. The crude material was taken up in EtOAc (100 mL) and washed with NaHCO₃ (sat. aq. 50 mL), H₂O (3 x 40 mL) and brine (40 mL); dried (MgSO₄), filtered and concentrated *in vacuo* to give an orange/brown sticky solid. Flash chromatography (applied in pet. ether; eluted 2:1 to 1:1 pet. ether/EtOAc) afforded the title compound as a white solid (689 mg, 2.30 mmol, 23%). Mpt: 128 °C; *R*_f = 0.41 (2:1 EtOAc/pet. ether); IR (ν_{max}/cm⁻¹, thin film): 3356, 3298, 3193, 3110, 2908 (C-H and N-H stretches), 1734 (C=O stretch), 1596 (C=C stretch), 1529 (N-H bend), 1336 (S=O asymmetric stretch), 1314, 1216, 1151 (S=O symmetric stretch), 1099, 1055 (C-O stretches); ¹H NMR (600 MHz, (CD₃)₂SO): δ_H = 3.63-3.64 (m, 2H, **5-H**), 3.99 (dt, *J* = 5.3, 1.6 Hz, 2H, **3-H**), 4.24-4.25 (m, 2H, **6-H**), 5.16 (dd, *J* = 10.4, 1.6 Hz, 1H, **1-H_b**), 5.27 (dd, *J* = 17.3, 1.6 Hz, 1H, **1-H_a**), 5.85-5.91 (m, 1H, **2-H**), 7.23 (s, 2H, **15-H**), 7.62 (d, *J* = 8.8 Hz, 2H, **11-H**), 7.72-7.72 (m, 2H, **12-H**), 10.17 (s, 1H, **9-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_C = 63.9 (C-6), 67.7 (C-5), 71.0 (C-3), 116.7 (C-1), 117.7 (C-11), 126.8 (C-12), 135.0 (C-2), 137.6 (C-13), 142.3 (C-10), 153.4 (C-8); LRMS *m/z* (EI⁺): 300 [M]⁺, 243 [M-OCH₂CH=CH₂]; HRMS *m/z* (EI⁺): Found 300.0781 [M]⁺; C₁₂H₁₆N₂O₅S requires 300.0774; Anal. Calcd. for C₁₂H₁₆N₂O₅S: C, 47.99; H, 5.37; N, 9.33. Found C, 48.24; H, 5.27; N, 9.57%.

2-(Prop-2-ynyloxy)ethyl 4-sulfamoylphenylcarbamate (325)



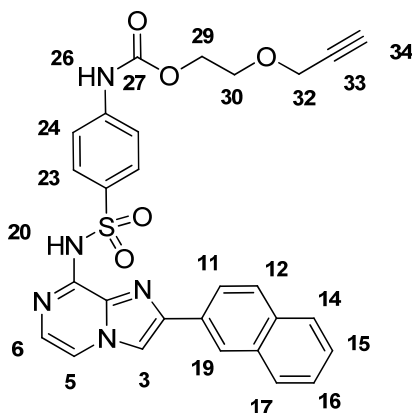
375 (557 mg, 5.57 mmol) was dissolved in anhydrous toluene (140 mL). **315** (1.51 g, 6.68 mmol) and molecular sieves (4Å, 10 sieves) were added and the reaction was stirred under reflux for 16 h. The solvent was removed and the reaction purified *via* flash chromatography (applied in toluene; eluted 2:1 toluene/EtOAc) to afford the title compound as a white solid (663 mg, 2.22 mmol, 40%). Mpt: 100-102 °C; R_f = 0.17 (2:1 toluene/EtOAc); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3350 ($\equiv\text{C-H}$ stretch), 3273 (aromatic C-H stretch), 2116 ($\text{C}\equiv\text{C}$ stretch), 1704 (C=O stretch), 1595, 1533 (N-H bends), 1314 (S=O asymmetric stretch), 1234 (COOR stretch), 1152 (S=O symmetric stretch), 1067 (C-O stretch); ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{H} = 3.48 (t, J = 2.3 Hz, 1H, **15-H**), 3.69-3.70 (m, 2H, **11-H**), 4.20 (d, J = 2.3 Hz, 2H, **13-H**), 4.24-4.26 (m, 2H, **10-H**), 7.23 (s, 2H, **1-H**), 7.61 (d, J = 8.8 Hz, 2H, **5-H**), 7.71-7.73 (m, 2H, **4-H**), 10.18 (s, 1H, **7-H**); ^{13}C NMR (150 MHz, $(\text{CD}_3)_2\text{SO}$): δ_{C} = 57.5 (**C-13**), 63.6 (**C-10**), 67.3 (**C-11**), 77.5 (**C-15**), 80.1 (**C-14**), 117.6 (**C-5**), 126.8 (**C-4**), 137.6 (**C-3**), 142.2 (**C-6**), 153.3 (**C-8**); LRMS m/z (EI^+): 298 $[\text{M}]^+$, 243 $[\text{M}-\text{OCH}_2\text{C}\equiv\text{CH}]^+$, 216 $[\text{M}-\text{CH}_2\text{CH}_2\text{OCH}_2\text{C}\equiv\text{CH}]^+$, 183 $[\text{M}-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{OCH}_2\text{C}\equiv\text{CH}]^+$; HRMS m/z (EI^+): Found 298.0625 $[\text{M}]^+$; $\text{C}_{12}\text{H}_{14}\text{N}_2\text{O}_5\text{S}$ requires 298.0618; Anal. Calcd. for $\text{C}_{12}\text{H}_{14}\text{N}_2\text{O}_5\text{S}$: C, 48.31; H, 4.73; N, 9.39. Found C, 48.60; H, 4.66; N, 9.12%.

2-(Allyloxy)ethyl-4-(N-(2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-yl)sulfamoyl)phenylcarbamate (298)



NaH was washed by stirring NaH (60% in Mineral Oil; 70.6 mg, 1.77 mmol) in anhydrous hexanes (50 mL) for 20 min, removing the solvent using a syringe and drying the contents under high vacuum. DMF (5 mL) was added followed by **324** (529 mg, 1.77 mmol) in DMF (7 mL) and the mixture was stirred at RT for 20 min. **227** (285 mg, 0.882 mmol) in DMF (13 mL) was added and the resulting dark brown solution was heated at 100 °C under Ar for 16 h. The mixture was then diluted with EtOAc (100 mL) and washed with NH₄Cl (sat. aq. 50 mL) and H₂O (5 x 40 mL). The combined aqueous layers were then re-extracted with EtOAc (2 x 40 mL), followed by washing the combined organics with brine (40 mL) drying (MgSO₄), filtering and concentrating *in vacuo*. Flash chromatography (applied in CH₂Cl₂; eluted 10% to 20% to 33% EtOAc/CH₂Cl₂) afforded the title compound as a light yellow solid (214 mg, 0.395 mmol, 45%). Mpt: >200 °C; *R_f* = 0.25 (1:1 CH₂Cl₂/EtOAc); IR (ν_{max} /cm⁻¹, thin film): 3223 (aromatic C-H stretch), 1720 (C=O stretch), 1583 (N-H bend and C=C stretch), 1392 (S=O asymmetric stretch), 1112 (S=O symmetric stretch), 1065 (C-O stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_{H} = 3.62 (dt, *J* = 4.6, 3.1 Hz, 2H, **30-H**), 3.97 (d, *J* = 6.7 Hz, 2H, **32-H**), 4.23 (ap.t, *J* = 4.4 Hz, 2H, **29-H**), 5.14 (dd, *J* = 10.5, 1.6 Hz, 1H, **34-H_b**), 5.25 (dd, *J* = 17.3, 1.6 Hz, 1H, **34-H_a**), 5.84-5.91 (m, 1H, **33-H**), 7.16 (d, *J* = 5.2 Hz, 1H, **6-H**), 7.50-7.55 (m, 2H, **15,16-H**), 7.65 (d, *J* = 8.7 Hz, 2H, **24-H**), 7.86 (d, *J* = 5.2 Hz, 1H, **5-H**), 7.92 (d, *J* = 8.5 Hz, 3H, **14,23-H**), 7.98 (d, *J* = 8.5 Hz, 1H, **12-H**), 8.03 (t, *J* = 8.9 Hz, 2H, **11,17-H**), 8.52 (s, 1H, **19-H**), 8.59 (s, 1H, **3-H**), 10.21 (s, 1H, **26-H**), 11.63 (bs, 1H, **7/20-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_{C} = 63.9 (**C-29**), 67.7 (**C-30**), 71.0 (**C-32**), 110.9 (**C-5**), 115.3 (**C-3**), 116.7 (**C-34**), 116.8 (**C-6**), 117.6 (**C-24**), 123.8 (**C-11**), 124.2 (**C-19**), 126.3 (**C-15**), 126.6 (**C-16**), 127.4 (**C-23**), 127.7 (**C-14**), 128.3 (**C-17**), 128.4 (**C-12**), 130.0 (**C-10**), 132.8 (**C-13**), 133.2 (**C-18**), 135.0 (**C-33**), 135.6 (**C-9**), 135.9 (**C-22**), 142.9 (**C-25**), 144.4 (**C-8**), 145.2 (**C-2**), 153.3 (**C-27**); LRMS *m/z* (ES⁺): 544 [M+H]⁺; HRMS *m/z* (ES⁺): Found 544.1631 [M+H]⁺; C₂₈H₂₆N₅O₅S requires 544.1655; Anal. Calcd. for C₂₈H₂₅N₅O₅S: C, 61.87; H, 4.64; N, 12.88. Found C, 61.61; H, 4.32; N, 12.85%.

2-(Prop-2-ynyloxy)ethyl-4-(N-(2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-yl)sulfamoyl)phenylcarbamate (299)

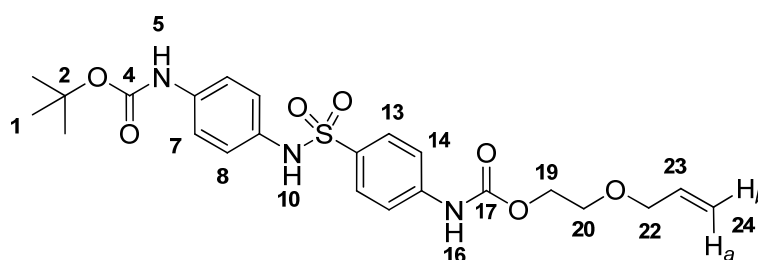


NaH was washed by stirring NaH (60% in Mineral Oil; 88.7 mg, 2.22 mmol) in anhydrous hexanes (25 mL) for 20 min, removing the solvent using a syringe and drying the contents under high vacuum. DMF (6 mL) was added followed by **325** (660 mg, 2.22 mmol) in DMF (8 mL) and the mixture was stirred at RT for 20 min. **227** (358 mg, 1.11 mmol) in DMF (20 mL) was added and the resulting dark brown solution was heated at 100 °C under Ar for 18 h. The solvent was removed and the resulting residue was then taken up in EtOAc (100 mL) and washed with NH₄Cl (sat. aq. 50 mL) and H₂O (5 x 40 mL). The combined aqueous layers were then re-extracted with EtOAc (2 x 40 mL), followed by washing the combined organics with brine (40 mL), drying (MgSO₄), filtering and concentrating *in vacuo*. Flash chromatography (applied in pet. ether; eluted 1:1 to 1:2 to 1:4 to 1:9 pet. ether/EtOAc) afforded the title compound as a yellow/orange solid (380 mg, 0.703 mmol, 64%). Mpt: 184-186 °C; *R_f* = 0.32 (9:1 EtOAc/pet. ether); IR (ν_{max} /cm⁻¹, thin film): 3227 (aromatic C-H and \equiv C-H stretch), 2112 (C \equiv C stretch), 1719 (C=O stretch), 1582, 1532 (N-H bends), 1391 (S=O asymmetric stretch), 1222 (COOR stretch), 1141 (S=O symmetric stretch), 1112 (C-O stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_{H} = 3.46 (t, *J* = 2.4 Hz, 1H, **34-H**), 3.67-3.69 (m, 2H, **30-H**), 4.18 (d, *J* = 2.4 Hz, 2H, **32-H**), 4.23-4.25 (m, 2H, **29-H**), 7.16 (t, *J* = 5.5 Hz, 1H, **6-H**), 7.51-7.54 (m, 2H, **15,16-H**), 7.65 (d, *J* = 8.9 Hz, 2H, **24-H**), 7.86 (d, *J* = 5.6 Hz, 1H, **5-H**), 7.92-7.93 (m, 3H, **14,23-H**), 7.98 (d, *J* = 8.6 Hz, 1H, **12-H**), 8.02-8.05 (m, 2H, **11,17-H**), 8.52 (s, 1H, **19-H**), 8.59 (s, 1H, **3-H**), 10.22 (s, 1H, **26-H**), 11.63 (bd, *J* = 4.7 Hz, **7/20-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_{C} = 57.5 (C-**32**), 63.7 (C-**29**), 67.3 (C-**30**), 77.5 (C-**34**), 80.1 (C-**33**), 110.9 (C-**5**), 115.3 (C-**3**), 116.8 (C-**6**), 117.7 (C-**24**), 123.8 (C-**11**), 124.2 (C-**19**), 126.3 (C-**15**), 126.6 (C-**16**), 127.4 (C-**23**), 127.7 (C-**14**), 128.3 (C-**17**), 128.4 (C-**12**), 130.0 (C-**10**), 132.8 (C-**13**), 133.2 (C-**18**), 135.6 (C-**9**), 136.9 (C-**22**), 142.9

(**C-25**), 144.4 (**C-8**), 145.2 (**C-2**), 153.3 (**C-27**); LRMS m/z (ES^+): 542 $[M+H]^+$; HRMS m/z (ES^+): Found 542.1512 $[M+H]^+$; $C_{28}H_{24}N_5O_5S$ requires 542.1498.

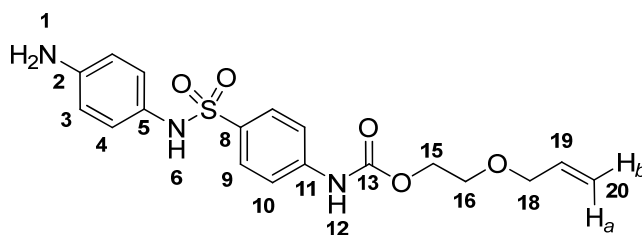
6.1.4.4.2 PEGylated-*N*-(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2-*a*]pyrazines

2-(Allyloxy)ethyl -4-(*N*-(4-Boc-aminophenyl)sulfamoyl)phenylcarbamate (**332**)



317 (66.5 mg, 0.159 mmol) was taken up in anhydrous toluene (5 mL) and 2-(allyloxy)ethanol (14.2 μ L, 0.133 mmol) was added and the reaction was stirred under reflux for 16 h. The reaction was then cooled to RT and purified *via* flash chromatography (applied in pet. ether; eluted 2:1 to 1:1 pet. ether/EtOAc) to afford the title compound as an off white solid (40.0 mg, 0.080 mmol, 61%). Mpt: 139-141 $^{\circ}C$; R_f = 0.37 (1:1 pet. ether; EtOAc); IR (ν_{max}/cm^{-1} , thin film): 3325 (N-H and C-H stretches), 1704 (C=O stretch), 1595, 1537 (N-H bends and C=C stretch), 1315 (S=O asymmetric stretch), 1155 (S=O symmetric stretch), 1061 (C-O stretch); 1H NMR (600 MHz, CD_3OD): δ_H = 1.48 (s, 9H, **1-H**), 3.68 (dd, J = 4.7, 3.2 Hz, 2H, **20-H**), 4.02 (dt, J = 5.5, 1.5 Hz, 2H, **22-H**), 4.28 (dd, J = 5.9, 4.7 Hz, 2H, **19-H**), 5.16 (dd, J = 10.4, 1.5 Hz, 1H, **24-H_b**), 5.28 (dq J = 17.3, 1.5 Hz, 1H, **24-H_a**), 5.87-5.93 (m, 1H, **23-H**), 6.95-6.97 (m, 2H, **8-H**), 7.24 (d, J = 8.5 Hz, 2H, **7-H**), 7.52 (d, J = 8.9 Hz, 2H, **14-H**), 7.59 (d, J = 7.1 Hz, 2H, **13-H**); ^{13}C NMR (150 MHz, CD_3OD): δ_C = 28.8 (**C-1**), 65.4 (**C-19**), 69.3 (**C-20**), 73.0 (**C-22**), 80.9 (**C-2**), 117.4 (**C-24**), 118.8 (**C-14**), 120.3 (**C-7**), 124.2 (**C-8**), 129.4 (**C-13**), 133.4 (**C-9**), 134.1 (**C-12**), 135.8 (**C-23**), 138.0 (**C-6**), 144.7 (**C-15**), 155.3 (overlapping signals, **C-4,17**); LRMS m/z (ES^+): 530 $[M+K]^+$, 514 $[M+Na]^+$, 458 $[M-^tBu+Na]^+$; HRMS m/z (ES^+): Found 514.1610 $[M+Na]^+$; $C_{23}H_{29}N_3O_7NaS$ requires 514.1624.

2-(Allyloxy)ethyl 4-(N-(4-aminophenyl)sulfamoyl)phenylcarbamate (328)



Method A:

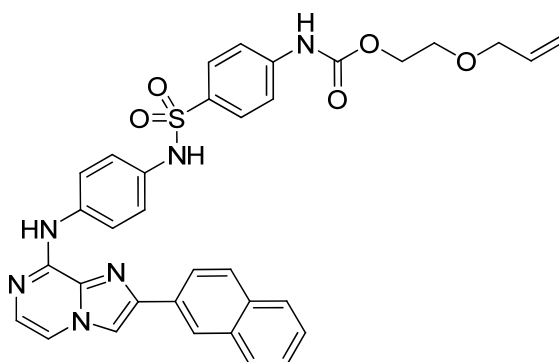
To a suspension of **310** (1.18 g, 0.466 mmol) in anhydrous toluene (5.35 mL) was added Et₃N (661 μ L, 4.75 mmol). Diphenylphosphoryl azide (1.03 mL, 0.545 mmol) in anhydrous toluene (4.75 mL) was added dropwise to the reaction mixture and stirred at RT for 2 h followed by heating at 90 °C for 90 min. 2-(allyloxy)ethanol (520 μ L, 4.87 mmol) in anhydrous DMF (2 mL) was then added and the reaction temperature was stirred at 90 °C for 16 h. The reaction was cooled to RT and solvent removed *in vacuo*. The crude material was taken up in EtOAc (75 mL) and washed with NaHCO₃ (sat. aq. 40 mL), H₂O (3 x 40 mL) and brine (40 mL); dried (MgSO₄), filtered and concentrated *in vacuo*. LCMS showed no product mass and so no further work carried out.

Method B:

332 (40.0 mg, 0.081 mmol) was dissolved in CH₂Cl₂ (3 mL), TFA (3 mL) was added and the reaction was stirred at RT for 1 h. The mixture was diluted with CH₂Cl₂ (20 mL) and was carefully washed with NaHCO₃ (sat. aq. 2 x 15 mL) and brine (15 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. The resulting crude material was purified *via* flash chromatography (applied in CH₂Cl₂; eluted 1% to 2% MeOH) to afford the title compound as a white solid (28.0 mg, 0.072 mmol, 89%). Mpt: 195 °C; *R_f* = 0.64 (0.29 for TFA Salt) (10% MeOH/CH₂Cl₂); IR (ν_{max} /cm⁻¹, thin film): 3401, 3334 (N-H stretches), 3200 (aromatic C-H stretch), 2849, 2791 (C-H stretches), 1704 (C=O stretch), 1595, 1509 (N-H bends), 1316 (S=O asymmetric stretch), 1249 (COOR stretch), 1160 (S=O symmetric stretch), 1059 (C-O stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_{H} = 3.61-3.63 (m, 2H, **16-H**), 3.98 (dt, *J* = 5.3, 1.4 Hz, 2H, **18-H**), 4.23 (t, *J* = 4.5 Hz, 2H, **15-H**), 4.95 (s, 2H, **1-H**), 5.15 (dd, *J* = 10.4, 1.8 Hz, 1H, **20-H_b**), 5.27 (dq, *J* = 17.3, 1.8 Hz, 1H, **20-H_a**), 5.85-5.91 (m 1H, **19-H**), 6.36-6.38 (m, 2H, **3-H**), 6.64 (d, *J* = 8.6 Hz, 2H, **4-H**), 7.51-7.55 (m, 4H, **9,10-H**), 9.32 (s, 1H, **6-H**), 10.17 (s, 1H, **12-H**); ¹³C NMR (150 MHz,

(CD₃)₂SO): δ_c = 63.9 (C-15), 67.7 (C-16), 71.0 (C-18), 114.0 (C-3), 116.7 (C-20), 117.4 (C-10), 124.7 (C-4), 128.0 (C-9), 125.5 (C-5), 132.8 (C-8), 135.0 (C-19), 142.8 (C-11), 146.5 (C-2), 153.3 (C-13); LRMS m/z (ES⁺): 414 [M+Na]⁺; HRMS m/z (ES⁺): Found 414.1082 [M+Na]⁺; C₁₈H₂₁N₃O₅NaS requires 414.1100; Anal. Calcd. for C₁₈H₂₁N₃O₅S: C, 55.23; H, 5.41; N, 10.73. Found C, 54.92; H, 5.28; N, 10.50%.

Attempted formation of 2-(allyloxy)ethyl 4-(N-(4-(2-(naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-ylamino)phenyl)sulfamoyl)phenylcarbamate (303)

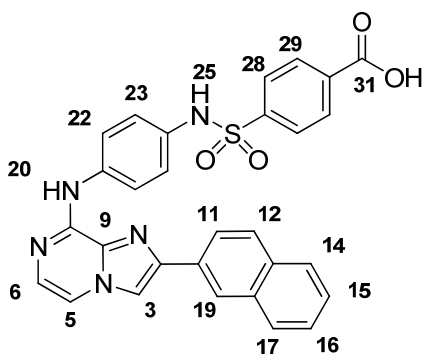


328 (25.0 mg, 0.064 mmol) and NaH (5.10 mg, 0.128 mmol) were dissolved in anhydrous DMF (1.5 mL). This yellow solution was stirred at RT for 20 min before **227** (10.3 mg, 0.032 mmol) in DMF (0.5 mL) was added. The resulting red solution was stirred at 100 °C for 16 h. After cooling to RT, the mixture was diluted with EtOAc (40 mL) and washed with NH₄Cl (sat. aq. 20 mL), H₂O (5 x 20 mL) and brine (20 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. LCMS on the crude material revealed no product mass and so no further work was carried out.

6.1.4.5 Alternative Synthesis of PEGylated Imidazo[1,2-a]pyrazines

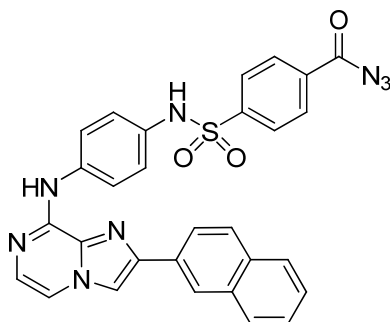
6.1.4.5.1 PEGylated-N-(4-aminophenyl)benzenesulfonamide-bound Imidazo[1,2-a]pyrazines

4-(N-(4-(2-(Naphthalen-2-yl)imidazo[1,2-a]pyrazin-8-ylamino)phenyl)sulfamoyl)benzoic acid (376)



233 (95.0 mg, 0.271 mmol) was taken up in acetone (10 mL) and 4-(chlorosulfonyl)benzoic acid (59.7 mg, 0.271 mmol) was added and the mixture was stirred at RT for 16 h. The solvent was removed and the crude material was purified *via* flash chromatography (applied in toluene; eluted 2:1 to 1:1 to 1:4 to 1:9 toluene/EtOAc to 1:1:8 toluene/MeOH/EtOAc) to afford the title compound as a green solid (38.9 mg, 0.073 mmol, 27%). Mpt: Decomposed before melting; R_f = 0.46 (20% MeOH/CH₂Cl₂); IR (ν_{\max} /cm⁻¹, thin film): 3363 (O-H and aromatic C-H stretches), 1537 (C=O stretch and N-H bend), 1508 (COO⁻ stretch), 1470, 1340 (S=O asymmetric stretch), 1161 (S=O symmetric stretch); ¹H NMR (600 MHz, (CD₃)₂SO): δ_H = 7.04 (d, J = 8.9 Hz, 2H, **23-H**), 7.40 (d, J = 4.6 Hz, 1H, **6-H**), 7.51-7.57 (m, 2H, **15,16-H**), 7.85 (d, J = 8.5 Hz, 2H, **28-H**), 7.93-7.95 (m, 2H, **14,22-H**), 7.97 (d, J = 8.0 Hz, 1H, **17-H**), 8.00 (d, J = 4.6 Hz, 1H, **5-H**), 8.02 (d, J = 8.5 Hz, 1H, **12-H**), 8.08 (d, J = 8.5 Hz, 2H, **29-H**), 8.17 (dd, J = 8.5, 1.6 Hz, 1H, **11-H**), 8.57 (s, 1H, **3-H**), 8.60 (s, 1H, **19-H**), 9.45 (s, 1H, **20-H**), 10.23 (s, 1H, **25-H**), 13.51 (bs, 1H, **32-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_C = 112.5 (**C-3,5**), 120.8 (**C-22**), 122.0 (**C-23**), 124.2 (**C-19**), 124.2 (**C-11**), 126.2 (**C-15**), 126.7 (**C-16**), 127.0 (**C-28**), 127.4 (**C-6**), 127.8 (**C-14**), 128.0 (**C-17**), 128.3 (**C-12**), 130.1 (**C-29**), 130.8 (**C-10**), 131.1 (**C-24**), 132.7 (**C-13**), 133.0 (**C-9**), 133.2 (**C-18**), 134.5 (**C-30**), 137.3 (**C-21**), 143.2 (**C-27**), 143.5 (**C-2**), 145.7 (**C-8**), 166.2 (**C-31**); LRMS m/z (ES⁺): 536 [M+H]⁺; HRMS m/z (ES⁺): Found 536.1373 [M+H]⁺; C₂₉H₂₂N₅O₄S requires 536.1393

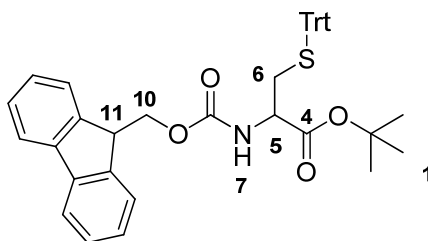
Attempted formation of 4-(N-(4-(2-(naphthalen-2-yl)imidazo[1,2-*a*]pyrazin-8-ylamino)-phenyl)sulfamoyl)benzoyl azide (377)



376 (35.0 mg, 0.065 mmol), PPh₃ (34.3 mg, 0.131 mmol) and NaN₃ (5.10 mg, 0.078 mmol) were suspended in anhydrous acetone (2 mL). Trichloroacetonitrile (13.1 μ L, 0.131 mmol) was added dropwise and the reaction was stirred at RT for 18 h. Removal of the reaction solvent *in vacuo* was followed by taking up the residual in CH₂Cl₂ (40 mL) and washing with H₂O (30 mL) and brine (30 mL), drying (MgSO₄), filtering and concentrating. Flash chromatography (applied in CH₂Cl₂; eluted 20% to 50% EtOAc) gave no desired product and so no further work was carried out.

6.1.5 Synthesis of S-Allyl Cysteine

Fmoc-Cys(Trt)-O^tBu (379)



*Method A.*²⁷⁵

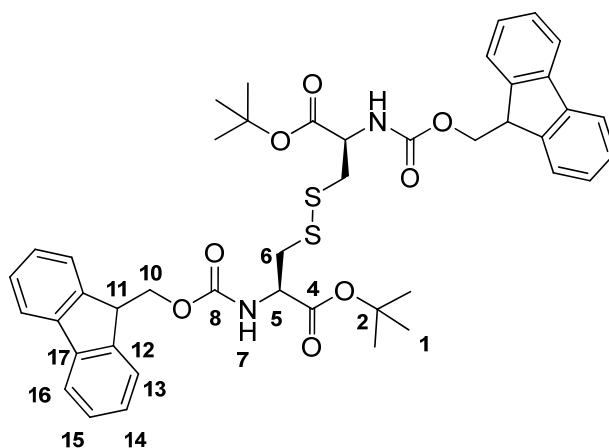
Fmoc-Cys(Trt)-OH (2.00 g, 3.41 mmol) was suspended in anhydrous CH₂Cl₂ (4 mL). *Tert*-Butyl-2,2,2-trichloroacetimidate (TBTA) (1.36 mL, 6.83 mmol), dissolved in anhydrous cyclohexane (8 mL) was added. BF₃.Et₂O (80 μ L) was added and the reaction was stirred at RT, under Ar for 4 hours. The resulting yellow solution was neutralised *via* the addition of Et₃N, upon which the appearance changed to milky white. Concentration *in vacuo* was followed

directly by flash chromatography (pet. ether; 0% to 10% EtOAc) which afforded the title compound as a sticky colourless solid (428 mg, 0.667 mmol, 20%) with NMR consistent with literature values.²⁷⁵ R_f = 0.76 (2:1 pet. ether/EtOAc); $[\alpha]_D^{20}$ +15.7 (c 0.10, MeOH); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3332 (N-H stretch), 3062, 2978 (C-H stretches), 1711 (C=O stretch), 1493 (N-H bend), 1447; ^1H NMR (500 MHz, CDCl_3): δ_{H} = 1.44 (s, 9H, **1-H**), 2.53-2.64 (m, 2H, **6-H**), 4.22-4.26 (m, 1H, **11-H**), 4.27-4.30 (m, 1H, **5-H**), 4.35 (d, J = 7.4 Hz, 2H, **10-H**), 5.33 (d, J = 8.3 Hz, 1H, **7-H**), 7.20-7.30 (m, 11H, **Trt-H/Fmoc Ar-H**), 7.40-7.42 (m, 8H, **Trt-H/Fmoc Ar-H**), 7.61 (d, J = 7.5 Hz, 2H, **13-H**), 7.76 (d, J = 7.5 Hz, 2H, **14-H**); ^{13}C NMR (125 MHz, CDCl_3): δ_{C} = 28.0 (**C-1**), 47.2, 120.0, 125.3, 126.9, 127.2, 127.4, 127.8, 128.0, 128.1, 129.6, 141.4, 143.9, 144.4; HRMS m/z (ES^+): Found 664.2469 $[\text{M}+\text{Na}]^+$; $\text{C}_{41}\text{H}_{39}\text{NO}_4\text{NaS}$ requires 664.2498;

Method B:

Fmoc-Cys(Trt)-OH (2.00 g, 3.41 mmol) was dissolved in $^t\text{BuOH}$ (40 mL). SiCl_4 (344 μL , 3.00 mmol) was added and the reaction was stirred under reflux for 48 h. Concentration *in vacuo* was followed directly by flash chromatography (applied in pet. ether; eluted 0% to 5% to 10% to 20% EtOAc) which afforded the title compound as a sticky colourless solid (49.6 mg, 0.077 mmol, 2%).

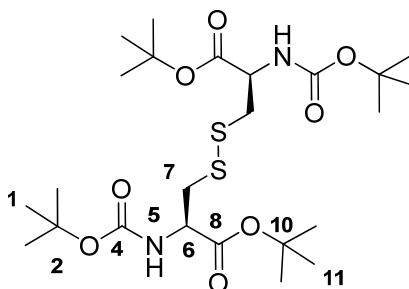
(Fmoc-Cys-O t Bu)₂ (**381**)²⁷⁸



Tert-butyl 3,3'-disulfanediyldis(2-aminopropanoate) (2.99 g, 8.49 mmol) and Fmoc-Cl (4.39 g, 17.0 mmol) were dissolved in anhydrous THF (100 mL) and the mixture was cooled on ice. *N*-methylmorpholine (1.87 mL, 17.0 mmol) was then added dropwise and the reaction was stirred

on ice for 3 h. The mixture was then diluted with EtOAc (100 mL), washed with KHSO₄ (5% aq w/v, 4 x 50 mL), H₂O (50 mL) and brine (50 mL); dried (MgSO₄), filtered and concentrated *in vacuo*. Flash chromatography (applied in hexane; eluted 30:1 to 15:1 to 2:1 to 1:1 hexane/Et₂O) afforded the title compound as a white solid (2.95 g, 3.70 mmol, 44%) with NMR consistent with literature values.²⁷⁸ Mpt: 150-155 °C [Lit.²⁷⁸ 146-148 °C]; *R_f* = 0.24 (5:1 pet. ether/EtOAc); [α]_D²⁰ -34.0 (c 0.10, MeOH); IR (ν_{max} /cm⁻¹, thin film): 3327 (N-H stretch), 2977 (C-H stretch), 1709 (C=O stretch), 1149 (COOR stretch); ¹H NMR (600 MHz, CDCl₃): δ_{H} = 1.48 (s, 18H, **1-H**), 3.16-3.26 (m, 4H, **6-H**), 4.06-4.23 (m, 2H, **11-H**), 4.33-4.37 (m, 4H, **10-H**), 4.56-4.59 (m, 2H, **5-H**), 5.74-5.76 (m, 2H, **7-H**), 7.27-7.34 (m, 4H, **12-H**), 7.36-7.42 (m, 4H, **15-H**), 7.57-7.63 (m, 4H, **13-H**), 7.73-7.79 (m, 4H, **16-H**); ¹³C NMR (150 MHz, CDCl₃): δ_{C} = 28.1 (**C-1**), 42.0 (**C-6**), 47.2 (**C-11**), 54.2 (**C-5**), 67.3 (**C-10**), 83.3 (**C-2**), 120.1 (**C-16**), 125.3 (**C-13**), 127.2 (**C-14**), 127.8 (**C-15**), 141.4 (**C-17**), 143.9 (**C-12**), 155.8 (**C-8**), 169.4 (**C-4**); LRMS *m/z* (ES⁺): 819 [M+Na]⁺; HRMS *m/z* (ES⁺): Found 819.2730 [M+Na]⁺; C₄₄H₄₈N₂O₈NaS₂ requires 819.2750.

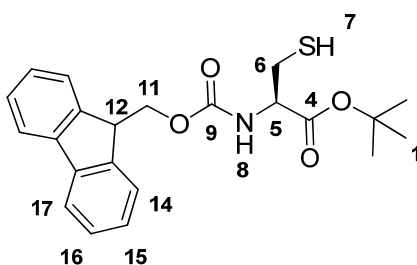
(Boc-Cys-O^tBu)₂ (**382**)



Tert-butyl 3,3'-disulfanediylbis(2-aminopropanoate) (2.30 g, 7.66 mmol) was dissolved in anhydrous CH₂Cl₂ (100 mL) and stirred under Ar. Et₃N (2.46 mL, 17.6 mmol) was added and mixture was stirred for 10 min before the portionwise addition of Boc₂O (3.68 g, 16.9 mmol). The reaction was stirred at RT for 30 h before washing with NaHCO₃ (sat. aq. 3 x 60 mL) and brine (60 mL), drying (MgSO₄), filtering and removal of solvent *in vacuo*. Flash chromatography (applied in pet. ether; eluted 30:1 to 20:1 to 9:1 pet. ether/EtOAc) afforded the title compound as a pale yellow oil (2.03 g, 3.68 mmol, 48%) with NMR consistent with literature values.²⁷⁹ *R_f* = 0.36 (9:1 pet. ether/EtOAc); [α]_D²⁰ -40.0 (c 0.07, MeOH); IR (ν_{max} /cm⁻¹, thin film): 3362 (N-H stretch), 2978, 2933 (C-H stretches), 1709 (C=O stretch), 1366, 1148 (COOR stretch); ¹H NMR (600 MHz, CDCl₃): δ_{H} = 1.44 (s, 18-H, **1-H**), 1.47 (s, 18H, **11-H**), 3.11-3.41 (m, 4H, **7-H**), 4.44-

4.52 (m, 2H, **6-H**), 5.35 (bd, $J = 6.2$ Hz, 2H, **5-H**); ^{13}C NMR (150 MHz, CDCl_3): $\delta_{\text{C}} = 28.1$ (**C-11**), 28.4 (**C-1**), 42.2 (**C-6**), 53.8 (**C-5**), 80.1 (**C-2**), 82.8 (**C-10**), 155.2 (**C-4**), 169.8 (**C-8**); LRMS m/z (ES^+): 575 $[\text{M}+\text{Na}]^+$; HRMS m/z (ES^+): Found 575.2420 $[\text{M}+\text{Na}]^+$; $\text{C}_{24}\text{H}_{44}\text{N}_2\text{O}_8\text{NaS}_2$ requires 575.2437.

Fmoc-(Cys)-O^tBu (**383**)



Method A:²⁷⁷

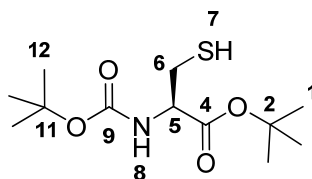
379 (312 mg, 0.486 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL). TFA (0.2 mL) added and a colour change was observed: colourless to yellow to orange to brown. TES (0.1 mL) was added and the colour change was from brown to yellow to colourless. The mixture was stirred at RT for 30 min, before evaporation (using MeOH to azeotrope TFA, care must be taken to keep dilute to avoid deprotection of *tert*-butyl protecting group). Flash chromatography (applied in pet. ether; eluted 5% to 10% to 20% EtOAc) afforded the title compound as a colourless oil (63.7 mg, 0.159 mmol, 33%).

Method B:²⁷⁸

381 (2.80 g, 3.51 mmol), was dissolved in anhydrous THF (20 mL). Tributylphosphine (0.876 mL, 3.51 mmol) was added followed by H_2O (0.320 mL) after 2 min. The reaction was stirred at RT for 24 h before the solvent was removed *in vacuo*, taken up in EtOAc (200 mL) and washed with citric acid (5% aq w/v, 150 mL) and brine (100 mL). Drying (MgSO_4), filtering and removal of solvent gave crude light yellow oil. Flash chromatography (applied in pet. ether; eluted 20:1 to 9:1 to 5:1 pet. ether/EtOAc) afforded the title compound as a viscous colourless oil (2.33 g, 5.83 mmol, 83%) with NMR values consistent with literature.²⁷⁸ $R_f = 0.52$ (5:1 pet.

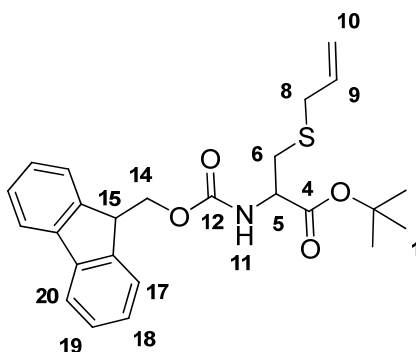
ether/EtOAc); $[\alpha]_D^{20}$ -19.9 (c 0.06, MeOH); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3326 (N-H stretch), 2978 (C-H stretch), 2575 (S-H stretch), 1717 (C=O stretch), 1510 (N-H bend), 1152 (COOR stretch); ^1H NMR (600 MHz, CDCl_3): δ_{H} = 1.34 (t, J = 8.9 Hz, 1H, **7-H**), 1.50 (s, 9H, **1-H**), 2.97-3.02 (m, 2H, **6-H**), 4.23 (t J = 7.0 Hz, 1H, **12-H**), 4.38-4.44 (m, 2H, **11-H**), 4.53-4.56 (m, 1H, **5-H**), 5.69 (d, J = 7.1 Hz, 1H, **8-H**), 7.32-7.34 (m, 2H, **15-H**), 7.41 (t, J = 7.5 Hz, 2H, **16-H**), 7.61 (d, J = 7.5 Hz, 2H, **14-H**), 7.77 (d, J = 7.5 Hz, 2H, **17-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = 27.5 (**C-6**), 28.1 (**C-1**), 47.3 (**C-12**), 55.5 (**C-5**), 67.2 (**C-11**), 83.2 (**C-2**), 120.1 (**C-17**), 125.2 (**C-14**), 127.2 (**C-15**), 127.9 (**C-16**), 141.4 (**C-13**), 143.8 (**C-18**), 155.7 (**C-9**), 169.1 (**C-4**); LRMS m/z (ES^+): 422 $[\text{M}+\text{Na}]^+$, 366 $[\text{M}-\text{SH}]^+$, 292 $[\text{M}-\text{SH}-\text{O}^t\text{Bu}]^+$; HRMS m/z (ES^+): Found 422.1392 $[\text{M}+\text{Na}]^+$; $\text{C}_{22}\text{H}_{25}\text{NO}_4\text{NaS}$ requires 422.1402.

Boc-Cys-O^tBu (**384**)²⁷⁹



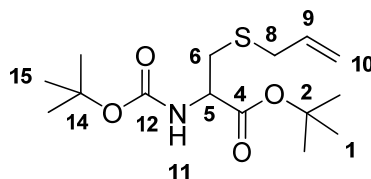
382 (2.03 g, 3.70 mmol) was dissolved in EtOH (100 mL). The pH of the solution was adjusted to 8 *via* the addition of NH_4OH and was saturated with Ar. Dithiothreitol, DTT (1.19 mL, 7.73 mmol) was added and the mixture was stirred at RT for 4 h. The pH of the resulting yellow solution was adjusted to 1 *via* the addition of conc. HCl and the solvent was removed *in vacuo*. The white solid was taken up in Et_2O (200 mL) and washed with 0.25 M HCl (2 x 75 mL) and brine (75 mL); dried (MgSO_4), filtered and concentrated *in vacuo*. Flash chromatography (applied in pet. ether; eluted 5:1 pet. ether/EtOAc) afforded the title compound as colourless oil (1.68 g, 6.05 mmol, 82%) with NMR consistent with literature values.²⁷⁹ R_f = 0.57 (5:1 pet. ether/EtOAc); $[\alpha]_D^{20}$ +15.7 (c 0.08, MeOH); IR ($\nu_{\max}/\text{cm}^{-1}$, thin film): 3355 (N-H stretch), 2978, 2933 (C-H stretches), 2571 (S-H stretch), 1711 (C=O stretch), 1495 (N-H bend), 1366, 1148 (COOR stretch); ^1H NMR (600 MHz, CDCl_3): δ_{H} = 1.36 (t, J = 8.8 Hz, 1H, **7-H**), 1.45 (s, 9H, **12-H**), 1.48 (s, 9H, **1-H**), 2.95 (dd, J = 8.8, 3.8 Hz, 2H, **6-H**), 4.47 (t, J = 3.8 Hz, 1H, **5-H**), 5.40 (bd, J = 6.4 Hz, 1H, **8-H**); ^{13}C NMR (150 MHz, CDCl_3): δ_{C} = 27.7 (**C-6**), 28.1 (**C-1**), 28.4 (**C-12**), 55.1 (**C-5**), 80.1 (**C-11**), 82.9 (**C-2**), 155.3 (**C-9**), 169.4 (**C-4**); LRMS m/z (ES^-): 276 $[\text{M}-\text{H}]^-$, 255; HRMS m/z (ES^-): Found 276.1256 $[\text{M}-\text{H}]^-$; $\text{C}_{12}\text{H}_{22}\text{NO}_4\text{S}$ requires 276.1270.

Fmoc-Cys(allyl)-O^tBu (385)



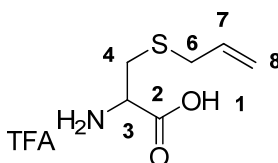
383 (1.80 g, 4.51 mmol) dissolved in anhydrous DMF (10 mL). K_2CO_3 (1.56 g, 11.3 mmol) added and the mixture was cooled on ice. Allyl bromide (1.95 mL, 22.6 mmol) was added and the reaction was stirred at RT for 16 h. The solvent was removed *in vacuo*, the resulting oil taken up in Et₂O (100 mL) and washed H₂O (3 x 60 mL) and brine (60 mL). The organic layer was then dried (MgSO₄) filtered and concentrated to give a crude yellow oil. Flash chromatography (applied in pet. ether; eluted 0% to 10 % to 25 % EtOAc) afforded the title compound as a pale yellow oil (1.63 g, 3.70 mmol, 82%). R_f = 0.45 (2:1 pet. ether/EtOAc); $[\alpha]_D^{20}$ +5.3 (c 0.08, MeOH); IR (ν_{max}/cm^{-1} , thin film): 3332, 3066, 2977 (C-H and N-H stretches), 1721 (C=O stretch), 1506 (N-H bend and C=C stretch), 1153 (COOR stretch); ¹H NMR (600 MHz, CDCl₃): δ_H = 1.49 (s, 9H, **1-H**), 2.86 (dd, J = 13.9, 5.6 Hz, 1H, **6-H**), 2.96 (dd, J = 13.9, 4.6 Hz, 1H, **6-H**), 3.15 (d, J = 7.0, 2H, **8-H**), 4.24 (t, J = 7.1 Hz, 1H, **15-H**), 4.39 (d, J = 7.2 Hz, 2H, **14-H**), 4.47-4.49 (m, 1H, **5-H**), 5.09-5.12 (m, 2H, **10-H**), 5.58 (d, J = 7.7 Hz, 1H, **11-H**), 5.72-5.76 (m, 1H, **9-H**), 7.32 (t, J = 7.4 Hz, 2H, **18-H**), 7.40 (t, J = 7.4 Hz, 2H, **19-H**), 7.61 (dd, J = 7.4, 3.1 Hz, 2H, **17-H**), 7.77 (d, J = 7.4 Hz, 2H, **20-H**); ¹³C NMR (150 MHz, CDCl₃): δ_C = 28.1 (**C-1**), 33.3 (**C-6**), 35.5 (**C-8**), 47.2 (**C-15**), 54.2 (**C-5**), 67.2 (**C-14**), 82.9 (**C-2**), 117.9 (**C-10**), 120.1 (**C-20**), 125.3 (**C-17**), 127.2 (**C-18**), 127.8 (**C-19**), 133.9 (**C-9**), 141.4 (**C-21**), 143.9 (**C-16**), 155.8 (**C-12**), 169.9 (**C-4**); LRMS m/z (ES⁺): 462 [M+H]⁺, 478 [M+K]⁺; HRMS m/z (ES⁺): Found 462.1698 [M+Na]⁺; C₂₅H₂₉NO₄NaS requires 462.1715.

Boc-Cys-(allyl)-O^tBu (386)



384 (1.67 g, 6.03 mmol) was dissolved in anhydrous DMF (10 mL). K_2CO_3 (2.08 g, 15.1 mmol) was added to the solution and cooled on ice. Allyl bromide (3.20 mL, 30.1 mmol) was added and the reaction was stirred at RT for 16 h. Diluting with Et_2O (100 mL) was followed by washing with H_2O (4 x 60 mL) and brine (60 mL). Drying ($MgSO_4$), filtering and concentrating *in vacuo* afforded the title compound as a light yellow oil (1.817 g, 5.73 mmol, 95%). No further purification was required. R_f = 0.66 (2:1 pet. ether/EtOAc); $[\alpha]_D^{20}$ -18.4 (c 0.13, MeOH); IR (ν_{max}/cm^{-1} , thin film): 3367 (C-H stretch), 2978, 2932 (N-H stretch), 1711 (C=O stretch), 1496 (C=C stretch), 1366, 1149; 1H NMR (600 MHz, $CDCl_3$): δ_H = 1.45 (s, 9H, **15-H**), 1.47 (s, 9H, **1-H**), 2.81 (dd, J = 13.8, 5.5 Hz, 1H, **6-H**), 2.91 (dd, J = 13.8, 4.6 Hz, 1H, **6-H**), 3.13-3.17 (m, 2H, **8-H**), 4.38-4.41 (m, 1H, **5-H**), 5.11-5.14 (m, 2H, **10-H**), 5.29 (bd, J = 7.5 Hz, 1H, **11-H**), 6.73-5.78 (m, 1H, **9-H**); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C = 28.1 (**C-1**), 28.4 (**C-15**), 33.2 (**C-6**), 35.4 (**C-8**), 53.9 (**C-5**), 80.0 (**C-14**), 82.6 (**C-2**), 117.9 (**C-10**), 133.9 (**C-9**), 155.3 (**C-12**), 170.2 (**C-4**); LRMS m/z (ES^+): 340 $[M+Na]^+$, 325, 269, 242; HRMS m/z (ES^+): Found 340.1557 $[M+Na]^+$; $C_{15}H_{27}NO_4NaS$ requires 340.1559.

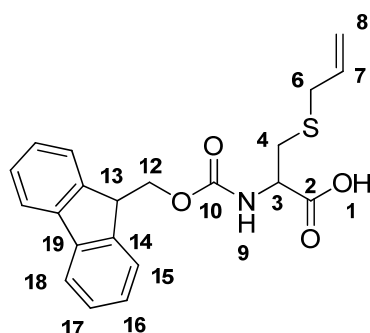
H-Cys(allyl)-OH.TFA (387)



386 (1.50 g, 4.76 mmol) was dissolved in CH_2Cl_2 (25 mL) and TFA (25 mL) was added. The reaction mixture was stirred at RT for 18 h. The solvent was removed *in vacuo via* trituration with toluene (the use of MeOH for trituration must be avoided or otherwise the methyl ester is formed) to afford a yellow solid (1.25 g, 4.56 mmol, 96%) with NMR consistent with literature values.³²⁰ Mpt: 150 °C; R_f = Baseline (2:1 pet. ether/EtOAc); $[\alpha]_D^{20}$ -5.4 (c 0.11, MeOH); IR (ν_{max}/cm^{-1} , thin film): 2914 (O-H stretch), 1741 (C=O stretch), 1656 (N-H bend), 1193; 1H NMR

(600 MHz, CD₃OD): δ_{H} = 2.92 (dd, J = 14.9, 8.2 Hz, 1H, **4-H**), 3.12 (dd, J = 14.9, 4.1 Hz, 1H, **4-H**), 3.24 (d, J = 7.2 Hz, 2H, **6-H**), 4.12 (dd, J = 8.2, 4.1 Hz, 1H, **3-H**), 5.17-5.23 (m, 2H, **8-H**), 5.78-5.85 (m, 1H, **7-H**); ^{13}C NMR (160 MHz, CD₃OD): δ_{C} = 31.5 (**C-4**), 35.3 (**C-6**), 53.5 (**C-3**), 118.7 (**C-8**), 134.7 (**C-7**), 170.7 (**C-2**); LRMS m/z (Cl^+): 162 [$\text{M}+\text{H}$]⁺, 145 [$\text{M}-\text{OH}$]⁺; HRMS m/z (Cl^+): Found 162.0592 [$\text{M}+\text{H}$]⁺; C₆H₁₂NO₂S requires 162.0589; Anal. Calcd. for C₈H₁₂F₃NO₄S: C, 34.91; H, 4.39; N, 5.09. Found C, 34.70; H, 4.26; N, 4.86%.

Fmoc-Cys(allyl)-OH (388)



Method A:

385 (1.63 g, 3.70 mmol) was dissolved in CH₂Cl₂ (25 mL) and followed by the addition of TFA (25 mL). The reaction mixture was stirred at RT for 16 h, followed by the removal of solvent *in vacuo* via trituration with toluene (the use of MeOH for trituration must be avoided or otherwise the methyl ester is formed). Flash chromatography (applied in CH₂Cl₂; eluted 0% to 5% MeOH) afforded the title compound as an off white solid (1.34g, 3.53 mmol, 95%). Mpt: 131-133 °C; R_f = 0.63 (CH₂Cl₂/5% MeOH); $[\alpha]_{\text{D}}^{20}$ -15.2 (c 0.40, MeOH); IR ($\nu_{\text{max}}/\text{cm}^{-1}$, thin film): 3067 (O-H stretch), 2950 (N-H stretch), 1709 (C=O stretch), 1520, 1450 (N-H bend and C=C stretch), 1228; ^1H NMR (600 MHz, CDCl₃): δ_{H} = 2.93 (dd, J = 14.1, 5.5 Hz, 1H, **4-H**), 2.99 (dd, J = 14.1, 4.3 Hz, 1H, **4-H**), 3.13 (d, J = 6.9 Hz, 2H, **6-H**), 4.23 (t, J = 6.9 Hz, 1H, **13-H**), 4.41 (d, J = 6.9 Hz, 2H, **12-H**), 4.61 (bs, 1H, **3-H**), 5.09-5.12 (m, 2H, **8-H**), 5.65 (bs, 1H, **9-H**), 5.70-5.77 (m, 1H, **7-H**), 7.31 (t, J = 7.3 Hz, 2H, **16-H**), 7.39 (t, J = 7.3 Hz, 2H, **17-H**), 7.53-7.61 (m, 2H, **15-H**), 7.76 (t, J = 7.3 Hz, 2H, **18-H**); ^{13}C NMR (150 MHz, CDCl₃): δ_{C} = 32.6 (**C-4**), 35.4 (**C-6**), 47.2 (**C-13**), 53.5 (**C-3**), 67.5 (**C-12**), 118.3 (**C-8**), 120.1 (**C-18**), 125.2 (**C-15**), 127.2 (**C-16**), 127.9 (**C-17**), 133.6 (**C-7**), 141.4 (**C-19**), 143.7 (**C-14**), 156.1 (**C-10**), 175.7 (**C-2**); LRMS m/z (ES^+): 406

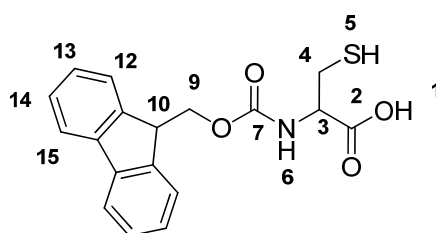
$[M+Na]^+$, 242 $[M-\text{Fluorene}+Na]^+$; HRMS m/z (ES^+): Found 406.1071 $[M+Na]^+$; $C_{21}H_{21}NO_4NaS$ requires 406.1089.

Method B:

387 (1.25 g, 4.53 mmol), was dissolved in acetone/ H_2O (1:1, 150 mL). $NaHCO_3$ (0.760 g, 9.05 mmol) was added followed by Fmoc-OSu (1.60 g, 4.75 mmol). The reaction was stirred at RT for 18 h. The reaction was acidified to pH 3 *via* the addition of HCl and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (applied in CH_2Cl_2 ; eluted 2% to 5% to 10% MeOH) afforded the title compound as a sticky colourless oil (680 mg, 1.77 mmol, 39%).

6.1.6 Trial Peptide-Small Molecule Conjugation

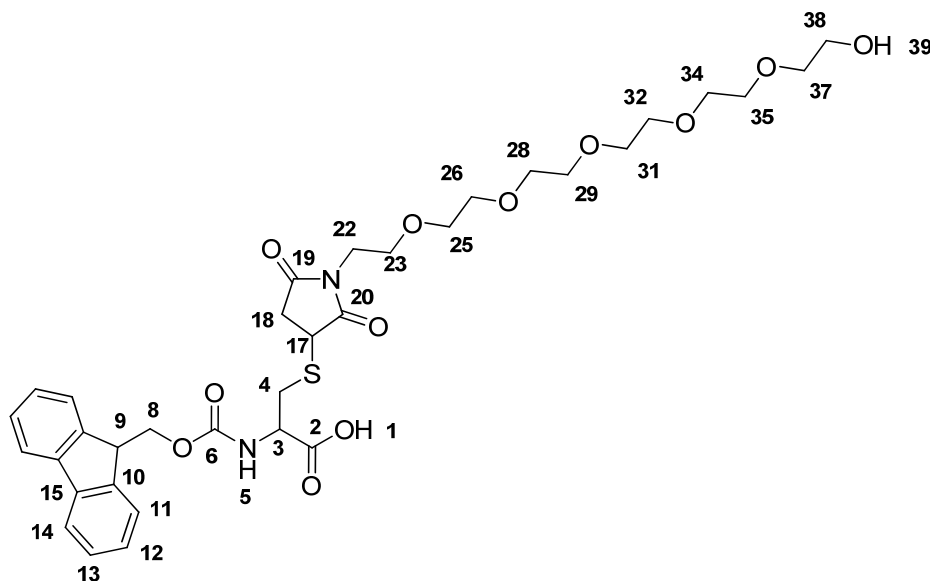
Fmoc-Cys-OH (398)



Fmoc-Cys(Trt)-OH (1.00 g, 1.70 mmol) was suspended in CH_2Cl_2 (20 mL) and TFA (2.5 mL) and TIPS (1 mL) were added. The reaction was stirred at RT for 16 h before the solvent was removed *in vacuo* *via* trituration with toluene (the use of MeOH for trituration must be avoided or otherwise the methyl ester is formed). Flash chromatography (applied in CH_2Cl_2 ; eluted 1% to 2% to 5% MeOH) afforded the title compound as an off white solid (371 mg, 1.08 mmol, 64%) with NMR consistent with literature values.³²¹ Mpt: 79-81 °C [Lit.³²¹ 96-98 °C]; R_f = 0.4 (10% MeOH/ CH_2Cl_2); $[\alpha]_D^{20}$ -20.9 (c 0.11, MeOH); IR (ν_{max}/cm^{-1} , thin film): 3310 (C-H and O-H stretches), 1686 (C=O stretch), 1528 (N-H bend), 1256 (COOR stretch), 1045; 1H NMR (600 MHz, CD_3OD): δ_H = 2.87 (dd, J = 13.9, 7.2 Hz, 1H, **4-H**), 2.96 (dd, J = 13.9, 4.1 Hz, 1H, **4-H**), 4.25 (t, J = 6.9 Hz, 1H, **10-H**), 4.35-4.38 (m, 3H, **3,9-H**), 7.31 (t, J = 7.4 Hz, 2H, **13-H**), 7.39 (t, J = 7.4 Hz, 2H, **14-H**), 7.62-7.70 (m, 2H, **12-H**), 7.80 (d, J = 7.4 Hz, 2H, **15-H**); ^{13}C NMR (150 MHz, CD_3OD): δ_C = 27.0 (C-4), 48.4 (C-10), 57.8 (C-3), 68.0 (C-9), 120.9 (C-15), 126.3 (C-12), 128.2 (C-13), 128.8 (C-14), 142.6 (C-16), 145.2 (C-11), 158.5 (C-7), 173.6 (C-2); LRMS m/z

(Cl⁺): 344 [M+H]⁺, 179 [M-Fluorene]⁺; HRMS m/z (Cl⁺): Found 344.0958 [M+H]⁺; C₁₈H₁₈NO₄S requires 344.0957.

2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(1-(17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)-2,5-dioxopyrrolidin-3-ylthio)propanoic acid (399)



Method A:

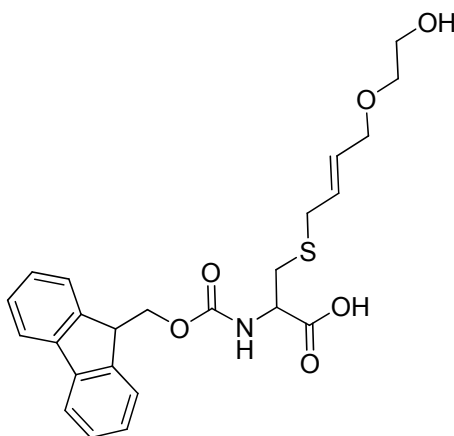
398 (73.9 mg, 0.215 mmol) was suspended in phosphate buffer (0.5 mL, pH 6.5, 50 mM), **355** (39 mg, 0.108 mmol) in DMF (0.5 mL) was added and the reaction was stirred at RT for 2½ h. After this time, the solvent was removed and purification *via* flash chromatography (applied in CH₂Cl₂; eluted 1% to 2% to 5% to 10% to 20% MeOH) afforded the title compound as a colourless oil (21.9 mg, 0.031 mmol, 29%).

Method B:

398 (76.0 mg, 0.222 mmol) was dissolved in DMF (0.5 mL), **355** (40.1 mg, 0.111 mmol) in DMF (0.5 mL) was added the mixture was stirred at RT for 2½ h. Removal of the solvent, followed by purification *via* flash chromatography (applied in CH₂Cl₂; eluted 0.5% to 1% to 2% to 5% to 10% to 20% MeOH) afforded the title compound as a colourless oil (62.9 mg, 0.089 mmol, 81%). [α]_D²⁰ -0.9 (c 0.11, MeOH); IR (ν_{max}/cm⁻¹, thin film): 3391 (O-H and N-H stretches), 2876 (C-H stretch), 1701 (C=O stretch), 1598 (N-H bend), 1399, 1104 (C-O stretch); ¹H NMR (600 MHz,

(CD₃)₂SO): δ_{H} = 3.05-3.14 (m, 2H, **4-H**), 3.16 (d, J = 3.5 Hz, 2H, **38-H**), 3.40 (t, J = 5.6 Hz, 2H, **23-H**), 3.46-3.49 (m, 18H, **25,26,28,29,31,32,34,35,17-H**), 3.53 (t, J = 5.6 Hz, 2H, **22-H**), 3.86 (bd, J = 11.0 Hz, 2H, **18-H**), 3.94-3.99 (m, 1H, **17-H**), 4.13 (bd, J = 4.7 Hz, 1H, **39-H**), 4.20-4.24 (m, 1H, **9-H**), 4.27-4.31 (m, 3H, **3,8-H**), 4.67 (bs, 1H, **1-H**), 6.80 (bs, 1H, **5-H**), 7.31-7.34 (m, 2H, **12-H**), 7.39-7.42 (m, 2H, **13-H**), 7.66-7.71 (m, 2H, **11-H**), 7.89 (d, J = 7.6 Hz, **14-H**); ¹³C NMR (150 MHz, (CD₃)₂SO): δ_{C} = 33.8 (**C-4**), 37.8 (**C-22**), 40.1 (**C-17**), 46.7 (**C-9**), 48.6 (**C-38**), 55.1 (**C-18**), 60.2 (**C-35**), 65.4 (**C-3**), 65.5 (**C-8**), 66.2 (**C-37**), 69.4-69.8 (**C-25,26,28,29,31,32,34**), 72.4 (**C-23**), 120.2 (**C-14**), 125.3 (**C-11**), 127.1 (**C-12**), 127.6 (**C-13**), 140.7 (**C-15**), 143.9 (**C-10**), 155.5 (**C-6**), 175.1 (**C-2**), 175.2 (**C-19**), 176.8 (**C-20**); LRMS m/z (ES⁺): 727 [M]⁺, 683; HRMS m/z (ES⁺): Found 727.2514; C₃₄H₄₄N₂O₁₂S requires 727.2513

(E)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(4-(2-hydroxyethoxy)but-2-enylthio)-propanoic acid (400)



To **388** (20.0 mg, 0.052 mmol) and 2-(allyloxy)ethanol (27.9 μ L, 0.261 mmol) was added ^tBuOH/H₂O (1:2; 1.5 mL), followed by a solution of Grubbs' 2nd generation catalyst (1.33 mg, 3 mol%) in ^tBuOH (500 μ L) and the reaction was stirred at 32 °C for 1 h. Further quantities of Grubbs' 2nd generation catalyst (1.33 mg, 3 mol%) in ^tBuOH (500 μ L), **388** (20 mg, 0.052 mmol), 2-(allyloxy)ethanol (27.9 μ L, 0.261 mmol) and H₂O (500 μ L) were added and the reaction was stirred at 32 °C for a further 2 h. LCMS showed 100% conversion to the desired product. No further purification was carried out.

6.2 Peptide Synthesis and PEGylated Imidazo[1,2-*a*]pyrazine Conjugation

Analytical HPLC traces and mass spectrometry spectra (LCMS and/or MALDI) for synthesised peptides and peptide conjugates are given in Appendix (Section 7.2)

6.2.1 General Methods

Amino acids and resins for peptide synthesis were purchased from Novobiochem, UK. HPLC grade solvents for peptide synthesis and HPLC purification were purchased from Sigma, UK and VWR.

All peptides were synthesized on a MultiSynTech Syro I automated system. Either pre-loaded Fmoc-Asp(O^tBu)-NovaSyn® TGT resin (0.200 mmol/g, 100 mg, 20.0 µmol) or pre-loaded Fmoc-Leu-NovaSyn® TGT resin (0.210 mmol/g, 100 mg, 21.0 µmol) were used in all cases. All resins were pre-swelled in DMF for at least 30 min prior to synthesis start.

Standard Fmoc solid phase peptide synthesis (SPPS) was employed. The total volume of all reagents in each step was 1.5 mL. All reagents were dissolved in HPLC grade DMF.

Fmoc Deprotection: To the reaction syringe containing *N*-terminal Fmoc-protected peptide was added piperidine in DMF (40% v/v, 1.5 mL). The mixture was agitated for 20 s every min for a total of 3 min. The reagents were removed by filtration under vacuum and the resin washed with DMF (4 x 1.5 mL). Piperidine in DMF solution (40% v/v, 0.75 mL) was added to the reaction syringe followed by DMF (0.75 mL) to make an overall 20% v/v solution of piperidine in DMF. This mixture was agitated for 20 s every min for a total of 10 min. The reagents were removed by filtration under vacuum and the resin washed with DMF (6 x 1.5 mL).

Amino Acid Coupling: To the reaction syringe was added Fmoc-protected amino acid (0.600 mL, 0.140 M, 4 eq.), HBTU (0.600 mL, 0.140 M, 4 eq.) and DIPEA (0.300 mL, 0.560 M, 8 eq.). The mixture was agitated for 20 s every 3 min for a total of 40 min. The reagents were removed by filtration under vacuum and the resin washed with DMF (4 x 1.5 mL).

Peptide Cleavage and Side Chain Deprotection: The resin was washed with CH₂Cl₂ (3 x 3 mL), MeOH (3 x 3 mL) and Et₂O (3 x 3 mL) and dried (desiccator) followed by adding TFA/EDT/H₂O/TIPS (88:5:4:2; 3 mL) to the reaction syringe. The syringe was then agitated for 3 h at RT. The cleavage cocktail was drained from the vessel under vacuum and Et₂O (~10-15 mL)

added to the filtrate. The resultant precipitate in solution was stored at -20 °C for 30 min prior to being spun at 4000 rpm for 10 min at 4 °C to produce a crude peptide pellet. The supernatant Et₂O was decanted off and the peptide washed a further three times with Et₂O. The crude peptide pellet was then re-dissolved in minimum water and freeze-dried for storage prior to purification.

Cysteine-Maleimide Conjugation (Solution): The purified peptide was taken up in DMF (approximately 2 mg/mL). An aliquot of a stock solution of **363** in DMF (2.87 mM) was added such that the volume added corresponded to 3 eq, and the reaction mixture stirred at RT for 3 h. The solvent was removed *in vacuo* and the crude material purified *via* reverse phase preparative HPLC.

Cross Metathesis (Solution): The purified peptide and **298** (5 eq) were taken up in H₂O/^tBuOH (2:1; 0.600 mL). An aliquot of a stock solution of Grubbs' 2nd generation catalyst in ^tBuOH (2.36 mM, 3 mol%) was added and the reaction mixture was stirred at 32 °C for 1 h. A further quantity of **298** (5 eq) and Grubb's II (3 mol%) were added and the reaction stirred for a further 2 h at 32 °C. The solvent was removed *in vacuo* and the crude material purified *via* reverse phase preparative HPLC.

Cysteine-Maleimide Conjugation (on Resin): The peptides were synthesised according to standard SPPS, with all side chain and the terminal Fmoc protecting groups left on. The peptide was also left bound to the resin and all subsequent reactions were carried out in the syringe. The S^tBu protecting group on R240C was selectively deprotected by: washing the resin-bound peptide with CH₂Cl₂ (3 x 3 mL); soaking in EtOH/CH₂Cl₂/H₂O (4:6:1; 3 mL); purging with Ar; adding nBu₃P (50.0 µL, 0.200 mmol, 10 eq); and agitating for 3 h at RT. The syringe was evacuated and washed with CH₂Cl₂ (2 x 3 mL), MeOH (2 x 3 mL), CH₂Cl₂ (2 x 3 mL) and DMF (2 x 3 mL). Maleimide conjugation to the free thiol in R240C was carried out by adding **363** (42.0 mg, 0.060 mmol, 3 eq) in DMF (3 mL) and agitating at RT for 16 h. The syringe was evacuated and washed with DMF (3 x 3 mL). The terminal Fmoc was deprotected, followed by cleavage of the peptide from the resin and deprotection of the side-chain protecting groups using conditions stated above.

Cross Metathesis (on Resin): The peptides were synthesised according to standard SPPS, with all side chain and the terminal Fmoc protecting groups left on. The peptide was also left bound to the resin and all subsequent reactions were carried out in the syringe. CHCl₃/H₂O (8:1; 540 µL) was added to the syringe, followed by **298** (21.7 mg, 0.040 mmol, 2 eq) in DMF (120 µL). An aliquot of a stock solution of Grubbs' 2nd generation catalyst in ^tBuOH (1 mM, 60.0 µL, 3 mol%)

was added giving CHCl₃/DMF/H₂O/^tBuOH (8:2:1:1; 720 μL). The mixture was agitated at 37 °C for 2 h. Further quantities of **298** (21.7 mg, 0.040 mmol, 2 eq) and Grubbs' 2nd generation catalyst (60.0 μL, 3 mol%) were added followed by maintaining solvent concentration by addition of CHCl₃/H₂O (8:1; 540 μL). The mixture was agitated at 37 °C for 16 h, followed by evacuating solvent from the syringe and washing DMF (3 x 3mL). The terminal Fmoc was deprotected, followed by cleavage of the peptide from the resin and deprotection of the side-chain protecting groups using conditions stated above.

“Click” Chemistry (on Resin): The peptides were synthesised according to standard SPPS, with all side chain and the terminal Fmoc protecting groups left on. The peptide was also left bound to the resin and all subsequent reactions were carried out in the syringe. **299** (43.3 mg, 0.080 mmol, 4 eq) in DMF (2 mL) was added to the reaction syringe followed by the addition of a suspension of CuI (76.0 mg, 20 eq) and sodium ascorbate (158 mg, 40 eq) in H₂O/^tBuOH (2:1; 750 μL) to give a total solvent composition of DMF/H₂O/^tBuOH (8:2:1). The mixture was agitated at RT for 16 h, followed by evacuating the solvent and washing with DMF (3 x 3 mL), MeOH (2 x 3 mL), CH₂Cl₂ (3 mL) and DMF (2 x 3 mL). The terminal Fmoc was deprotected, followed by cleavage of the peptide from the resin and deprotection of the side-chain protecting groups using conditions stated above.

General peptide purification: The peptides were analyzed and purified *via* reverse phase HPLC using a Varian ProStar system with a Model 210 solvent delivery module and a Model 320 UV detector. The preparative purification was performed using either a Discovery®BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 μm beads, flow rate of 10 mL/min), Discovery®BIO Wide Pore C18 (Varian; 25 cm x 21.2 mm, 10 μm beads, flow rate of 8 mL/min) or an Onyx Monolithic Semi-Prep C18 (Phenomenex®; 100 x 10 mm, 2μm macropore size, 13 nm mesopore size, flow rate 10 mL/min), loaded with 200-400 μL aliquots of a 10-20 mg/mL solution of peptide dissolved in 0.1% TFA containing H₂O. The mobile phase was a decreasing gradient of H₂O/0.1% TFA (A) in MeCN/0.1% TFA (B). Precise gradients are reported for each peptide.

The fractions containing the correct peak were pooled, the solvent removed under reduced pressure to approximately 2 mL and the solution freeze-dried.

The purified peptide was analyzed by analytical HPLC using an Discovery®BIO Wide Pore C18 (Varian; 25 cm x 4.6 mm, 10 μm beads, flow rate of 1 mL/min) or Onyx monolithic C18 column

(Phenomenex[®]; 100 x 3.0 mm, 2 µm macropore size, 13 nm mesopore size, flow rate 1.0 mL/min). Precise gradients reported for each peptide. The analysis of the chromatograms was conducted using Star Chromatography Workstation software Version 1.9.3.2.

ES-MS analysis was performed on a Waters Acquity Ultra Performance LC/MS machine. MALDI analysis was carried out using a Waters MALDI MICRO MX, Micromass Technologies.

6.2.1.1 HPLC Methods

6.2.1.1.1 Preparative HPLC

Method A: Discovery[®]BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Linear gradient: 2-98% B over 18 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method B: Discovery[®]BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Linear gradient: 20-30% B over 8 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method C: Discovery[®]BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Linear gradient: 20-98% B over 14 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method D: Discovery[®]BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Linear gradient: 25-35% B over 8 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method E: Discovery[®]BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Linear gradient: 25-40% B over 8 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method F: Discovery[®]BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Linear gradient: 25-45% B over 10 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method G: Discovery®BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Isocratic: 30% B over 20 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method H: Discovery®BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Linear gradient: 30-40% B over 8 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method I: Discovery®BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Linear gradient: 32-37% B over 8 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method J: Discovery®BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Linear gradient: 33-38% B over 15 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method K: Discovery®BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Isocratic: 35% B over 25 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method L: Discovery®BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Linear gradient: 35-45% B over 8 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method M: Discovery®BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Linear gradient: 40-50% B over 15 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method N: Discovery®BIO Wide Pore C18 (Varian; 100 x 21.2 mm, 5 µm beads) flow rate of 10 mL/min, and UV detection at 215 and 254 nm. Linear gradient: 38-42% B over 10 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method O: Discovery®BIO Wide Pore C18 (Varian; 25 cm x 21.2 mm, 10 µm beads) flow rate of 8 mL/min, and UV detection at 215 and 254 nm. Isocratic: 20% B over 15 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method P: Onyx Monolithic Semi-Prep C18 (Phenomenex[®]; 100 x 10 mm, 2µm macropore size, 13nm mesopore size) flow rate 10 mL/min; UV detection at 215 and 254 nm. Linear gradient: 25-50% B over 10 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method Q: Onyx Monolithic Semi-Prep C18 (Phenomenex[®]; 100 x 10 mm, 2µm macropore size, 13nm mesopore size) flow rate 10 mL/min; UV detection at 215 and 254 nm. Linear gradient: 30-35% B over 10 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method R: Onyx Monolithic Semi-Prep C18 (Phenomenex[®]; 100 x 10 mm, 2µm macropore size, 13nm mesopore size) flow rate 10 mL/min; UV detection at 215 and 254 nm. Linear gradient: 35-45% B over 10 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

6.2.1.1.2 Analytical HPLC

Method S: Discovery[®]BIO Wide Pore C18 (Varian; 25 cm x 4.6 mm, 10 µm beads), flow rate 1.0 mL/min, UV detection at 214 nm. Linear gradient: 5-95% B over 20 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method T: Discovery[®]BIO Wide Pore C18 (Varian; 25 cm x 4.6 mm, 10 µm beads), flow rate 1.0 mL/min, UV detection at 214 nm. Linear gradient: 5-95% B over 30 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method U: Onyx monolithic C18 column (Phenomenex[®]; 100 x 3.0 mm, 2µm macropore size, 13nm mesopore size), flow rate 1.0 mL/min, UV detection at 214 nm. Linear gradient: 5-95% B over 20 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA).

Method V: Discovery[®]BIO Wide Pore C18 (Varian; 25 cm x 4.6 mm, 10 µm beads), flow rate 1.0 mL/min, UV detection at 214 nm. Linear gradient: 5-95% B over 20 min (A = H₂O/0.1% TFA, B = MeCN/0.1% TFA). Preparative HPLC machine used with 2 ml injection loop.

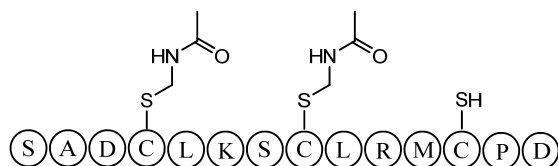
6.2.2 Synthesis of Non-conjugated Peptides

α F-loop WT (389)



Standard side-chain protecting groups were used. *Purification*: Method E; *Analysis*: Method S, R_T = 4.81 min; m/z (ES⁺): 798.20 [M+2H]²⁺, 532.14 [M+3H]³⁺, 399.46 [M+4H]⁴⁺; MALDI TOF⁺: 1595 (M⁺).

α F-loop R240C (390)



Standard side-chain protecting groups were used with the exception of Fmoc-Cys(Acm)-OH for residues C232 and C236. *Purification*: Method B; *Analysis*: Method S, R_T = 8.41 min; m/z (ES⁺): 842.30 [M+2H]²⁺, 561.84 [M+3H]³⁺; MALDI TOF⁺: 1684 (M⁺).

α F-loop R240SAC (391)



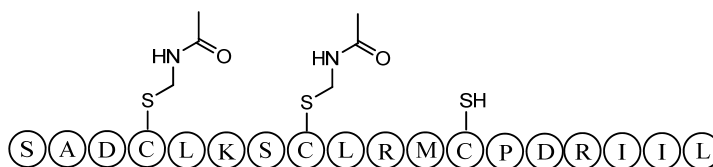
Standard side-chain protecting groups were used. S-Allyl Cysteine used as synthesised (388). *Purification*: Method D; *Analysis*: Method S, R_T = 9.39 min; m/z (ES⁺): 791.13 [M+2H]²⁺, 527.85 [M+3H]³⁺; MALDI TOF⁺: 1582 (M⁺).

α F- β 10 WT (392)



Standard side-chain protecting groups were used. *Purification*: Method G; *Analysis*: Method T, $R_T = 17.52$ min; m/z (ES⁺): 697.57 [M+3H]³⁺; MALDI TOF⁺: 2091 (M⁺).

α F- β 10 R240C (393)



Standard side-chain protecting groups were used with the exception of Fmoc-Cys(Acm)-OH for residues C232 and C236. *Purification*: Method D; *Analysis*: Method S, $R_T = 9.60$ min; m/z (ES⁺): 727.10 [M+3H]³⁺, 545.65 [M+4H]⁴⁺; MALDI TOF⁺: 2180 (M⁺).

α F- β 10 R240SAC (394)



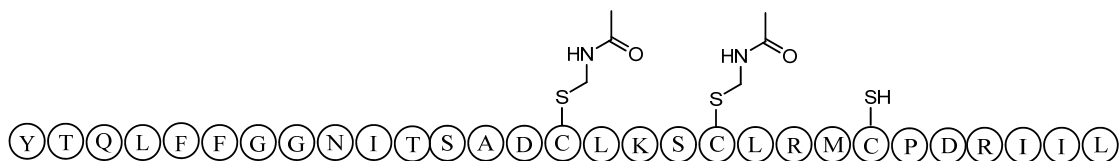
Standard side-chain protecting groups were used. S-Allyl Cysteine used as synthesised (**388**). *Purification*: Method K; *Analysis*: Method T, $R_T = 18.47$ min; m/z (ES⁺): 693 [M+3H]³⁺.

B9- α F- β 10 WT (395)



Standard side-chain protecting groups were used. *Purification*: Method I; *Analysis*: Method S, $R_T = 10.25$ min; m/z (ES⁺): 1111.75 [M+3H]³⁺, 833.93 [M+4H]⁴⁺, 667.34 [M+5H]⁵⁺; MALDI TOF⁺: 3332 (M⁺).

B9- α F- β 10 R240C (396)



Standard side-chain protecting groups were used with the exception of Fmoc-Cys(Acm)-OH for residues C232 and C236. *Purification*: Method J; *Analysis*: Method U, $R_T = 6.80$ min; m/z (ES⁺): 1141.25 [M+3H]³⁺, 856.13 [M+4H]⁴⁺; MALDI TOF⁺: 3421 (M⁺).

B9- α F- β 10 R240SAC (397)

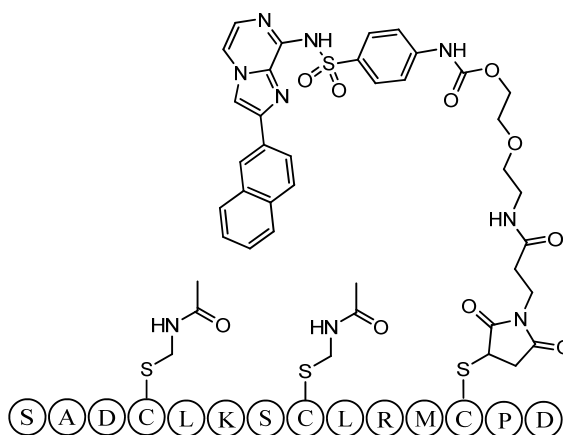


Standard side-chain protecting groups were used. S-Allyl Cysteine used as synthesised (**388**). *Purification*: Method N; *Analysis*: Method S, $R_T = 11.02$ min; m/z (ES⁺): 1107.40 [M+3H]³⁺, 830.52 [M+4H]⁴⁺; MALDI TOF⁺: 3319 (M⁺).

6.2.3 Solution-Based Conjugation

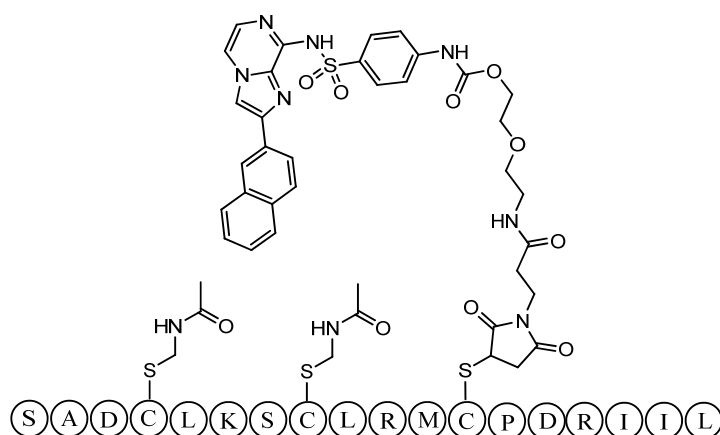
6.2.3.1 Solution-Based R240C-Maleimide Conjugation

α F-loop (401)



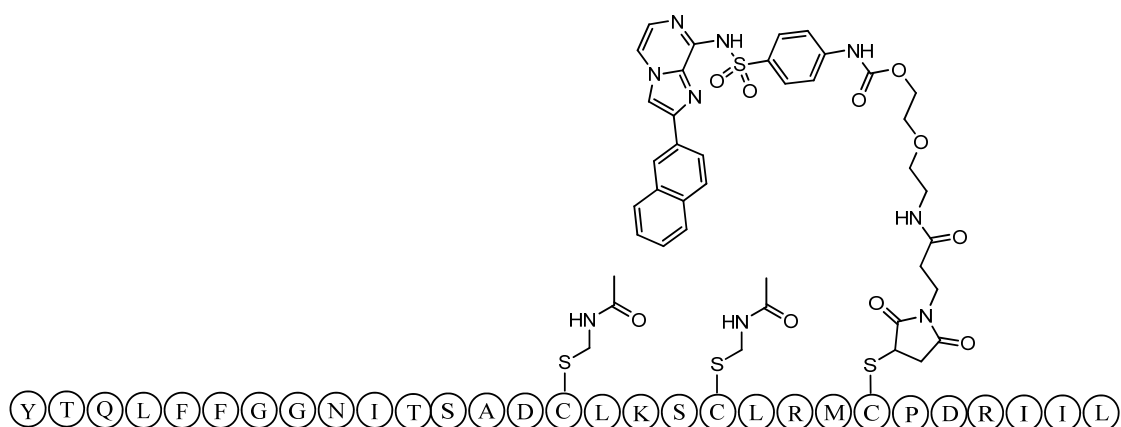
Conjugation between **390** and **363** was carried out in solution as previously described. *Purification*: Method A; *Analysis*: Method S, $R_T = 10.82$ min; m/z (ES⁺): 1192.18 [M+2H]²⁺, 794.35 [M+3H]³⁺, 605.63 [M+K+3H]⁴⁺; MALDI TOF⁺: 2382 (M⁺).

α F- β 10 (402)



Conjugation between **393** and **363** was carried out in solution as previously described. *Purification*: Method A; *Analysis*: Method S, $R_T = 11.28$ min; m/z (ES⁺): 959.86 [M+3H]³⁺, 720.13 [M+4H]⁴⁺.

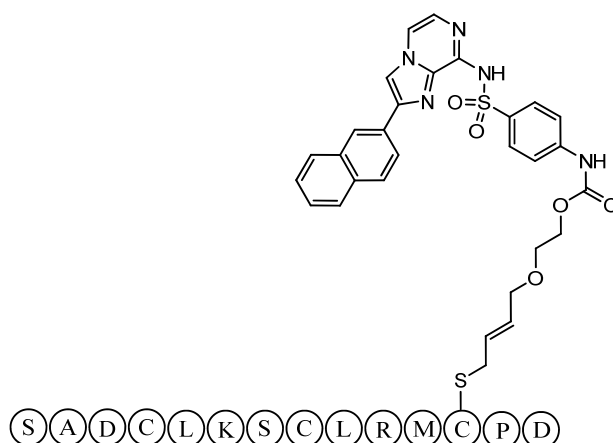
B9- α F- β 10 (403)



Conjugation between **396** and **363** was carried out in solution as previously described. *Purification*: Method A; *Analysis*: Method V, $R_T = 5.03$ min; m/z (ES⁺): 1374 [M+3H]³⁺, 1031.07 [M+4H]⁴⁺, 832.29 [M+K+4H]⁵⁺, 824.35 [M+5H]⁵⁺, 694.03 [M+K+5H]⁶⁺.

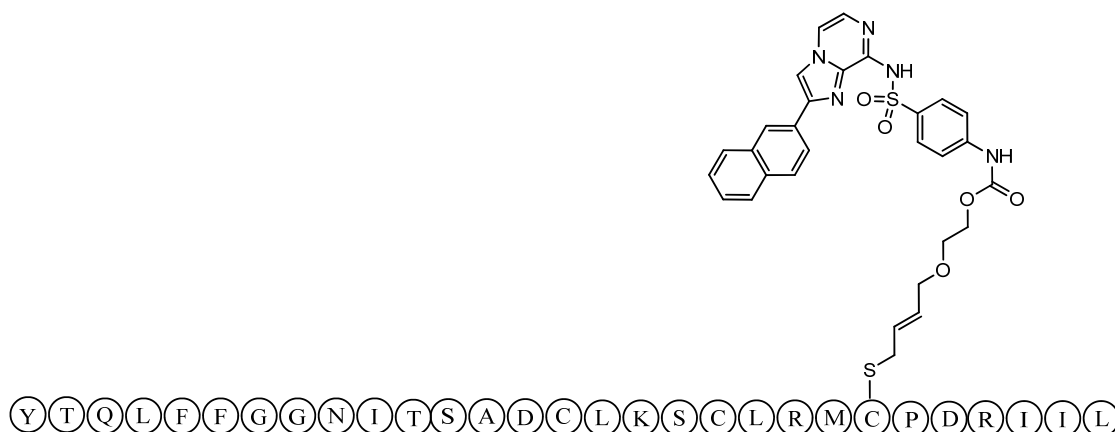
6.2.3.2 Solution-Based R240SAC-Allyl Cross Metathesis

Attempted cross metathesis with α F-loop (404)



Attempted solution phase cross metathesis between **391** and **298** was carried out as previously described. *Purification*: Method A.

Attempted cross metathesis with B9- α F- β 10 (405)

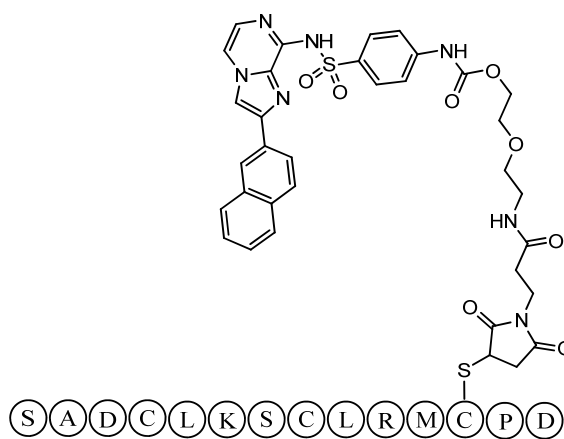


Attempted solution phase cross metathesis between **391** and **298** was carried out as previously described. *Purification*: Method A.

6.2.4 Resin-Bound Conjugation

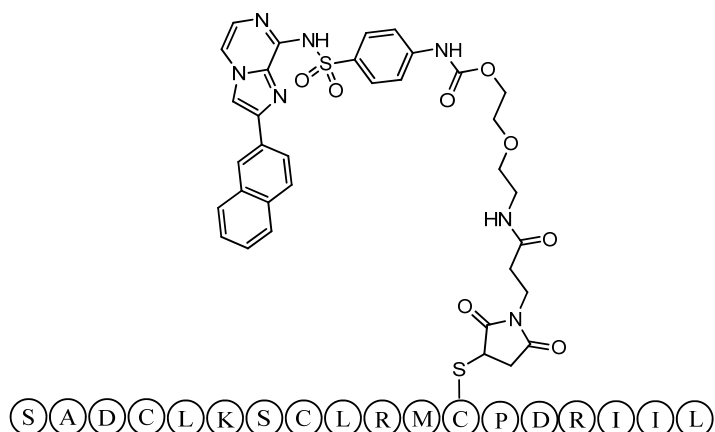
6.2.4.1 Resin-Based R240C-Maleimide Conjugation

α F-loop (406)



Standard side-chain protecting groups were used with the exception of Fmoc-Cys(S^tBu)-OH for residue C240. Conjugation on resin was carried out as described previously. *Purification*: Method H; *Analysis*: Method S, $R_T = 10.94$ min; m/z (ES⁺): 1120.62 [M+2H]²⁺, 747.24 [M+3H]³⁺; MALDI TOF⁺: 2240 (M⁺).

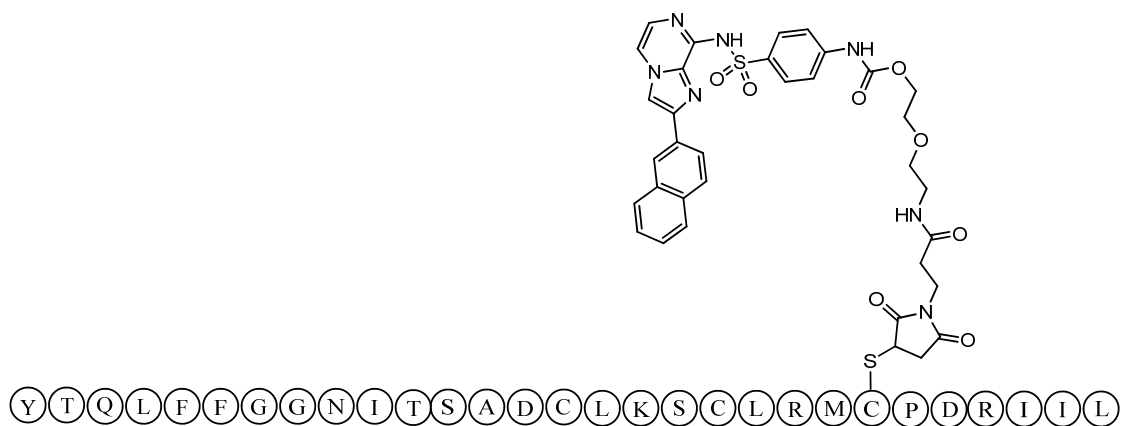
Attempted conjugation with α F- β 10 (407)



Standard side-chain protecting groups were used with the exception of Fmoc-Cys(S^tBu)-OH for residue C240. Attempted conjugation on resin was carried out as described previously.

Purification: Method D.

Attempted conjugation with B9- α F- β 10 (408)

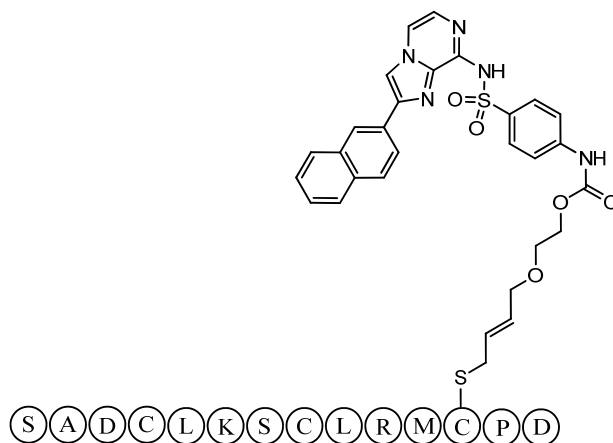


Standard side-chain protecting groups were used with the exception of Fmoc-Cys(S^tBu)-OH for residue C240. Attempted conjugation on resin was carried out as described previously.

Purification: Method P.

6.2.4.2 Resin-Based R240SAC-Allyl Cross Metathesis

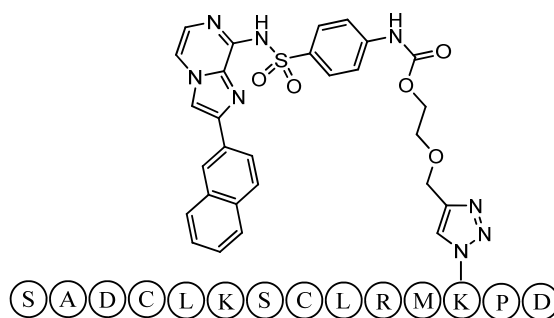
Attempted cross metathesis with α F-loop (409)



Standard side-chain protecting groups were used. S-Allyl Cysteine used as synthesised (**388**). Attempted cross metathesis on resin was carried out as described previously. *Purification*: Method G.

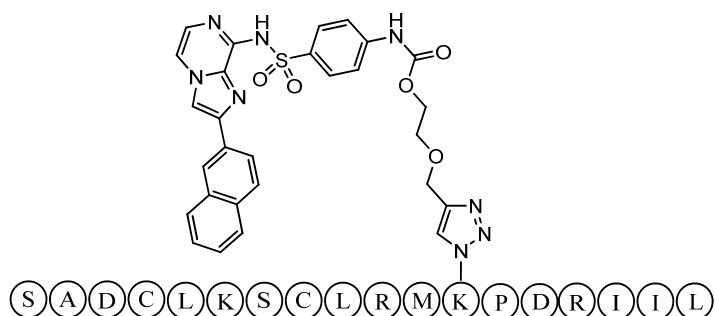
6.2.4.3 Resin-Based R240AzLys-Alkyne Click

α F-loop (410)



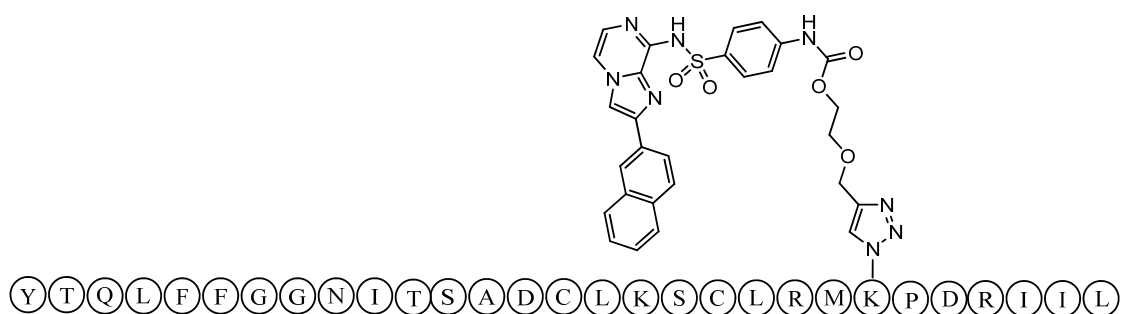
Standard side-chain protecting groups were used. Azidolysine was used from available sources within the laboratory. Click Chemistry on resin carried out as previously described. *Purification*: Method M followed by Method P; *Analysis*: Method S, $R_T = 10.72$ min; m/z (ES⁺): 1067 [M+2H]²⁺, 711.49 [M+3H]³⁺; MALDI TOF⁺: 2132 (M⁺).

α F- β 10 (411)



Standard side-chain protecting groups were used. Azidolysine was used from available sources within the laboratory. Click Chemistry on resin carried out as previously described. *Purification*: Method N followed by Method Q; *Analysis*: Method S, $R_T = 11.44$ min; m/z (ES⁺): 877.36 [M+3H]³⁺, 847.90 [M-(Ser)+3H]³⁺, 658.27 [M+4H]⁴⁺; 636.17 [M-(Ser)+4H]⁴⁺.

Attempted Click with B9- α F- β 10 (412)



Standard side-chain protecting groups were used. Azidolysine was used from available sources within the laboratory. Click Chemistry on resin carried out as previously described. *Purification*: Method N.

6.3 Biochemical Assay

The HP0525 protein was produced^s in the *E. coli* strain BL21 Star(DE3) (Invitrogen) as described previously.³⁸ The protein concentration was estimated spectroscopically using a NanoDrop (Thermo Scientific) and a calculated extinction coefficient at 280 nm, based on the amino acid

^s HP0525 produced and characterised by Dr K. Wallden

composition. The ATPase activity of HP0525 was measured, with and without a specific amount of compound present, using an *in vitro* ATPase colorimetric assay kit (Innova Biosciences). The assay was performed in 96-well ELISA microplates (Greiner Bio-One), using a multipipett/robot.

A volume of 49 μL of a substrate/buffer solution (200 mM tris(hydroxymethyl)aminomethane (TRIS), pH 7.5; 5 mM MgCl_2 ; 250 μM ATP; and 10% DMSO) was added to each assigned well, followed by the addition of 1 μL of compound (at 0.5; 5 or 50 mM to achieve the final concentrations of 5; 50 and 500 μM in the reaction, respectively) in DMSO (or 1 μL DMSO to controls). The solutions were mixed carefully by pipetting. The reaction was started by the addition of 50 μL of 0.106 μM HP0525 to each well (except the negative control, see text below), and the reaction plate was directly transferred to 37 °C for 30 min of incubation. The reaction was stopped by the addition of the Gold mix according to the standard protocol of the kit. The absorbance at 620 nm was measured after 30 min at RT. For each compound, the percentage of absorbance relative non-inhibited HP0525 was calculated, after subtracting the absorbance value of the negative control. In the negative control, the protein was added after the Gold mix, as described in the standard protocol of the kit, which when used as a blank corrects for all free P_i not produced by the enzyme during the 30 min incubation at 37 °C. A known inhibitor of HP0525 (CHIR02)³⁹ was used as a control inhibitor. All measurements were made in duplicate.

A selection of the compounds was assayed as above at additional concentrations ranging between 5 and 200 μM (the measuring points were optimized so that they cover the range to fit a sigmoidal dose response curve) from which IC_{50} values were calculated. Each compound was screened on 3 separate plates and each plate was read twice and a mean was calculated for each concentration. The data was normalised by subtracting the negative control (0% active) and relating it to the positive control (100% active). The software GraphPad Prism 5 was used to generate 2 dose-response curves (Log [inhibitor] vs. normalised response (Standard and Variable Slope)) and the IC_{50} values were calculated from each.

The same assay format was used for the enzyme kinetics experiments, where the activity of HP0525 was tested at various concentrations of ATP (0-500 μM). The data was plotted and fitted to the Michaelis-Menten equation to obtain V_{max} , from which K_m can be calculated.

6.4 Molecular Modelling

AutoDock 4.0 and Vina were used for the *in silico* docking of the compounds into PDB File: 1G6O (ADP-HP0525). All heteroatoms (H₂O, PEG and ADP molecules) were removed from the .pdb file, which was converted into .pdbqt format using AutoDock Tools (ADT), with the addition of polar hydrogen's and Kollman charges.

AutoDock 4.0 requires the following files: *enzyme.pdbqt*, *ligand.pdbqt*, *GridFile.gpf* and *DockingFile.dpf*. AutoDock Vina requires the *enzyme.pdbqt*, *ligand.pdbqt* and *configuration.txt* files. ADT was used to generate the ligand .pdbqt file from a .pdb or .mol2 input which was subjected to no initial energy minimisations. When using PyRx, the program automatically converts .pdb or .mol2 files to .pdbqt files.

Table 35 shows the coordinates of the Grid Box used for the docking into sites A and B. The size of the grid box used is as follows: x (14 Å), y (16 Å), z (24 Å). In the case of AutoDock 4.0, the grid file and docking file was generated using ADT. In the case of AutoDock Vina, the coordinates were used in the configuration text file and a value of 8 was used for the exhaustiveness.

	Site A	Site B
Centre x	-40.36	-12.034
Centre y	53.652	24.627
Centre z	22.3	22.363

Table 35: Coordinate of Grid Boxes used for docking into Sites A and B of 1G6O

Linux was used to run the AutoDock 4.0 program and either cmd or PyRx was used to run AutoDock Vina. The output file from 4.0 is in .dlg format which can be converted to .pdbqt using ADT which can be viewed in PyMOL. In order to visualise the docking file using Chimera, the file needs to be converted further to the .pdb, using PyMOL. Vina exports the docking files as .pdbqt.

MOE 2009 was used for evaluation of the bifunctional reagents. The R240 residue was mutated to the relevant residue and the molecule was built using the 'Build Molecule' function. For example: select residue, add H, convert to C, add H, convert to C, add H etc.

References

-
- ¹ Warren, J. R. and B. J. Marshall., Unidentified curved bacilli on gastric epithelium in active chronic gastritis, *Lancet*, **1983**, 321, 8336, 1273-1275.
- ² Kusters, J. G.; van Vliet, A. H. and Kuipers, E. J., Pathogenesis of *Helicobacter pylori* Infection, *Clin. Microbiol. Rev.*, **2006**, 19, 3, 449-490.
- ³ Covacci, A.; Telford, J. L.; Del Giudice, G.; Parsonnet, J. and Rappuoli, R., *Helicobacter pylori* Virulence and Genetic Geography, *Science*, **1999**, 284, 1328-1333.
- ⁴ Dicken, B. J.; Bigam, D. L.; Cass, C.; Mackay, J. R.; Joy, A. A. and Hamilton, S. M., Gastric adenocarcinoma. Review and Considerations for Future Directions, *Ann. Surg*, **2005**, 241, 27-39.
- ⁵ Brown, L. M., *Helicobacter pylori*: Epidemiology and Routes of Transmission, *Epidemiol. Rev.*, **2000**, 22, 283-297.
- ⁶ International Agency for Research on Cancer (IARC), Monographs on the evaluation of carcinogenic risks to humans, vol. 61. Infection with *Helicobacter pylori*, IARC, Lyon, France, **1994**. 177-240.
- ⁷ Selbach, M.; Moese, S.; Backert, S.; Jungblut, P. R. and Meyer, T., The *Helicobacter pylori* CagA protein induces tyrosine dephosphorylation of ezrin, *Proteomics*, **2004**, 4, 2961-2968.
- ⁸ Blaser, M. J. and Berg, D. E., *Helicobacter pylori* genetic diversity and risk of human disease, *J. Clin. Invest.*, **2001**, 107, 767-773.
- ⁹ Viala, J.; Chaput, C.; Boneca, I. G.; Cardona, A.; Girardin, S. E.; Moran, A. P.; Athman, R.; Memet, S.; Huerre, M. R.; Coyle, A. J.; DiStefano, P. S.; Sansonetti, P. J.; Labigne, A.; Bertin, J.; Philpott, D. J. and Ferrero, R. L., Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island, *Nat. Immunol.*, **2004**, 5, 1166-1174.
- ¹⁰ Backhed, F.; Torstensson, E.; Seguin, D.; Richter-Dahlfors, A. and Rokbi. B., *Helicobacter pylori* infection induces interleukin-8 receptor expression in the human gastric epithelium, *Infect. Immun.*, **2003**, 71, 3357-3360.
- ¹¹ Cover, T. L. and Blaser, M. J., Purification and characterization of the vacuolating toxin from *Helicobacter pylori*, *J. Biol. Chem.*, **1992**, 267, 10570-10575.
- ¹² Wada, A.; Yamasaki, E. and Hirayama, T., *Helicobacter pylori* vacuolating cytotoxin, VacA, is responsible for gastric ulceration, *J. Biochem.*, **2004**, 136, 741-746.
- ¹³ Atherton, J. C.; Cao, P.; Peek Jr.; R. M.; Tummuru, M. K.; Blaser, M. J. and Cover, T. L., Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific vacA types with cytotoxin production and peptic ulceration, *J. Biol. Chem.*, **1995**, 270, 17771-17777.
- ¹⁴ Segal, E. D.; Cha, J.; Lo, J.; Falkow, S. and Tompkins. L. S., Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*, *Proc. Natl. Acad. Sci. USA*, **1999**, 96, 14559-14564.

-
- ¹⁵ Selbach, M.; Moese, S.; Hurwitz, R.; Hauck, C. R.; Meyer T. F. and Backert, S., The *Helicobacter pylori* CagA protein induces cortactin dephosphorylation and actin rearrangement by c-Src inactivation, *EMBO J.*, **2003**, 22, 3, 515-528.
- ¹⁶ Christie, P. J. and Vogel, J.P., Bacterial type IV secretion: conjugation systems adapted to deliver effector molecules to host cells, *Trends Microbiol.*, **2000**, 8, 354-360.
- ¹⁷ Hatakeyama, M., Oncogenic Mechanisms of the *Helicobacter pylori* CagA Protein, *Nature Rev. Cancer*, **2004**, 4, 688-694.
- ¹⁸ Walsh, J. H. and Peterson, W. L., The Treatment of *Helicobacter pylori* Infection in the Management of Peptic Ulcer Disease, *N. Engl. J. Med.*, **1995**, 333, 984-991.
- ¹⁹ Chey, W. D. and Wong, B. C. Y., American College of Gastroenterology Guideline on the Management of *Helicobacter pylori* Infection, *Am. J. Gastroenterol.*, **2007**, 102, 1808-1825.
- ²⁰ Luther, J.; Higgins, P. D. R.; Schoenfeld, P. S.; Moayyedi, P.; Vakil, N. and Chey, W. D., Empiric Quadruple vs. Triple Therapy for Primary Treatment of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis of Efficacy and Tolerability, *Am. J. Gastroenterol.*, **2010**, 105, 65-73.
- ²¹ Rêgo, A. T.; Chandran, V. and Waksman, G., Two-step and one-step secretion mechanisms in Gram-negative bacteria: contrasting the type IV secretion system and the chaperone-usher pathway of pilus biogenesis, *Biochem. J.*, **2010**, 425, 475-488.
- ²² Alvarez-Martinez, C. E. and Christie, P. J., Biological Diversity of Prokaryotic Type IV Secretion Systems, *Microbiol. Mol. Biol. Rev.*, **2009**, 73, 4, 775-808.
- ²³ Remaut, H. and Waksman, G., Structural biology of bacterial pathogenesis, *Curr. Opin. Struct. Biol.*, **2004**, 14, 161-170.
- ²⁴ Filloux, A., The underlying mechanisms of type II protein secretion, *Biochim. Biophys. Acta*, **2004**, 1694, 163-179.
- ²⁵ Cornelis, G. R. and van Gijsegem, F., Assembly and function of type III secretory systems, *Annu. Rev. Microbiol.*, **2000**, 54, 735-774.
- ²⁶ Wallden, K.; Rivera-Calzada, A. and Waksman, G., Type IV secretion systems: versatility and diversity in function, *Cell. Microbiol.*, **2010**, 12, 9, 1203-1212.
- ²⁷ Fronzes, R.; Christie, P. J. and Waksman, G., The structural biology of the type IV secretion systems, *Nat. Rev. Microbiol.*, **2009**, 7, 703-714.
- ²⁸ Terradot, L. and Waksman, G., Architecture of the *Helicobacter pylori* Cag-type IV secretion system, *FEBS J.*, **2011**, 278, 1213-1222.
- ²⁹ Henderson, I. R.; Navarro-Garcia, F.; Desvaux, M.; Fernandez, R. C. and Ala'Aldeen, D., Type V Protein Secretion Pathway: the Autotransporter Story, *Microbiol. Mol. Biol. Rev.*, **2004**, 68, 692-744.
- ³⁰ Filloux, A.; Hachani, A. and Bleves, S., The bacterial type VI secretion machine: yet another player for protein transport across membranes, *Microbiology*, **2008**, 154, 1570-1583.
- ³¹ Waksman, G. and Holmgren, S. J., Structural biology of the chaperone-usher pathway of pilus biogenesis, *Nat. Rev. Microbiol.*, **2009**, 7, 765-774.

-
- ³² Yeo, H-J. and Waksman, G., Unveiling Molecular Scaffolds of the Type IV Secretion System, *J. Bacteriol.*, **2004**, *186*, 7, 1919-1926.
- ³³ Fronzes, R.; Schäfer, E.; Wang, L.; Saibil, H. R.; Orlova, E. V. and Waksman, G., Structure of a Type IV Secretion System Core Complex, *Science*, **2009**, *323*, 266-268.
- ³⁴ Chandran, V.; Fronzes, R.; Duquerroy, S.; Cronin, N.; Navaza, J. and Waksman, G., Structure of the outer membrane complex of a type IV secretion system, *Nature*, **2009**, *462*, 1011-1015.
- ³⁵ Yeo, H. J.; Savvides, S. N.; Herr, A. B.; Lanka, E. and Waksman, G., Crystal Structure of the Hexameric Traffic ATPase of the *Helicobacter pylori* Type IV Secretion System, *Mol. Cell.*, **2000**, *6*, 1461-1472.
- ³⁶ Savvides, S. N.; Yeo, H-J.; Beck, M. R.; Blaesing, F.; Lurz, R.; Lanka, E.; Buhrdorf, R.; Fischer, W.; Haas, R. and Waksman, G., VirB11 ATPases are dynamic hexameric assemblies: new insights into bacterial type IV secretion, *EMBO J*, **2003**, *22*, 1969-1980.
- ³⁷ Pettersen E. F.; Goddard T. D.; Huang C. C.; Couch G. S.; Greenblatt D. M.; Meng E. C. and Ferrin T. E., UCSF Chimera--a visualization system for exploratory research and analysis.. *J Comput Chem.*, **2004**, *25*, 13, 1605-12.
- ³⁸ Hare, S.; Fischer, W.; Williams, R.; Terradot, L.; Bayliss, R.; Haas, R. and Waksman, G., Identification, structure and mode of action of a new regulator of the *Helicobacter pylori* HP0525 ATPase, *EMBO J.*, **2007**, *26*, 4926-4934.
- ³⁹ Hilleringmann, M.; Pansegrau, W.; Doyle, M.; Kaufman, S.; MacKichan, M. L.; Gianfaldoni, C.; Ruggiero, P. and Covacci, A., Inhibitors of *Helicobacter pylori* ATPase Cag α block CagA transport and cag virulence, *Microbiol.*, **2006**, *152*, 2919-2930.
- ⁴⁰ Irwin, J. J. and Shoichet, B. K., ZINC – A Free Database of Commercially Available Compounds for Virtual Screening, *J. Chem. Inf. Model.*, **2005**, *45*, 177-182.
- ⁴¹ *SciFinder Scholar*, version 2012; Chemical Abstracts Service: Columbus, OH, 2012.
- ⁴² von Baeyer, A., Systematik und Nomenclatur bicyclischer Kohlenwasserstoffe, *Ber. Dtsch. Chem. Ges.*, **1900**, *33*, 3771-3775.
- ⁴³ Panico, R.; Powell, W. H.; Richer, J. C.; A Guide to IUPAC Nomenclature of Organic Compounds, *Blackwell Science*, **1993**, ISBN 0632034882.
- ⁴⁴ Basiuk, V. A., Imidazo[1,2-*a*]pyrazines, *Russ. Chem. Rev.*, **1997**, *66*, 3, 187-204.
- ⁴⁵ Hori, K.; Charbonneau, H.; Hart, R. C. and Cormier, M. J., Structure of native *Renilla reniformis* luciferin, *Proc. Natl. Acad. Sci. USA*, **1977**, *74*, 10, 4285-4287.
- ⁴⁶ Martin, R. H. and Tarasiejska, Z., Synthèse de Derives de l'Imidazo(4,5- β)pyrazine et de l'Imidazolino(1,2- α)pyrazine (nouveau système cyclique), *Bull. Soc. Chim. Belg.* **1957**, *66*, 136.
- ⁴⁷ Sablayrolles, C.; Cros, G. H.; Milhavet, J. C.; Rechenq, E.; Chapat, J. P.; Boucard, M.; Serrano, J. J. and McNeill, J. H., Synthesis of Imidazo[1,2-*a*]pyrazine Derivatives with Uterine-Relaxing, Antibronchospastic, and Cardiac-Stimulating Properties, *J. Med. Chem.*, **1984**, *27*, 206-212.

- ⁴⁸ Kaminski, J. J.; Hilbert, J. M.; Pramanik, B. N.; Solomon, D. M.; Conn, D. J.; Rizvi, R. K.; Elliot, A. J.; Guzik, H.; Lovey, R. G.; Domalski, M. S.; Wong, S.-C.; Puchalski, C.; Gold, E. H.; Long, J. F.; Chiu, P. J. S. and McPhail, A. T., Antiulcer Agents. 2. Gastric Antisecretory, Cytoprotective, and Metabolic Properties of Substituted Imidazo[1,2-a]pyridines and Analogues, *J. Med. Chem.*, **1987**, *30*, 2031-2046.
- ⁴⁹ Spitzer, W. A.; Victor, F.; Pollock, G. D. and Hayes, J. S., Imidazo[1,2-*a*]pyrimidines and Imidazo[1-2-*a*]pyrazines: The Role of Nitrogen Position in Inotropic Activity, *J. Med. Chem.*, **1988**, *31*, 1590-1595.
- ⁵⁰ Lumma, W. C., Jr.; Randall, W. C.; Cressen, E. L.; Huff, J. R.; Hartman, R. D. and Lyon, T. F., Piperazinylimidazo[1,2-*a*]pyrazines with Selective Affinity for in Vitro α -Adrenergic Receptor Subtypes, *J. Med. Chem.*, **1983**, *26*, 3, 357-363.
- ⁵¹ Bonnet, P. A.; Michel, A.; Laurent, F.; Sablayrolles, C.; Rechencq, E.; Mani, J. C.; Boucard, M. and Chapat, J. P., Synthesis and Antibronchospastic Activity of 8-Alkoxy- and 8-(Alkylamino)imidazo[1,2-*a*]pyrazines, *J. Med. Chem.*, **1992**, *35*, 3353-3358.
- ⁵² Bradač, J.; Furek, Z.; Janežič, D.; Molan, S.; Smerkolj, I.; Stanovnik, B.; Tišler, M. and Verček, B., Telesubstitution and other transformations of imidazo[1,2-*a*]- and s-Triazolo[4,3-*a*]pyrazines, *J. Org. Chem.*, **1977**, *42*, 4197-4201.
- ⁵³ Barlin, G. B.; Brown, D. J.; Kadunc, Z.; Petrič, A.; Stanovnik, B. and Tišler, M., Imidazo[1,2-*b*]pyridazines and an Imidazo[1,2-*a*]pyrazine from Pyridazin- and Pyrazin-amines, *Aust. J. Chem.*, **1983**, *36*, 1215-1220.
- ⁵⁴ Lumma, Jr., W. C. and Springer, J. P., Novel Condensation of 2,3-Epoxybutanal with 2-Aminopyridine and 2-Aminopyrazine. Synthesis and Stability of 3-(1-Hydroxyethyl)imidazo[1,2-*a*]azines, *J. Org. Chem.*, **1981**, *46*, 3735-3736.
- ⁵⁵ Parkanyi, C.; Abdelhamid, A. O.; Cheng, J. C. S. and Shawali, A. S., Convenient Synthesis of Fused Heterocycles from α -Ketohydroximoyl Chlorides and Heterocyclic Amines, *J. Heterocycl. Chem.*, **1984**, *21*, 1029-1032.
- ⁵⁶ Meurer, L. C.; Tolman, R. L.; Chapin, E. W.; Saperstein, R.; Vicario, P. P.; Zrada, M. and MacCoss, M., Synthesis and Hypoglycemic Activity of Substituted 8-(1-Piperaziny)imidazo[1,2-*a*]pyrazines, *J. Med. Chem.*, **1992**, *35*, 3845-3857.
- ⁵⁷ MacCoss, M.; Meurer, L. C.; Hoogsteen, K.; Springer, J. P.; Koo, G.; Peterson, L. B. and Tolman, R. L., Synthesis and Biological Evaluation of Nucleosides Containing 8-Aminoimidazo[1,2-*a*]pyrazine as an Isosteric Replacement for Adenine, *J. Heterocyclic Chem.*, **1993**, *30*, 1213-1220.
- ⁵⁸ Slepukhin, P. A.; Kim, D. G.; Rusinov, G. L.; Charushin, V. N. and Chupakhin, O. N., Simple Synthesis of Imidazo[1,2-*a*]pyrazines, *Chem. Heterocycl. Compd.*, **2002**, *38*, 1142-1143.
- ⁵⁹ Macleod, A.; Mitchell, D. R.; Palmer, N. J.; Parsy, C. C.; Goldsmith, M. D. and Harris, C. J., Imidazopyrazine Compounds, PCT Int. Appl. WO 2009/024585, Feb 26, 2009.
- ⁶⁰ Contour-Galcéra, M. O.; Poitout, L.; Moinet, C.; Morgan, B.; Gordon, T.; Roubert, P. and Thurieau, C., Synthesis of Substituted Imidazopyrazines as Ligands for the Human Somatostatin Receptor Subtype 5, *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 741-745.

- ⁶¹ Kayağıl, I. and Demirayak, S., Synthesis of some 2,3,6,8-tetraarylimidazo[1,2-*a*]pyrazine derivatives by using either reflux or microwave irradiation method, and investigation their anticancer activities, *Turk. J. Chem.*, **2011**, 35, 13-24.
- ⁶² Davey, D. D., Synthesis of 6-Phenylimidazo[1,2-*a*]pyrazine-8-one and 1-Methyl-6-Phenylimidazo[1,5-*a*]pyrazine-8-one via Quaternary Intermediates, *J. Org. Chem.*, **1987**, 52, 4379-4381.
- ⁶³ Legroux, D.; Schoeni, J. P.; Pont, C. and Fleury, J. P., Aza-2-diènes-1,3. Partie 5: Préparation de *N*-aminoimidazoles, 3*H*-pyrroles, triazolo[1,2,4][1,5-*a*]pyrazines et imidazo[1,2-*a*]pyrazines., *Helv. Chim. Acta.*, **1987**, 70, 187-195.
- ⁶⁴ Lang, M.; Lacroix, A.; Pont, C. and Fleury, J. P., Aza-2-diènes-1,3. Préparation et propriétés d'alkyl-1-pyrazone-imines et de leurs isomères, *Helv. Chim. Acta.*, **1986**, 69, 1025-1033.
- ⁶⁵ Jones, D. S.; Kenner, G. W.; Preston, J. and Sheppard, R. C., The Isomerization of some Oxazolones derived from Tripeptides, *Tetrahedron*, **1965**, 21, 3209-3218.
- ⁶⁶ Goto, T., Cyridina Bioluminescence IV. Synthesis and Chemiluminescence of 3,7-dihydroimidazo[1,2-*a*]pyrazine-3-one and its 2-methyl derivative, *Tetrahedron Lett.*, **1968**, 9, 36, 3873-3876.
- ⁶⁷ Blackburn, C.; Guan, B.; Fleming, P.; Shiosaki, K. and Tsai, S., Parallel Synthesis of 3-Aminoimidazo[1,2-*a*]pyridines and pyrazines by a New Three-Component Condensation, *Tetrahedron Lett.*, **1998**, 39, 3635-3638.
- ⁶⁸ Bienaymé, H. and Bouzid, K., A New Heterocyclic Multicomponent Reaction for the Combinatorial Synthesis of Fused 3-Aminoimidazoles, *Angew. Chem. Int. Ed.*, **1998**, 37, 2234-2237.
- ⁶⁹ Groebke, K.; Weber, L. and Mehlin, F., Synthesis of Imidazo[1,2-*a*] annulated Pyridines, Pyrazines and Pyrimidines by a Novel Three-Component Condensation, *Synlett*, **1998**, 661-663.
- ⁷⁰ Guasconi, M.; Lu, X.; Massarotti, A.; Caldarelli, A.; Ciruolo, E.; Tron, G. C.; Hirsch, E.; Sorba, G. and Pirali, T., Groebke multicomponent reaction and subsequent nucleophilic aromatic substitution for a convenient synthesis of 3,8-diaminoimidazo[1,2-*a*]pyrazines as potential kinase inhibitors, *Org. Biomol. Chem.*, **2011**, 9, 4144-4149.
- ⁷¹ Polyakov, A. I.; Eryomina, V. A.; Medvedeva, L. A.; Tihonova, N. I.; Listratova, A. V. and Voskressensky, L. G., Silica-sulfuric acid: a highly efficient catalyst for the synthesis of imidazo[1,2-*a*]pyridines using trimethylsilyl cyanide or cyanohydrins, *Tetrahedron Lett.*, **2009**, 50 4389-4393.
- ⁷² Lyon, M. A. and Kercher, T. S., Glyoxylic Acid and MP-Glyoxylate: Efficient Formaldehyde Equivalents in the 3-CC of 2-Aminoazines, Aldehydes, and Isonitriles, *Org. Lett.*, **2004**, 6, 4989-4992.
- ⁷³ Adib, M.; Sheibani, E.; Bijanzadeh, H. R. and Zhu, L-G., A new, one-pot, multicomponent synthesis of imines of 3-amino-2-arylimidazo[1,2-*a*]pyridines, 3-amino-2-arylimidazo[1,2-*a*]pyrazines, and 3-amino-2-arylimidazo[1,2-*a*]pyrimidines, *Tetrahedron*, **2008**, 64, 10681-10686.
- ⁷⁴ Pastor, J.; Rodríguez-Arístegui, S.; Hernández, A. I.; Varela, C.; Salgado, A. and Martinez, S., New use of bis(benzotriazolyl)-1,2-(dialkylamino)ethanes for the synthesis of 2-*H*-3-dialkylamino imidazo[1,2-*a*]pyrazine derivatives, *Tetrahedron Lett.*, **2012**, 53, 1082-1084.

- ⁷⁵ Katritzky, A. R.; Xu, Y.-J. and Tu, H., Regiospecific Synthesis of 3-Substituted Imidazo[1,2-*a*]pyridines, Imidazo[1,2-*a*]pyrimidines and Imidazo[1,2-*c*]pyrimidines, *J. Org. Chem.*, **2003**, *68*, 4935-4937.
- ⁷⁶ Harris, A. R.; Nason, D. M.; Collantes, E. M.; Xu, W.; Chi, Y.; Wang, Z.; Zhang, B. Zhang, Q.; Gray, D. L. and Jennifer E. Davoren, Synthesis of 5-bromo-6-methyl imidazopyrazine, 5-bromo and 5-chloro-6-methylimidazopyridine using electron density surface maps to guide synthetic strategy, *Tetrahedron*, **2011**, *67*, 9063-9066.
- ⁷⁷ Kaminski, J. J.; Perkins, D. G.; Frantz, J. D.; Solomon, D. M.; Elliot, A. J.; Chiu, P. J. S. and Long, J. F., Antiulcer Agents 3. Structure-Activity-Toxicity Relationships of Substituted Imidazo[1,2-*a*]pyridines and a Related Imidazo[1,2-*a*]pyrazine, *J. Med. Chem.*, **1987**, *30*, 2047-2051.
- ⁷⁸ Zimmermann, P. J.; Brehm, C.; Buhr, W.; Palmer, A. M.; Volz, J. and Simon, W.-A., Novel imidazo[1,2-*a*]pyrazine derivatives as potent reversible inhibitors of the gastric H⁺/K⁺ ATPase, *Bioorg. Med. Chem.*, **2008**, *16*, 536-541.
- ⁷⁹ Vitse, O.; Bonnet, P. A.; Bompert, J.; Viols, H.; Subra, G.; Chapat, J. P. and Grassy, G., Nitration of the Imidazo[1,2-*a*]pyrazine Series. Experimental and Computational Results., *J. Heterocycl. Chem.*, **1997**, *34*, 701-707.
- ⁸⁰ Myadaraboina, S.; Alla, M.; Saddanapu, V.; Bommena, V. R. and Addlagatta, A., Structure activity relationship studies of imidazo[1,2-*a*]pyrazine derivatives against cancer cell lines, *Eur. J. Med. Chem.*, **2010**, *45*, 5208-5216.
- ⁸¹ Vitse, O.; Laurent, F.; Pocock, T. M.; Bénézech, V.; Zanic, L.; Elliot, K. R. F.; Subra, G.; Portet, K.; Bompert, J.; Chapat, J.-P.; Small, R. C.; Michel, A. and Bonnet, P.-A., New Imidazo[1,2-*a*]pyrazine Derivatives with Bronchodilatory and Cyclic Nucleotide Phosphodiesterase Inhibitory Activities, *Bioorg. Med. Chem.*, **1999**, *7*, 1059-1065.
- ⁸² Barraclough, P.; Firmin, D.; Lindon, J. C.; Nobbs, M. S.; Sanderson, P. N.; Smith, S. and Gillam, J. M., Sites of Protonation in Cardiotonic Polyazaindolizines by NMR Spectroscopy, *Magn. Reson. Chem.*, **1991**, *29*, 468-475.
- ⁸³ Sanderson, P. N.; Farrant, R. D.; Lindon, J. C. and Barraclough, P., A Multinuclear Magnetic Resonance Study of Protonation in Imidazo[1,2-*a*]pyrazine, *Magn. Reson. Chem.*, **1990**, *28*, 874-877.
- ⁸⁴ Mitchell, S. A.; Danca, M. D.; Blomgren, P. A.; Darrow, J. W.; Currie, K. S.; Kropf, J. E.; Lee, S. H.; Gallion, S. L.; Xiong, J.-M.; Pippin, D. A.; DeSimone, R. W.; Brittelli, D. R.; Eustice, D. C.; Bourret, A.; Hill-Drzewi, M.; Maciejewski, P. M. and Elkin, L. L., Imidazo[1,2-*a*]pyrazine diaryl ureas: Inhibitors of the receptor tyrosine kinase EphB4, *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 6991-6995.
- ⁸⁵ Matthews, T. P.; McHardy, T.; Klair, S.; Boxall, K.; Fisher, M.; Cherry, M.; Allen, C. E.; Addison, G. J.; Ellard, J.; Aherne, G. W.; Westwood, I. M.; van Monfort, R.; Garrett, M. D.; Reader, J. C. and Collins, I., Design and evaluation of 3,6-di(hetero)aryl imidazo[1,2-*a*]pyrazines as inhibitors of checkpoint and other kinases, *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 4045-4049.
- ⁸⁶ Voss, M. E.; Rainka, M. P.; Fleming, M.; Peterson, L. H.; Belanger, D. B.; Siddiqui, M. A.; Hruza, A.; Voigt, J.; Gray, K. and Basso, A. D., Synthesis and SAR studies of imidazo-[1,2-*a*]pyrazine Aurora

kinase inhibitors with improved off-target kinase selectivity, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3544-3549.

⁸⁷ Belanger, D. B.; Curran, P. J.; Hruza, A.; Voigt, J.; Meng, Z.; Mandal, A. K.; Siddiqui, M. A.; Basso, A. D. and Gray, K., Discovery of imidazo[1,2-*a*]pyrazine-based Aurora kinase inhibitors, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5170-5174.

⁸⁸ Nic, M.; Jirat, J. and Kosata, B., Tele-substitution, *IUPAC Compendium of Chemical Terminology (Online ed.)*, Version 2.3.2 (Aug 19, 2012), doi: 10.1351/goldbook.T06256.

⁸⁹ Bonnet, P. A.; Sablayrolles, C. and Chapat, J. P., Imidazo[1,2-*a*]pyrazine, Bromo and Methoxy Derivatives: a ¹³C N.M.R. Determination Applied to Nucleophilic Substitution Studies, *Aust. J. Chem.*, **1984**, *37*, 1357-1361.

⁹⁰ Gueiffier, A.; Viols, H.; Galtier, C.; Y. Blache, Y.; Chavignon, O.; Teulade, J-C.; Debouzy, J-C. and Chapat, J. P., *Heterocyclic Commun.*, **1994**, *1*, 83-87.

⁹¹ Gueiffier, A.; Viols, H.; Y. Blache, Y.; Chavignon, O.; Teulade, J. C.; Aumelas, A. and Chapat, J. P., Reactivity of Phenyllithium toward Bridgehead Nitrogen Heterocycles, *Heterocycles*, **1994**, *38*, 551-557.

⁹² Vitse, O.; Bompard, J.; Subra, G.; Viols, H.; Escalé, R.; Chapat, J-P. and Bonnet, P-A., Aza-Indolizine with Bridgehead Nitrogen. Metalation, Halogen-Metal Exchange and Directed *Ortho*-Lithiation in the Imidazo[1,2-*a*]pyrazine Series, *Tetrahedron*, **1998**, *54*, 6485-6496.

⁹³ Kercher, T.; Rao, C.; Bencsik, J. R. and Josey, J. A., Diversification of the Three-Component Coupling of 2-Aminoheterocycles, Aldehyde, and Isonitriles: Efficient Parallel Synthesis of a Diverse and Druglike Library of Imidazo- and Tetrahydroimidazo[1,2-*a*] Heterocycles, *J. Comb. Chem.*, **2007**, *9*, 1177-1187.

⁹⁴ Blackburn, C. and Guan, B., A novel dealkylation affording 3-imidazo[1,2-*a*]pyridines: access to new substitution patterns by solid phase synthesis, *Tetrahedron Lett.*, **2000**, *41*, 1495-1500.

⁹⁵ Yu, T.; Tagat, J. R.; Kerekes, A. D.; Doll, R. J.; Zhang, Y.; Xiao, Y.; Esposito, S.; Belanger, D. B.; Curran, P. J.; Mandal, A. K.; Siddiqui, M. A.; Shih, N-Y.; Basso, A. D.; Liu, M.; Gray, K.; Tevar, S.; Jones, J.; Lee, S.; Liang, L.; Ponery, S.; Smith, E. B.; Hruza, A.; Voigt, J.; Ramanathan, L.; Prosis, W. and Hu, M., Discovery of a Potent, Injectable Inhibitor of Aurora Kinases Based on the Imidazo[1,2-*a*]pyrazine core, *ACS Med. Chem. Lett.*, **2010**, *1*, 214-218.

⁹⁶ Belanger, D. B.; Williams, M. J.; Curran, P. J.; Mandal, A. K.; Meng, Z.; Rainka, M. P.; Yu, T.; Shih, N-Y.; Siddiqui, M. A.; Liu, M.; Tevar, S.; Lee, S.; Liang, L.; Gray, K.; Yaremko, B.; Jones, J.; Smith, E. B.; Prelusky, D. B. and Basso, A. D., Discovery of orally bioavailable imidazo[1,2-*a*]pyrazine-based Aurora kinase inhibitors, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6739-6743.

⁹⁷ Kerekes, A. D.; Esposito, S.; Doll, R. J.; Tagat, J. R.; Yu, T.; Xiao, Y.; Zhang, Y.; Prelusky, D. B.; Tevar, S.; Gray, K.; Terracina, G. A.; Lee, S.; Jones, J.; Liu, M.; Basso, A. D. and Smith, E. B., Aurora Kinase Inhibitors Based on the Imidazo[1,2-*a*]pyrazine Core: Fluorine and Deuterium Incorporation Improve Oral Absorption and Exposure, *J. Med. Chem.*, **2011**, *54*, 201-210.

⁹⁸ Meng, Z.; Kulkarni, B. A.; Kerekes, A. D.; Mandal, A. K.; Esposito, S.; Belanger, D. B.; Reddy, P. A.; Basso, A. D.; Tevar, S.; Gray, K.; Jones, J.; Smith, E. B.; Doll, R. J. and Siddiqui, M. A., Bioisosteric approach to the discovery of imidazo[1,2-*a*]pyrazine as potent Aurora kinase inhibitors, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 592-598.

-
- ⁹⁹ Andrews, P. D., Aurora kinases: shining lights on the therapeutic horizon?, *Oncogene*, **2005**, *24*, 5005-5015.
- ¹⁰⁰ Bouloc, N.; Large, J. M.; Kosmopoulou, M.; Sun, C.; Faisal, A.; Matteucci, M.; Reynisson, J.; Brown, N.; Atrash, B.; Blagg, J.; McDonald, E.; Linardopoulos, S.; Bayliss, R. and Bavetsias, V., Structure-based design of imidazo[1,2-*a*]pyrazine derivatives as selective inhibitors of Aurora-A kinase in cells, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5988-5993.
- ¹⁰¹ González, S. M.; Hernández, A. I.; Varela, C.; Rodríguez-Aristegui, S.; Alvarez, R. M.; García, A. B.; Lorenzo, M.; Rivero, V.; Oyarzabal, J.; Rabal, O.; Bischoff, J. R.; Albarrán, M.; Cebrián, A.; Alfonso, P.; Link, W.; Fominaya, J. and Pastor, J., Imidazo[1,2-*a*]pyrazines as novel PI3K inhibitors *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1874-1878.
- ¹⁰² González, S. M.; Hernández, A. I.; Varela, C.; Lorenzo, M.; Ramos-Lima, F.; Cendón, E.; Cebrián, D.; Aguirre, E.; Gomez-Casero, E.; Albarrán, M. I.; Alfonso, P.; García-Serelde, B.; Mateos, G.; Oyarzabal, J.; Rabal, O.; Mulero, F.; Gonzalez-Granda, T.; Link, W.; Fominaya, J.; Barbacid, M.; Bischoff, J. R.; Pizcueta, P.; Blanco-Aparicio, C. and Pastor, J., Rapid identification of ETP-46992, orally bioavailable PI3K inhibitor, selective versus mTOR, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5208-5214.
- ¹⁰³ Zeng, H.; Belanger, D. B.; Curran, P. J.; Shipps Jr, G. W.; Miao, H.; Bracken, J. B.; Siddiqui, M. A.; Malkowski, M. and Wang, Y., Discovery of novel imidazo[1,2-*a*]pyrazin-8-amines as Brk/PTK6 inhibitors, *Bioorg. Med. Chem. Lett.*, **2011**, *21*, 5870-5875.
- ¹⁰⁴ Guzi, T. J.; Paruch, K.; Dwyer, M. P.; Zhao, L.; Curran, P. J.; Belanger, D. B.; Hamman, B.; Reddy, P. A. and Siddiqui, M. A., Novel Imidazopyrazines as Cyclin Dependent Kinase Inhibitors, US Patent Appl. US 2006/016023, May 18, 2006.
- ¹⁰⁵ Reddy, P. A. P.; Siddiqui, M. A.; Tadikonda, P. K.; Mansoor, U. F.; Shipps, G. W. Jr.; Belanger, D. B. and Zhao, L., Novel JNK Inhibitors, PCT Int. Appl. WO 2008/082490, Jul 10, 2008.
- ¹⁰⁶ Birault, V.; Harris, C. J. and Harrison, S. A., Imidazo[1,2-*a*]pyrazine compounds which interact with protein kinases, PCT Int. Appl. WO 2005/085252, Sep 15, 2005.
- ¹⁰⁷ Zimmermann, P. J.; Buhr, W.; Chiesa, M. V.; Palmer, A.; Brehm, C.; Grundler, G.; Senn-Bilfinger, J.; Simon, W. A.; Postius, S. and Kromer, W., 6-Substituted Imidazopyrazines, PCT Int. Appl. WO 2004/074289, Sept 2, 2004.
- ¹⁰⁸ Shimokawa, H. and Yamasishi, T., Imidazo[1,2-*a*]pyrazine Derivatives and their use as Acid Pump Antagonists, PCT Int. Appl. WO 2008/059373, May 22, 2008.
- ¹⁰⁹ Du, X.; Gustin, D. J.; Chen, X.; Duquette, J.; McGee, L. R.; Wang, Z.; Ebsworth, K.; Henne, K.; Lemon, B.; Ma, J.; Miao, S.; Sabalan, E.; Sullivan, T. J.; Tonn, G.; Collins, T. L. and Medina, J. C., Imidazo-pyrazine derivatives as potent CXCR3 antagonists, *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 5200-5204.
- ¹¹⁰ Burke, J. R.; Townsend, R. M.; Qui, Y.; Zusi, F. C.; Nadler, S. G. and Davis, S. B., Methods of Treating Inflammatory and Immune Diseases Using Inhibitors of IκB Kinase, US Patent Appl. 2003/0022898, Jan 30, 2003.
- ¹¹¹ Hynes, J.; De Lucca, G V. and Wu, H., Imidazopyridine and Imidazopyrazine Compounds Useful as Kinase Inhibitors, US Patent Appl. US 2011/0288085, Nov 24, 2011.

-
- ¹¹² Andrews, M. J. I.; Chambers, M. S.; Van De Poël, H. and Bar, G. L. J., Fused Pyrazine Compounds Useful for the Treatment of Degenerative and Inflammatory Diseases, PCT Int. Appl. WO 2009/135885, Nov 12, 2009.
- ¹¹³ Koppitz, M.; Klar, U.; Jautelat, R.; Kosemund, D.; Bohlmann, R.; Bader, B.; Lienau, P. and Siemeister, G., 6-Substituted Imidazopyrazines for the use as Mps-1 and TKK Inhibitors in the Treatment of Hypoproliferative Disorders, PCT Int. Appl WO 2012/080236, Jun 21, 2012.
- ¹¹⁴ Dean, D. K.; Munoz-Murieddas, J.; Sime, M.; Steadman, J. G. A.; Thewlis, R. E. A.; Trani, G.; Wall, I. D. and Walter, D. S., 5,6,7,8-Tetrahydroimidazo[1,2-*a*]pyrazine Derivatives as P2X7 Modulators, PCT Int. Appl WO 2010/125101, Nov 4, 2010.
- ¹¹⁵ Mitchell, S. A.; Currie, K. S.; Blomgren, P. A.; Armistead, D. M. and Raker, J., 6-Aryl-Imidazo[1,2-*a*]pyrazine Derivatives, Methods of Making, and Methods of Use Thereof, PCT Int. Appl WO 2009/102468, Aug 20, 2009.
- ¹¹⁶ Crilley, T. K.; Wanstall, J. C. and Bonnet, P. A., Vasorelaxant Effects of SCA40 (A Phosphodiesterase III Inhibitor) in Pulmonary Vascular Preparations in Rats, *Clin. Exp. Pharmacol. Physiol.*, **1998**, 25, 355-360.
- ¹¹⁷ Cook, S. J.; Archer, K.; Martin, A.; Bucheit, K. H.; Fozard, J. R.; Müller, T.; Miller, A. J.; Elliot, K. R. F.; Foster, R. W. and Small, R. C., Further analysis of the mechanisms underlying the tracheal relaxant action of SCA40, *Br. J. Pharmacol*, **1995**, 114, 143-151.
- ¹¹⁸ Laurent, F.; Michel, A.; Bonnet, P. A.; Chapat, J.P. and Boucard, M., Evaluation of the relaxant effects of SCA40, a novel charybdotoxin-sensitive potassium channel opener, in guinea-pig isolated trachealis, *Br. J. Pharmacol*, **1993**, 108, 622-626.
- ¹¹⁹ Bartolomé-Nebreda, J. M.; Conde-Ceide, S.; MacDonald, G. J.; Pastor-Fernández, J.; Van Gool, M. L. M.; Martín-Martín, M. L. and Vanhoof, G. C. P., Imidazo[1,2-*a*]pyrazine Derivatives and their use for the Prevention or Treatment of Neurological, Psychiatric and Metabolic Disorders and Diseases, PCT Int. Appl. WO 2011/110545, Sep 15, 2011.
- ¹²⁰ Gijssen, H. J. M.; MacDonald, G. J.; Bischoff, G. J.; Trabanco-Suárez, A. A.; Van Brandt, S. F. A. and Berthelot, D. J-C., Substituted Bicyclic Imidazo Derivatives as Gamma Secretase Modulators, PCT Int. Appl. WO 2010/070008, Jun 24, 2010.
- ¹²¹ Zurbonsen, K.; Michel, A.; Bonnet, P. A.; Mathieu, M. N. and Chevillard, C., Antiproliferative, Differentiating and Apoptotic Effects Elicited by Imidazo[1,2-*a*]pyrazine Derivatives, *Gen. Pharmac.*, **1999**, 32, 135-141.
- ¹²² Barraclough, P.; Black, J. W.; Cambridge, D.; Gerskowitch, P.; Giles, H.; Glen, R. C.; Hull, R. A. D.; Iyer, R.; King, W. R.; Nobbs, M. S.; Randall, P.; Shah, G. P.; Stone, D.; Vine, S. J. and Whiting, M. V., Synthesis and pharmacological properties of BW315C and other inotropic 2-arylimidazo[1,2-*a*]pyrazines, *Bioorg. Med. Chem. Lett.*, **1993**, 3, 4, 509-514.
- ¹²³ Brown, A.; Henderson, A.; Lane, C.; Lansdell, M.; Maw, G. and Monaghan, S., Small molecule inhibitors of IgE synthesis, *Bioorg. Med. Chem. Lett.*, **2006**, 16, 4697-4699.

-
- ¹²⁴ Edmondson, S. D.; Fisher, M. H.; Dooseop, K.; Maccoss, M. and Permee, E. R., Beta-amino tetrahydroimidazo[1,2-*a*]pyrazines and tetrahydrotriazolo[4,3-*a*]pyrazines for the use in the treatment of diabetes type II, Eu Patent Appl. EP 2,292,232, Sep 3, 2001.
- ¹²⁵ Ruel, R.; Chantigny, Y.; Marinier, A.; René, P. and Bouvier, M., Imidazopyridine, Imidazopyrimidine and Imidazopyrazine Derivatives as Melanocortin-4 Receptor Modulators, PCT Int. Appl. WO 2012/003576, Jan 12, 2012.
- ¹²⁶ Bakthavatchalam, R.; Wilde, R. G.; Gilligan, P. J. and Davis, S. B., Imidazo[1,2-*a*]pyrazines for the Treatment of Irritable Bowel Syndrome, US Patent Appl. US 2003/0220342, Nov 27, 2003.
- ¹²⁷ Alvaro, G.; Amantini, D. and Belvedere, S., Piperidine Derivatives useful as Orexin Receptor Antagonists, PCT Int. Appl. WO 2010/060470, Jun 3, 2010.
- ¹²⁸ Starke, I.; Wallmark, B. and Väänänen, K., Imidazo[1,2-*a*]pyridazines or pyrazines for the Treatment of Diseases Related to Bone Loss, PCT Int. Appl. WO 89/03833, May 5, 1989.
- ¹²⁹ Wren, S. P.; Wynne, G. M.; Lecci, C.; Wilson, F. X.; Price, P. D. and Middleton, P., Compounds for Treatment of Duchenne Muscular Dystrophy, PCT Int. Appl. WO 2010/069684, Jun 24, 2010.
- ¹³⁰ Prileschajew, N., Oxydation ungesättigter Verbindungen mittels organischer Superoxyde, *Chem. Ber.*, **1909**, 42, 4811-4815.
- ¹³¹ Imuta, M. and Ziffer, H., Convenient Method for the Preparation of Reactive Oxiranes by Direct Epoxidation, *J. Org. Chem.*, **1979**, 44, 1351-1352.
- ¹³² Corey, E.J. and Chaykovsky, M., Dimethyloxosulfonium Methylide ((CH₃)₂SOCH₂) and Dimethylsulfonium Methylide ((CH₃)₂SCH₂), *J. Am. Chem. Soc.*, **1965**, 87, 1353-1363.
- ¹³³ Favretto, L.; Nugent, W. A. and Licini, G., Highly Regioselective Microwave-assisted Synthesis of Enantiopure C3-Symmetric Trialkanolamine, *Tetrahedron Lett.*, **2002**, 43, 2581-2584.
- ¹³⁴ Fringuelli, F.; Piermatti, O.; Pizzo, F. and Vaccaro, L., Ring Opening of Epoxides with Sodium Azide in Water. A Regioselective pH-Controlled Reaction, *J. Org. Chem.*, **1999**, 64, 6094-6096.
- ¹³⁵ Blazevic, N., Hexamethylenetetramine, A Versatile Reagent in Organic Synthesis, *Synthesis*, **1979**, 161-176.
- ¹³⁶ Sabitha, G., Microwave promoted synthesis of β-aminoalcohols in dry media, *Green Chemistry (RSC)*, **1999**, 251-252.
- ¹³⁷ Lindstrom, U. M., Microwave-assisted aminolysis of vinylepoxides, *Tetrahedron Lett.*, **1999**, 40, 9273-9276.
- ¹³⁸ Boruwa, J.; Borah, J. C.; Kalita, B. and Barua, N. C., Highly regioselective ring opening of epoxides using NaN₃: a short and efficient synthesis of (-)-cytoxazone, *Tetrahedron Lett.*, **2004**, 45, 7355-7358.
- ¹³⁹ Azizi, N., and Saidi, M. R., Highly Chemoselective Addition of Amines to Epoxides in Water, *Org. Lett.*, **2005**, 7, 3649-3651.
- ¹⁴⁰ Koufaki, M.; Kiziridi, C.; Nikoloudaki, F. and Alexis, M. N., Design and Synthesis of 1,2-dithiolane Derivatives and Evaluation of their Neuroprotective Activity, *Bioorg. Med. Chem. Lett.*, **2007**, 17, 4223-4227.

- ¹⁴¹ Ganesh, T.; Kumar, C. H. and Krupadanam, D. G. L., A Facile Synthesis of 2-Hydroxy-2-aryl 1,4-benzodioxanes, *Syn. Comm.*, **1999**, 29, 12, 2069-2078.
- ¹⁴² Shang, G.; Liu, D.; Allen, S. E.; Yang, Q. and Zhang, X., Asymmetric Hydrogenation of α -Primary and Secondary Amino Ketones: Efficient Asymmetric Synthesis of (-)-Arbutamine and (-)-Denopamine, *Chem. Eur. J.*, **2007**, 13, 7780-7784.
- ¹⁴³ Benaissa, T.; Hamman, S. and Beguin, C. G. Synthesis of β -fluoroazides: A Route to Primary β -fluoroamines. *J. Fluorine Chem.*, **1988**, 38, 163-173.
- ¹⁴⁴ Kamal, A.; Shaik, A. A.; Sandbhor, M. and Malik, M.S., Chemoselective synthesis of (R)- and (S)-tembamide, aegeline and denopamine by a one-pot lipase resolution protocol, *Tetrahedron: Asymm.*, **2004**, 15, 3939-3944.
- ¹⁴⁵ Bunnage, M. E.; Burke, A. J.; Davies, S. G.; Millican, N. L.; Nicholson, R. L.; Roberts, P. M. and Smith, A. D., Asymmetric Synthesis of *anti*-(2S,3S)- and *syn*-(2R,3S)-diaminobutanoic acid, *Org. Biomol. Chem.*, **2003**, 1, 3708-3715.
- ¹⁴⁶ Omura, K. and Swern, D. Oxidation of alcohols by activated dimethyl sulfoxide. A preparative, steric and mechanistic study, *Tetrahedron*, **1978**, 34, 1651-1660.
- ¹⁴⁷ Paul, F.; Patt, J.; Hartwig, J. F., Palladium-catalyzed formation of carbon-nitrogen bonds. Reaction intermediates and catalyst improvements in the hetero cross-coupling of aryl halides and tin amides, *J. Am. Chem. Soc.*, **1994**, 116, 13, 5969-5970.
- ¹⁴⁸ Surry, D. S. and Buchwald, S. L., Dialkylbiaryl phosphines in Pd-catalyzed amination: a user's guide, *Chem. Sci.*, **2011**, 2, 27-50.
- ¹⁴⁹ Ikawa, T.; Barder, T. E.; Biscoe, M. R. and Buchwald, S. L., Pd-Catalysed Amidations of Aryl Chlorides Using Monodentate Biaryl Phosphine Ligands: A Kinetic, Computational and Synthetic Investigation, *J. Am. Chem. Soc.*, **2007**, 129, 13001-13007.
- ¹⁵⁰ Parikh, J. R. and Doering, W. von E., Sulfur Trioxide in the Oxidation of Alcohols by Dimethyl Sulfoxide, *J. Am. Chem. Soc.*, **1967**, 89, 5505-5507.
- ¹⁵¹ Lindley, J., Copper assisted nucleophilic substitution of aryl halogen, *Tetrahedron*, **1984**, 40, 1433-1456.
- ¹⁵² Razzuk, A. and Biehl, E. R., The reaction of various methoxy-substituted haloarenes with amines and nitriles under aryne-forming conditions, *J. Org. Chem.*, **1987**, 52, 2619-2622.
- ¹⁵³ Bunnett, J. F., Aromatic substitution by the SRN1 mechanism, *Acc. Chem. Res.*, **1978**, 11, 413-420.
- ¹⁵⁴ Kosugi, M.; Kameyama, M. and Migita, T., Palladium-Catalyzed Aromatic Amination of Aryl Bromides with N,N-Di-Ethylamino-Tributyltin, *Chem. Lett.*, **1983**, 927-928.
- ¹⁵⁵ Guram, A. S.; Rennels, R. A. and Buchwald, S. L., A Simple Catalytic Method for the Conversion of Aryl Bromides to Arylamines, *Angew. Chem. Int. Ed.*, **1995**, 34, 1348-1350.
- ¹⁵⁶ Louie, J. and Hartwig, J. F., Palladium-catalyzed synthesis of arylamines from aryl halides. Mechanistic studies lead to coupling in the absence of tin reagents, *Tetrahedron Lett.*, **1995**, 36, 21, 3609-3612.

-
- ¹⁵⁷ Wolfe, J. P.; Wagaw, S. and Buchwald, S. L., An Improved Catalyst System for Aromatic Carbon–Nitrogen Bond Formation: The Possible Involvement of Bis(Phosphine) Palladium Complexes as Key Intermediates, *J. Am. Chem. Soc.*, **1996**, *118*, 7215–7216.
- ¹⁵⁸ Driver, M. S. and Hartwig, J. F., A Second-Generation Catalyst for Aryl Halide Amination: Mixed Secondary Amines from Aryl Halides and Primary Amines Catalyzed by (DPPF)PdCl₂, *J. Am. Chem. Soc.*, **1996**, *118*, 7217–7218.
- ¹⁵⁹ Guari, Y.; van Es, D. S.; Reek, J. N. H.; Kamer, P. C. J. and van Leeuwen, P., An efficient, palladium-catalysed, amination of aryl bromides, *Tetrahedron Lett.*, **1999**, *40*, 3789–3790.
- ¹⁶⁰ Shen, Q.; Ogata T. and Hartwig, J. F., Highly Reactive, General and Long-Lived Catalysts for Palladium-Catalyzed Amination of Heteroaryl and Aryl Chlorides, Bromides, and Iodides: Scope and Structure–Activity Relationships, *J. Am. Chem. Soc.*, **2008**, *130*, 6586–6596.
- ¹⁶¹ Marion, N.; Navarro, O.; Mei, J. G.; Stevens, E. D.; Scott, N. M. and Nolan, S. P., Modified (NHC)Pd(allyl)Cl (NHC = N-Heterocyclic Carbene) Complexes for Room-Temperature Suzuki–Miyaura and Buchwald–Hartwig Reactions, *J. Am. Chem. Soc.*, **2006**, *128*, 4101–4111.
- ¹⁶² Fleckenstein, C. A.; Plenio, H., Sterically demanding trialkylphosphines for palladium-catalyzed cross coupling reactions—alternatives to PtBu₃, *Chem. Soc. Rev.*, **2010**, *39*, 694–711.
- ¹⁶³ Old, D. W.; Wolfe, J. P. and Buchwald, S. L., A Highly Active Catalyst for Palladium-Catalyzed Cross-Coupling Reactions: Room-Temperature Suzuki Couplings and Amination of Unactivated Aryl Chlorides, *J. Am. Chem. Soc.*, **1998**, *120*, 9722–9723.
- ¹⁶⁴ Kataoka, N.; Shelby, Q.; Stambuli, J. P. and Hartwig, J. F., Air Stable, Sterically Hindered Ferrocenyl Dialkylphosphines for Palladium Catalysed C–C, C–N and C–O Bond-Forming Cross-Couplings, *J. Org. Chem.*, **2002**, *67*, 5553–5566.
- ¹⁶⁵ Burton, G.; Cao, P.; Li, G. and Rivero, R., Palladium-Catalysed Intermolecular Coupling of Aryl Chlorides and Sulfonamides under Microwave Irradiation, *Org. Lett.*, **2003**, *5*, 23, 4373–4376.
- ¹⁶⁶ Baffoe, J.; Hoe, M. Y. and Touré, B. B., Copper-Mediated *N*-Heteroarylation of Primary Sulfonamides: Synthesis of Mono-*N*-heteroaryl Sulfonamides, *Org. Lett.*, **2010**, *12*, 7, 1532–1535.
- ¹⁶⁷ Deng, W.; Liu, L.; Zhang, C.; Liu, M.; Guo, Q-X., Copper-catalysed cross-coupling of sulfonamides with aryl iodides and bromides facilitated by amino acid ligands, *Tetrahedron Lett.*, **2005**, *46*, 7295–7298.
- ¹⁶⁸ Schirmacher, R.; Wängler, B.; Schirmacher, E.; August, T. and Rösch, F., Dimethylpyridin-4-ylamino-Catalysed Alcoholysis of 2-Amino-*N,N,N*-trimethyl-9*H*-purine-6-ylammonium Chloride: An Effective Route to O⁶-Substituted Guanine Derivatives from Alcohols with Poor Nucleophilicity, *Synthesis*, **2002**, *4*, 538–542.
- ¹⁶⁹ Huang, X. and Buchwald, S. L., New Ammonia Equivalents for the Pd-Catalyzed Amination of Aryl Halides, *Org. Lett.*, **2001**, *3*, 21, 3417–3419.
- ¹⁷⁰ Patrick, G. L., *An Introduction to Medicinal Chemistry*, 4th ed.; Oxford University Press, 2009.
- ¹⁷¹ Miller, A. and Tanner, J., *Essentials of Chemical Biology: Structure and Dynamics of Biological Macromolecules*, Wiley, 2009.

- ¹⁷² Tetko, I. V.; Gasteiger, J.; Todeschini, R.; Mauri, A.; Livingstone, D.; Ertl, P.; Palyulin, V. A.; Radchenko, E. V.; Zefirov, N. S.; Makarenko, A. S.; Tanchuk, V. Y. and Prokopenko, V. V., Virtual computation chemistry laboratory – design and description, *J. Comput. Aid. Mol. Des.*, **2005**, 19, 453-63.
- ¹⁷³ Johnson, K. A. and Goody, R. S., The Original Michaelis Constant: Translation of the 1913 Michaelis-Menten Paper, *Biochem.*, **2011**, 50, 8264-8269.
- ¹⁷⁴ Young, D. C., *Computational Drug Design, A Guide for Computational and Medicinal Chemists*, Wiley, 2009.
- ¹⁷⁵ <http://www.rcsb.org/pdb/home/home.do> accessed 21st August 2012
- ¹⁷⁶ Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Goodsell, D. S. and Olson, A. J., Autodock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility, *J. Comput. Chem.*, **2009**, 30, 2785-2791.
- ¹⁷⁷ Trott, O. and Olson, A. J., AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization and Multithreading, *J. Comput. Chem.*, **2010**, 31, 455-461.
- ¹⁷⁸ Wolf, L. K., New Software and Websites for the Chemical Enterprise, *Chem. Eng. News*, **2009**, 87, (11), 56.
- ¹⁷⁹ The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.
- ¹⁸⁰ Jackson, S. K.; Banfield, S. C. and Kerr, M. A., Total Synthesis of (±)-Herbindole B and (±)-*cis*-Triketrin B, *Org Lett*, **2005**, 7, 1215-1218.
- ¹⁸¹ Bevers, S.; Schutte, S. and McLaughlin, L. W., Naphthalene- and Perylene-Based Linkers for the Stabilisation of Hairpin Triplexes, *J. Am. Chem. Soc.*, **2000**, 122, 5905-5915.
- ¹⁸² Lin, Y. A.; Chalker, J. M.; Floyd, N.; Bernardes, G. J. L.; Davis, B. G., Allyl Sulfides are Privileged Substrates in Aqueous Cross Metathesis: Application to Site-Selective Protein Modification, *J. Am. Chem. Soc.*, **2008**, 130, 9642-9643.
- ¹⁸³ Fontana, F.; Minisci, F.; Barbosa, M. and Vismara, E., Homolytic Acylation of Protonated Pyridines and Pyrazines with α -Keto Acids: The Problem of Monoacylation, *J. Org. Chem.*, **1991**, 56, 8, 2866-2869.
- ¹⁸⁴ Rees, D. C.; Congreve, M.; Murray, C. W. and Carr, R., Fragment-Based Lead Discovery, *Nat. Rev. Drug. Discovery*, **2004**, 3, 660-672.
- ¹⁸⁵ *Reaxys*, version 1.0.5986; Elsevier Properties SA: Frankfurt, Germany, 2010.
- ¹⁸⁶ Djung, J. F. J.; Golebiowski, A.; Hunter, J. A. and Shrum, G. P., 2-Anilino-4-(Heterocyclic) Amino-Pyrimidines, US Patent Appl US2007/0293494, Dec 20, 2007.
- ¹⁸⁷ Bromidge, S. M.; Dabbs, S.; Davies, D. T.; Davies, S.; Duckworth, D. M.; Forbes, I. T.; Gaster, L. M.; Ham, P.; Jones, G. E.; King, F.; D.; Mulholland, K. R.; Saunders, D. V.; Wyman, P. A.; Blaney, F. E.; Clarke, S. E.; Blackburn, T. P.; Holland, V.; Kennett, G. A.; Lightowler, S.; Middlemiss, D. N.; Trail, B.; Riley, G. J. and Wood, M. D., Biarylcarbamoylindolines Are Novel and Selective 5-HT_{2C} Receptor Inverse Agonists: Identification of 5-Methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-6-trifluoromethylindoline (SB-243213) as a Potential Antidepressant/Anxiolytic Agent, *J. Med. Chem.*, **2000**, 43, 6, 1123-1134.

-
- ¹⁸⁸ Teuber, L. and Larsen, J. S., Benzimidazole Derivatives and their use for Modulating the GABA_A Receptor Complex, PCT Int. Appl. WO2004/087137, Oct 14, 2004.
- ¹⁸⁹ Carpino, L. A.; Chao, H. G. and Tien, J. H., Investigation of the reaction between amino acids or amino acid esters and 9-formylfluorene and its equivalents. Possible utility of the derived enamines as amino group protectants, *J. Org. Chem.*, **1989**, *54*, 4302-4313.
- ¹⁹⁰ To, K.; Spears, G. W.; Takahashi, F.; Yamada, A.; Tomishima, M. and Miyake, H., Amide Compounds, US Patent Appl. US6770667, Aug 3, 2004.
- ¹⁹¹ Broom, A. D., Rational Design of Enzyme Inhibitors: Multisubstrate Analogue Inhibitors, *J. Med. Chem.*, **1989**, *32*, 2-7.
- ¹⁹² Parang, K. and Cole, P. A., Designing bisubstrate analog inhibitors for protein kinases, *Pharmacol. Ther.*, **2002**, *93*, 145-157.
- ¹⁹³ Stebbins, J. L.; De, S. K.; Pavlickova, P.; Chen, V.; Machleidt, T.; Chen, L-H.; Kuntzen, C.; Kitada, S.; Karin, M. and Pellecchia, M., Design and Characterisation of a Potent and Selective Dual ATP- and Substrate-Competitive Subnanomolar Bidentate c-Jun N-Terminal Kinase (JNK) Inhibitor, *J. Med. Chem.*, **2011**, *54*, 6206-6214.
- ¹⁹⁴ Lienhard, G. E. and Secemski, I. I., P¹,P⁵-Di(adenosine-5')pentaphosphate, a Potent Multisubstrate Inhibitor of Adenylate Kinase, *J. Biol. Chem.*, **1973**, *248*, 1121-1123.
- ¹⁹⁵ Fletcher, S. and Hamilton, A. D., Targeting protein-protein interactions by rational design: mimicry of protein surfaces, *J. R. Soc. Interface*, **2006**, *3*, 215-233.
- ¹⁹⁶ Jekle, A.; Chow, E.; Kopetzki, E.; Ji, C.; Yan, M. J.; Nguyen, R.; Sankuratri, S.; Cammack, N. and Heilek, G., CD4-BFFI: A novel, bifunctional HIV-1 entry inhibitor with high and broad antiviral potency, *Antiviral Res.*, **2009**, *83*, 257-266.
- ¹⁹⁷ Tamburino, R.; Pizzo, E.; Sarcinelli, C.; Poerio, E.; Tedeschi, F.; Ficca, A. G.; Parente, A. and Di Maro, A., Enhanced cytotoxic activity of a bifunctional chimeric protein containing a type 1 ribosome-inactivating protein and a serine protease inhibitor *Biochimie*, **2012**, *94*, 1990-1996 .
- ¹⁹⁸ de Koning, M. C.; van der Marel, G. A. and Overhand, M., Synthetic developments towards PNA-peptide conjugates, *Curr. Opin. Chem. Biol.*, **2003**, *7*, 734-740.
- ¹⁹⁹ Parang, K.; Till, J. H.; Ablooglu, A. J.; Kohanski, R. A.; Hubbard, S. R. and Cole, P. A., Mechanism-based design of a protein kinase inhibitor, *Nat. Struct. Biol.*, **2001**, *8*, 37-41.
- ²⁰⁰ Ablooglu, A. J.; Till, J. H.; Kim, K.; Parang, K.; Cole, P. A.; Hubbard, S. R. and Kohanski, R. A., Probing the Catalytic Mechanism of the Insulin Receptor Kinase with a Tetrafluorotyrosine-containing Peptide Substrate, *J. Biol. Chem.*, **2000**, *275*, 30394-30398.
- ²⁰¹ Hines, A. C. and Cole, P. A., Design, synthesis and characterization of an ATP-peptide conjugate inhibitor of protein kinase A, *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 2951-2954.
- ²⁰² de la Houssaye, B. and Masaracchia, R. A., Standardization of the Assay for the Catalytic Subunit of Cyclic AMP-Dependent Protein Kinase Using a Synthetic Peptide Substrate, *Anal. Biochem.*, **1983**, *128*, 54-59.

-
- ²⁰³ Shen, K. and Cole, P. A., Conversion of a Tyrosine Kinase Protein Substrate to a High Affinity Ligand by ATP Linkage, *J. Am. Chem. Soc.*, **2003**, *125*, 16172-16173.
- ²⁰⁴ Schneider, T. L.; Mathew, R. S.; Rice, K. P.; Tamaki, K.; Wood, J. L. and Schepartz, A., Increasing the Kinase Specificity of K252a by Protein Surface Recognition, *Org. Lett.*, **2005**, *7*, 1695-1698.
- ²⁰⁵ Kase, H.; Iwahashi, K.; Nakanishi, S.; Matsuda, Y.; Yamada, K.; Takahashi, M.; Murakata, C.; Sata, A. and Kaneko, M., K-252 Compounds, Novel and Potent Inhibitors of Protein Kinase C and Cyclic Nucleotide-dependent Protein Kinases, *Biochem. Biophys. Res. Commun.*, **1987**, *142*, 436-440.
- ²⁰⁶ Bain, J.; McLauchlan, H.; Elliot, M. and Cohen, P., The Specificities of Protein Kinase Inhibitors: an Update, *Biochem. J.*, **2003**, *371*, 199-204.
- ²⁰⁷ Enkvist, E.; Lavogina, D.; Raidaru, G.; Vaasa, A.; Viil, I.; Lust, M.; Viht, K. and Uri, A., Conjugation of Adenosine and Hexa-(D-arginine) Leads to a Nanomolar Bisubstrate-Analog Inhibitor of Basophilic Protein Kinases, *J. Med. Chem.*, **2006**, *49*, 7150-7159.
- ²⁰⁸ Lavogina, D.; Lust, M.; Viil, I.; König, N.; Raidaru, G.; Rogozina, J.; Enkvist, E.; Uri, A. and Bossemeyer, D., Structural Analysis of ARC-Type Inhibitor (ARC-1034) Binding to Protein Kinase A Catalytic Subunit and Rational Design of Bisubstrate Analogue Inhibitors of Basophilic Protein Kinases, *J. Med. Chem.*, **2009**, *52*, 308-321.
- ²⁰⁹ Enkvist, E.; Kriisa, M.; Roben, M.; Kadak, G.; Raidaru, G. and Uri, A., Effect of the Structure of Adenosine Mimic of Bisubstrate-Analog Inhibitors on their activity Towards Basophilic Protein Kinases, *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 6098-6101.
- ²¹⁰ Ricouart, A.; Gesquiere, J. C.; Tartar, A. and Sergheraert, C., Design of Potent Protein Kinase Inhibitors Using the Bisubstructure Approach, *J. Med. Chem.*, **1991**, *34*, 73-78.
- ²¹¹ Enkvist, E.; Raidaru, G.; Vaasa, A.; Pehk, T.; Lavogina, D. and Uri, A., Carbocyclic 3'-deoxyadenosine-based Highly Potent Bisubstrate-analog Inhibitor of Basophilic Protein Kinases, *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 5336-5339.
- ²¹² Hill, Z. B.; Perera, G. K. and Maly, D. J., A Chemical Genetic Method for Generating Bivalent Inhibitors of Protein Kinases, *J. Am. Chem. Soc.*, **2009**, *131*, 6686-6688.
- ²¹³ Hill, Z. B.; Perera, G. K. and Maly, D. J., Bivalent Inhibitors of the Tyrosine Kinases ABL and SRC: Determinants of Potency and Selectivity, *Mol. Biosyst.*, **2011**, *7*, 447-456.
- ²¹⁴ Hill, Z. B.; Perera, G. K.; Andrews, S. S. and Maly, D. J., Targeting Diverse Signalling Interaction Sites Allows the Rapid Generation of Bivalent Kinase Inhibitors, *ACS Chem. Biol.*, **2012**, *7*, 487-495.
- ²¹⁵ Profit, A. A.; Lee, T. R. and Lawrence, D. S., Bivalent Inhibitors of Protein Tyrosine Kinases, *J. Am. Chem. Soc.*, **1999**, *121*, 280-283.
- ²¹⁶ Meyer, S. C.; Shomin, C. D.; Gaj, T. and Ghosh, I., Tethering Small Molecules to a Phage Displayed Library: Discovery of a Selective Bivalent Inhibitor of Protein Kinase A, *J. Am. Chem. Soc.*, **2007**, *129*, 13812-13813.
- ²¹⁷ Ladner, R. C.; Sato, A. K.; Gorzelany, J. and de Souza, M., Phage Display-Derived Peptides as Therapeutic Alternatives to Antibodies, *Drug Discovery Today*, **2004**, *9*, 525-529.

-
- ²¹⁸ Shomin, C. D.; Restituyo, E.; Coz, K. J. and Ghosh, I., Selection of Cyclic-Peptide Inhibitors Targeting Aurora Kinase A: Problems and Solutions, *Bioorg. Med. Chem.*, **2011**, *19*, 6743-6749.
- ²¹⁹ Tate, E. W.; Bowyer, P. W.; Brown, K. A.; Smith, D. F.; Holder, A. A. and Leatherbarrow, R. J., Peptide-based inhibitors of N-myristoyl transferase generated from a lipid/combinatorial peptide chimera library, *Signal Transduction*, **2006**, *6*, 160-166.
- ²²⁰ Edman, P., Method for Determination of the Amino Acid Sequence in Peptides, *Acta Chem. Scand.*, **1950**, *4*, 283-293.
- ²²¹ Walsh, C. T.; Garneau-Tsodikova, S.; Gatto, G. J.; Protein Posttranslational Modifications: The Chemistry of Proteome Diversifications, *Angew. Chem. Int. Ed.*, **2005**, *44*, 7342-7372.
- ²²² Chalker, J. M.; Bernardes, G. J. L. and Davis, B. G., A Tag-and-Modify Approach to Site-Selective Protein Modification, *Acc. Chem. Res.*, **2011**, *44*, 99, 730-7410.
- ²²³ Lin, Y. A.; Chalker, J. M. and Davis, B. G., Olefin Metathesis for Site-selective Protein Modification, *ChemBioChem.*, **2009**, *10*, 959-969.
- ²²⁴ Carrico, I. S., Chemoselective modification of proteins: hitting the target, *Chem. Soc. Rev.*, **2008**, *37*, 1423-1431.
- ²²⁵ Foley, T. L.; Burkart, M. D., Site-specific protein modification: advances and applications, *Curr. Opin. Chem. Biol.*, **2007**, *11*, 12-19.
- ²²⁶ Astronomo, R. D.; Lee, H. K.; Scanlan, C. N.; Pantophlet, R.; Huang, C. Y.; Wilson, I. A.; Blixt, O.; Dwek, R. A.; Wong, C. H.; Burton, D. R., A Glycoconjugate Antigen Based on the Recognition Motif of a Broadly Neutralizing Human Immunodeficiency Virus Antibody, 2G12, Is Immunogenic but Elicits Antibodies Unable To Bind to the Self Glycans of gp120, *J. Virol*, **2008**, *82*, 6359-6368.
- ²²⁷ Krug, L. M.; Ragupathi, G.; Ng, K. K.; Hood, C.; Jennings, H. J.; Guo, Z.; Kris, M. G.; Miller, V.; Pizzo, B.; Tyson, L.; Baez, V.; Livingston, P. O., Vaccination of Small Cell Lung Cancer Patients with Polysialic Acid or N-Propionylated Polysialic Acid Conjugated to Keyhole Limpet Hemocyanin, *Clin. Cancer. Res.* **2004**, *10*, 916-923.
- ²²⁸ Seeberger, P. H.; Wertz, D. B., Synthesis and medical applications of oligosaccharides, *Nature*, **2007**, *446*, 1046-1051.
- ²²⁹ Herrerías, C. I.; Yao, X.; Li, Z.; Li, C. J., Reactions of C–H Bonds in Water, *Chem. Rev.*, **2007**, *107*, 2546-2562.
- ²³⁰ Li, C. J., Organic Reactions in Aqueous Media with a Focus on Carbon–Carbon Bond Formations: A Decade Update, *Chem. Rev.*, **2005**, *105*, 3095-3166.
- ²³¹ Antos, J. M.; Francis, M. B., Transition metal catalyzed methods for site-selective protein modification, *Curr. Opin. Chem. Biol.*, **2006**, *10*, 253-262.
- ²³² Antos, J. M.; Francis, M. B., Selective Tryptophan Modification with Rhodium Carbenoids in Aqueous Solution, *J. Am. Chem. Soc.*, **2004**, *126*, 10256-10257.
- ²³³ Tilley, D. D.; Francis, M. B., Tyrosine-Selective Protein Alkylation Using π -Allylpalladium Complexes, *J. Am. Chem. Soc.*, **2006**, *128*, 1080-1081.

- ²³⁴ Kodama, K.; Fukuzawa, S.; Nakayama, H.; Sakamoto, K.; Kigawa, T.; Yabuki, T.; Matsuda, N.; Shirouzu, M.; Takio, K.; Yokoyama, S.; Tachibana, K., Site-Specific Functionalization of Proteins by Organopalladium Reactions, *ChemBioChem.*, **2007**, *8*, 232-238.
- ²³⁵ Kodama, K.; Fukuzawa, S.; Nakayama, H.; Kigawa, T.; Sakamoto, K.; Yabuki, T.; Matsuda, N.; Shirouzu, M.; Takio, K.; Tachibana, K.; Yokoyama, S., Regioselective Carbon–Carbon Bond Formation in Proteins with Palladium Catalysis; New Protein Chemistry by Organometallic Chemistry, *ChemBioChem.*, **2006**, *7*, 134-139.
- ²³⁶ Brustad, E.; Bushey, M. L.; Lee, J. W.; Groff, D.; Liu, W.; Schultz, P. G., A Genetically Encoded Boronate-Containing Amino Acid, *Angew. Chem. Int. Ed.*, **2008**, *47*, 8220-8223.
- ²³⁷ Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G., Bioconjugation by Copper(I)-Catalysed Azide-Alkyne [3+2] Cycloaddition *J. Am. Chem. Soc.*, **2003**, *125*, 3192-3193.
- ²³⁸ Gupta, S. S.; Kuzulka, J.; Singh, P.; Lewis, W. G.; Manchester, M.; Finn, M. G., Accelerated Bioorthogonal Conjugation: A Practical Method for the Ligation of Diverse Functional Molecules to a Polyvalent Virus Scaffold, *Bioconj. Chem.*, **2005**, *16*, 1572-1579.
- ²³⁹ Lin, P.-C.; Ueng, S.-H.; Tseng, M.-C.; Ko, J.-L.; Huang, K.-T.; Yu, S.-C.; Adak, A. K.; Chen, Y.-J.; Lin, C.-C., Site-Specific Protein Modification through Cu^I-Catalysed 1,2,3-Triazole Formation and its Implementation in Protein Microarray Fabrication, *Angew. Chem.*, **2006**, *118*, 4392-4396.
- ²⁴⁰ van Kasteren, S. I.; Kramer, H. B.; Jensen, H. H.; Campbell, S. J.; Kirkpatrick, J.; Oldham, N. J.; Anthony, D. C.; Davis, B.G., Expanding the diversity of chemical protein modification allows post-translation mimicry, *Nature*, **2007**, *446*, 1105-1109.
- ²⁴¹ Lin, Y. A.; Chalker, J. M.; Boutureira, O.; Davis, B. G., Enabling olefin metathesis on proteins: chemical methods for installation of *S*-allyl cysteine, *Chem. Commun.*, **2009**, 3714-3716.
- ²⁴² Henchey, L. K.; Jochim A. L.; Arora, P. S., Contemporary strategies for the stabilization of peptides in the α -helical conformation *Curr. Opin. Chem. Biol.*, **2008**, *12*, 692-697.
- ²⁴³ Miller, S. J.; Grubbs, R. H., Synthesis of Conformationally Restricted Amino Acids and Peptides Employing Olefin Metathesis, *J. Am. Chem. Soc.*, **1995**, *117*, 5855-5836.
- ²⁴⁴ Miller, S. J.; Blackwell, H. E.; Grubbs, R. H., Application of Ring-Closing Metathesis to the Synthesis of Rigidified Amino Acids and Peptides, *J. Am. Chem. Soc.*, **1996**, *118*, 9606-9614.
- ²⁴⁵ Ghalit, N.; Reichwein, J. F.; Hilbers, H. W.; Breukink, E.; Rijkers D. T. S.; Liskamp, R. M. J., Synthesis of Bicyclic Alkene-/Alkane-Bridged Nisin Mimics by Ring-Closing Metathesis and their Biochemical Evaluation as Lipid II Binders: toward the Design of Potential Novel Antibiotics, *ChemBioChem* **2007**, *8*, 1540–1554.
- ²⁴⁶ Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H., Efficient and Recyclable Monomeric and Dendritic Ru-Based Metathesis Catalysts, *J. Am. Chem. Soc.*, **2000**, *122*, 8168-8179.
- ²⁴⁷ Kolb, H. C.; Finn, M. G.; Sharpless, K. B., Click Chemistry: Diverse Chemical Function from a Few Good Reactions, *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021.
- ²⁴⁸ Sletten, E. M and Bertozzi, C. R., Bioorthogonal Chemistry: Fishing for Selectivity in a Sea of Functionality, *Angew. Chem. Int. Ed.*, **2009**, *48*, 6974-6998.

-
- ²⁴⁹ Saxon, E. and Bertozzi, C. R., Cell Surface Engineering by a Modified Staudinger Reaction, *Science*, **2000**, 287, 2007-2010.
- ²⁵⁰ Hangauer, M. J and Bertozzi, C. R., A FRET-Based Fluorogenic Phosphine for Live-Cell Imaging with the Staudinger Ligation, *Angew. Chem. Int. Ed.*, **2008**, 47, 2394-2397.
- ²⁵¹ Huisgen, R., 1,3-Dipolar Cycloadditions, *Proc. Chem. Soc.*, **1961**, 357-369.
- ²⁵² Sletten, E. M. and Bertozzi, C. R., A Hydrophilic Azacyclooctyne for Cu-Free Click Chemistry, *Org. Lett.*, **2008**, 10, 3097-3099.
- ²⁵³ Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A. and Bertozzi, C. R., *Proc. Nat. Acad. Sci.*, **2007**, 104, 16793-16797.
- ²⁵⁴ Laughlin, S. T.; Baskin, J. M.; Sharon L. Amacher, S. L. and Bertozzi, C. R., In Vivo Imaging of Membrane-Associated Glycans in Developing Zebrafish, *Science*, **2008**, 320, 664-667.
- ²⁵⁵ Roberts, M. J.; Bentley, M. D.; Harris, J. M.; Chemistry for peptide and protein PEGylation, *Adv. Drug Deliv. Rev.*, **2002**, 54, 459-476.
- ²⁵⁶ Veronese, F. M.; Peptide and protein PEGylation: a review of problems and solutions, *Biomaterials*, **2001**, 22, 405417.
- ²⁵⁷ Smith, M. E. B.; Schumacher, F. F.; Ryan, C. P.; Tedaldi, L. M.; Papaioannou, D.; Waksman, G.; Caddick, S.; Baker, J. R.; Protein modification, bioconjugation, and disulfide bridging using bromomaleimides, *J. Am. Chem. Soc.*, **2010**, 132, 1960-1965.
- ²⁵⁸ Scriven, E. F. V. and Turnbull, K., Azides: Their Preparation and Synthetic Uses, *Chem. Rev.*, **1988**, 88, 297-368.
- ²⁵⁹ *Molecular Operating Environment (MOE)*, 2011.10; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, **2011**.
- ²⁶⁰ Jung, M. E.; Ouk, S.; Yoo, D.; Sawyers, C. L.; Chen, C.; Tran, C. and Wongvipat, J., Structure-Activity Relationship for Thiohydantoin Androgen Receptor Antagonists for Castration-Resistant Prostate Cancer (CRPC), *J. Med. Chem.*, **2010**, 53, 7, 2779-2796.
- ²⁶¹ Hu, B.; Ellingboe, J.; Han, S.; Largis, E.; Lim, K.; Malamas, M.; Mulvey, R.; Niu, C.; Oliphant, A.; Pelletier, J.; Singanalore, T.; Sum, F. W.; Tillett, J. and Wong, V., Novel (4-Piperidin-1-yl)-phenyl Sulfonamides as Potent and Selective Human β_3 Agonists, *Bioorg. Med. Chem.*, **2001**, 9, 2045-2059.
- ²⁶² Kim, J. G. and Jang, D. O., Direct Synthesis of Acyl Azides from Carboxylic Acids by the Combination of Trichloroacetonitrile, Triphenylphosphine and Sodium Azide, *Synlett*, **2008**, 13, 2072-2074.
- ²⁶³ Naito, R.; Takeuchi, M.; Morihira, K.; Hayakawa, M.; Ikeda, K.; Shibamura, T. and Isomura, Y., Selective Muscarinic Antagonists. II. Synthesis and Antimuscarinic Properties of Biphenylcarbamate Derivatives, *Chem. Pharm. Bull.* **1998**, 46, 8, 1286-1294.
- ²⁶⁴ Woo, Y. H.; Fernandes, R. P. M. and Proteau, P. J., Evaluation of fosmidomycin analogs as inhibitors of the *Synechocystis* sp. PCC6803 1-deoxy-D-xylulose 5-phosphate reductoisomerase, *Bioorg. Med. Chem.*, **2006**, 14, 2375-2385.

- ²⁶⁵ Cavelier, F. And Enjalbal, C., Studies of Selective Boc Removal in the Presence of Silyl Ethers, *Tetrahedron. Lett.*, **1996**, 37, 29, 5131-5134.
- ²⁶⁶ Bernardo, P. H.; Wan, K. F.; Sivaranman, T.; Xu, J.; Moore, F. K.; Hung, A. W.; Mok, H. Y. K.; Yu, V. C. and Chai, C. L. L., Structure-Activity Relationship Studies of Phenanthridine-Based Bcl-X_L Inhibitors, *J. Med. Chem.*, **2008**, 51, 6999-6710.
- ²⁶⁷ Hurley, C. A.; Wong, J. B.; Ho, J.; Writer, M.; Irvine, S. A.; Lawrence, M. J.; Hart, S. L.; Tabor, A. B. and Hailes, H. C., Mono- and dicationic short PEG and methylene dioxyalkylglycerols for use in synthetic gene delivery systems. *Org. Biomol. Chem.*, **2008**, 6, 2554-2559.
- ²⁶⁸ Zhu, J.; Kell, A. J. and Workentin, M. S., A Retro-Diels-Alder Reaction to Uncover Maleimide-Modified Surfaces on Monolayer-Protected Nanoparticle for Reversible Covalent Assembly, *Org Lett*, **2006**, 8, 22, 4993-4996.
- ²⁶⁹ Yeo, W. S.; Min, D. H.; Hsiesh, R. W.; Greene, G. L. and Mrksich, M., Label-Free Detection of Protein-Protein Interactions on Biochips, *Angew. Chem. Int. Ed.*, **2005**, 44, 5480-5483.
- ²⁷⁰ Gill, H. S.; Tinianow, J. N.; Ogasawara, A.; Flores, J. E.; Vanderbilt, A. N.; Raab, H.; Scheer, J. M.; Vandlen, R.; Williams, S. P. and Marik, J., A Modular Platform for the Rapid Site-Specific Radiolabelling of Proteins with ¹⁸F Exemplified by Quantitative Positron Emission Tomography of Human Epidermal Growth Factor Receptor 2, *J. Med. Chem.*, **2009**, 52, 5816-5825.
- ²⁷¹ De, P.; Li, M.; Gondi, S. R. and Sumerlin, B. S., Temperature-Regulated Activity of Responsive Polymer-Protein Conjugates Prepared by Grafting-from via RAFT Polymerization, *J. Am. Chem. Soc.*, **2008**, 130, 11288-11289.
- ²⁷² Sakagami, M.; Horie, K.; Nakamoto, K.; Kawaguchi, T. and Hamana, H., Synthesis of Sialyl Lewis X-Polysaccharide Conjugates, *Chem. Pharm. Bull.*, **2000**, 48, 1256-1263.
- ²⁷³ Greenwald, R. B.; Choe, Y. H.; Conover, C. D.; Shum, K.; Wu, D. and Royzen, M., Drug Delivery Systems Based on Trimethyl Lock Lactonization: Poly(ethylene glycol) Prodrugs of Amino-Containing Compounds, *J. Med. Chem.* **2000**, 43, 475-487.
- ²⁷⁴ Nieman, J. A.; Nair, S. K.; Heasley, S. E.; Schultz, B. L.; Zerth, H. M.; Nugent, R. A.; Chen, K.; Stephanski, K. J.; Hopkins, T. A.; Knechtel, M. L.; Oien, N. L.; Wieber, J. L. and Wathen, M. W., Modifications of C-2 on the pyrroloquinoline template aimed at the development of potent herpesvirus antivirals with improved aqueous solubility, *Bioorg. Med. Chem. Lett.*, **2010**, 20, 3039-3042.
- ²⁷⁵ Haag, T.; Hughes, R. A.; Ritter, G. and Schmidt, R., Carbohydrate-Bases VEGF Inhibitors, *Euro. J. Org. Chem.*, **2007**, 6016-6033.
- ²⁷⁶ Srinivas, K. V. N. S.; Mahender, I. and Das, B., Silica Chloride: A Versatile Heterogeneous Catalyst for Esterification and Transesterification, *Synthesis*, **2003**, 16, 2479-2482.
- ²⁷⁷ Bregant, S. and Tabor, A. B., Orthogonally Protected Lanthionines: Synthesis and Use for the Solid-Phase Synthesis of an Analogue of Nisin Ring C, *J. Org. Chem.* **2005**, 70, 7, 2430-2438.
- ²⁷⁸ Mustapa, M. F. M.; Harris, R.; Bulic-Subanovic, N.; Elliot, S. L.; Bregant, S.; Groussier, M. F. A.; Mould, J.; Schultz, D.; Chubb, N. A. L.; Gaffney, P. R. J.; Driscoll, P. C. and Tabor, A. B., Synthesis of Orthogonally Protected Lanthionines, *J. Org. Chem.*, **2003**, 68, 8185-8192.

-
- ²⁷⁹ Bergeron, R. J.; Wollenweber, M. and Wiegand J., An Investigation of Desferrithiocin Metabolism, *J. Med. Chem.*, **1994**, *37*, 2889-2895.
- ²⁸⁰ Schafmeister, C. E.; Po, J. and Verdine, G.L., An all-hydrogen crosslinking system for enhancing the helicity and metabolic stability of peptides, *J. Am. Chem. Soc.*, **2000**, *122*, 5891-5892.
- ²⁸¹ His, T., *Cleavage, Deprotection, and Isolation of Peptides after Fmoc Synthesis*; Technical Bulletin; Applied Biosystems, 1998.
- ²⁸² Chapman, R.; Jolliffe, K. A. and Perrier, S., Synthesis of self assembling cyclic peptide-polymer conjugates using click chemistry, *Aust. J. Chem.*, **2010**, *63*, 1169-1172
- ²⁸³ Beekman, N. J. C. M.; Schaaper, W. M. M.; Tesser, G. I.; Dalsgaard, K.; Kamstrup, S.; Langeveld, J. P. M.; Boshuizen, R. S. and Meloen, R. H., Synthetic peptide vaccines: palmitoylation of peptide antigens by a thioester bond increases immunogenicity, *J. Peptide Res.*, **1997**, *50*, 357-364.
- ²⁸⁴ Holub, J. M.; Jang, H. and Kirshenbaum, K., Clickity-click: highly functionalized peptoid oligomers generated by sequential conjugation reactions on solid-phase support, *Org. Biomol. Chem.*, **2006**, *4*, 1497-1502.
- ²⁸⁵ Turner, R. A.; Oliver, A. G. and Lokey, R. S., Click Chemistry as a Macrocyclization Tool in the Solid-Phase Synthesis of Small Cyclic Peptides, *Org. Lett.*, **2007**, *9*, 5011-5014.
- ²⁸⁶ Macmillan, D. and Blanc, J., A novel neoglycopeptide linkage compatible with native chemical ligation, *Org. Biomol. Chem.*, **2006**, *4*, 2847-2850.
- ²⁸⁷ Kuijpers, B. H. M.; Groothuys, S.; Hawner, C.; ten Dam, J.; Quaedflieg, P. J. L. M.; Schoemaker, H. E.; van Delft, F. L. and Rutjes, F. P. J. T., Cu-Catalysed Formation of Triazole-Linked Glycoamino Acids and Application in Chemoenzymatic Peptide Synthesis, *Org. Process Res. Dev.*, **2008**, *12*, 504-511.
- ²⁸⁸ Brood, Version, 2.0, 2010; OpenEye Scientific Software, Santa Fe, New Mexico.
- ²⁸⁹ Andrews, M. J. I. and Tabor, A. B., Forming stable helical peptides using natural and artificial amino acids, *Tetrahedron*, **1999**, *55*, 11711-11743.
- ²⁹⁰ Kutchukian, P. S.; Yang, J. S. and Verdine, G. L.; Shakhnovich, E.I., All-Atom Model for Stabilization of α -Helical Structure in Peptides by Hydrocarbon Staples, *J. Am. Chem. Soc.*, **2009**, *131*, 4622-4627.
- ²⁹¹ Walensky, L. D.; Kung, A. L.; Escher, I.; Malia, T. J.; Barbuto, S.; Wright, R. D.; Wagner, G.; Verdine, G. L. and Korsmeyer, S. K., Activation of Apoptosis in Vivo by a Hydrocarbon-stapled BH3 Helix, *Science*, **2004**, *305*, 1466-1470.
- ²⁹² Walensky, L. D.; Pitter, K.; Morash, J.; Oh, K. J.; Barbuto, S.; Fisher, J.; Smith, E.; Verdine, G. L. and Korsmeyer, S. K., A Stapled BID BH3 Helix Directly Binds and Activates BAX, *Mol. Cell*, **2006**, *24*, 199-210.
- ²⁹³ Pangborn, A. B.; Ciardello, M. A.; Grubbs, R. H.; Rosen, R. K. and Timmers, F. J., Safe and Convenient Procedure for Solvent Purification, *Organometallics*, **1996**, *15*, 1518-1520.
- ²⁹⁴ Kulasegaram, S. and Kulawiec, R. J., Palladium-Catalysed Isomerisation of Aryl-Substituted Epoxides: A Selective Synthesis of Substituted Benzylic Aldehydes and Ketones, *J. Org. Chem.*, **1997**, *62*, 6547-6561.

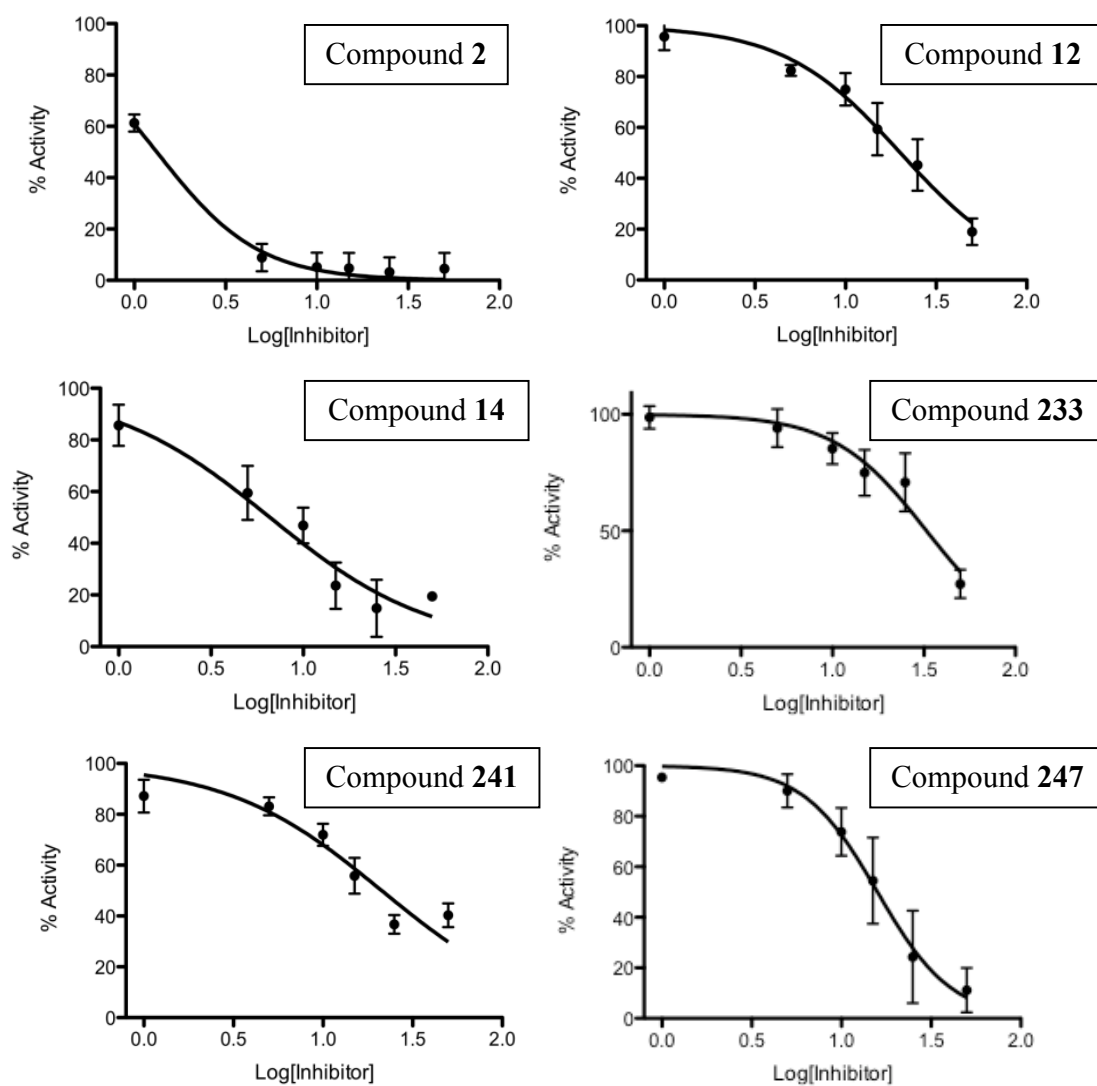
- ²⁹⁵ Von Roman, U., Ruhdorfer, J., Knorr, R., Alkenyl Bromides by Brominative Deoxygenation of Ketones in One or Two Steps, *Synthesis*, **1993**, 10, 985-992.
- ²⁹⁶ La Regina, G.; Bai, R.; Rensen, W.; Coluccia, A.; Piscitelli, F.; Gatti, V.; Bolognesi, A.; Lavecchia, A.; Granata, I.; Porta, A.; Maresca, B.; Soriani, A.; Iannitto, M. L.; Mariani, M.; Santoni, A.; Brancale, A.; Ferlini, C.; Dondio, G.; Varasi, M.; Mercurio, C.; Hamel, E.; Lavia, P., Novellino, E. and Silvestri, R., Design and Synthesis of 2-Heterocyclyl-3-arylthio-1*H*-indoles as Potent Tubulin Polymerisation and Cell Growth Inhibitors with Improved Metabolic Stability, *J. Med. Chem.*, **2011**, 54, 8394-8406.
- ²⁹⁷ Moumne, R.; Larue, V.; Seijo, B.; Lecourt, T.; Micouin, L. and Tisne, C., Tether Influence on the Binding Properties of tRNA^{Lys}₃ Ligands Designed by a fragment-based approach, *Org. Biomol. Chem.*, **2010**, 8, 1154-1159.
- ²⁹⁸ Gong, P.K.; Blough, B.E.; Brieady, L.E.; Huang, X.; Kuhar, M.J.; Navarro, H.A. and Carroll, I., Synthesis, Monoamine Transporter Binding, Properties, and Functional Monoamine Uptake Activity of 3*α*-[4-Methylphenyl and 4-Chlorophenyl]-2*α*-[5-(Substituted phenyl)thiazol-2-yl]tropanes, *J. Med. Chem.*, **2007**, 50, 3686-3695.
- ²⁹⁹ Cho, B.T.; Kang, S.K. and Shin, S.H., Application of optically active 1,2-diol monotosylates for synthesis of β -azido and β -amino alcohols with very high enantiomeric purity. Synthesis of enantiopure (*R*)-octopamine, (*R*)-tembamide and (*R*)-aegeline, *Tetrahedron: Asymm.*, **2002**, 13, 1209-1217
- ³⁰⁰ Ina, S.; Yamana, K. and Noda, K., Use of 2-Phenylmorpholin-5-one Derivatives, US Patent Appl. US6265402, Jul 24, 2001.
- ³⁰¹ Ruff, B. M.; Bräse, S. and O'Connor, S. E., Biocatalytic production of tetrahydroisoquinolines, *Tetrahedron Lett.*, **2012**, 53, 1071-1074.
- ³⁰² Togashi, M.; Urano, Y.; Kojima, H.; Terai, T. and Hanaoka, K., Sensitive Detection of Acrolein in Serum Using Time-resolved Luminescence, *Org. Lett.*, **2010**, 12, 1704-1707.
- ³⁰³ Troisi, F.; Russo, A.; Gaeta, C.; Bifulco, G. and Neri, P., Aramidocalix[4]arenes as New Anion Receptors, *Tetrahedron Lett.*, **2007**, 48, 7986-7989.
- ³⁰⁴ Zeng, H.; Li, Y. and Shao, H., Simple and Efficient Method for N-Boc Protection of Amines Using PEG-400 as a Reaction Medium Under Mild Conditions, *Synth. Commun.*, **2012**, 42, 25-32.
- ³⁰⁵ Dragovich, P. S.; Murphy, D. E., Tran, C. V. and Ruebsam, F., Efficient Large-Scale Synthesis of 2-Amino-5-Methanesulfonylaminobenzenesulfonamide, *Synth. Commun.*, **2008**, 38, 1909-1916.
- ³⁰⁶ Moreno, H. R. and Schultz, H. P., Quinoxaline Studies. XV. Potential Antimalarials. Some (*RS*)- α -(Dialkylaminomethyl)-2-quinoxalinemethanols, *J. Med. Chem.*, **1970**, 13, 119-121.
- ³⁰⁷ Rowe, D. J.; Garner, C. D. and Joule, J. A., The Preparation of 1-Aryl- and 1-Heteroaryl-alkene-1,2-dithiolates, *J. Chem. Soc. Perkin Trans. 1*, **1985**, 1907-1910.
- ³⁰⁸ Manolikakes, G.; Hernandez, C. M.; Schade, M. A.; Metzger, A.; Knochel, P., Palladium- and Nickel-Catalysed Cross-Couplings of Unsaturated Halides Bearing Relatively Acidic Protons with Organozinc Compounds, *J. Org. Chem.*, **2008**, 73, 8422-8436.
- ³⁰⁹ Kim, S. H. and Rieke, R. D., 2-Pyridyl and 3-pyridylzinc bromides: direct preparation and coupling reaction, *Tetrahedron*, **2010**, 66, 3135-3146.

- ³¹⁰ Anderson, K. W. and Buchwald, S. L., General Catalysts for the Suzuki-Miyaura and Sonogashira Coupling Reactions of Aryl Chlorides and for the Coupling of Challenging Substrate Combinations in Water, *Angew. Chem. Int. Ed.*, **2005**, *44*, 6173-6177.
- ³¹¹ Guram, A. S.; Wang, X.; Bunel, E. E.; Faul, M. M.; Larsen, R. D. and Martinelli, M. J., New Catalysts for Suzuki-Miyaura Coupling Reactions of Heteroatom-Substituted Heteroaryl Chlorides, *J. Org. Chem.*, **2007**, *72*, 5104-5112.
- ³¹² Zajdel, P.; Nomezine, G.; Masurier, N.; Amblard, M.; Pawlowski, M.; Martinez, J. and Subra, G., A New Highly Versatile Handle for Chemistry on a Solid Support: The Pipecolic Linker, *Chem. Eur. J.* **2010**, *16*, 7547-7553.
- ³¹³ Gersbach, P.; Jantsch, A.; Feyen, F.; Scherr, N.; Dangy, J. P.; Pluschke, G. and Altman, K. H., A Ring-Closing-Metathesis (RCM)-Based Approach to Mycolactones A/B, *Chem. Eur. J.*, **2011**, *17*, 13017-13031.
- ³¹⁴ Lautens, M.; Paquin, J. F. and Piguel S., Palladium-Catalysed Sequential Alkylation-Alkenylation Reactions. Application to the Synthesis of 2-Substituted-4-Benzoxepines and 2,5-Disubstituted-4-Benzoxepines, *J. Org. Chem.*, **2002**, *67*, 3972-3974.
- ³¹⁵ Jose, J.; Ueno, Y. and Burgess, K., Water-Soluble Nile Blue Derivatives: Synthesis and Photophysical Properties, *Chem. Eur. J.*, **2009**, *15*, 418-423.
- ³¹⁶ Kung, H. F.; Kung, M. P. and Zhuang, Z. P., Stilbene Derivatives and Their Use for Binding and Imaging Amyloid Plaques. PCT Int. Appl. WO 2006/066104, Jun 22, 2006.
- ³¹⁷ Hervé, G.; Haln, D. U.; Hervé, A. C.; Goodsworth, K. J.; Hill, A. M. Hailes, H. C., The selective functionalisation and difunctionalisation of *p*-substituted calix[6]arene and calyx[8]arenes using hydrophilic moieties, *Org. Biomol. Chem.*, **2003**, *1*, 427-435.
- ³¹⁸ Hynes, M. J. and Maurer, J. A., Unmasking Photolithography: A Versatile Way to Site-Selective Pattern Gold Substrates, *Angew. Chem. Int. Ed.*, **2012**, *51*, 2151-2154.
- ³¹⁹ Paulick, M. G.; Wise, A. R.; Forstner, M. B.; Groves, J. T. and Bertozzi, C. R., Synthetic Analogues of Glycosylphosphatidylinositol-Anchored Proteins and Their Behaviour in Supported Lipid Bilayers, *J. Am. Chem. Soc.*, **2007**, *129*, 11543-11550.
- ³²⁰ Nazir, S.; Anwar, J. and Munawar, M. A., Chromium, manganese, and zinc complexes of deoxyalliin and their bioactivity, *J. Coord. Chem.*, **2010**, *63*, 4145-4152.
- ³²¹ Han, Y. and Barany, G., Novel *S*-Xanthenyl Protecting Groups for Cysteine and Their Applications for the *N*^α-9-Fluorenylmethoxycarbonyl (Fmoc) Strategy of Peptide Synthesis, *J. Org. Chem.*, **1997**, *62*, 3841-3948.

Chapter 7: Appendix

7.1 Dose Response Curves

Figure 71 shows selected dose response curves of compounds synthesised and tested for inhibition against HP0525.



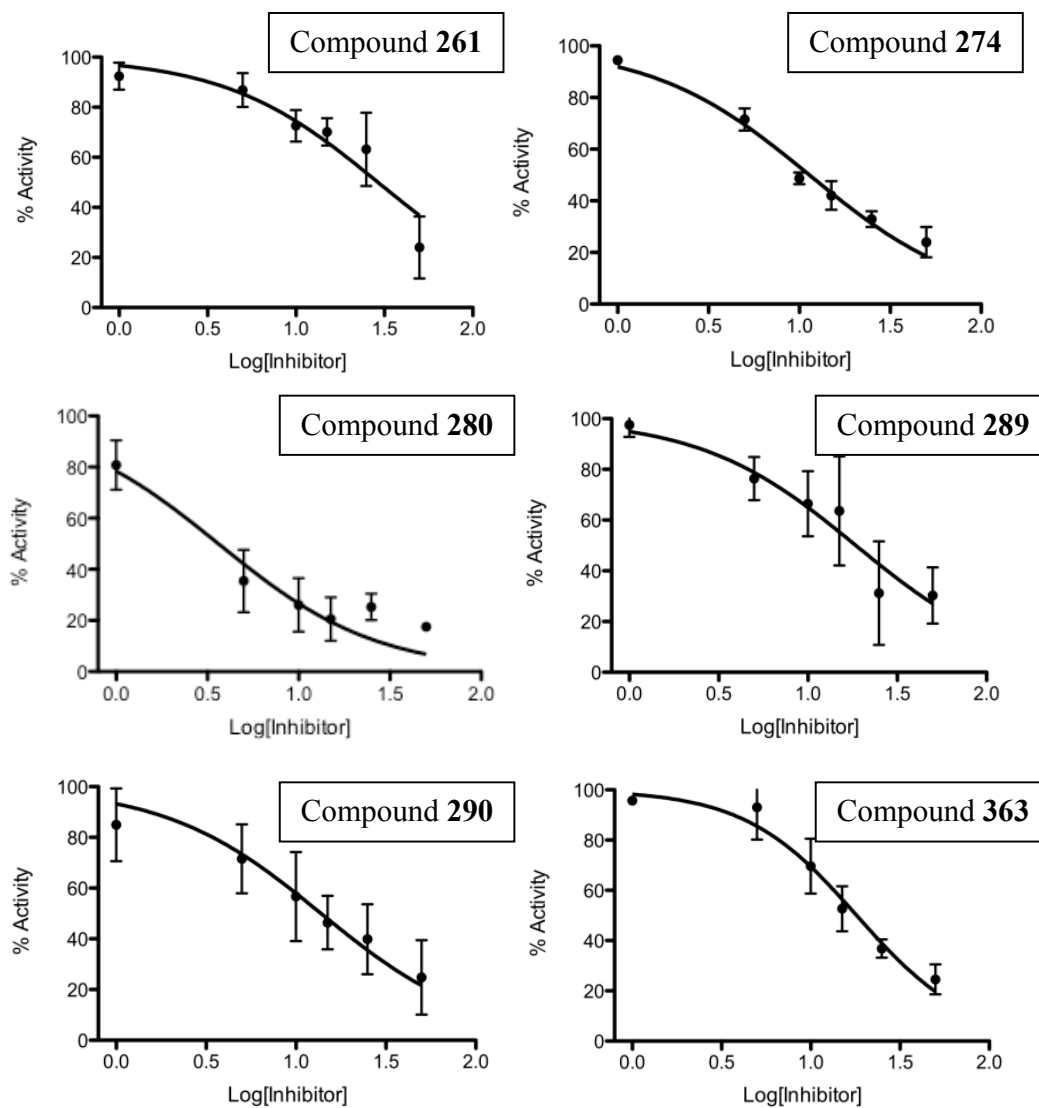


Figure 71: Selected dose response curves for compounds synthesised in Chapters 2-4. % Activity = % ATP hydrolysis compared to positive and negative controls. [Inhibitor] (μM)

7.2 Characterisation of Peptides and Peptide Conjugates

Figures X-X illustrates the characterisation of peptides and peptide conjugated synthesised in Chapter 4 (Sections 4.4 and 4.5)

α F-loop WT (389)

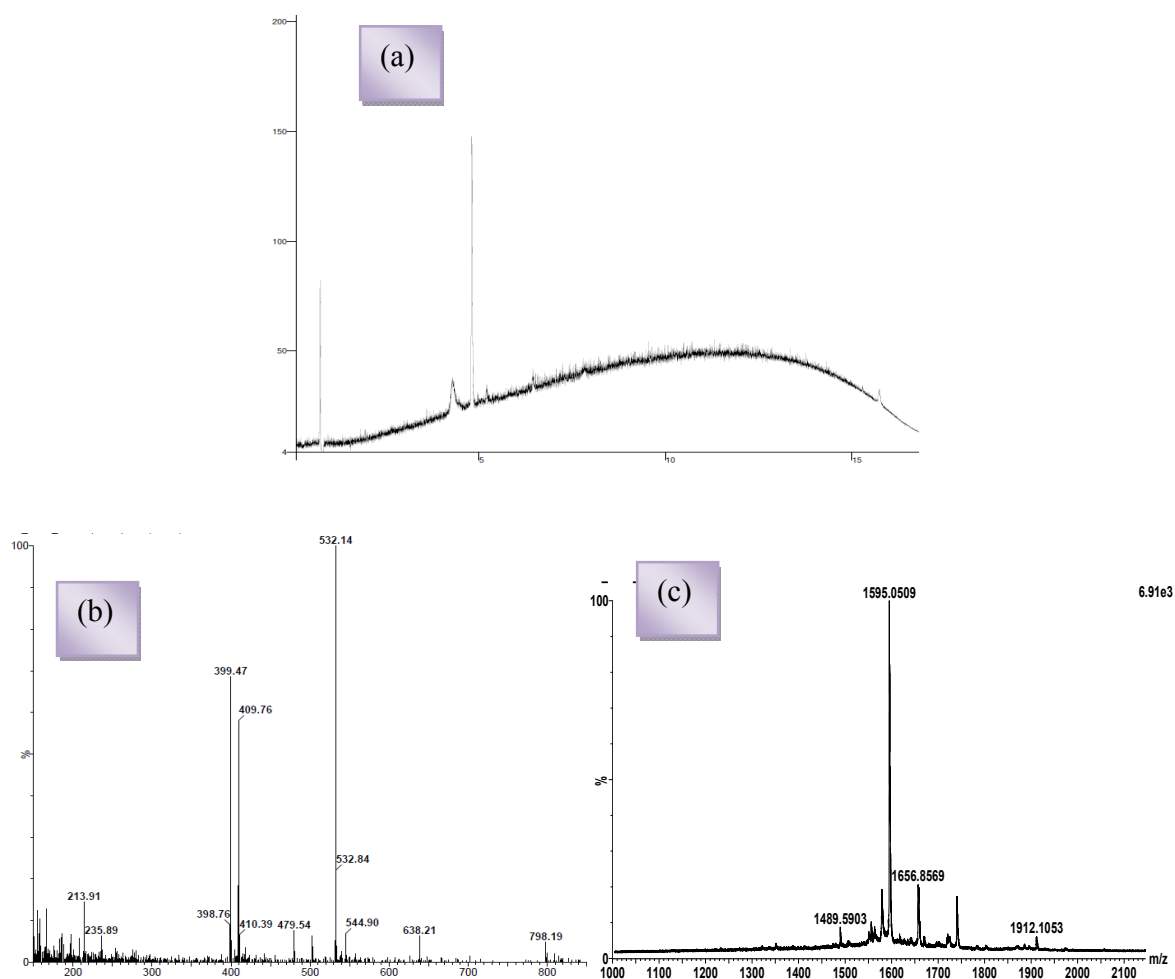


Figure 72: 389: (a) Analytical HPLC trace; (b) ES+; (c) MALDI

α F-loop R240C (390)

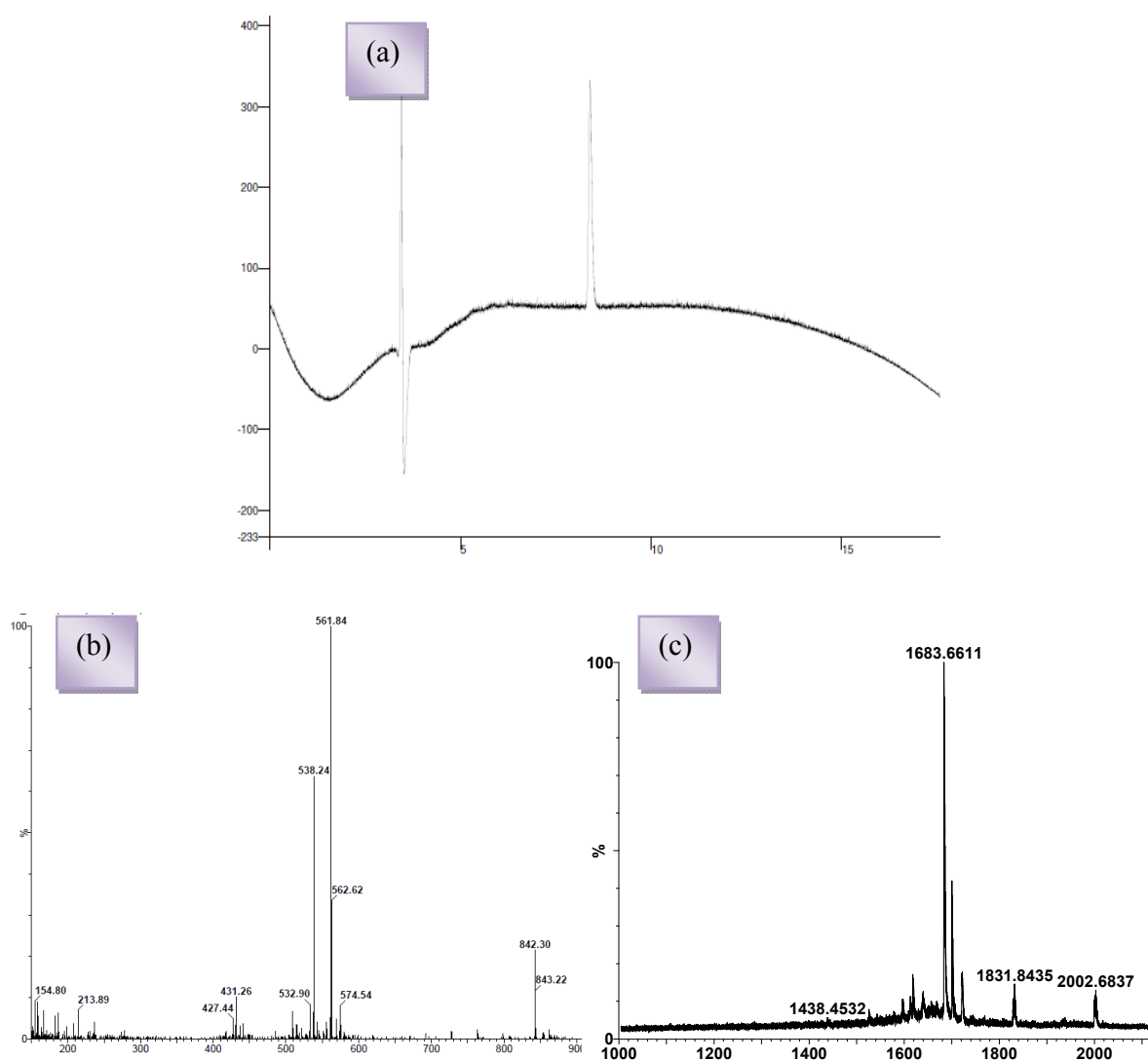
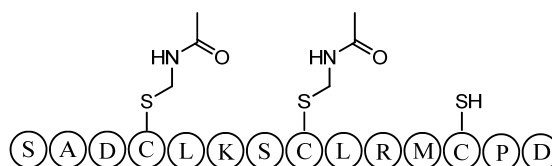


Figure 73: 390: (a) Analytical HPLC trace; (b) ES⁺; (c) MALDI

α F-loop R240SAC (391)

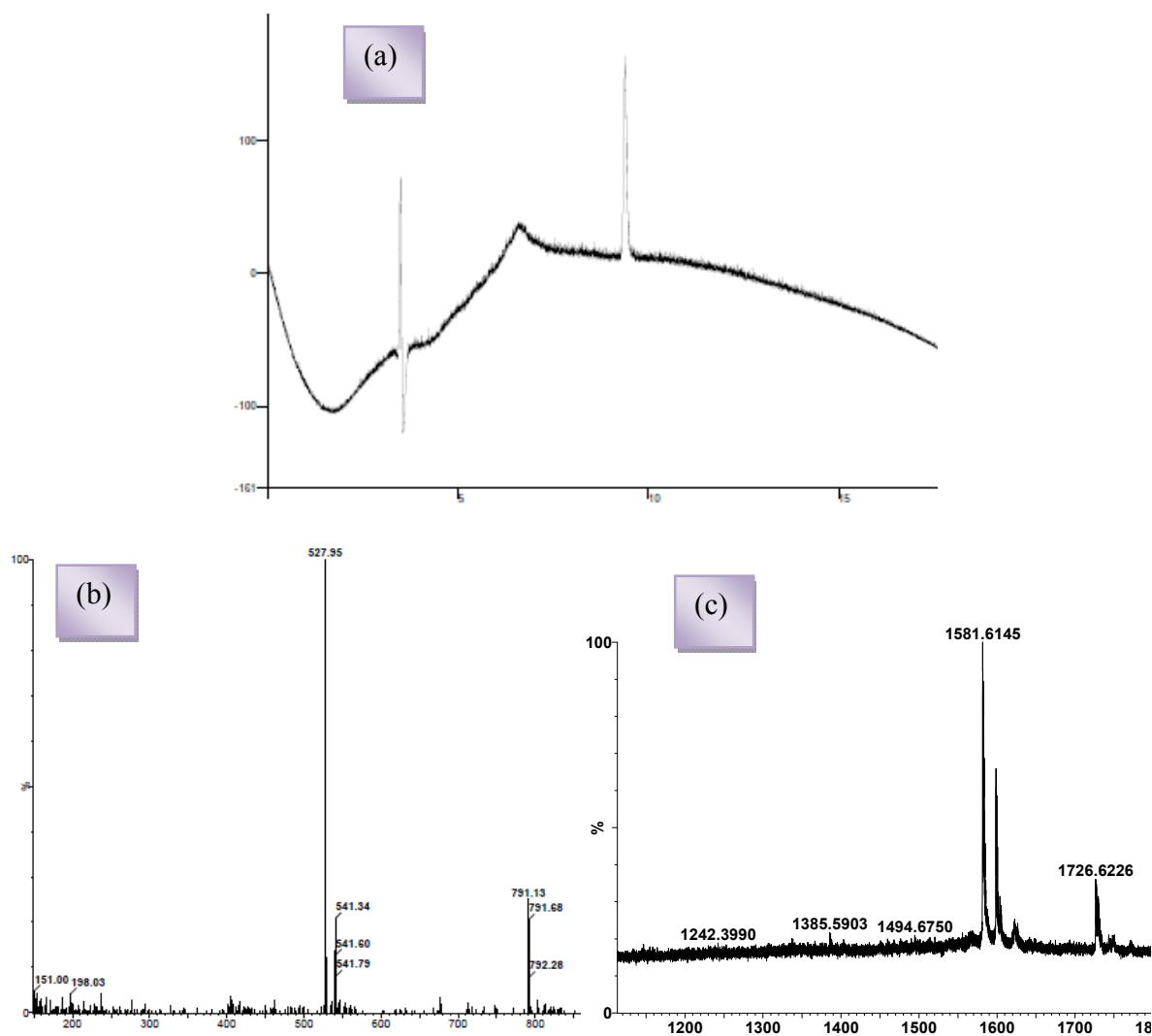


Figure 74: 391: (a) Analytical HPLC trace; (b) ES+; (c) MALDI

α F- β 10 WT (392)

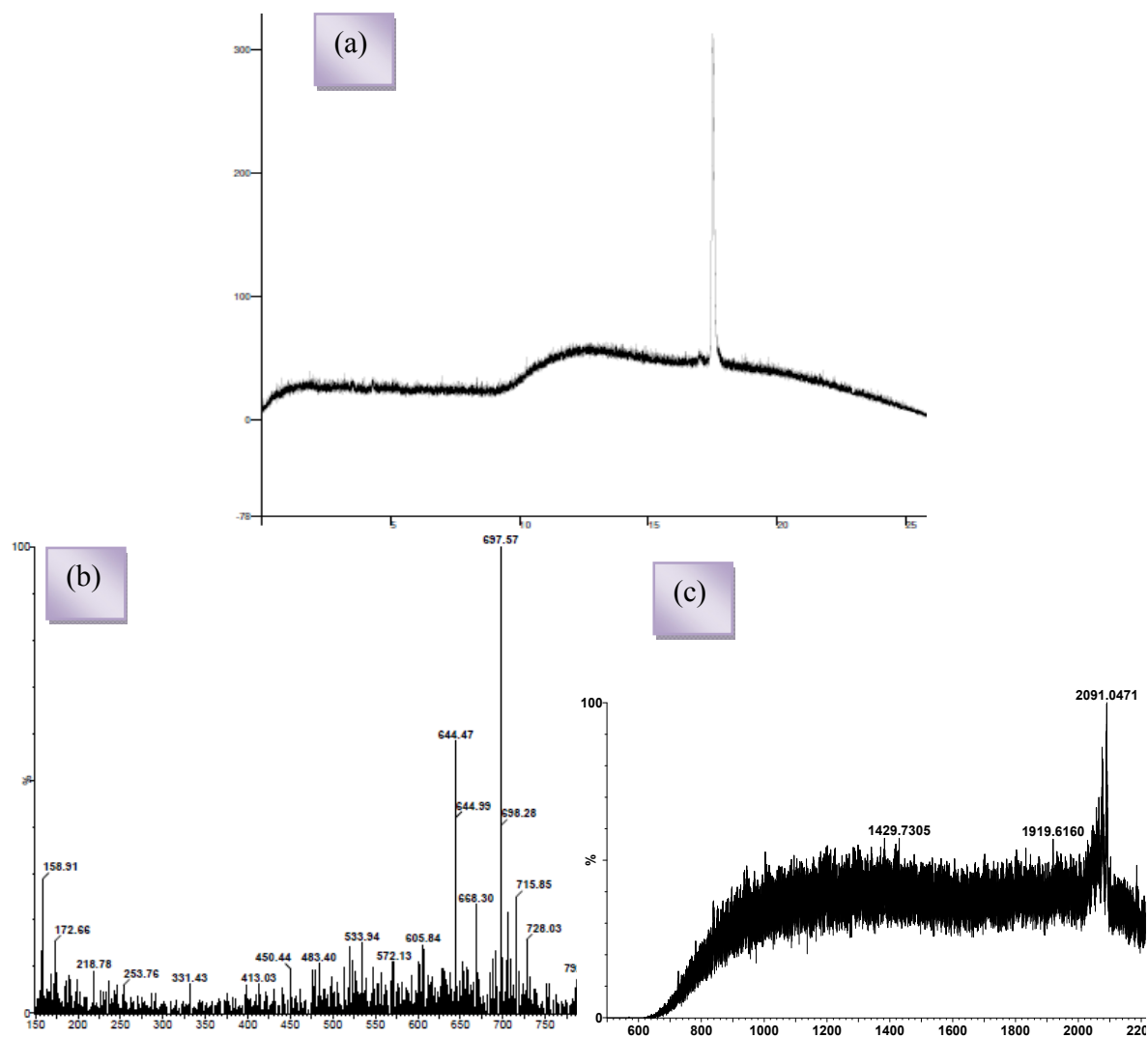


Figure 75: 392: (a) Analytical HPLC trace; (b) ES+; (c) MALDI

α F- β 10 R240C (393)

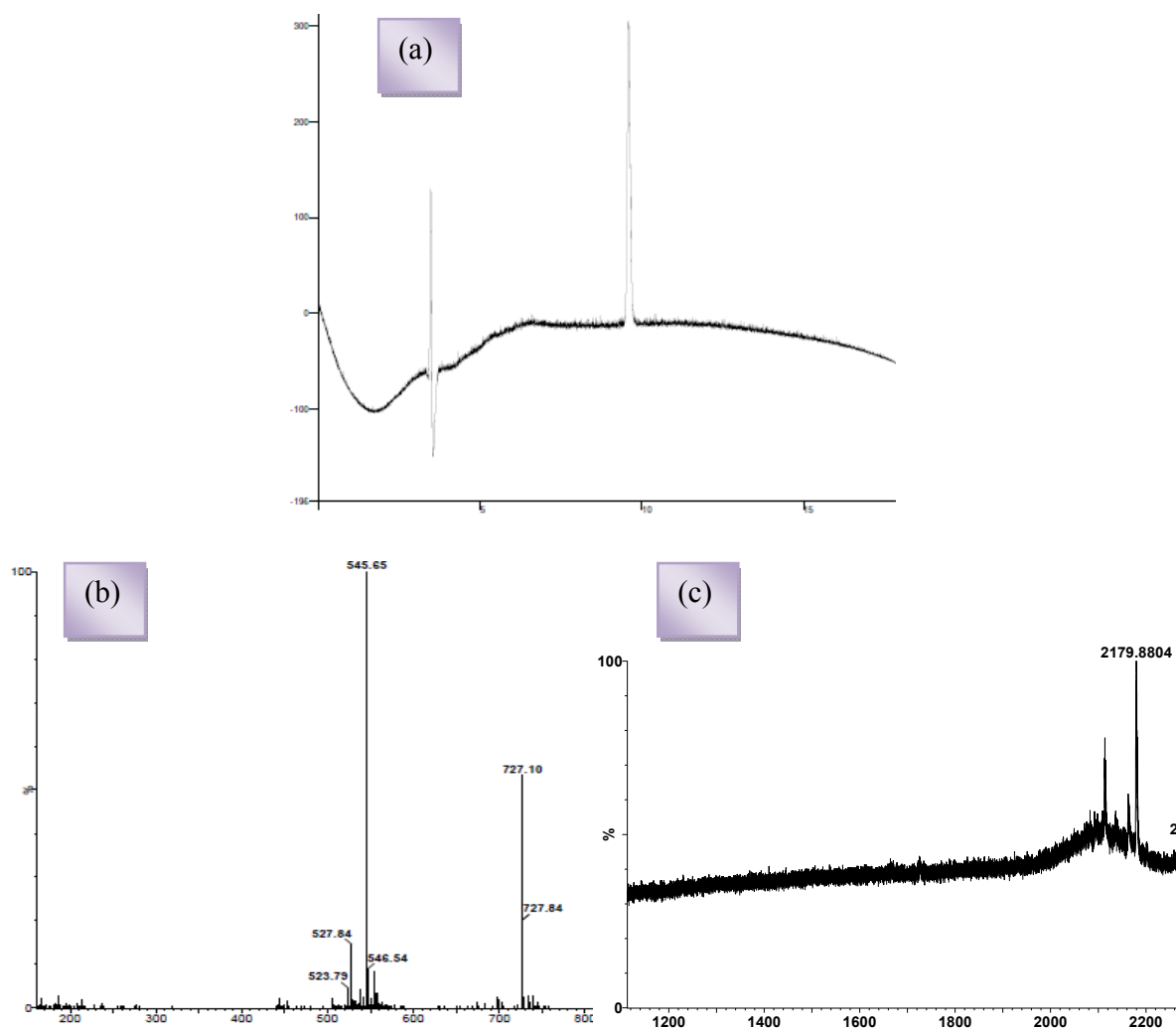
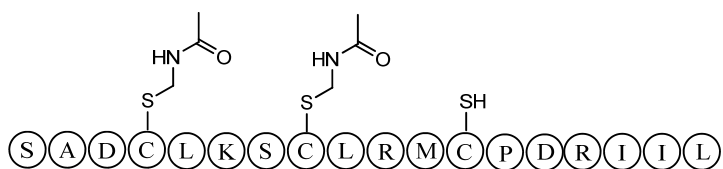


Figure 76: 393: (a) Analytical HPLC trace; (b) ES⁺; (c) MALDI

α F- β 10 R240SAC (394)

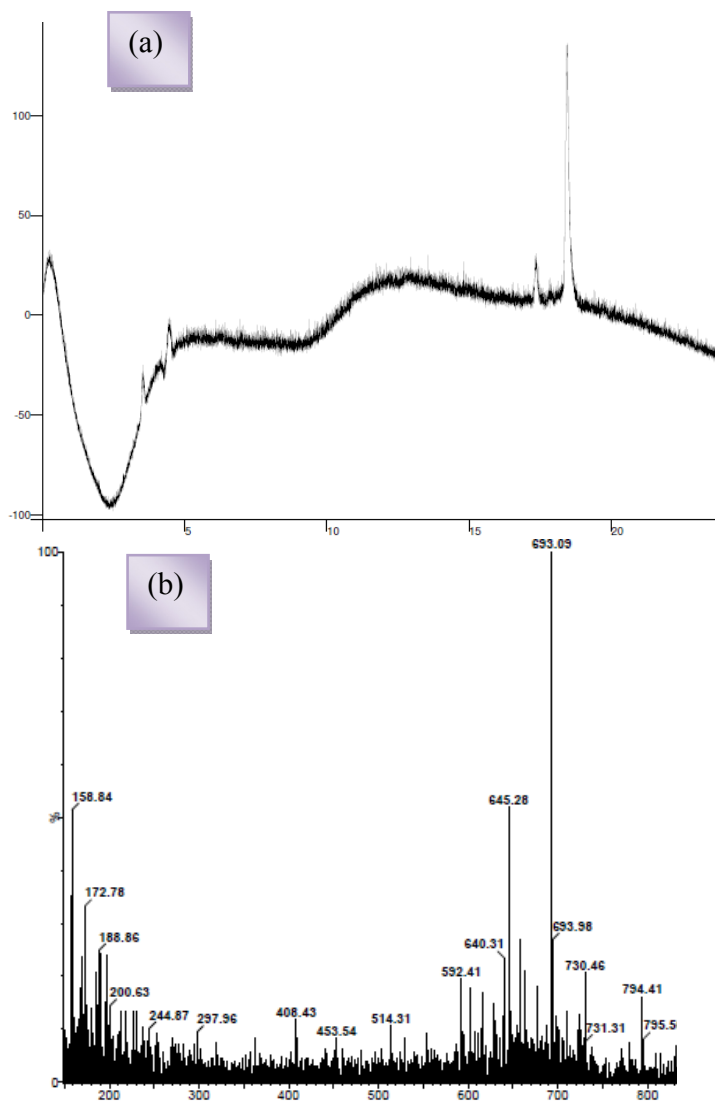


Figure 77: 394: (a) Analytical HPLC trace; (b) ES+

B9- α F- β 10 WT (395)

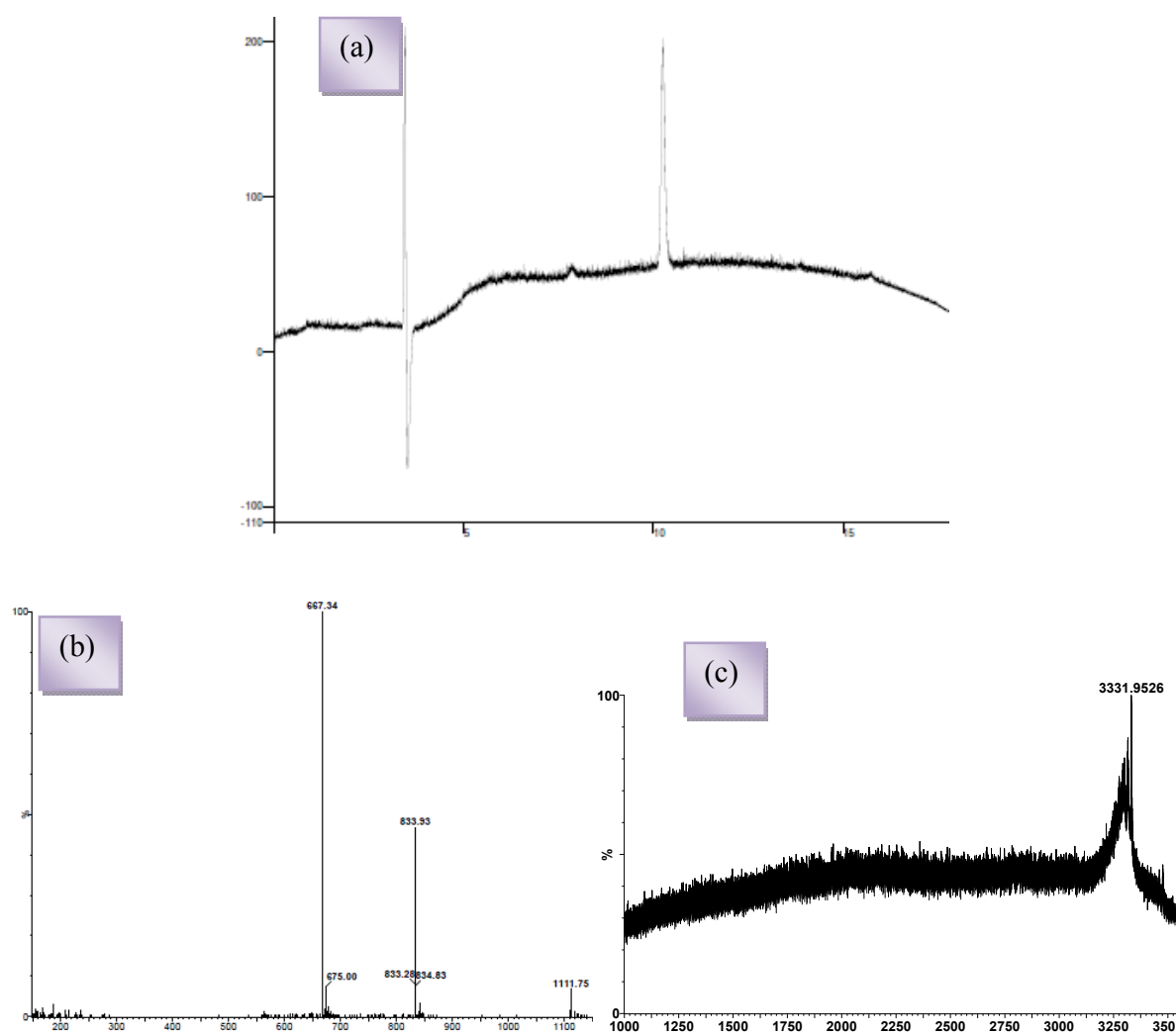


Figure 78: 395: (a) Analytical HPLC trace; (b) ES+; (c) MALDI

B9- α F- β 10 R240C (396)

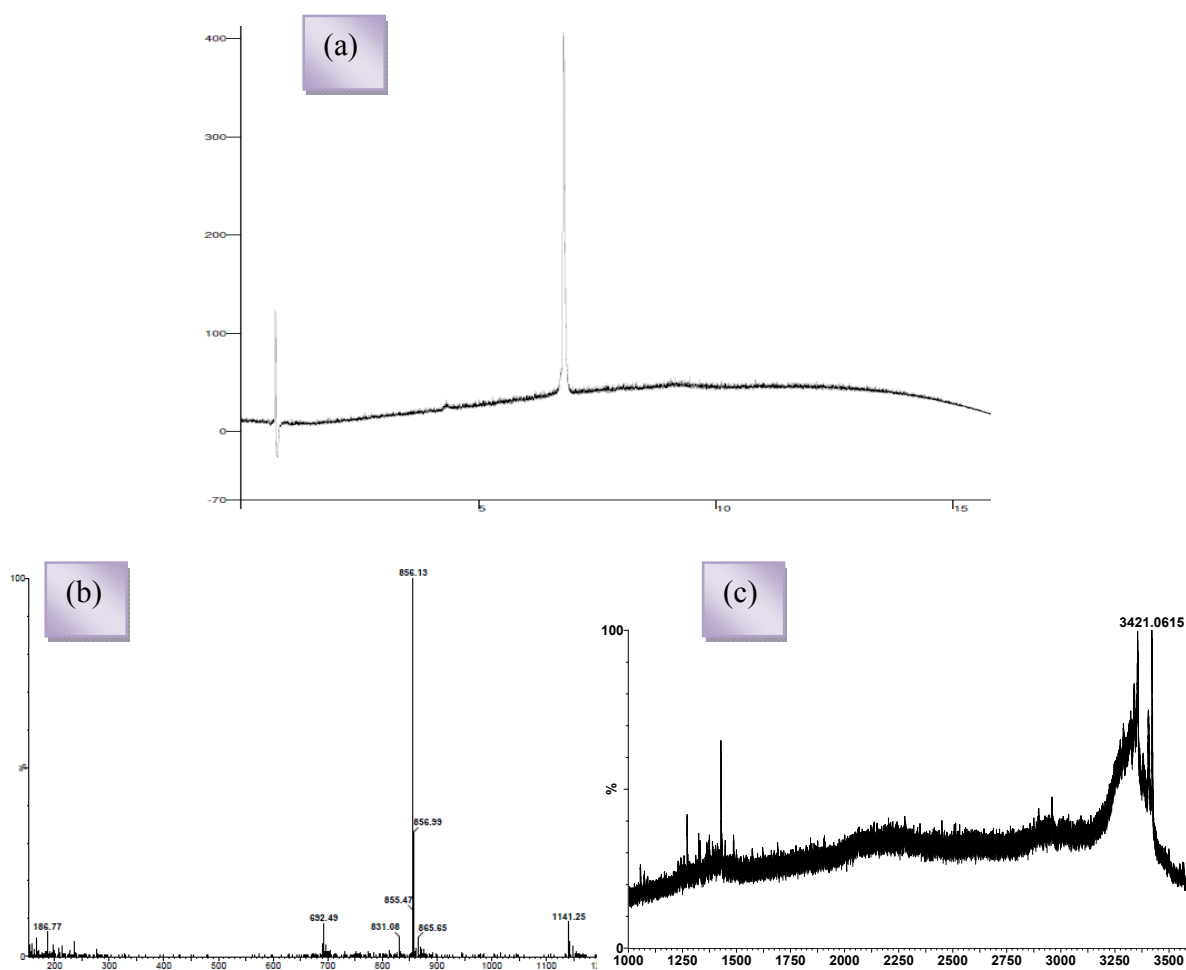
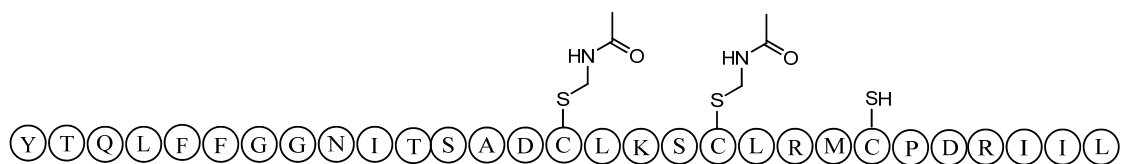


Figure 79: 396: (a) Analytical HPLC trace; (b) ES+; (c) MALDI

B9- α F- β 10 R240SAC (397)

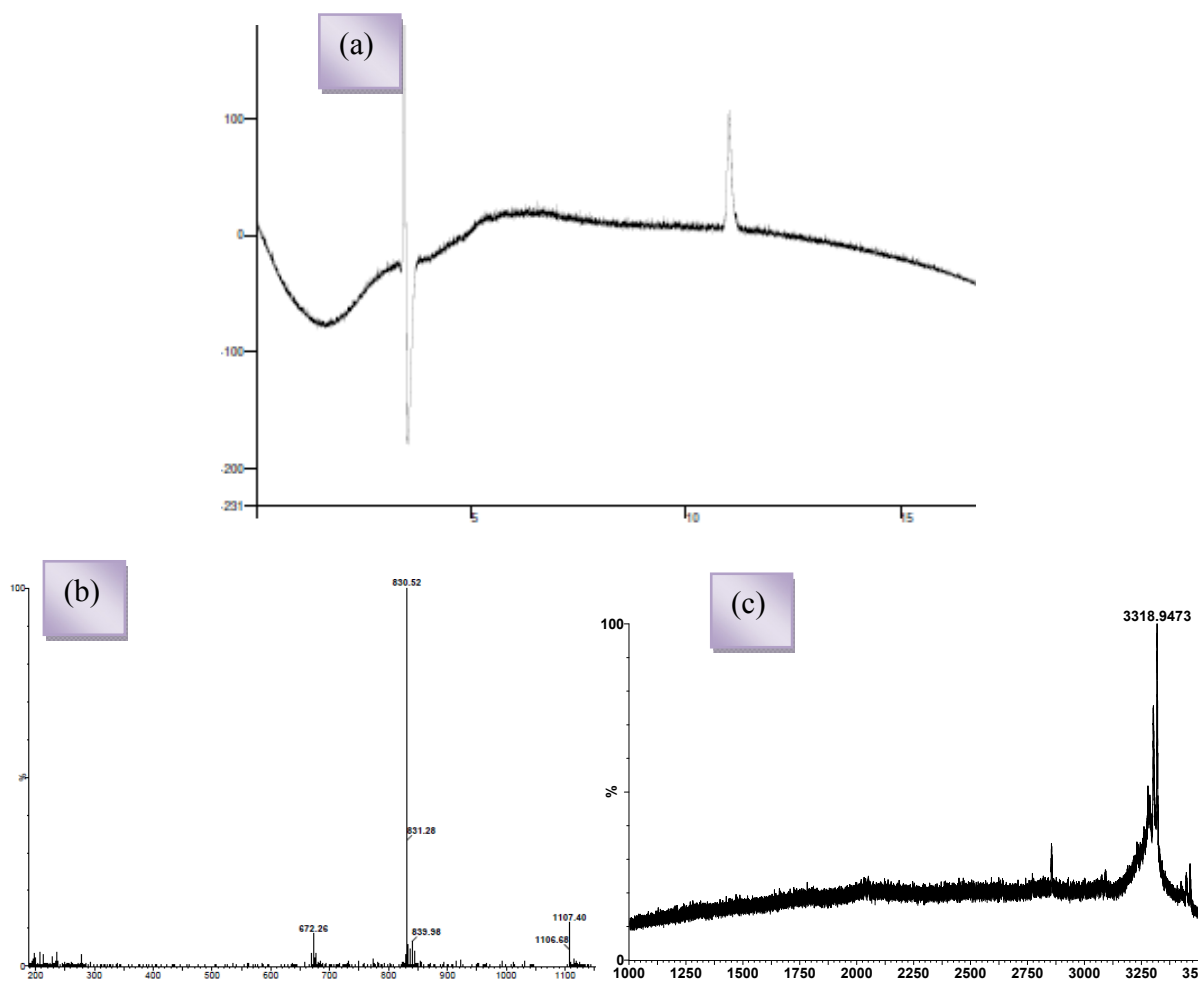


Figure 80: 397: (a) Analytical HPLC trace; (b) ES+; (c) MALDI

α F-loop (401)

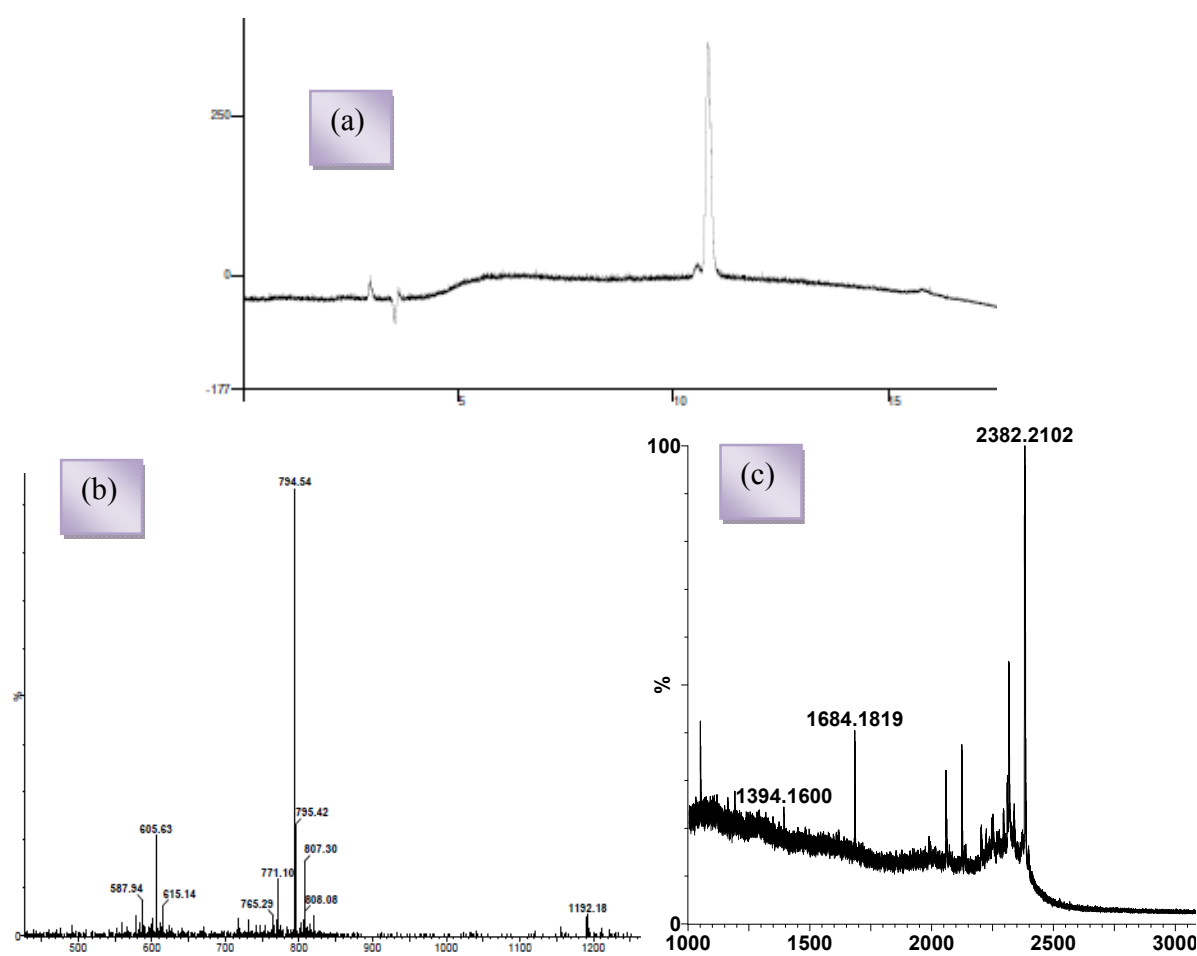
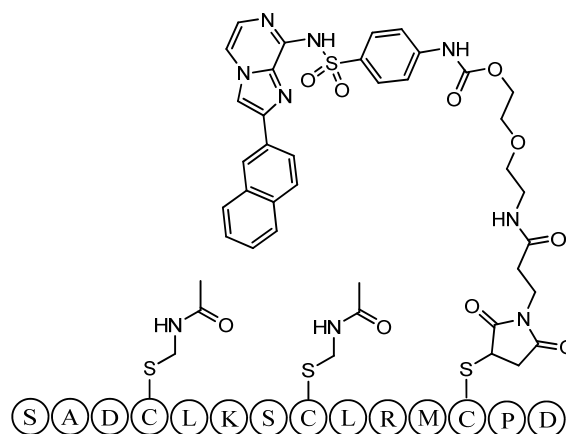


Figure 81: 401: (a) Analytical HPLC trace; (b) ES⁺; (c) MALDI

α F- β 10 (402)

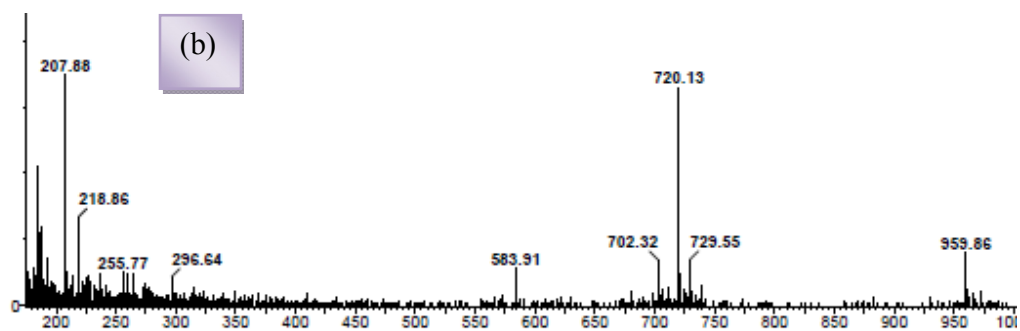
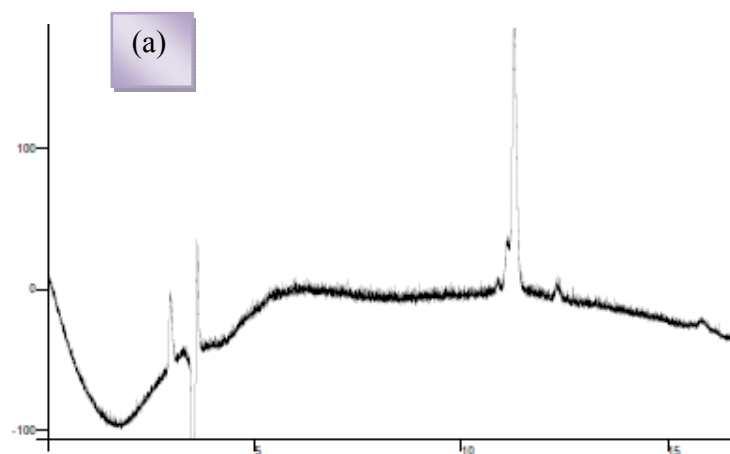
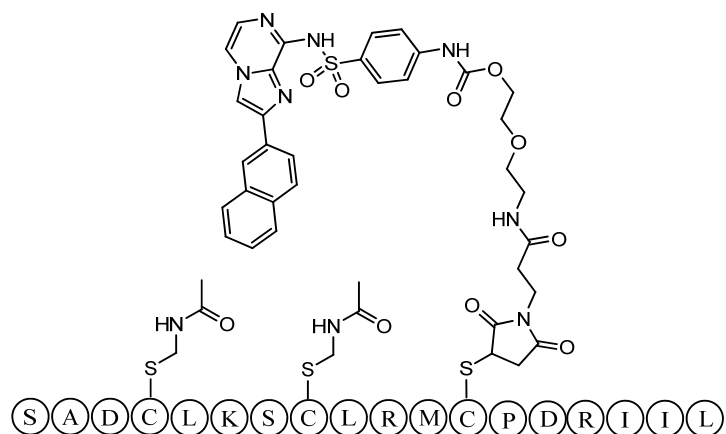


Figure 82: 402: (a) Analytical HPLC trace; (b) ES+

B9- α F- β 10 (403)

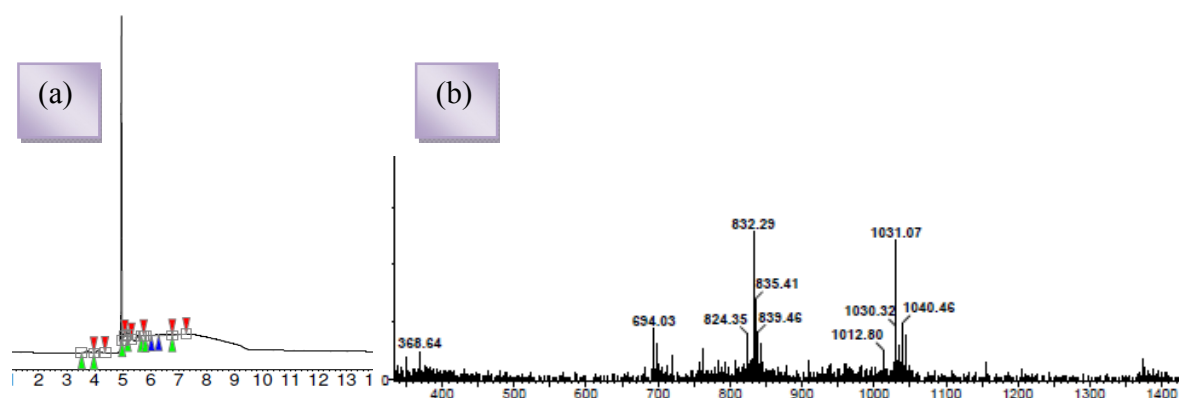
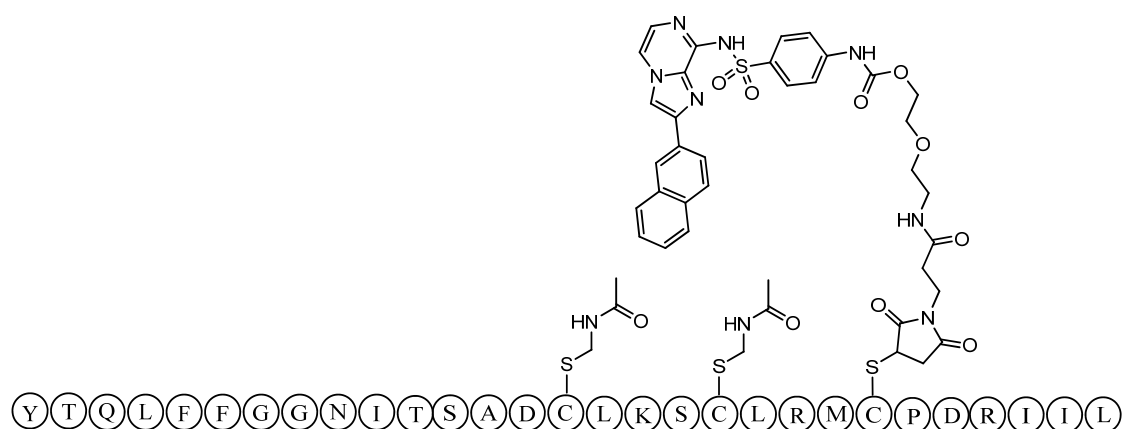


Figure 83; 403: (a) Analytical HPLC trace; (b) ES+

α F-loop (406)

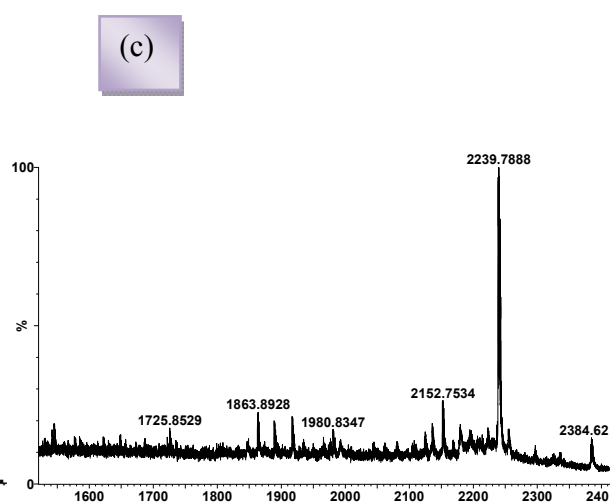
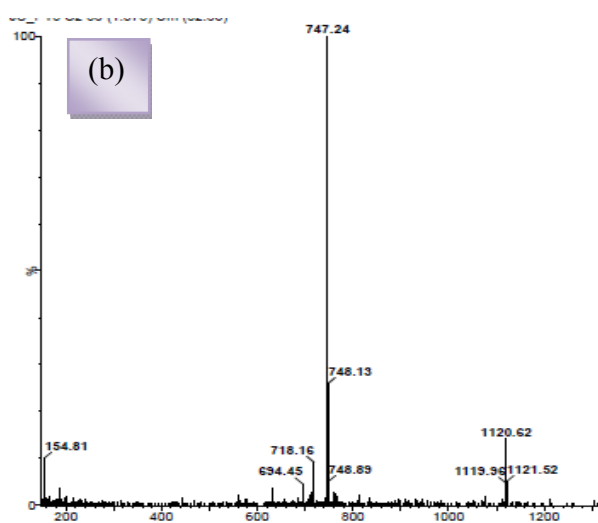
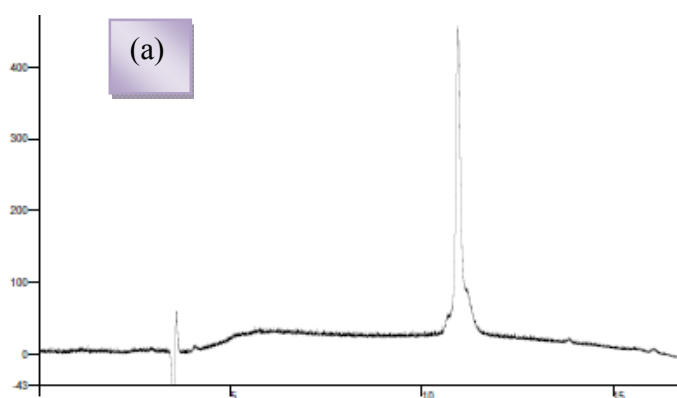
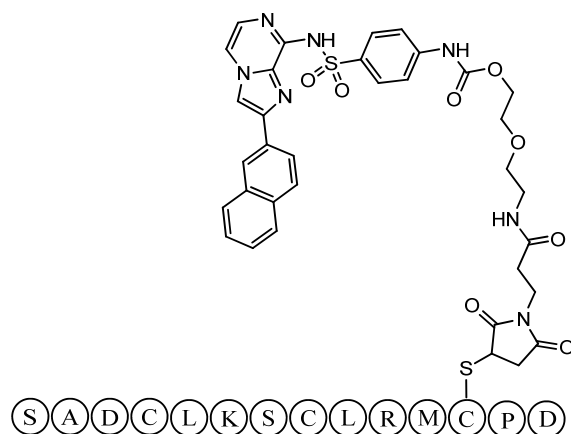


Figure 84: 406: (a) Analytical HPLC trace; (b) ES⁺; (c) MALDI

α F-loop (410)

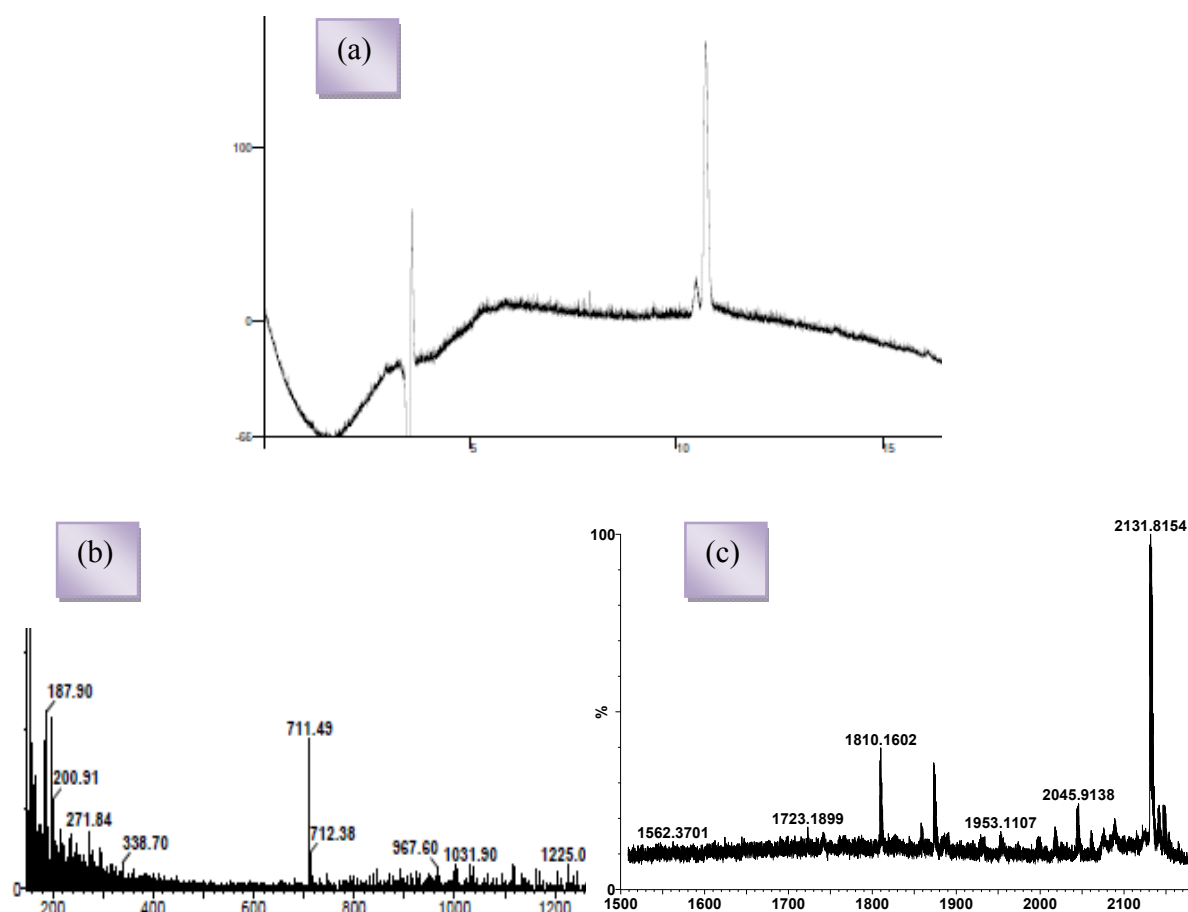
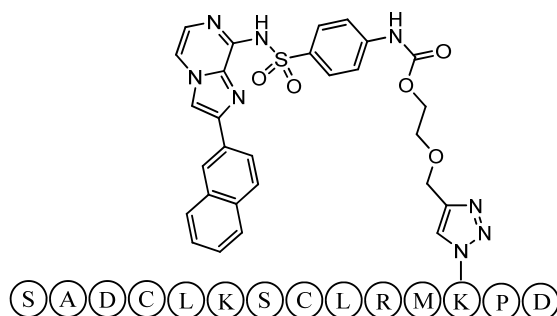


Figure 85: 410: (a) Analytical HPLC trace; (b) ES⁺; (c) MALDI

α F- β 10 (411)

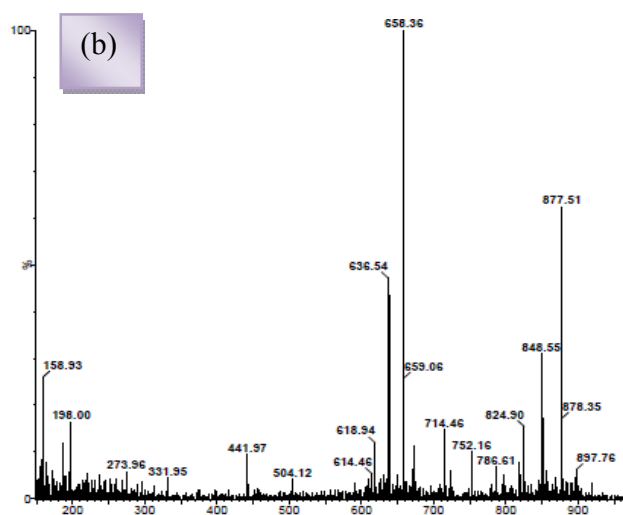
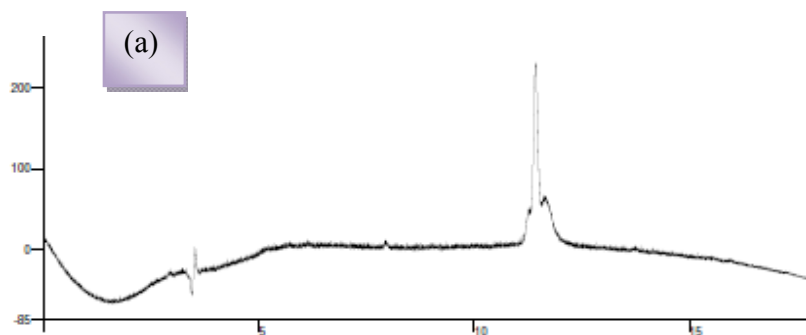
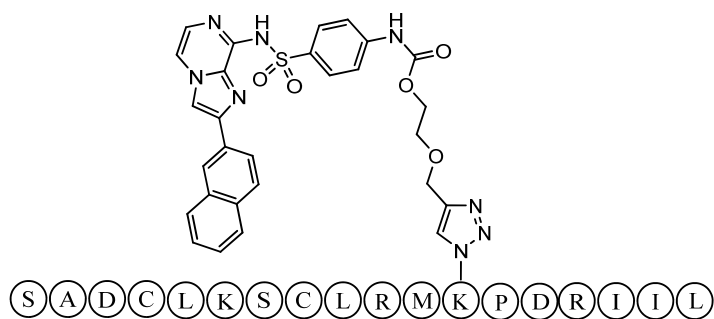


Figure 86: 411: (a) Analytical HPLC trace; (b) ES+