Zinc Ejectors – Synthesis of Epi-3,6-dithio-2,5-diketopiperazines as therapeutically active agents

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In loving memory of my brother Carlos Manuel da Silva Nunes Lucas

(1980 – 2011)

DECLARATION

This thesis describes research conducted in University College London School of Pharmacy between April 2009 and January 2013 under the supervision of Dr. Stephen T. Hilton. I, Bruno César da Silva Sil dos Santos, 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. I also certify that I have written all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

Signature:_____ **Date:**_____

ABSTRACT

This present thesis focuses on compounds related to the epidithiodiketopiperazine (ETP) family of natural products, their synthesis and biological effects. The domain of natural products has long been recognized as an invaluable source of lead structures for the discovery and development of novel therapeutic agents, with the motif being one example that has long been recognized for its potent biological effects which are directly related to the disulfide bridge at the central core.

The first chapter provides a detailed analysis of the ETP family of natural products with both the monomeric and dimeric family members being described to provide an overview of this diverse family of compounds and their corresponding biological effects. The second section in this chapter highlights the various synthetic strategies adopted by a number of different research groups and attempts to classify these into the varying approaches towards the central core. Finally the section closes with a description of dimeric natural products and their biological effects in relation to the potent activity observed from the dimeric ETP congeners.

The results of our own approach towards the ETP core are described in the third chapter which commences with the synthesis of disubstituted ETPs and expands towards our efforts in synthesizing their tri and tetrasubstituted counterparts. Efforts to extend this chemistry towards dimeric ETPs are also described and our successful routes towards these complex compounds are discussed. The following section of this chapter outlines the successful biological evaluation of the ETP compounds that were synthesised with relevance to their effects in a surrogate model of tuberculosis, cancer cells via inhibition of the hypoxia pathway and activity against the feline immunodeficiency virus.

The final chapter provides a formal description of the experimental results and procedures with full characterisation of the data for each compound.

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ABBREVIATIONS

Ac	Acetyl
ACC	Automatic Column Chromatography
Ac_2O	Acetic anhydride
AD-mix-α	Asymmetric dihydroxylation-mix-alpha
AIBN	2,2′-Azobis(2-methylpropionitrile)
ARNT	Aryl hydrocarbon receptor nuclear translocator
Bn	Benzyl
BOM	Methoxybenzyl
BOP-Cl	Bis(2-oxo-3-oxazolidinyl)phosphinic chloride
b.p.	Boiling point
Brucin-OL	*cis*-Bicyclo(3.1.0)hexan-2-ol brucine salt
Bu	Butyl
br	Broad
CFP	Cyan fluorescent protein
CH1	Cysteine-histidine rich 1 domain
CLSI	Clinical and Laboratory Standards Institute
CM	Cross metathesis
CRFK	Crandell Rees feline kidney
C-TAD	C-Terminal activation domain
d	Doublet
DACA	*N*-[(2-Dimethylamino)ethyl]acridine-4-carbomaxide

DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
$(DHQ)_2PHAL$	Hydroquinine 1,4-phthalazinediyl diether
DIAD	Diisopropylazodicarboxylate
DIBAL	Diisobutylaluminum hydride
DKP	Diketopiperazine
DMAP	4-*N,N'*-Dimethylaminopyridine
DME	Dimethoxyethane
DMF	Dimethylformamide
DMS	Dimethyl sulfide
DMSO	Dimethylsulfoxide
DTNB	5,5′-Dithiobis(2-nitrobenzoic acid)
D-Trp-OMe.HCl	D-Tryptophane methyl ester hydrochloride
EDC	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
EDCl	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
ETA	Ethanolamine
ETP	Epi-3,6-dithio-2,5-diketopiperazine
FCC	Flash Column Chromatography
FHT	Fixed Hold Temperature
FIV	Feline Immunodeficiency Virus
FRET	Fluorescence Resonance Energy Transfer
FT-IR	Fourier Transform Infrared Spectroscopy
HIF-1	Hypoxia Inducible Factor-1

HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HRE	Hypoxia Response Element
IC_{50}	Half maximum inhibitory concentration
i-PrOH	Isopropanol
$La(OTf)_3$	Lanthanum(III) trifluoromethanesulfonate
LDA	Lithium diisopropylamide
LiHMDS	Lithium bis(trimethylsilyl)amide
L-Trp-OMe	L-Tryptophan methyl ester
m-CPBA	3-Chloroperbenzoic acid
MeCN	Acetonitrile
MIC	Minimal inhibitory concentration
MMP	Matrix metalloproteinases
MOM	Methoxymethyl
m.p.	Melting point
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass Spectroscopy
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate-oxidase
NCS	*N*-Chlorosuccinimide
NCp	Nucleocapsid protein
NBS	*N*-Bromosuccinimide
N-Cbz-N-Me-D-Ser	*N*-Benzyloxycarbonyl-*N*-methyl-D-serine

NMR	Nuclear Magnetic Resonance
PCR	Polymerase Chain Reaction
PMB	*para*-Methoxybenzyl
PPy	Polypyrrole
pVHP	Von Hippel-Lindau protein
RCM	Ring-closing metathesis
RFU	Relative Fluorescence Units
$[Rh(cod)Cl]_2$	Chloro(1,5-cyclooctadiene)rhodium(I) dimer
RP-HPLC	Reverse Phase – High Performance Liquid Chromatography
RPMI	Roswell Park Memorial Institute
RT	Room Temperature
s	Singlet
$Sc(OTf)_3$	Scandium(III) triflate
SRA	Structure Relationship Activity
t	Triplet
TBAF	Tetrabutylammonium fluoride
TBDPS	*tert*-Butyldiphenylsilyl
TBS/TBDMS	*tert*-Butyldimethylsilyl
TBSCl	*tert*-Butyldimethylsilyl chloride
Teoc-OSu	*N*-[2-(Trimethylsilyl) ethoxycarbonyloxy]succinimide
TEV	Tobacco etch virus
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran

TLC	Thin Layer Chromatography
TNA	Total Nucleic Acid
TPP	5,10,15,20-Tetraphenyl-21*H*,23*H*-porphine zinc
Triton B	Benzyltrimethylammonium hydroxide
V-70	2,2'-Azobis(4-methoxy-2.4-dimethyl valeronitrile)
VEGF	Vascular endothelial growth factor
VRE	Vancomycin-resistant *Enterococcus*
YFP	Yellow fluorescent protein

1. Introduction

1.1 Epi-3,6-dithio-2,5-diketopiperazines Overview

This present thesis is concerned with the synthesis and biological activity of compounds known as the epi-3,6-dithio-2,5-diketopiperazines (ETPs). As such, this introduction outlines the diversity of these compounds with the structures of the natural products as well as the synthetic approaches to both the core structures and the parent natural products.

To date, the ETP core has been reported in over 120 natural products, comprising a minimum of 14 different sub-classes with minor modifications to the primary ETP core structure. The ETP core is characterised by a diketopiperazine ring bridged by two sulfur atoms at the three and six positions. Variation amongst the natural products occurs at the remaining four positions around the ring – at the two bridgehead carbon atoms and the amidic nitrogens as shown below (Figure **1**).[1]

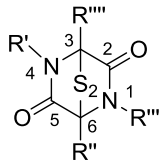


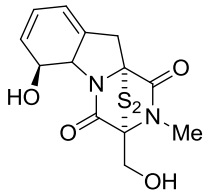
Figure 1 – ETP core

The large array of interest in these compounds stems from their diverse potent reported antibacterial, antiviral, antifungal and anticancer activities. Studies by a number of groups have confirmed that the biological activity shown by the ETP family is a result of the unique disulfide bridge.[1] As a result of this unique structure and potent activity, the ETP family are attractive targets for chemical synthesis and consequential pharmacological analysis.[2] In order to define the different sub-families of natural products containing the ETP core, they were grouped firstly by the presence of one or more epidithiol core and secondly from the structure and biosynthetic origin. Accordingly, the ETPs can be divided into main groups of monomeric and dimeric ETPs. By definition, monomeric ETPs clearly contain one epidithiodiketopiperazine moiety in their basic structure. The dimeric ETP family of compounds contain more than one epidithiodiketopiperazine moiety or include one ETP core with structural similarity to family congeners as the original group member. Dimeric ETPs contain more variation in the ETP core with both tri- and tetra-sulfide bridges reported in one of the diketopiperazine motifs.

1.1.1 Monomeric ETPs

1.1.1.1 Gliotoxin and dehydrogliotoxin

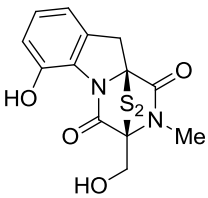
Gliotoxin (**1**) is perhaps the most extensively studied member of the ETP family as a result of its widely studied biological activity in a number of areas and presumably in addition, its commercial availability (Figure **2**).[3] It was first isolated in 1936 by Weindbling and Emerson [4] from the fungus *Gliocladium fimbriatum*.



Gliotoxin (1)

Figure 2 – Gliotoxin

It was also subsequently isolated from other fungal sources, namely *Aspergillus fumigatus*, *Trichoderma viride*, *Gliocladium* spp., *Penicillium* spp. and *Candida albicans*.[5, 6, 7, 8, 9, 10] The *Penicillium* species were found to produce not only gliotoxin (**1**) but also dehydrogliotoxin (**2**) (Figure **3**).[11, 12] The first elemental analysis of gliotoxin (**1**) was reported in 1944 which produced its empirical formula: $C_{13}H_{14}O_4N_2S_2{\cdot}_{1/2}H_2O$.[13] After further studies from Johnson and Woodward [14] to elucidate the correct absolute configuration of gliotoxin (**1**) it was Beecham *et al*, [15] that first reported the *S* configuration of the alcohol on the 1,3-cyclohexadiene moiety and the *R,R* configuration of the two disulfide bridgehead carbons. However, it took a further three years till Benedetti *et al* [16] demonstrated that the proposed rigid structure of ETPs was in fact flexible from its X-ray structure, which raised further interest in this family of natural products.



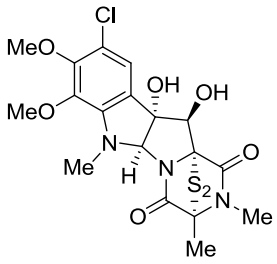
Dehydrogliotoxin (2)

Figure 3 – Dehydrogliotoxin

The enormous range of studies behind the characterization of gliotoxin (**1**) can only be matched by the wide variety of reported biological properties that it exhibits. Initial studies showed potent bacteriostatic activity against *Bacillus subtilis*, [17] however, both gliotoxin (**1**) and its related analogue, dehydrogliotoxin (**2**), also demonstrated the ability to block viral RNA replication by reverse transcriptase inhibition in infected cells. [18, 19] Infection deterrence against *Plasmodium falciparum* [20] was also seen by these compounds which demonstrated the great biological potential as both an antiviral and antimalarial agent. Despite widespread interest in gliotoxin (**1**) as a possible panacea therapeutic, its toxicity and allergic side effects when used in high concentrations proved to dampen the initial enthusiasm.[18] Studies on gliotoxin (**1**) were later broadened to an enzymatic level in the works of Waring which concluded that inhibition of both alcohol dehydrogenase and creatine kinase activity in mammals could be observed from analysis of the effects of this natural product.[21] Further studies were carried out on gliotoxin (**1**) which found it to be apoptotic, necrotic and immunosuppressive to cells.[22, 23] These results were found to be fundamental to its activity during *in vitro* and *in vivo* studies, due to the discovery of its immunosuppressive effects that led to the discovery of its inactivation and consequent decrease of T and B cells in rats.[23] Studies by Pahl *et al* [24] highlighted the inhibition of nuclear factor-kappa B by gliotoxin, which acts as a regulator for transcription of inflammatory related cytokines that display crucial importance in inflammatory bowel disease. However, non-specific necrotic response occured at concentrations above 10 μM resulting in osmotic swelling and plasma membrane destruction due to an increase in intracellular calcium concentration.[25, 26, 27, 28, 29] Gliotoxin (**1**) also activates Bak, a pro-apoptotic gene belonging to the Bcl-2 class of proteins. Activation of this gene leads to generation of reactive oxygen species and apoptosis, particularly of immunomodulating cells.[30] Redox cycling reactions generate reactive oxygen species that cause single and double strand breakages in DNA, ultimately resulting in an apoptotic outcome. [3, 31]

1.1.1.2 Sporidesmin

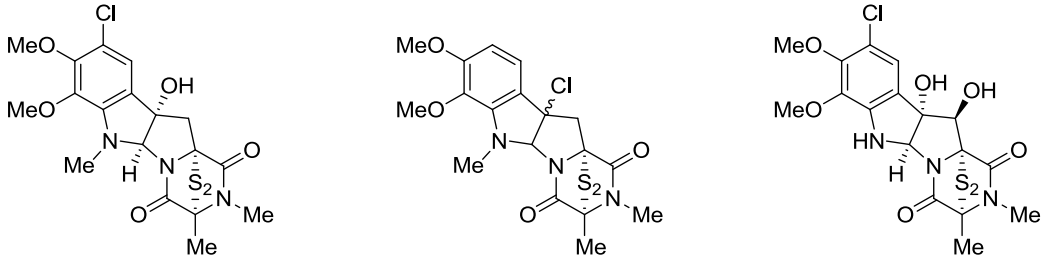
The sporidesmin group of ETP natural products was first characterized by Synge and White [32, 33] when sporidesmin A (**3**) (Figure **4**) was isolated from the fungi *Sporidesmium bakeri* (current denomination: *Phytomyces chartarum*). In 1962 Fridrichsons *et al* [34] reported the X-ray structure of the natural product proving the absolute configuration at the disulfide bridge carbons as *R,R*.



Sporidesmin A (3)

Figure 4 – Sporidemisn A

Related compounds were isolated by Ronaldson [35, 36, 37, 38] and Shannon, [39] with sporidesmin B (**4**) isolated as a derivative of sporidesmin A (**3**). Sporidesmin H (**5**) and sporidesmin J (**6**) were isolated by Taylor and co-workers in 1978 [40] in which the first was described as an analogue of the original sporidesmin with the inclusion of a 3-chloroindole moiety and the second as a N^6-desmethylsporidesmin derivative, respectively (Figure **5**).



Sporidesmin B (4) **Sporidesmin H (5)** **Sporidesmin J (6)**

Figure 5 – Sporidesmins B, H and J

The importance of the sporidesmin group of natural products arises from a pathological state known as facial eczema which affects sheep in New Zealand.[33, 34] This type of infection caused early hepatic dysfunction which led to the animal's death.[41, 42] Interestingly literature reports by Smith *et al* [43] demonstrated that the use of oral zinc sulfate for a period of five days protected sheep from the effects of sporidesmin. These results were later adapted by Towers [44] who carried out a similar test using sporidesmin poisoned rats, where zinc sulfate was added to their diets demonstrating that it protected them against the fungal metabolite. These findings would later prove crucial when determining the mechanism of action associated with ETPs. Much like gliotoxin (**1**), sporidesmin A (**3**) was also found to inhibit mitogen stimulation of T lymphocytes which showed the immunomodulating capability of these compounds.[45]

1.1.1.3 Aranotin

In 1968 Nagarajan *et al* [46, 47, 48, 49] and Moncrief [50] were the first to characterize the antiviral metabolites aranotin (**7**) and acetylaranotin (**8**) from *Arachniotus aureus* and the pentacyclic structures are shown below (Figure **6**).

Figure 6 – Aranotin and Acetylaranotin

In the same year, Nagarajan and Neuss [49, 51] identified two new members of this family of natural products: apoaranotin (**9**) and acetylapoaranotin (**10**) (Figure **7**). The structures of these two metabolites were quite peculiar since one of the terminal parts of the molecules contained the already reported dihydrooxepin ring, with the other formed by a cyclohexadiene moiety similar to gliotoxin's (**1**) structure.

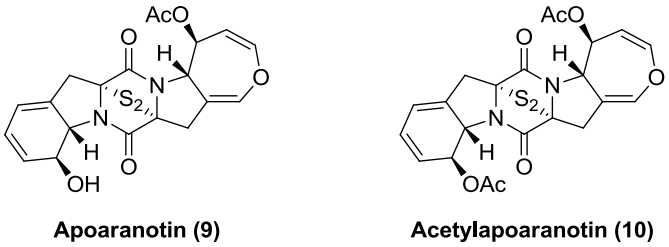


Figure 7 – Apoaranotin and Acetylapoaranotin

In 1970 De Long *et al* [52] described the antiviral activity of aranotin (**7**) and its analogues and hypothesised that the mode of action was associated with inhibition of virus-induced RNA-dependent RNA polymerase. In the same year Ho and co-workers [53] assigned the antiviral action of these compounds to cellular DNA-dependent rather than viral RNA-dependent RNA polymerase. Another important conclusion from the same authors was acknowledgment that the reduced form of aranotin lacking the sulfur bridge was completely inactive. This important report indicated that the disulfide bond was essential for its inhibitory activity. Trown renamed acetylaranotin (**8**) to LL-S88α in 1970 [54] after isolating the metabolite from a different source, *Aspergillus terreus*. LL-S88α (aranotin's 5-acetate analogue) and showed that it exhibited antiviral properties with specific inhibition of viral RNA replication. In 1997 Hegde *et al* [55] identified SCH 64874 (**11**) from an unknown source of fungi compound (Figure **8**). The core structure

was identical to aranotin (**7**) and this new compound also showed anticancer properties. However, whilst the ester group stereochemistry was not assigned by the authors further studies on this sub-class of compounds have not been reported till date.

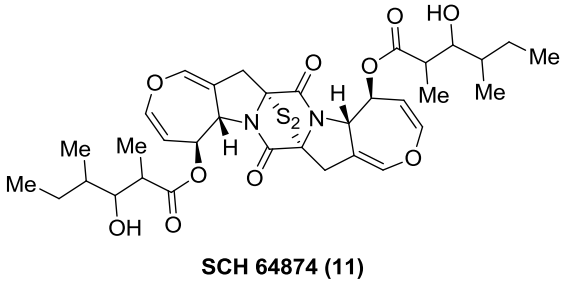


Figure 8 – SCH 64874

1.1.1.4 Emethallicin

Kawahara *et al* [56, 57] isolated and characterized the emethallicin family of compounds from *Emericella heterothallica* which are structurally similar to the aranotin family of natural products. Amongst the reported examples, emethallicin A (**12**), E (**13**) and F (**14**) contain the dithiodiketopiperazine moiety (Figure **9**).[58]

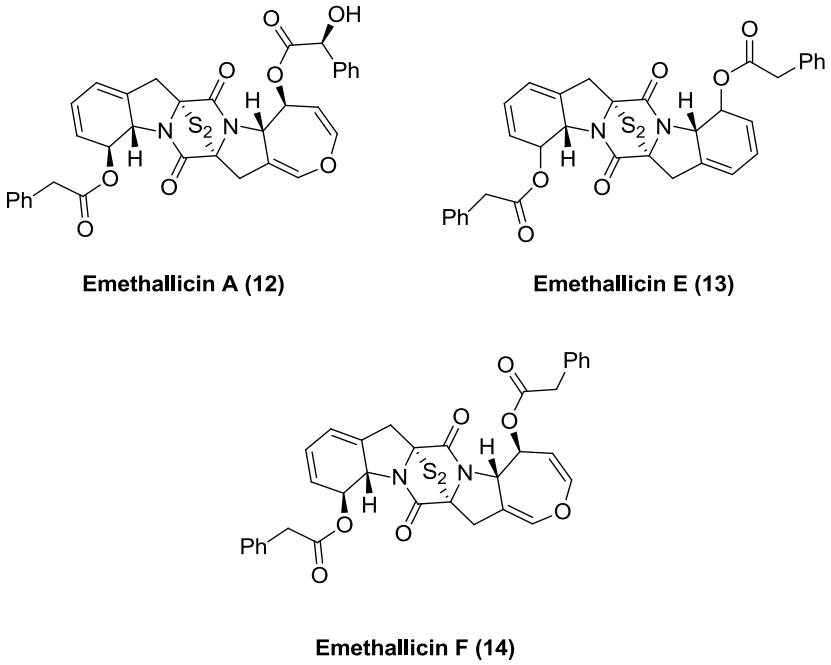


Figure 9 – Emethallicins A, E and F

These compounds were shown to have a potent anti-allergic effects from their inhibitory activity on histamine release.[59] In 1995, Ueno *et al* [60] demonstrated that these same molecules could also induce apoptosis in cancer cell lines.

1.1.1.5 Epicorazine

Baute [61, 62] and Deffieux [63, 62] were the first to identify two members from the epicorazine family of natural products: epicorazine A (**15**) and B (**16**) (Figure **10**); isolated from the fungi *Epicoccum nigrum*. Later, in the works of Mallea, [64] epicorazine B (**16**) was also identified from a different species of fungi, *Epicoccum purpurascens*. More recently, Epicorazine A (**15**) was also found to be produced by *Capnodium* sp.[65]

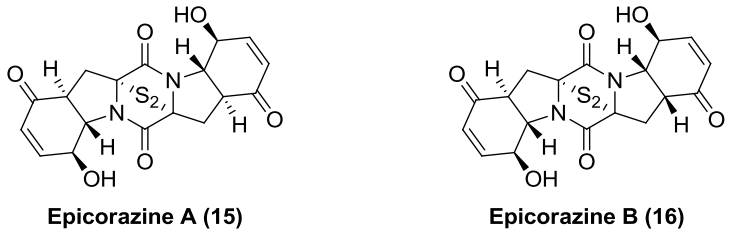


Figure 10 – Epicorazines A and B

Studies by Deffieux defined the *R,R* configuration of the disulfide bridgehead carbons and the authors also concluded that the source of derivatization comes from the oxygenated indole moieties.[66, 67] X-ray models showed that the only difference between these two epicorazines arises from the stereochemistry of one ring junction as can be seen above.[68] The same author also reported the antibacterial activity of these molecules against *Staphylococcus aureus*.[62] In 2001, Dornberger and co-workers [69] isolated epicorazine C (**17**) (Figure **11**) as well as the other two already known epicorazines A (**15**) and B (**16**) from *Stereum hirsutum* fungi. This new compound was found to be active against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VREF) and *Candida albicans*. Epicorazine C (**17**) was later isolated by Lindequist *et al* [70] in cultures of the fungi *Podaxis pistillaris*.

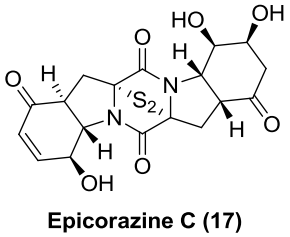
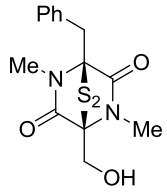


Figure 11 – Epicorazine C

1.1.1.6 Hyalodendrin

In 1973 Strunz *et al* [71] isolated hyalodendrin (**18**) (Figure **12**) from the *Hyalodendron* sp. fungus which is perhaps the simplest structural member of the ETP family. The

same author defined the absolute configuration of the disulfide bridgehead carbons as *S,S*.[72, 73] Hyalodendrin (**18**) has been studied for its antifungal and antibacterial activity in a number of models and has been shown to inhibit *Staphylococcus pyogenes*, *Klebsiella pneumoniae* and *Candida albicans* with 12.5 µg/mL, 50.0 µg/mL and 25.0 µg/mL MIC's, respectively.[74, 75]

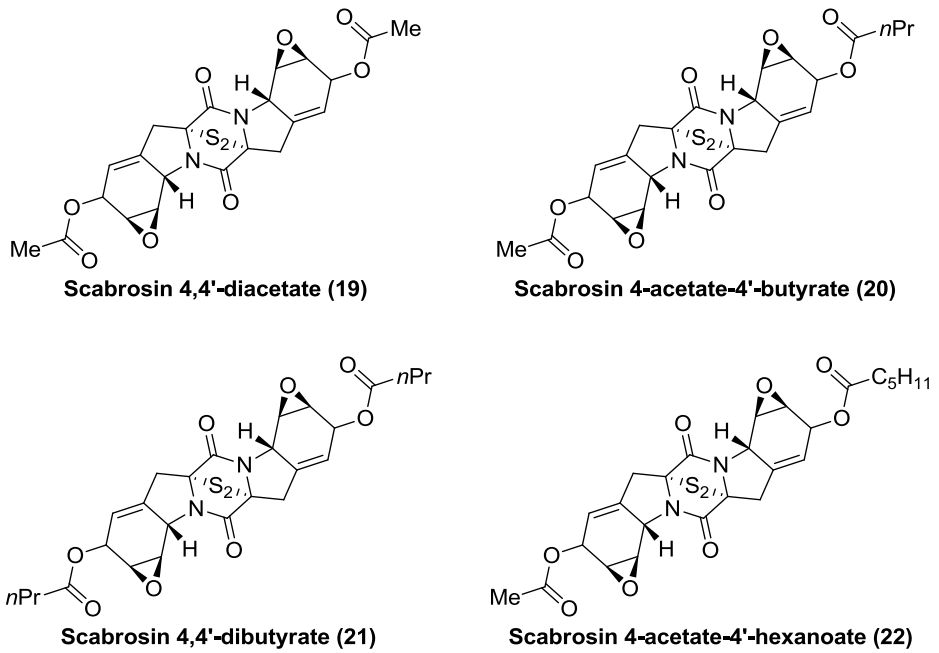


Hyalodendrin (18)

Figure 12 – Hyalodendrin

1.1.1.7 Scabrosin

In 1978, Begg *et al* [76] isolated several compounds from *Xanthoparmelia scabrosa* which they named scabrosin esters. Compounds (**19**) to (**22**) illustrate the examples of this group of natural products with differences lying in the ester alkyl substituents for each member of this ETP sub-family of compounds, as shown below (Figure **13**).



Scabrosin 4,4'-diacetate (19) **Scabrosin 4-acetate-4'-butyrate (20)**

Scabrosin 4,4'-dibutyrate (21) **Scabrosin 4-acetate-4'-hexanoate (22)**

Figure 13 - Scabrosins

These scabrosin derivatives previously isolated from *Parmelia pulla* by Culberson [77] were reanalysed some years after their discovery by Helix and co-workers [76,78] who had also catalogued another member of this family, scabrosin butyrate hexanoate (**23**)

(Figure **14**). These authors and others, were also responsible for establishing the potent cytotoxic activity against cancer lines that these products displayed.[76,79,80]

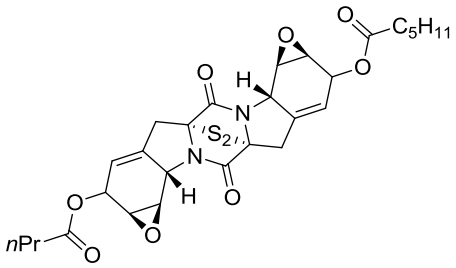


Figure 14 – Scabrosin butyrate hexanoate

In 1998 Williams *et al* [81] using the lichen *Usnea* sp. were able to isolate two identical products that shared the same structural complexity displayed by the scabrosins family. These were called Ambewelamides A (**24**) and B (**25**) (Figure **15**) and both molecules possessed analogous properties exhibited by their former congeners.[82]

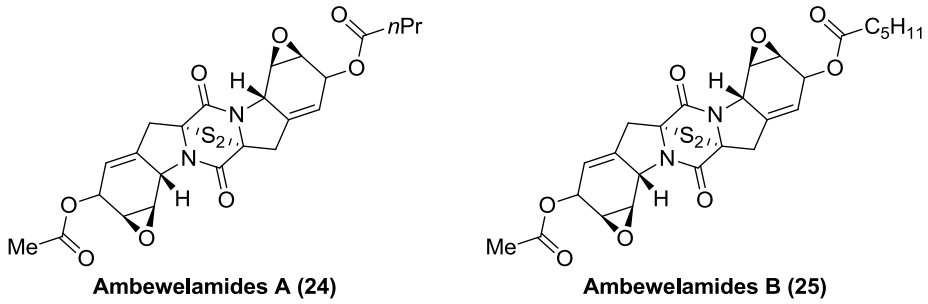
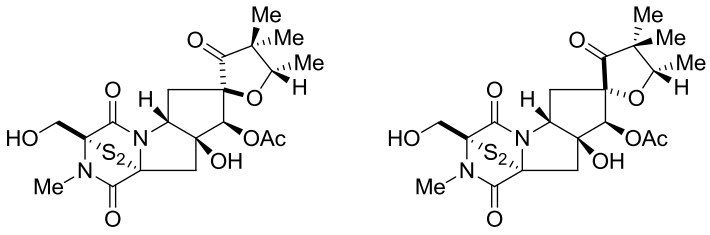


Figure 15 – Ambewelamides' A and B

In 2004, studies by Chai [83] re-iterated the importance of the disulfide bridge in the biological activities of this class of natural products. They were clearly able to obviate the importance of the epoxide rings on the scabrosin esters anticancer activity.

1.1.1.8 Sirodesmin

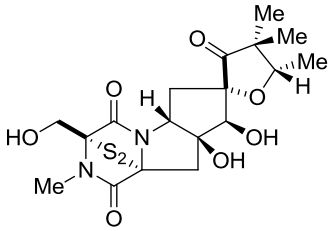
In 1974, Broadbent *et al* [84] isolated sirodesmins from the *Sirodesmium diversum* fungi, which they described as being antiviral agents against rhinoviruses. Amongst these extracts, three compounds were found to incorporate the dithiodiketopiperazine moiety: sirodesmin A (**26**) and G (**27**). The difference between these two compounds lies in the stereochemistry of the methyl groups in the four position of the dyhidrofuran-one ring, as shown below (Figure **16**).



Sirodesmin A (26) **Sirodesmin G (27)**

Figure 16 – Sirodesmins A and G

In 1977, Curtis and co-workers [85] characterised these compounds, establishing the configuration of the disulfide bridgehead carbons as *R,R*. In the same year, Ferezou *et al* [86] reported sirodesmin G (**27**) as sirodesmin PL. The name relates to the source of isolation, the fungi *Phoma lingam*.[87] The same group also isolated deacetylsirodesmin PL (**28**) shown below, which is a biological precursor of sirodesmin E (Figure **17**).



Deacetylsirodesmin PL (28)

Figure 17 – Deacetylsirodesmin PL

Sirodesmins were also found in isolates of *Leptosphaeria maculans* and tests developed by Rouxel *et al* [88] in the early 1990s unveiled important information regarding their infectious role. The addition of zinc sulfide to *Leptosphaeria maculans* growth medium decreased the production of these toxic metabolites by 75%.[89] Other studies by the same group reported the inhibition of RNA polymerase activity as an outcome of the antimetabolic activity induced by these compounds.[88] Other sirodesmins were also isolated from the fungi *Microsphaeropsis* sp. by Funabashi and co-workers.[90] These compounds TAN-1496 A (**29**), TAN-1496 B (identical to sirodesmin A (**26**)) and TAN-1496 D (deacetylsirodesmin A derivative (**30**)) (Figure **18**) were found to have anticancer activity suppressing tumour growth in various cell lines.

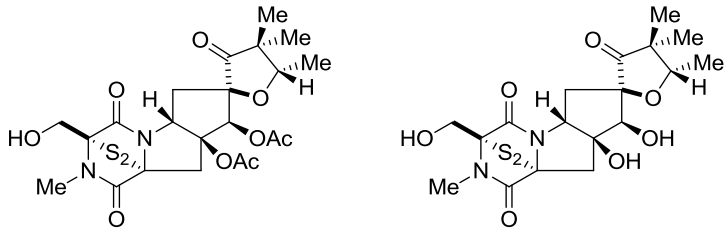


Figure 18 – TAN-1496 A and D

1.1.1.9 Dithiosilvatin

In 1987, Kawai *et al* [56] isolated dithiosilvatin (**31**) (Figure **19**) from *Aspergillus silvaticus*. Its structural backbone led the authors to conclude that the biochemical synthetic pathway would have glycine, tyrosine and phenylalanine as intermediates in its biosynthetic origins.

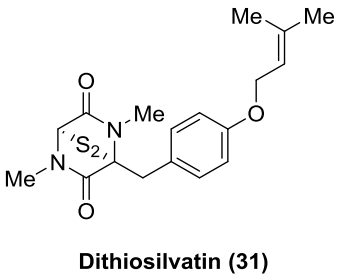


Figure 19 – Dithiosilvatin

1.1.1.10 Emestrin

Isolated from *Emericella striata* in 1985, emestrin (**32**) (Figure **20**) was described as a macrocycle which incorporates a dithiodiketopiperazine motif in its core structure.[91] The configuration of both disulfide bridgehead carbons was identified as *R*,*R*.[92]

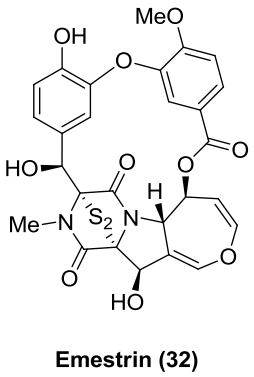


Figure 20 – Emestrin

Later, emestrin (**32**) was also isolated from different sources: *Emericella quadrilineata*, *Emericella foveolata*, *Emericella acristata* and *Emericella parvathecia*.[92, 93, 94] Works by Kawai on the cytotoxic effects of emestrin (**32**) showed that it inhibited ATP synthesis in mitochondria, restricting the respiratory process.[93] However, it also showed potent antifungal as well as anticancer activity.[92, 60] In 2004, Kanda *et al* [95] isolated three new compounds from *Cladorrhinum* sp. which contained the identical skeleton structure as emestrin (**32**): MPC1001 (**33**), MPC1001 B (**34**) and MPC1001 C (**35**) (Figure **21**).

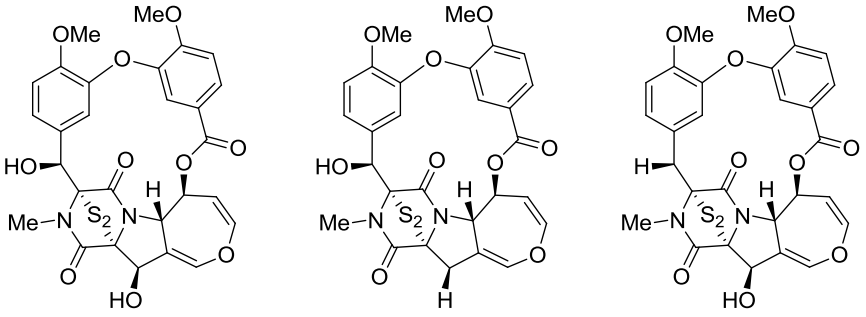


Figure 21 – MPCs 1001

These compounds proved to have highly potent anticancer activity and till now, are considered as important research models for both chemical and biological studies.[96, 97, 98, 99]

1.1.1.11 Rostratin

In 2004, Fenical *et al* [100] isolated four new examples of ETP containing molecules from *Exserohilum rostratum* which were named rostratins. Rostratins A (**36**), B (**37**), C (**38**) and D (**39**) (Figure **22**) all demonstrated potent *in vitro* cytotoxicity against human colon carcinoma cell lines with IC50's of 8.50, 1.90, 0.76, and 16.5 g/mL, respectively. In terms of absolute configuration the disulfide bridgehead carbons were reported as *R, R'*.

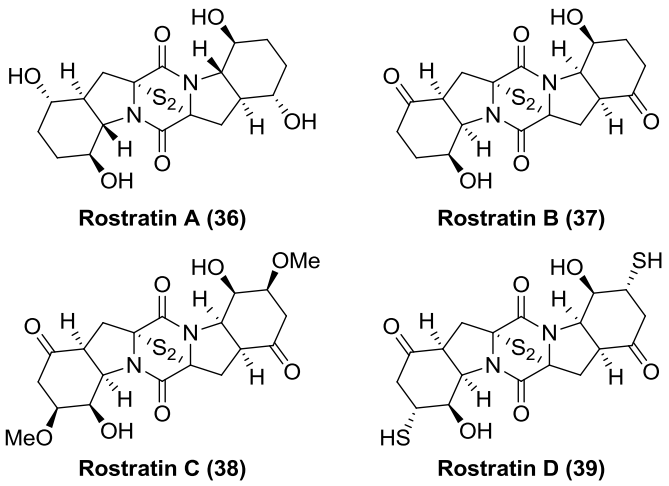


Figure 22 – Rostratins A, B, C and D

1.1.1.12 Epicoccin

In 2007, Zhang *et al* [101] isolated a new class of natural products from *Epicoccum nigrum* which were named epicoccins. From this diverse group of products, one contained the epidithiodiketopiperazine moiety – epicoccin T (**40**) (Figure **23**). This compound showed similar structural properties as rostratins A (**36**) and B (**37**) in terms of its absolute configuration.[102, 103]

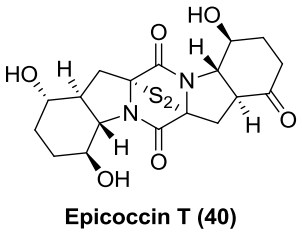
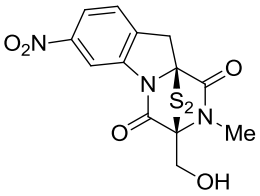


Figure 23 – Epicoccin T

1.1.1.13 Glionitrin

In 2009, Park *et al* [104] were able to isolate an unusual nitro-aromatic compound similar to gliotoxin (**1**) from the *Sphingomonas* bacterial strain KMK-001 and *Aspergillus fumigatus* fungal strain KMC-901, which was named Glionitrin A (**41**) (Figure **24**). Studies proved the *S,S* configuration of the two disulfide bridgehead carbons.



Glionitrin A (41)

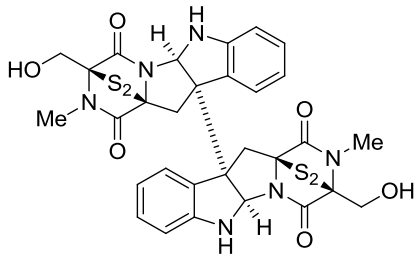
Figure 24 – Glionitrin A

The same group reported that the biological properties of this compound included antibiotic activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *in vitro* submicromolar cytotoxicity activity against four human cancer cell lines: HCT-116, A549, AGS, and DU145.[105, 106]

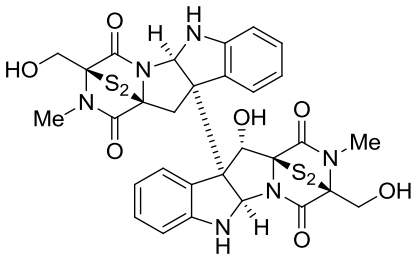
1.1.2 Dimeric ETPs

1.1.2.1 Chaetocin

In the early 1970', Hauser *et al* [107, 108] isolated both chaetocin A (**42**) and 11α,11'α-dihydroxychaetocin (**43**) from *Chaetomiun minutum* (Figure **25**). Studies by Weber [109] showed that the two disulfide bridgehead carbons configuration was *S,S*. Later that decade, chaetocin A (**42**) was also isolated from *Chaetomiun thielavioideum*.[110]



Chaetocin A (42)

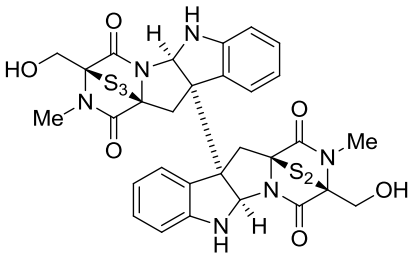


11α,11'α-dihydroxychaetocin (43)

Figure 25 – Chaetocin A and 11α,11'α-Dihydroxychaetocin

In 1988, Saito *et al* [111] isolated chetocin B (**44**) from one of the *Chaetomiun* subspecies, *Chaetomiun virescens thielavioideum* (Figure **26**). This family of compounds was widely studied for their biological activity that included antibacterial, antiviral and currently their more important anticancer activity.[112, 113, 114, 115, 116] As anticarcinogenic agents, chetocins were proven to not only act as possible therapeutic agents against

cancer, but also in enviorments where oxygen levels are low such as seen in hypoxic cancer cells.[117]

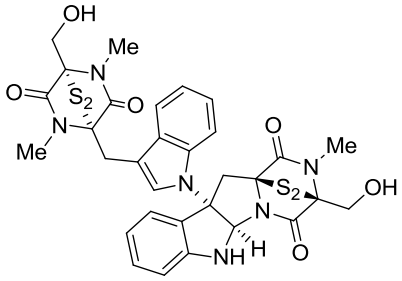


Chetocin B (44)

Figure 26 – Chetocin B

1.1.2.2 Chaetomin

In 1944, Bugie and Waksman [118, 119] isolated the first known natural product with two epidithiodiketopiperazine moieties present in the same structure from *Chaetomium cochliodes* and they named this compound chaetomin (**45**) (Figure **27**). It was later renamed chetomin (**45**) [120] and this compound was found to have a related origin from the *Chaetomium* species, *Chaetomium globosum*.[121]



Chaetomin (45)

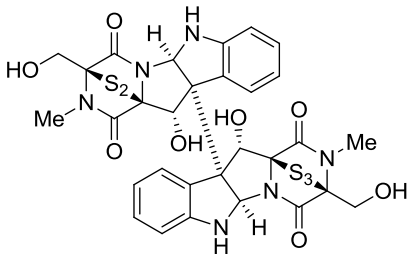
Figure 27 – Chaetomin

Chetomin (**45**) was shown to have potent antibacterial activity against *Mycobacterium tuberculosis*,[122] as well as antiviral activity against the hepatitis C virus in rat models [123] and anticancer activity.[124, 125] Studies by Spirig *et al* demonstrated that chetomin (**45**) can also induce vasodilatation in *in vitro* human monocyte stimulation models. [126]

1.1.2.3 Chetracin

In 2012, Zhu *et al* [127] characterised the only known example to date of the chetracin group of natural products with a trisulfide and disulfide bridged ETP in its structure and

it was named chetracin B (**46**) as shown below (Figure **28**). It is structurally related to the chaetocin family, with an additional *OH* group.



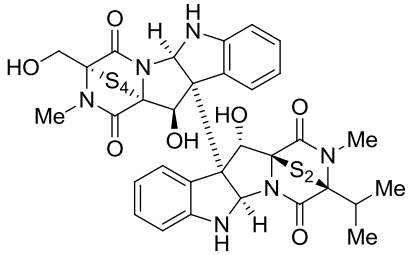
Chetracin B (46)

Figure 28 – Chetracin B

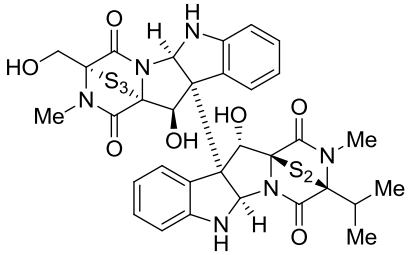
Isolated from *Oidiodendron truncatum*, chetracin B (**46**) displayed potent cytotoxicity against cancer with IC50's ranging from 0.003 µM for Bel-7402 cell lines to 0.028 µM for A2780 cell lines.

1.1.2.4 Leptosin

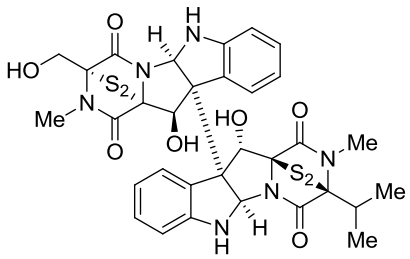
In 1994, Takahashi *et al* [128] were able to isolate several examples of the leptosin class of ETP containing natural products from *Leptosphaeria* sp.. Leptosins A (**47**), B (**48**), C (**49**) and D (**50**) (Figure **29**) displayed antitumor activity for P-388 cancer cell line with activities ranging from 1.75 mg/mL for leptosin C (**49**) to 86.0 mg/mL for leptosin D (**50**).[129, 130]



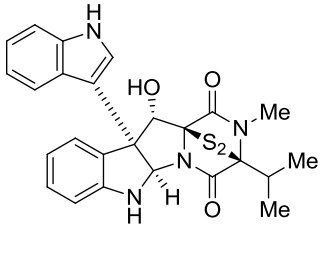
Leptosin A (47)



Leptosin B (48)



Leptosin C (49)



Leptosin D (50)

Figure 29 – Leptosins A, B, C and D

The same group was able to characterise two new compounds similar in structure to leptosins A (**47**) and B (**48**) that incorporated at least one epidithiodiketopiperazine moiety – leptosins G_2 (**51**) and H (**52**) (Figure **30**).[131]

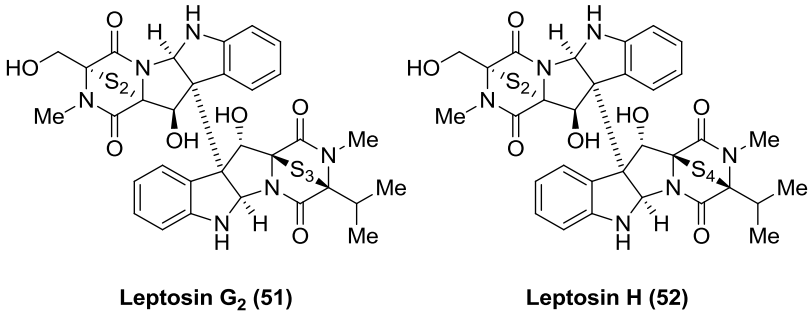


Figure 30 – Leptosins G_2 and H

In 1995, Takahashi and co-workers [132] introduced three new members of the leptosin family of natural products – leptosins K (**53**), K_1 (**54**) and K_2 (**55**) (Figure **31**), in which leptosin K (**53**) was the only example that included two ETP cores. All these isolates were demonstrated to be cytotoxic metabolites.

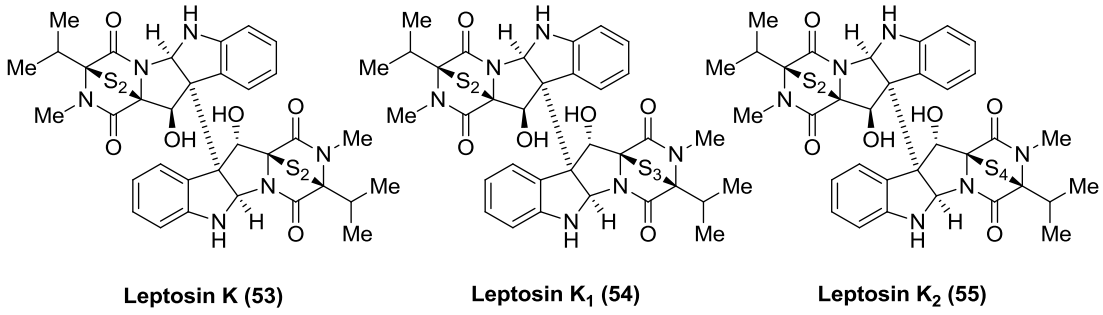
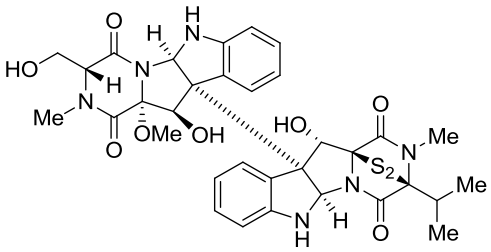


Figure 31 – Leptosins K, K_1 and K_2

The leptosin sub-family of ETP natural products contain perhaps the largest number of members and to date, the last isolated leptosin from the *Leptosphaeria* species that contains an epidithiol bridged moiety is leptosin M_1 (**56**) (Figure **32**),[133] which was shown to have potent anticancer activity.

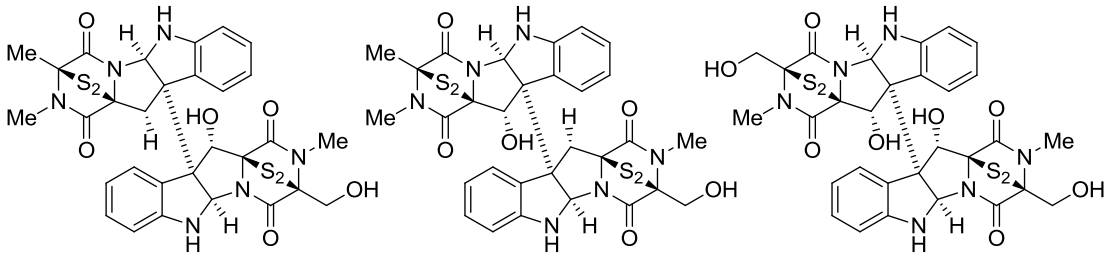


Leptosin M_1 (56)

Figure 32 – Leptosin M_1

1.1.2.5 Melinacidin

Reusser [134] described the isolation of a mixture of compounds from *Acrostalagmus cinnabarinus* var. *melinacidinus* with antibacterial activity in 1968 which was named melinacidin. Later in 1977, Argoudelis [135, 136, 137] described the structures of melinacidins II (**57**) and III (**58**) as related forms of chaetocin A (**42**) and verticillins; and melinacidin IV (**59**) was considered to be identical to 11α,11'α-dihydroxychaetocin (**43**) (Figure **33**).



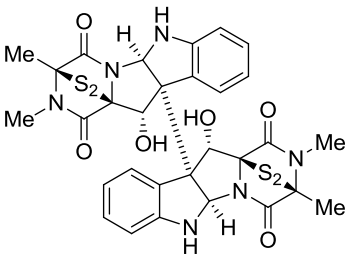
Melinacidin II (57) **Melinacidin III (58)** **Melinacidin IV (59)**

Figure 33 – Melinacidins II, III and IV

Melinacidin III (**58**) and IV (**59**) were later isolated from *Corollospora pulchella* by Furuya *et al*.[138] In the works of Feng,[139] melinacidin IV (**59**) was also found in the isolates of *Cladobotryum* sp.

1.1.2.6 Verticillin

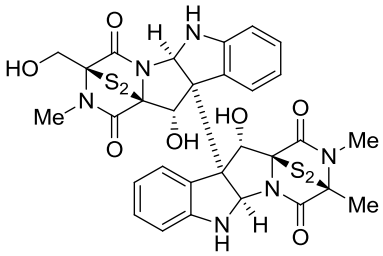
Verticillin A (**60**) (Figure **34**) was isolated from *Verticillium dahlia* by Minato *et al* [140] in 1970 and the authors demonstrated that this metabolite functioned as an antibacterial agent. In 2011, Liu [141] concluded that this compound was biologically active against different resistant human carcinoma cells with IC50's ranging from 62 nM for HepG2 hepatoma cell lines to 122 nM for HeLa cell cultures.



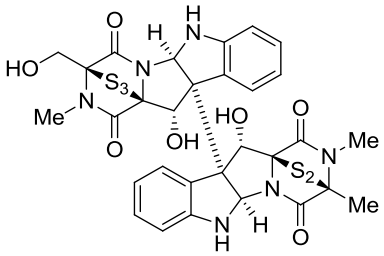
Verticillin A (60)

Figure 34 – Verticillin A

Minato isolated two new compounds from this family of natural products – verticillins B (**61**) and C (**62**) (Figure **35**).[142] In contrast to verticillins A (**60**) and B (**61**) that contain two epidithiodiketopiperazines, verticillin C (**62**) possesses one epitrithiodiketopiperazine and one epidithiodiketopiperazine core. All verticillins were shown to possess antibacterial and anticancer activity.[142, 143]



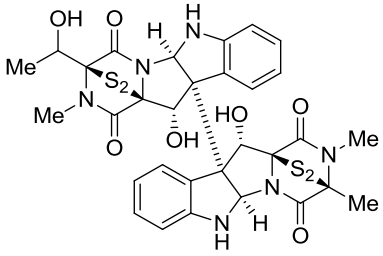
Verticillin B (61)



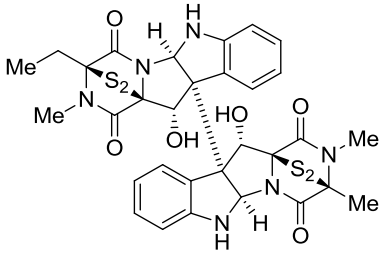
Verticillin C (62)

Figure 35 – Verticillins B and C

In 1995, Chu *et al* [144] isolated two new members of this group of molecules, Sch52900 (**63**) and Sch52901 (**64**) from *Gliocladium* sp. (Figure **36**), as well as verticillin A (**60**), and reported that all compounds isolated possessed antitumorogenic activity when tested *in vitro*.[145]



Sch52900 (63)



Sch52901 (64)

Figure 36 – Sch52900 and Sch52901

A marine isolate of the *Penicillium* sp. by Byeng and co-workers [146] led to the identification of two new compounds with identical core structures to verticillin A (**60**) – 11,11'-dideoxyverticillin A (**65**) and 11'-deoxyverticillin A (**66**) (Figure **37**). 11,11'-Dideoxyverticillin A (**65**) was later isolated from the herb *Shiraia bambusicola*.[147] Both compounds demonstrated *in vitro* cytotoxicity against colon and human breast carcinoma cell lines.[146, 147]

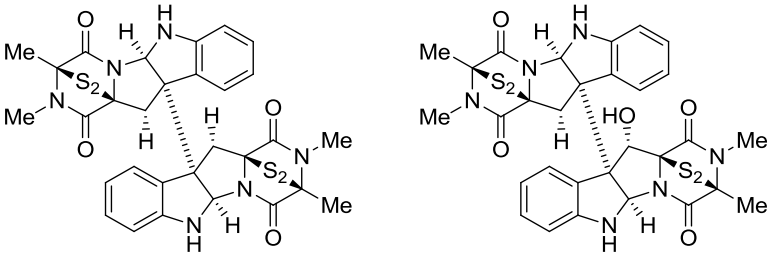


Figure 37 – 11,11'-Dideoxyverticillin A and 11'-Deoxyverticillin A

From the same family, Joshi *et al* [148] isolated verticillins D (**67**), E (**68**) and F (**69**) (Figure **38**) from the mycoparasite *Gliocladium catenulatum*. Stereochemistry for both the alcohol (**67** and **69**) and acetate groups (**68** and **69**) was not described by the authors.

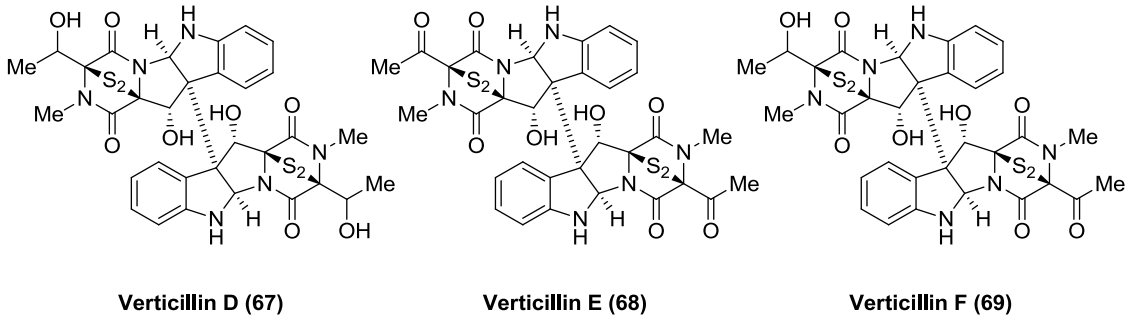


Figure 38 – Verticillins D, E and F

A different species of the previous parasite isolates – *Gliocladium roseum* – by Zhang *et al* [101] led to the characterisation of three new examples of structurally related verticillin analogues: gliocladines A (**70**), B (**71**) and C (**72**) (Figure **39**). These compounds showed antiparasitic activity against *Caenorhabditis elegans* and *Panagrellus redivivus*.[149,]

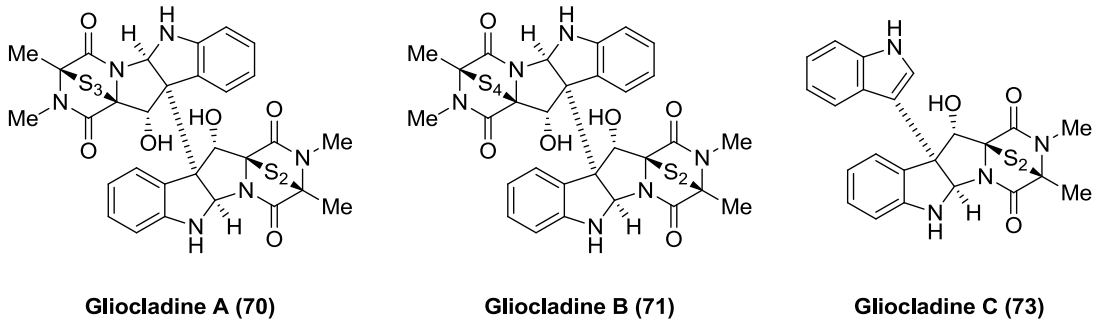


Figure 39 – Gliocladines A, B and C

Kim *et al* [150] isolated three new compounds from *Bionectra byssicola* with structural similarity to the verticillin group of natural products: bionectins A (**73**), B (**74**) and verticillin G (**75**) (Figure **40**). These compounds were found to have potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA) as well as the quinolone-resistant species.[150, 151]

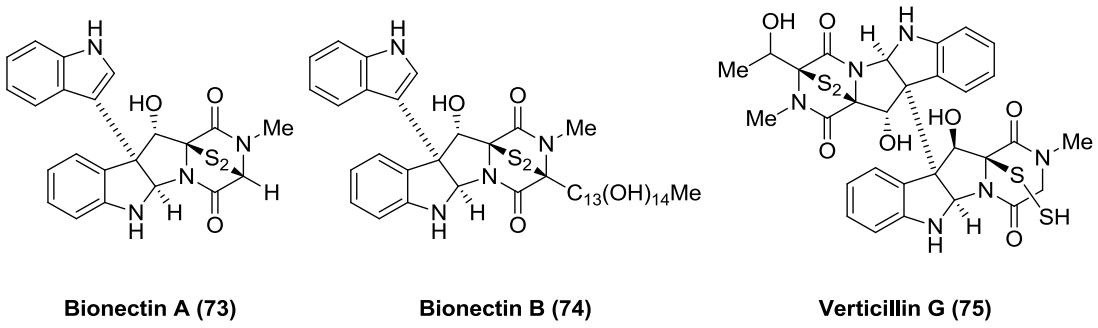
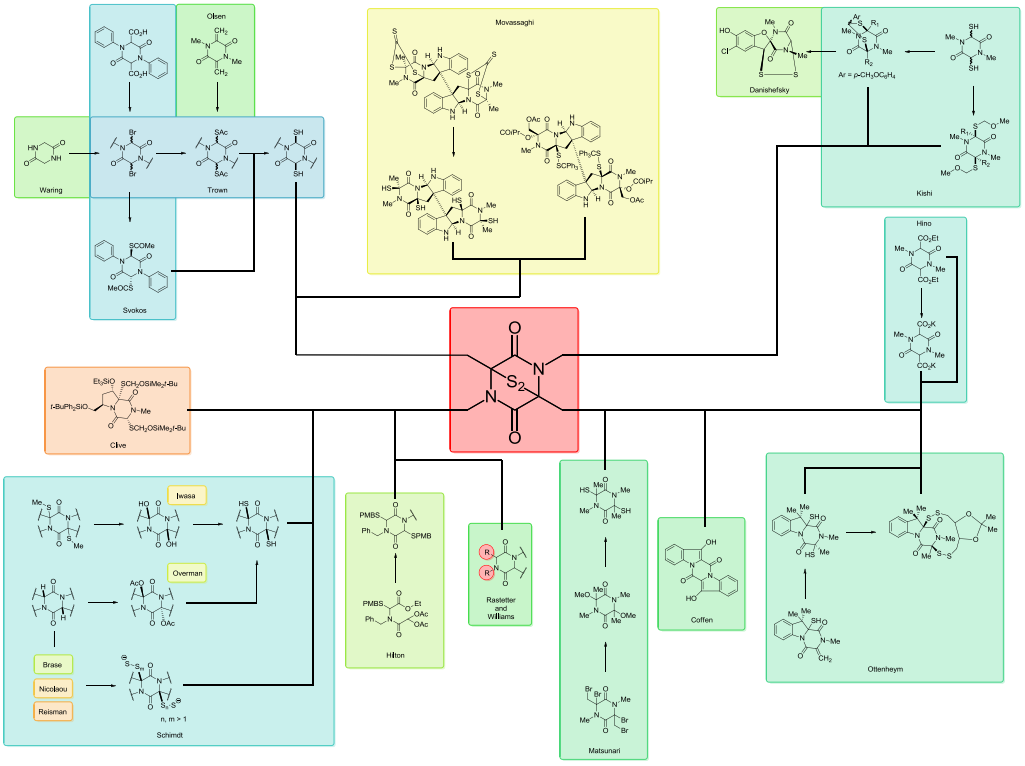


Figure 40 – Bionectins A, B and G

In 2011, Schenke *et al* contested the origin of the verticillin group of compounds by presenting data that suggested that verticillin A (**60**) is not produced by the *Verticillium* species but possibly from *Gliocladium roseum*, a known mycoparasite of the *Verticillium* sp.[152]

1.2 Synthetic approaches towards the epi-3,6-dithio-2,5-diketopiperazine core and related natural products

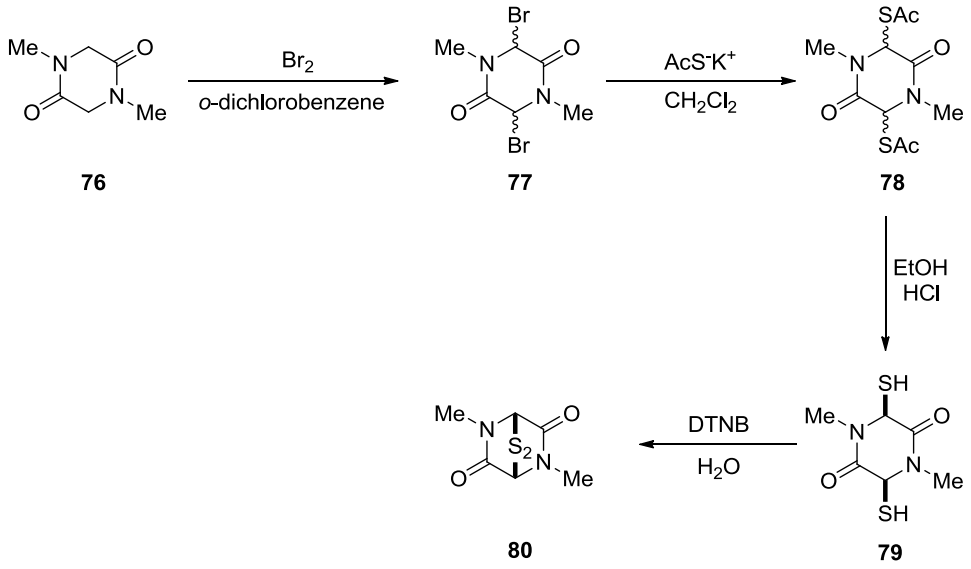
Despite their structural complexity, the ETP family of compounds all have a common origin, as amino acids have clearly been identified as their biosynthetic precursors, with cyclic dipeptides suggested as obvious reaction intermediates from radiolabelling experiments.[153, 154] These biosynthetic pathways have clearly inspired ETPs synthetic approaches using amino acids as starting points for the synthetic process.[154] Following the initial works by Trown in the late 1960's,[155] there have been a number of advances made to access either natural or synthetic ETPs using several different strategies as outlined below (Scheme **1**).[156] Incorporation of sulfur into a pre-formed diketopiperazine core or the introduction of sulfur in an initial pathway allows a classification of the numerous approaches. Rastetter and Williams [157] also highlighted the relevance and innovation that the work around the ETP core can bring to chemistry. Many authors attempted to find the best pathway to achieve a facile method towards the ETP core and synthesise structural analogues with the various approaches to this shown below (Scheme **1**).



Scheme 1 – Schematic representation of the different approaches used to achieve the ETP core including the corresponding authors

1.2.1 Trown's approach

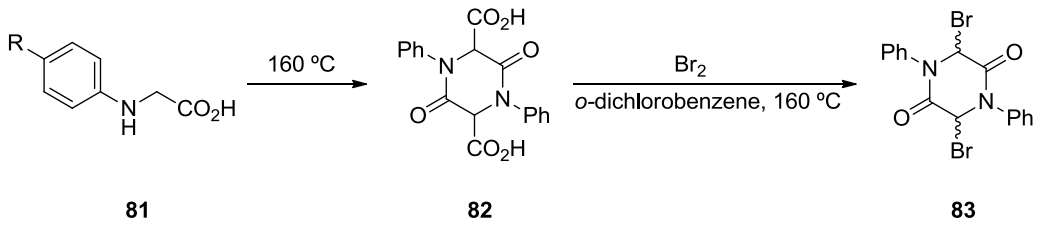
In 1968 Trown *et al* [54, 155, 158, 159, 160, 161] was the first group that clearly introduced a synthetic methodology to access epi-3,6-dithio-diketopiperazines using the nucleophilic propensity of sulfur. According to their method, sarcosine anhydride (**76**) – a dimethyl substituted 2,5-piperazinedione – was first dibrominated at the *3*- and *6*- positions to form the 3,6-dibromo-1,4-dimethylpiperazine-2,5-dione intermediate (**77**). Treatment with potassium thioacetate led to the displacement of both bromine atoms to yield the resulting dithioacetate (**78**). Trown and co-workers already highlighted a concern at this stage of the synthesis which was to try to obtain the maximum yield of the *cis* isomer since it was the only one that could undergo the subsequent transformation to the disulfide. The next step involved the acidic hydrolysis of (**78**) in ethanol to remove the acetate groups giving compound (**79**). The disulfide bridged compound (1R,4R)-6,8-dimethyl-2,3-dithiabicyclo[2.2.2]octane-5,7-dione (**80**) was achieved after oxidation with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (Scheme **2**).[162]



Scheme 2 – Trown's approach

1.2.2 Svokos' approach

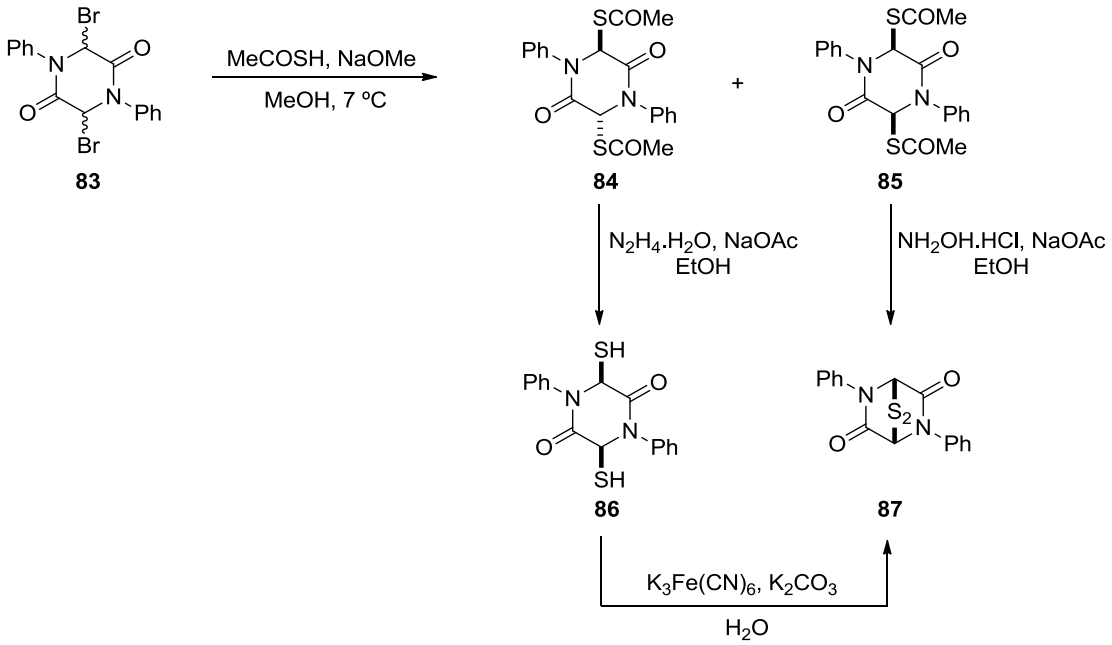
In 1970 Svokos' *et al* [163, 164] patented their approach to the ETP core. The first step of the synthesis followed the works of Meyer [165] and Halberkann [166] to achieve the functionalized 1,4-diphenyl-2,5-piperazinedione (**82**) from dimerization of the initial substituted 4-phenylamino acetic acid (**81**) at high temperatures followed by bromination in positions *3*- and *6*- giving (**83**) (Scheme **3**).



81 **82** **83**

Scheme 3 – Svoko's approach (1)

Treatment of (**83**) with thioacetic acid and sodium methoxide in methanol yielded the thiol intermediate in both the *cis* (**85**) and *trans* (**84**) isomeric forms. The *cis* (**85**) isomer was directly converted to (**87**) by treatment with hydroxylamine; whereas the *trans* isomer (**84**) had to be converted to the dithiol form (**86**) using hydrazine hydrate and was later oxidized with potassium ferricyanide to give the desired ETP (**87**) (Scheme **4**).[163]



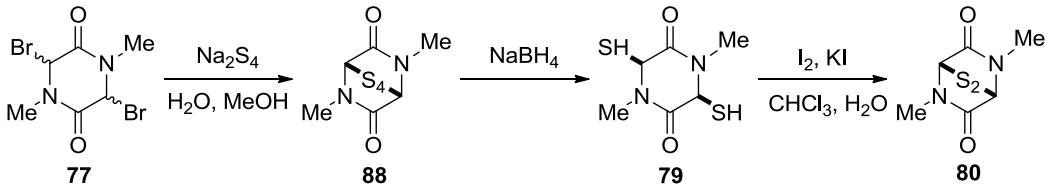
83 **84** **85** **86** **87**

Scheme 4 - Svoko's approach (2)

1.2.3 Schmidt's approach

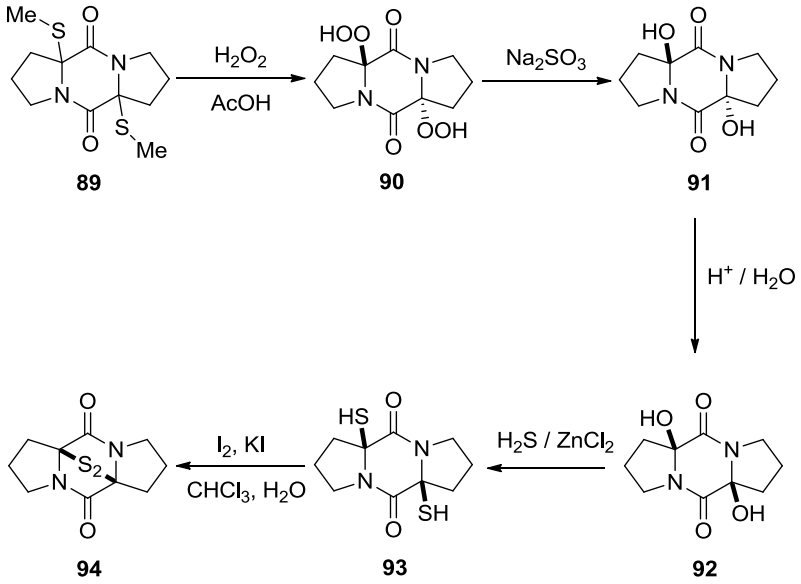
In 1971 Schmidt *et al* [167, 168] achieved an ETP synthesis by direct introduction of sulfur into the 2,5-piperazine ring using the same nucleophilic substitution principle as Trown.[155] Treatment of 3,6-dibromo-1,4-dimethyl-2,5-piperazinedione with sodium tetrasulfide led to the stable compound (**80**). The epi-tetrasulfide (**88**) was then reduced using sodium tetrahydridoborate to the *cis*-dithiol compound (**79**) which could be dehydrogenated in a two-phase system of dithiol in chloroform / aqueous potassium iodide solution giving compound (**80**).[169, 170] Schmidt developed this new method of

oxidation as they were unable to reproduce the system previously developed by Trown using Ellmans's reagent.[155]



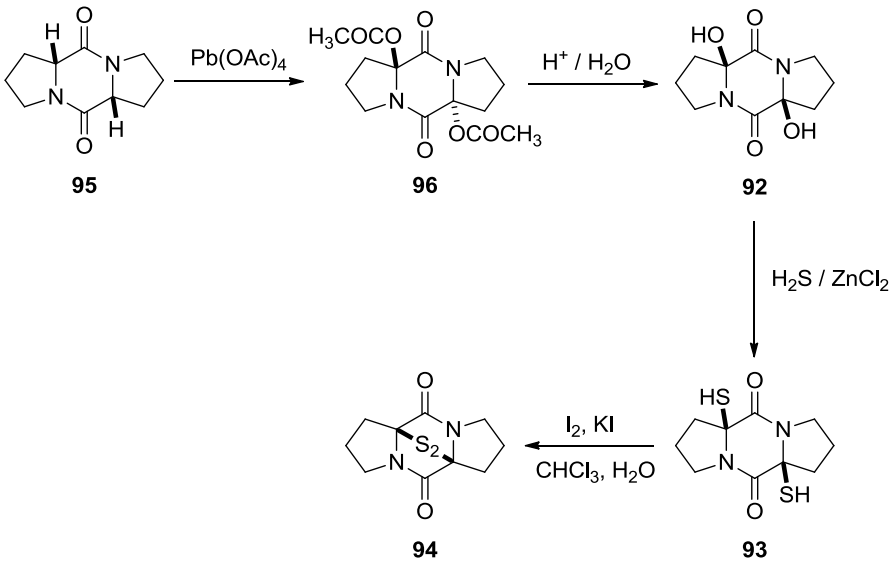
Scheme 5 – Schmidt's approach (1)

Another method reported by Schmidt was nucleophilic introduction of sulfur using sulfones and hydroxy derivatives. The synthesis of epidithiopropyl-proline anhydride (**94**) involved the oxidation of compound (**89**) affording the corresponding hydroperoxide (**90**) which could be reduced to the corresponding hydroxide (**91**) using sodium sulfite and hydrogenation of (**91**) gave *cis* compound (**92**). Displacement of the hydroxides using zinc chloride and attack by hydrogen sulfide gave compound (**93**) that after oxidation led to the desired product (**94**) (Scheme **6**).[167, 171, 172, 173, 174]



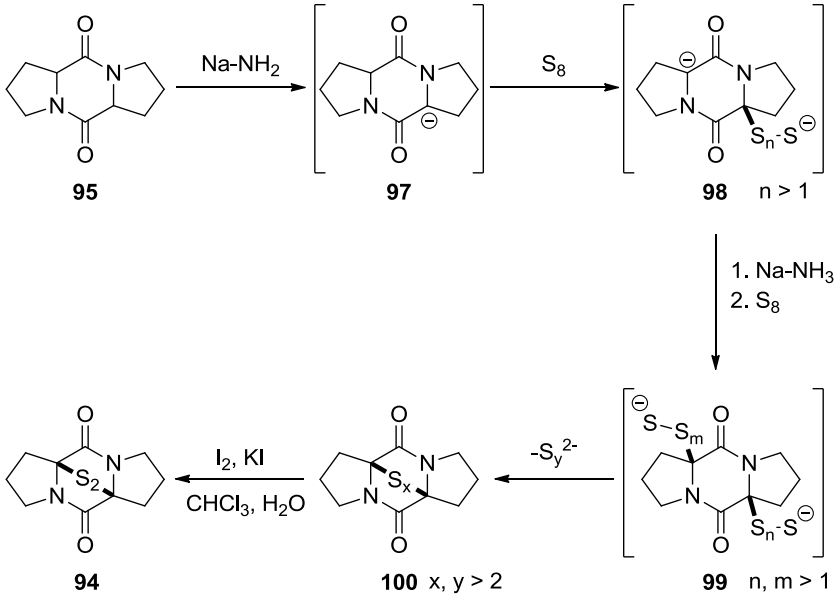
Scheme 6 – Schmidt's approach (2)

Using the same principles as before, octahydrodipyrrolo[1,2-a:1',2'-d]pyrazine-5,10-dione (**95**) was oxidised by lead tetraacetate to give intermediate (**96**) that led to the known compound (**92**) using dilute aqueous acid (Scheme **7**). These new approaches were important as they introduced the ability to interconvert mixtures of isomeric intermediates to racemic ETPs.[172, 173, 175, 174]



Scheme 7 – Schmidt's approach (3)

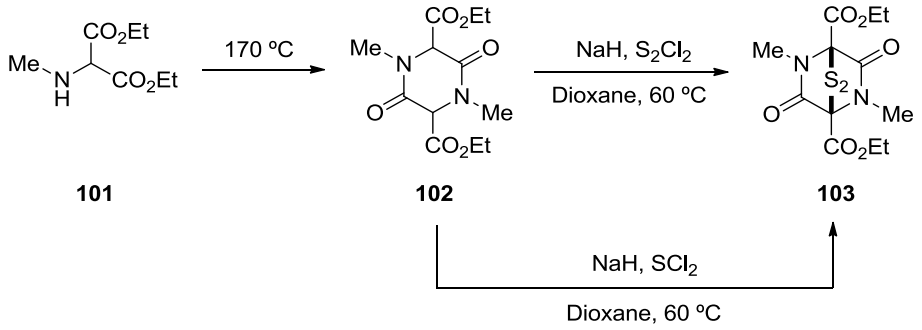
However, despite these advances, this new methodology never led to highly functionalised ETPs. An alternative strategy developed by Schmidt was to incorporate both sulfurs as electrophiles into a 2,5-diketopiperazine scaffold using a strong base.[172, 173, 175, 174] Deprotonation of dione (**95**) with sodamide or lithium diisopropylamide (LDA) afforded anion (**97**). The reaction of elemental sulfur with (**97**) gave the polysulfide thiolate anion (**98**), thus repeating these steps led to (**99**) forming the polysulfide bridged product. Compound (**100**) was first reduced and further oxidized to give the disulfide bridged product (**94**) (Scheme **8**).



Scheme 8 – Schmidt's approach (4)

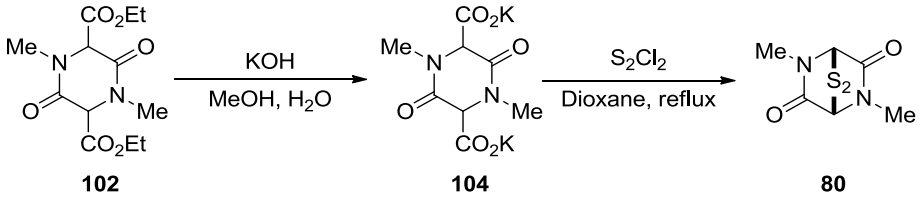
1.2.4 Hino's approach

In 1971 Hino *et al* [176, 177] described a new way to achieve the ETP ring system in two steps. First, diethyl *N*-amino malonate (**101**) was heated to high temperatures to yield compound (**102**). Secondly, the diethoxy carbonyl precursor (**102**) was treated with a strong base to form the dicarbanion which, when treated with either disulfur dichloride or sulfur dichloride, gave ETP (**103**) (Scheme **9**).



Scheme 9 – Hino's approach (1)

Similar C-S bond formation was studied by Hino and co-workers using dicarboxylated salts.[178] Hydrolysis of the already known (**102**) using potassium hydroxide led to the corresponding potassium salt (**104**) that was subsequently reacted with disulfur dichloride to yield the desired ETP (**80**) (Scheme **10**). Intriguingly, the group noted that the decarboxylation process did not occur in boiling dioxane unless disulfur dichloride was added, indicating that the sulfur containing reactant was involved in the process.

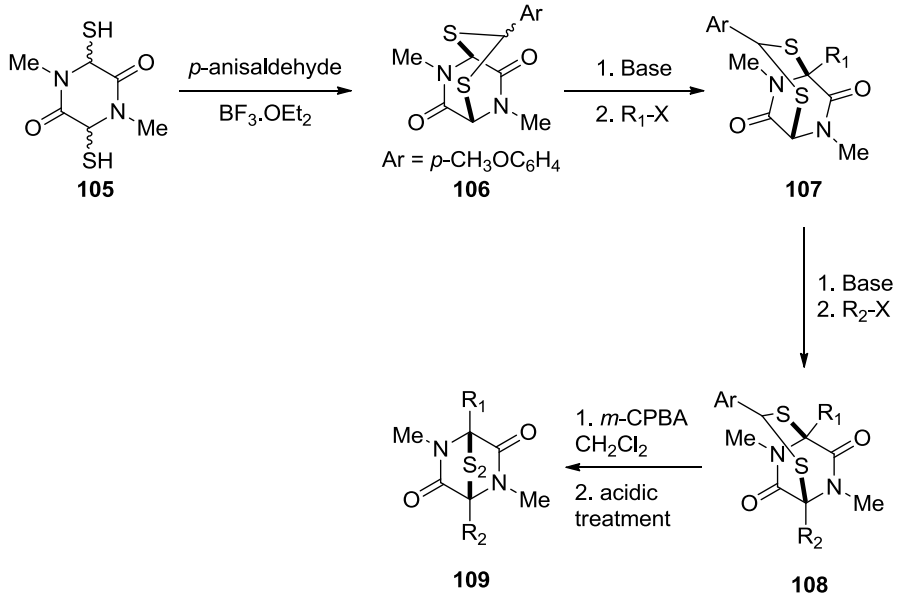


Scheme 10 – Hino's approach (2)

1.2.5 Kishi's approach

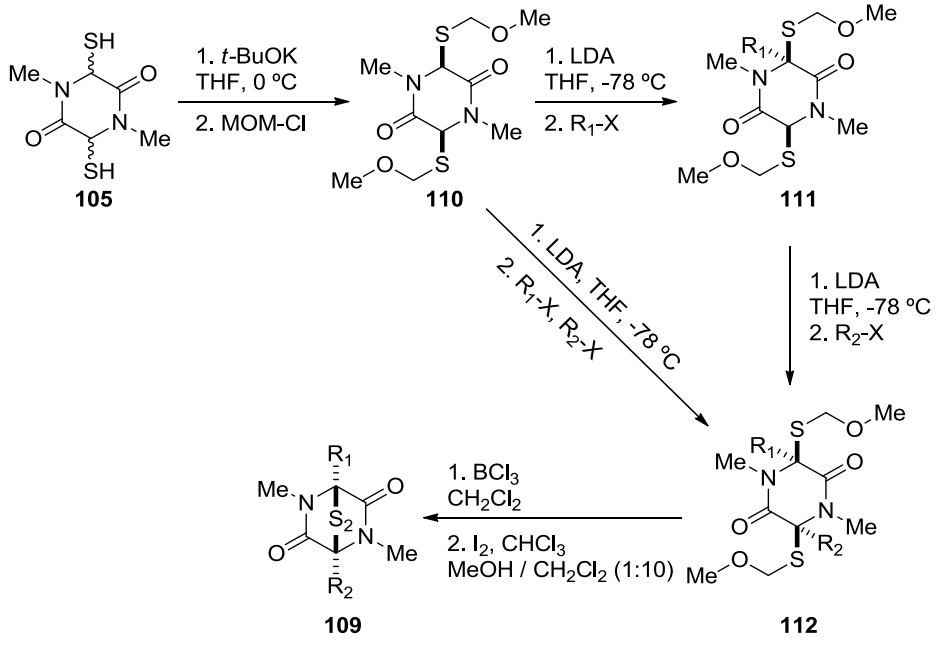
Kishi *et al* [179] in 1973 developed two methods in order to synthesise both natural and synthetic ETP containing products: one was to introduce a protected disulfide bridge at an early stage; the second complemented the other end of the synthesis introducing the disulfide bridge at a later stage.

The starting and key point of the first synthetic route was to introduce a protected precursor of the ETP ring; this would enable functionalization of both *3*- and *6*-positions of the 3,6-dithio-2,5-diketopiperazine using carbanion chemistry.[179, 180] The resulting intermediate would become useful since it was stable under acidic, basic or reductive conditions. In the first step of the proposed synthetic pathway it was interesting to note that both *cis*- and *trans*-dithiol materials (**105**) would react with *para*-anisaldehyde in the presence of boron trifluoride etherate to produce the same thioacetal intermediate (**106**).[181] Deprotonation on the bridge-head position of (**106**) was achieved using a strong base such as *n*-butyllithium (*n*BuLi) or LDA giving the carbanion. The next step involved alkylation using primary halides, acid halides and aldehydes to give mono-substituted thioacetals (**107**) and a small amount of disubstituted thioacetals. The disubstituted ETP precursor (**108**) could be obtained if the same procedure of deprotonation / alkylation was conducted once more under the same conditions. Kishi explained the need of this two-step reaction due to the differing acidities that both hydrogens on the *3*- and *6*- positions displayed depending on the position of the anisaldehyde residue.[179] The disulfide was regenerated in two steps: reaction of the thioacetal with *meta*-chloroperbenzoic acid (*m*-CPBA) to give a sulfoxide intermediate followed by acid treatment, using Lewis or protic acids, leading to the ETP (**109**) (Scheme **11**). According to Kishi, the *para*-methoxybenzene ring was proven to be of crucial importance since the cleavage of the sulfoxide did not take place using thioacetals derived from formaldehyde, acetaldehyde or benzaldehyde.[179]



Scheme 11 – Kishi's approach (1)

Although this synthetic route proved to be very useful to achieve a large variety of compounds, Kishi faced two problems: the short half-life of the bridgehead carbanion and the reaction conditions for acid cleavage of the thioacetal monosulfoxide (**108**). In order to overcome these potential problems, another methodology was developed in which the key intermediate, a dimethoxymethylthio compound (**110**), was prepared from (**105**) using potassium *t*-butoxide and chloromethyl methyl ether. This new compound (**110**) was functionalised in two separate stages since during alkylation studies, a difference in reactivity between both dicarbanion and carbanion formed from the monoalkylated derivative (**111**) was observed. When treated with a strong base, the *cis*-monoalkylated piperazinedione compound (**111**) was obtained in high yields. In light of these results, two approaches were used, based on the differences in reactivity associated with the alkylating agents to achieve unsymmetrically disubstituted dimethoxymethylthio compounds (**112**); or alkylation of the mono-substituted derivative (**111**) to give compound (**112**). After consecutive alkylation, compound (**112**) was converted to the corresponding *cis*-dithiol using boron trichloride which was then oxidized using iodine to yield the ETP (**109**) (Scheme **12**).[182]

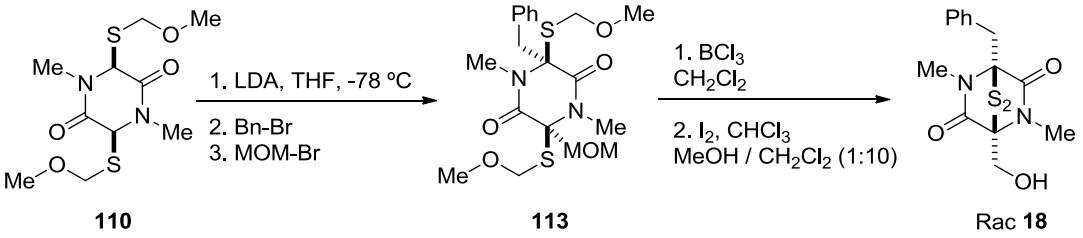


Scheme 12 – Kishi's approach (2)

Using these two different synthetic pathways, Kishi and co-workers were able to synthesise natural products from the ETP family. Their accomplishments are described below.

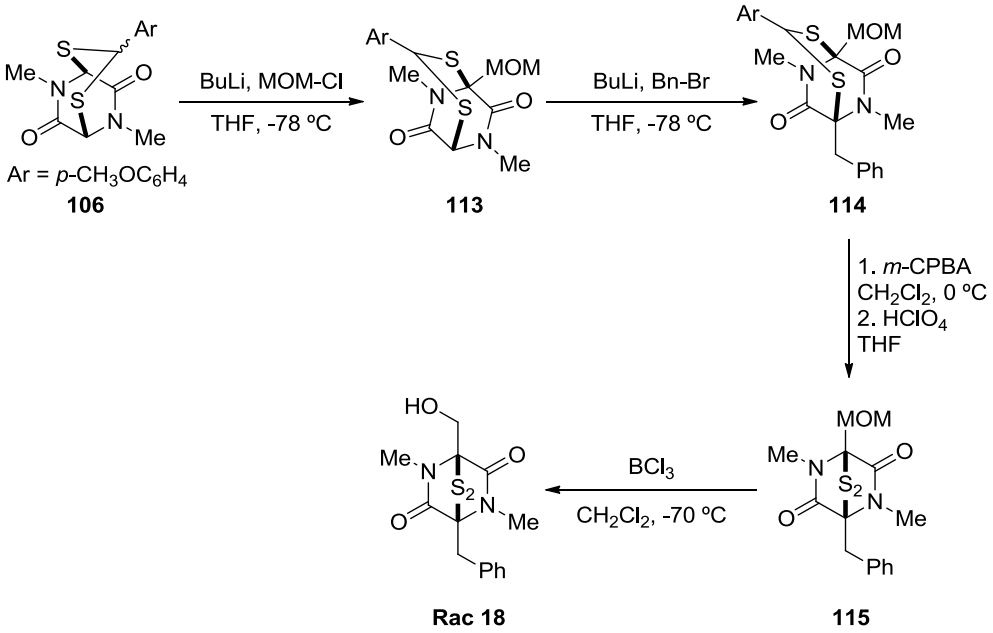
1.2.5.1 Hyalodendrin

Using the dimethoxymethylthio derivative (**110**), Kishi [182] was able to use different alkylating agents reactivity to form the unsymmetrical disubstituted compound (**113**). This was followed by *cis*-dithiol formation and oxidation to give a racemic mixture of hyalodendrin (**18**) (Scheme **13**).



Scheme 13 – Kishi's synthesis of hyalodendrin

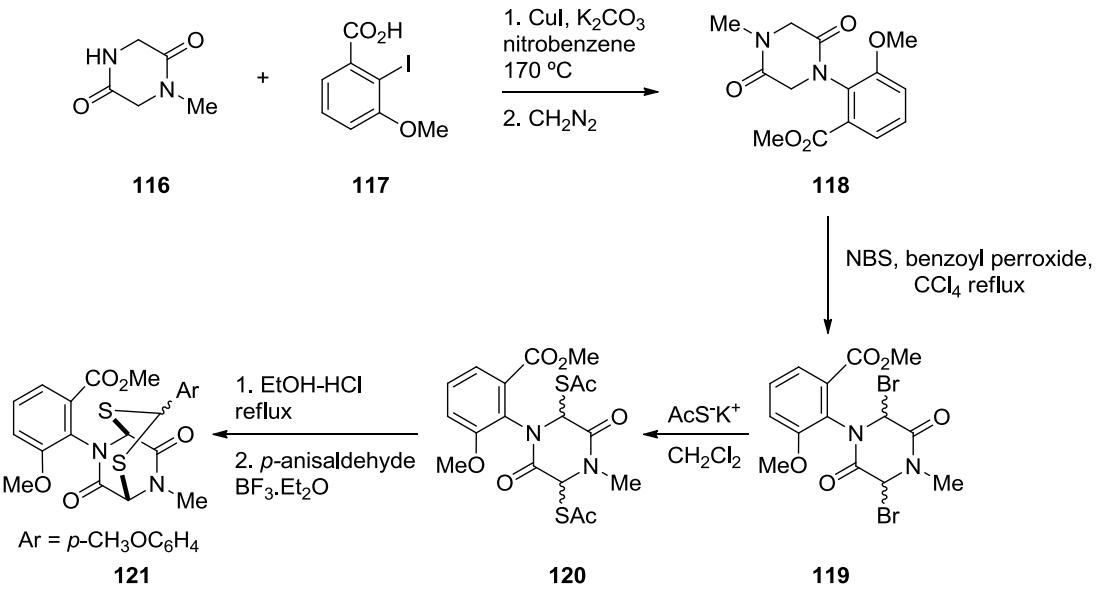
In 1974 using thioacetal intermediate (**106**), Strunz and Kakushima [73] were able to synthesise a racemic mixture of hyalodendrin.[71, 72, 74] Promoting carbanion formation using *n*-BuLi and treatment with chloromethyl methyl ether gave (**113**) which yielded (**114**) after alkylation with benzyl bromide. The resultant product was then treated with *m*-chloroperbenzoic acid and perchloric acid rendering (**115**). The last step involved reduction of the methyl ether to the corresponding alcohol using boron trichloride to give the desired racemic mixture of ETP (**18**) (Scheme **14**).



Scheme 14 – Strunz and Kakushima's synthesis of hyalodendrin

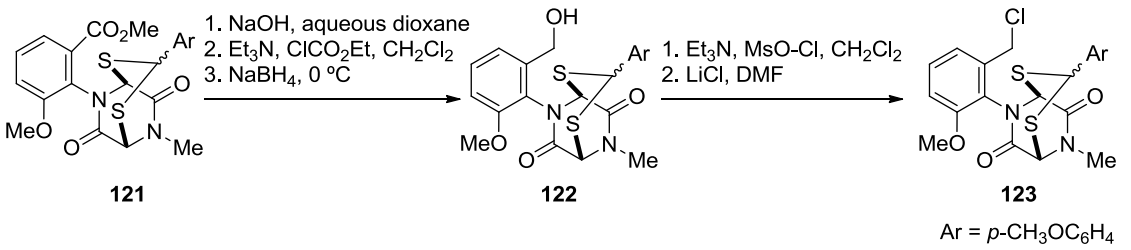
1.2.5.2 Dehydrogliotoxin

Using the previously described thioacetal intermediate chemistry, Kishi *et al* were able to synthesise (**2**).[179, 182, 183, 184] The chosen starting material for this experiment was the piperazinedione (**118**) achieved by coupling 2-iodo-3-methoxybenzoic acid (**117**) to glycine-sarcosine anhydride (**116**) in the presence of cuprous iodide and potassium carbonate. The subsequent product of this reaction was treated with diazomethane to yield the esterified compound (**118**). Taking the previous product (**118**) in carbon tetrachloride and reacting it with *N*-bromosuccinimide and benzoyl peroxide gave Trown's brominated intermediate analogue (**119**). Treatment with potassium thioacetate and displacement of both bromine atoms gave dithioacetate (**120**) in a 1:1 *cis*/*trans* mixture. Acid hydrolysis followed by treatment with *para*-anisaldehyde and boron trifluoride etharate gave thioacetals (**121**) in a 1:1 diastereomeric mixture (Scheme **15**).



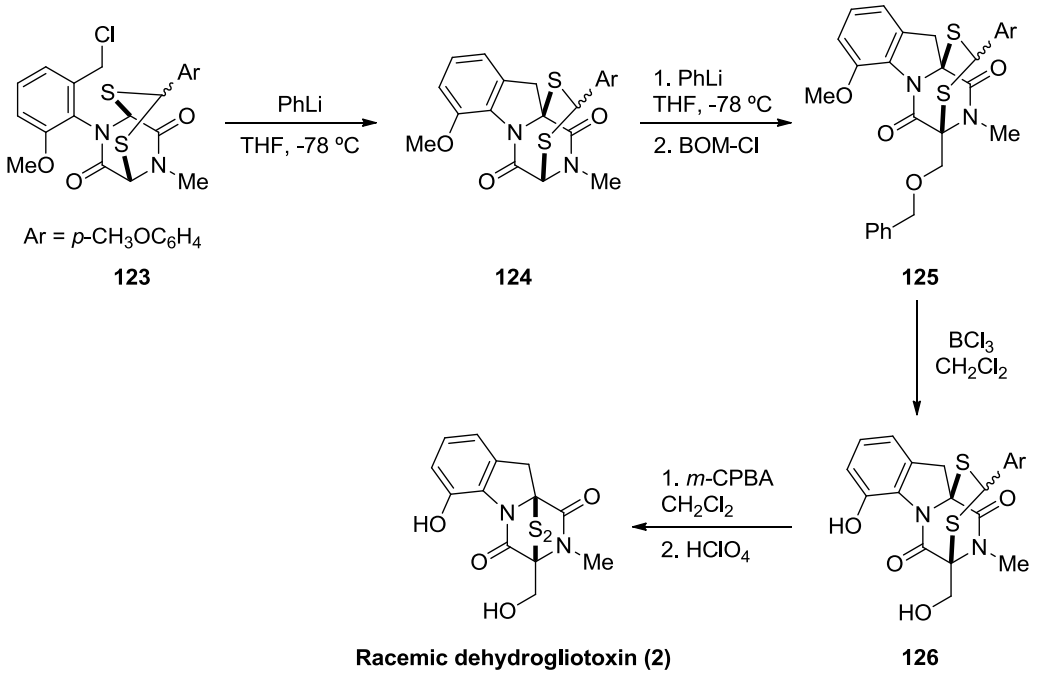
Scheme 15 – Kishi's synthesis of dehydrogliotoxin (1)

The ester was then converted to the alcohol (**122**) using sodium hydroxide followed by triethylamine and ethyl chloroformate treatment. A methanolic solution of sodium borohydride was then added to form the desired compound (**122**). This thioacetal alcohol (**122**) was then converted to the corresponding thioacetal chloride (**123**) by treatment with triethylamine and methanesulfonylchloride. Conversion of this intermediate with lithium chloride gave the desired compound (**123**) in 89% yield (Scheme **16**).[183]



Scheme 16 – Kishi's synthesis of dehydrogliotoxin (2)

The diastereomeric chloride (**123**) was regiospecifically metallated at the bridgehead position using phenyllithium to yield dihydroindole moiety (**124**) which was converted to (**125**) using the carbanion resulting from the addition of further phenyllithium and benzyl chloromethylether. The dehydrogliotoxin thioacetal (**126**) was obtained using boron trichloride and the product of this reaction treated with *m*-chloroperbenzoic acid and perchloric acid to render the racemic mixture of dehydrogliotoxin (**2**) (Scheme **17**).[183]

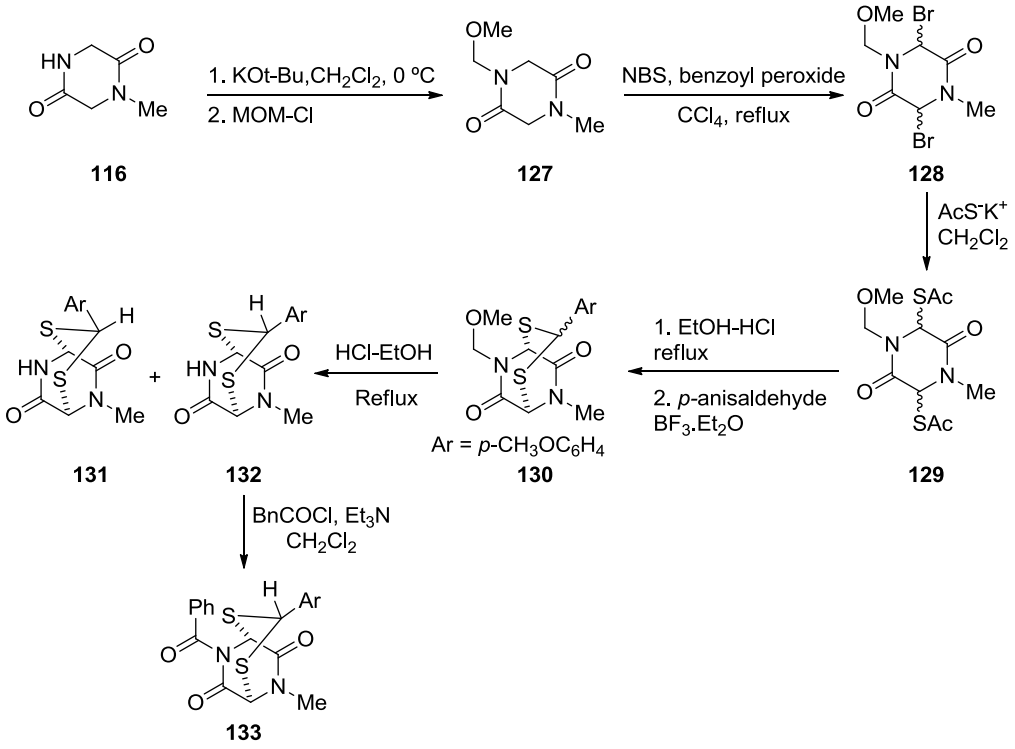


Scheme 17 – Kishi's synthesis of dehydrogliotoxin (3)

1.2.5.3 Gliotoxin

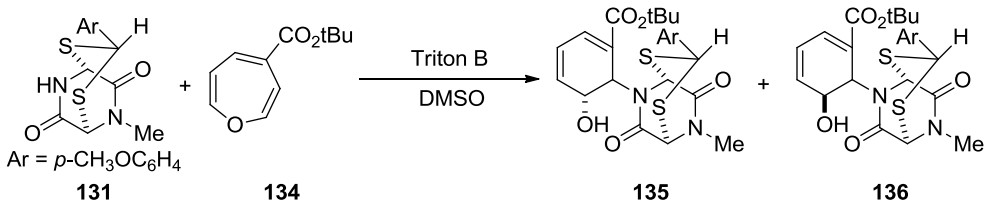
For the synthesis of gliotoxin (**1**), Kishi *et al* [179, 182, 185] commenced with the protection of known glycine-sarcosine anhydride (**116**) with a methoxymethyl group, using a strong base and chloromethyl methyl ether. Compound (**127**) was converted to the diastereomeric mixture of dithioacetates (**128**), followed by conversion to the corresponding methoxymethyl thioacetal (**129**) to form intermediate (**130**). The

methoxymethyl group was removed using a 1:1 ethanolic-acid mixture to form (**131**) and (**132**) (Scheme **18**).



Scheme 18 – Kishi's synthesis of gliotoxin (1)

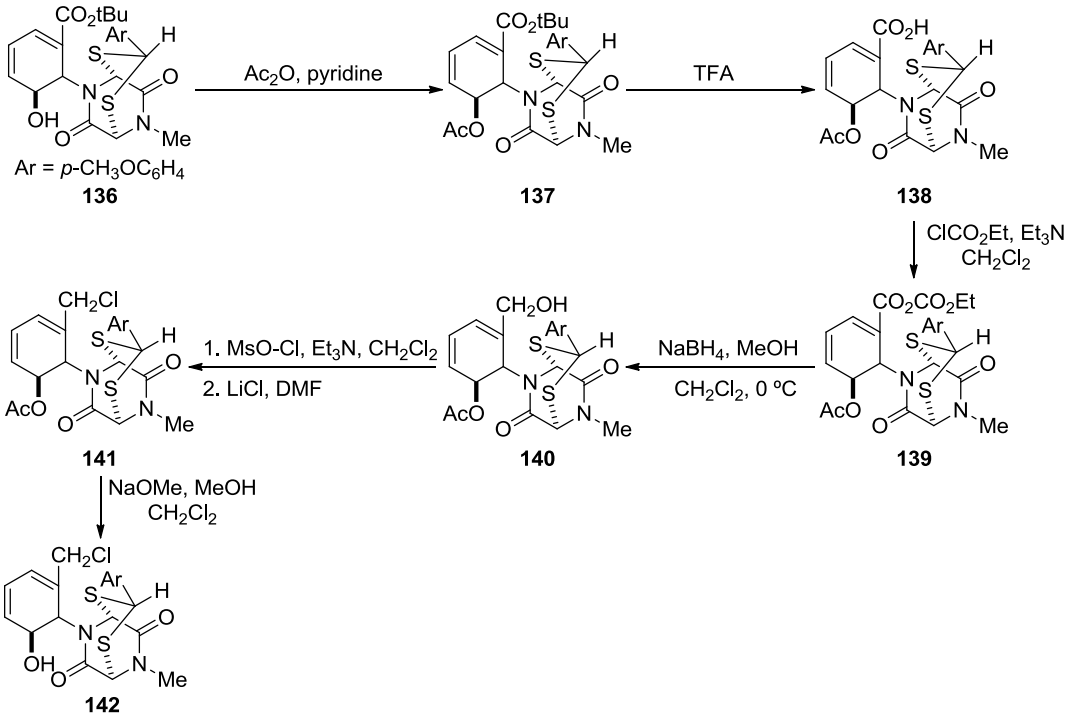
Isomer (**132**) was found to be more reactive to *N*-benzoylation than (**131**) enabling them to be separated by silica gel chromatography. Treatment of the preferred diastereomer (**131**) with 4-carbo-*t*-butoxyoxepin (**134**) and Triton B yielded a mixture of Michael adducts (**135** and **136**). This reaction was found to be solvent dependent since the preferred configuration of product (**136**) could be achieved in a better ratio using dimethyl sulfoxide instead of dichloromethane as a solvent (Scheme **19**).[179, 185]



Scheme 19 – Kishi's synthesis of gliotoxin (2)

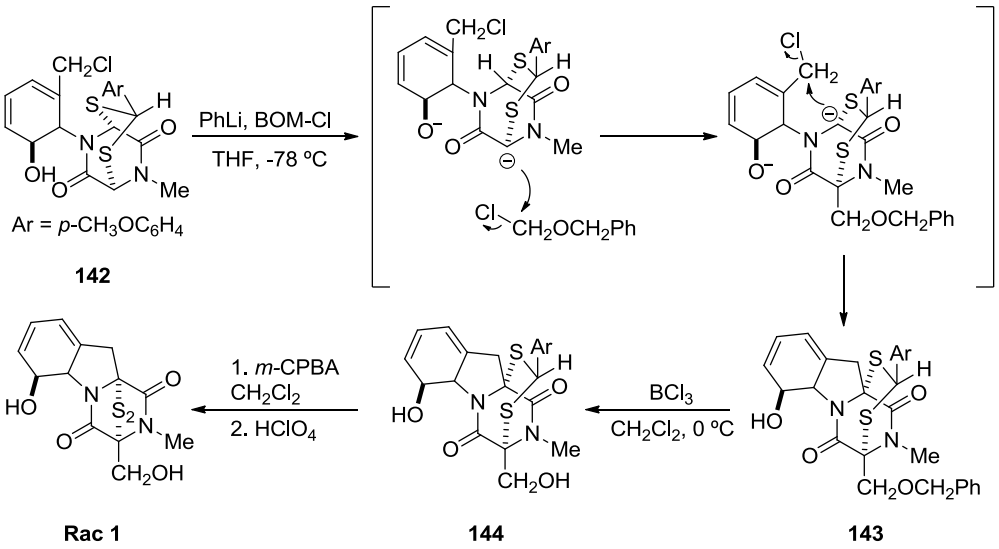
Compound (**136**) was subsequently treated with base and acetic anhydride to give acetate (**137**) followed by carboxylic acid formation using trifluoroacetic acid. The resultant product (**138**) was converted to the anhydride (**139**) by base and ethyl chloroformate treatment and further reduced to the alcohol (**140**) using sodium

borohydride. Mesylation and chlorination yielded the chloride acetate (**141**), which was reduced to chloride alcohol (**142**) on treatment with sodium methoxide (Scheme **20**).[185]



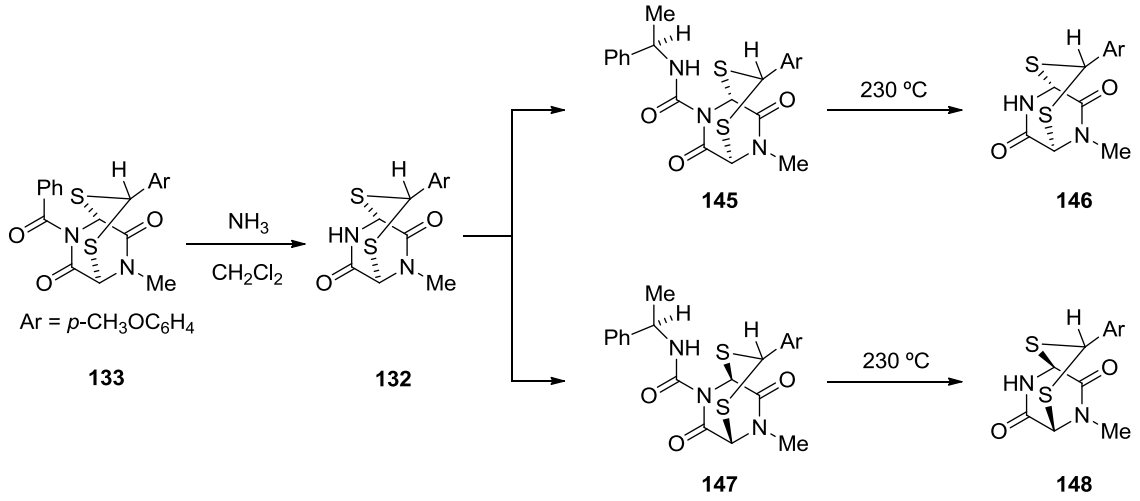
Scheme 20 – Kishi's synthesis of gliotoxin (3)

The decisive step that involved alkylation-cyclization of (**142**) was skilfully achieved by addition of a strong base and benzyl chloromethyl ether to give the desired product (**143**). The final steps of this synthesis are equivalent to the described route that achieved dehydrogliotoxin (**2**),[183] with benzyl ether cleavage to give (**144**) followed by epidithiol formation that rendered racemic gliotoxin (**1**) (Scheme **21**).



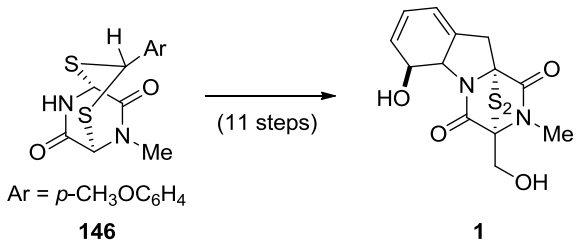
Scheme 21 – Kishi's synthesis of gliotoxin (4)

After separation of both isomeric forms of (**131**) and (**132**), Kishi and co-workers were able to synthesise optically active gliotoxin (**1**).[179, 182, 184, 185] Compound (**132**) was recovered by exposing the *N*-benzoyl thioacetal (**133**) through treatment with ammonia solution. Thioacetal ureas (**145** and **147**) were obtained from compound (**132**) using α-(-)-methylbenzyl isocyanate. The two diastereomers were separated by silica gel chromatography and the resolving agent removed by pyrolysis, yielding enantiomers (**146**) and (**148**) (Scheme **22**).



Scheme 22 – Kishi's synthesis of gliotoxin (5)

Use of the desired optically active thioacetal (**146**) by Kishi as starting material and the previously established synthetic route, enabled them to obtain optically active gliotoxin (**1**) as shown below (Scheme **23**).

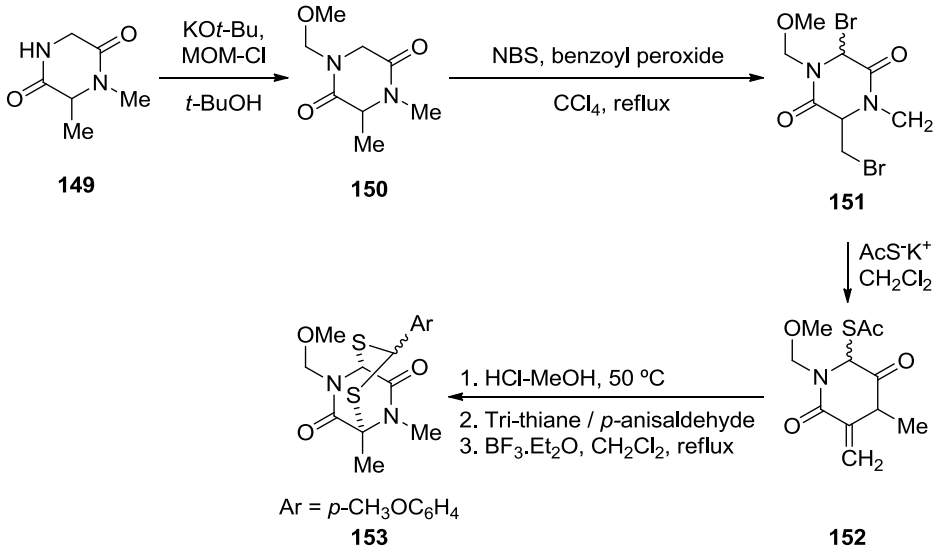


Scheme 23 – Kishi's synthesis of gliotoxin (6)

1.2.5.4 Sporidesmin A

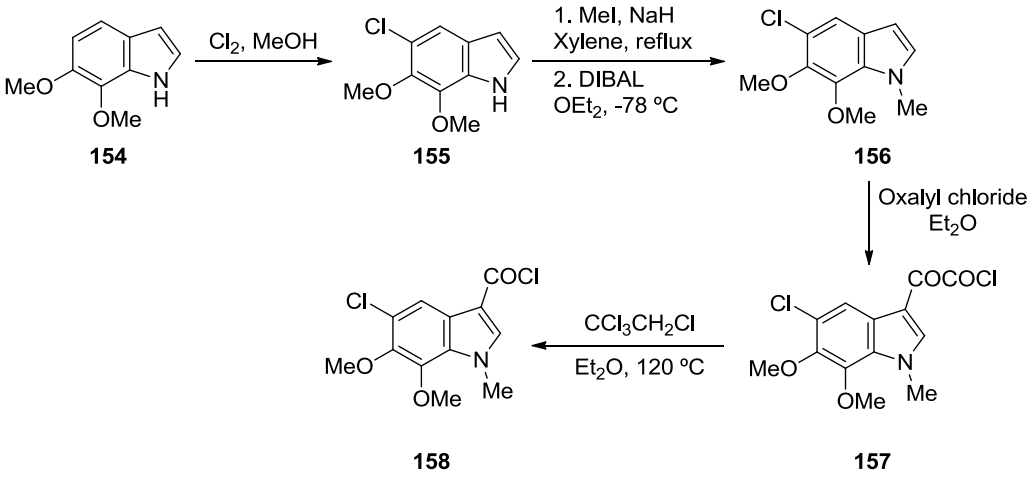
Following the synthesis of gliotoxin (**1**),[185] Kishi *et al* turned their attention to the total synthesis of sporidesmin A (**3**).[179, 186, 187] This involved a thioacetal precursor (**153**) that, after treatment with the indole moiety (**158**), would give desired compound (**159**). Thioacetal intermediate synthesis started by treatment of 1,6-dimethylpiperazine-2,5-dione (**149**) with base and chloromethyl methyl ether to give (**150**). Bromination /

dehydrobromination followed to give compound (**151**) and thioacetate formation afforded intermediate (**152**). The thioacetate was then transformed into the thioacetal (**153**) using a different method from the one described before since it used a trithiane derivative of *p*-anisaldehyde to form a *syn*- and *anti*- mixture of (**153**) to the anisaldehyde and methoxymethyl residues, respectively (Scheme **24**).



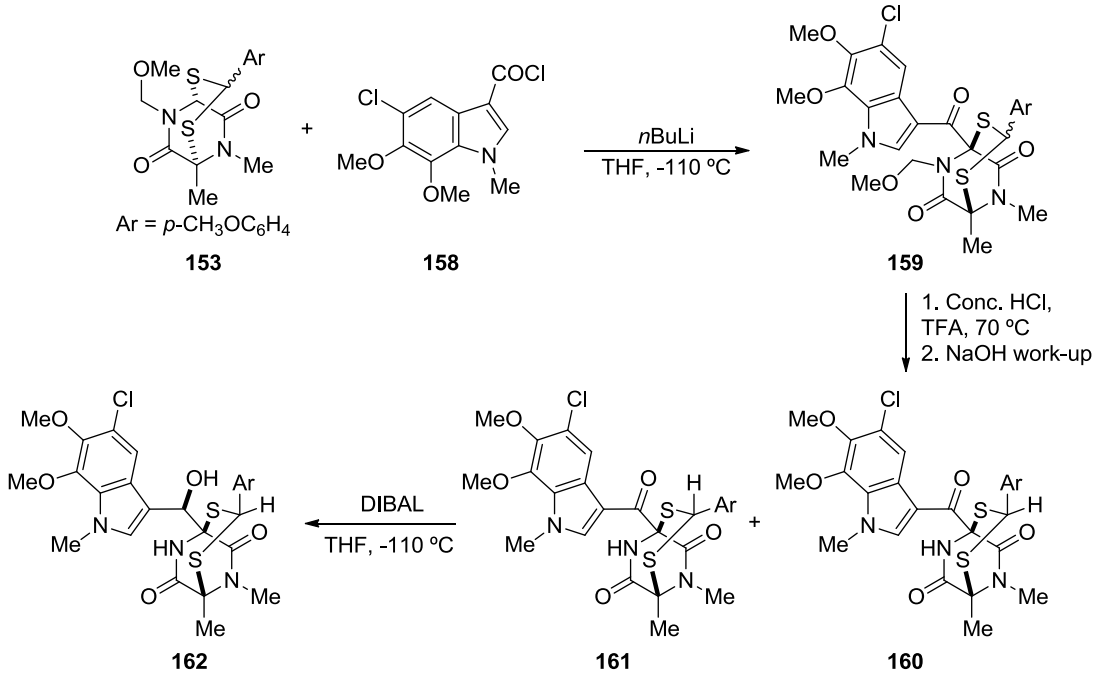
Scheme 24 – Kishi's synthesis of sporidesmin A (1)

6,7-Dimethoxyoxindole (**154**) chlorination was the first step in the indole moiety synthesis. This compound (**155**) was then *N*-methylated using methyl iodide in the presence of a strong base and reduced using diisobutylaluminium hydride (DIBAL) to give (**156**). Treatment of (**156**) with oxalyl chloride afforded the oxamic acid chloride (**157**) that when submitted to high temperatures yielded (**158**) (Scheme **25**).[186]



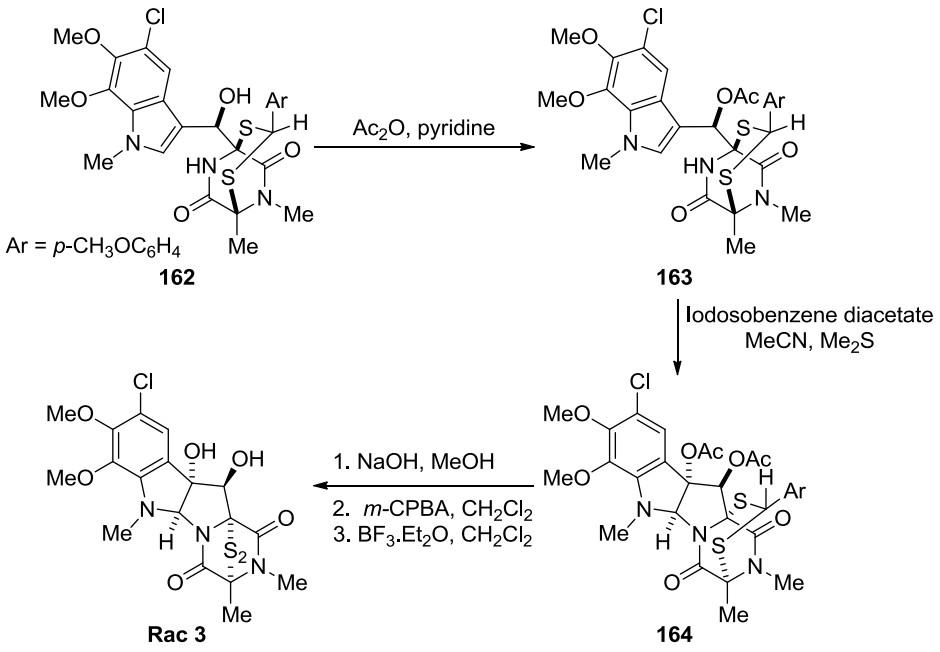
Scheme 25 – Kishi's synthesis of sporidesmin A (2)

The next step in the synthesis gave the carbanion derived from (**153**) using a strong base. Alkylation in the presence of pre-formed acid chloride (**158**) gave a *syn* / *anti* mixture of (**159**). Acidic treatment afforded compounds (**160**) and (**161**) that were separated using preparative thin layer chromatography (TLC). Ketone (**160**) was stereoselectively reduced using DIBAL yielding alcohol (**162**) (Scheme **26**).



Scheme 26 – Kishi's synthesis of sporidesmin A (3)

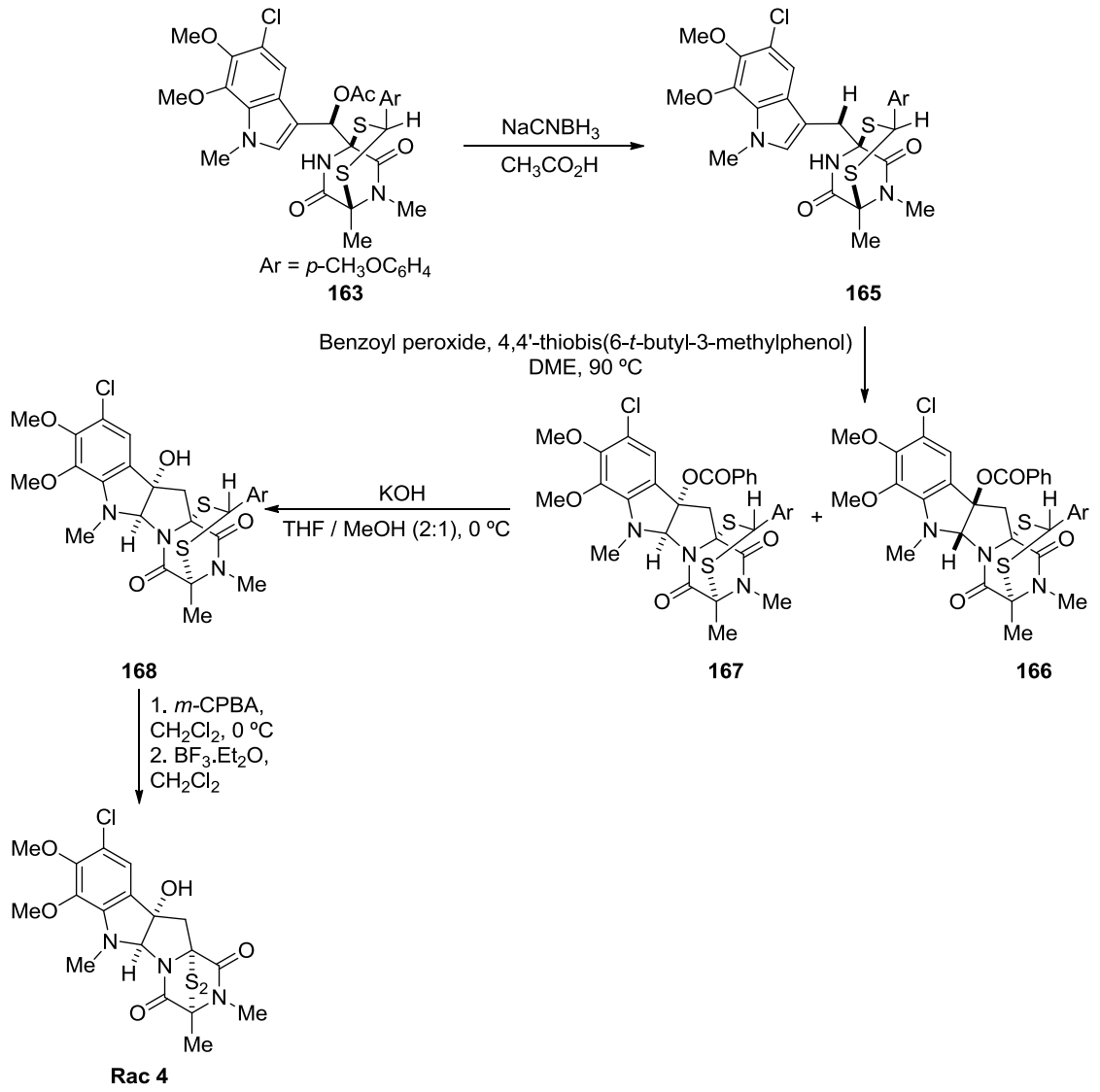
Alcohol (**162**) was oxidized using acetic anhydride in a basic environment to give (**163**) that after treatment with iodosobenzene diacetate and dimethyl sulfide, yielded the cyclized diacetate (**164**). Using the previously described synthetic route (sodium hydroxide treatment; followed by *m*-chloroperbenzoic acid and boron trifluoride etherate reaction), a racemic mixture of sporidesmin A (**3**) was achieved (Scheme **27**).



Scheme 27 – Kishi's synthesis of sporidesmin A (4)

1.2.5.5 Sporidesmin B

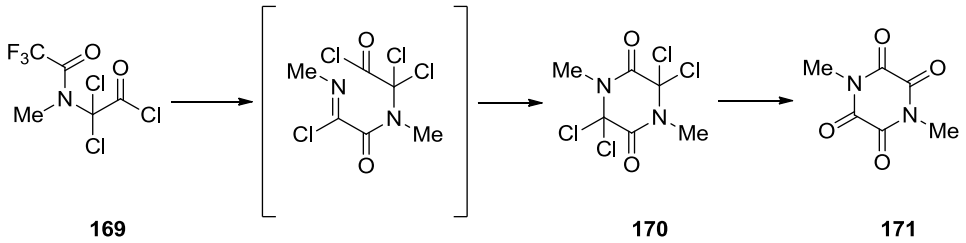
Using the same acetate intermediate (**163**)[186] used for the synthesis of racemic sporidesmin A (**3**) as starting material, Kishi *et al* [179, 188] were able to produce sporidesmin B (**4**) as a racemate. Reduction of (**163**) to the subsequent methylene derivative (**165**) followed by oxidation using benzoyl peroxide with traces of 4,4'-thiobis(6-*t*-butyl-3-methylphenol) gave a mixture of both cyclized products (**166**) and (**167**). After purification, benzoate (**167**) was converted to alcohol (**168**) and reacted with *m*-chloroperbenzoic acid and boron trifluoride etherate to yield the desired monomeric ETP sporidesmin B (**4**) as a racemic mixture (Scheme **28**).



Scheme 28 – Kishi's synthesis of sporidesmin B (1)

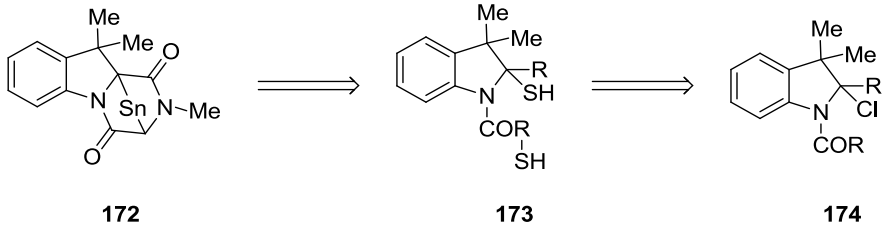
1.2.6 Ottenheym's approach

Before establishing a new route for ETP synthesis, Ottenheym *et al* [189, 190, 191, 192, 193, 194] made remarkable breakthroughs towards the ETP core structure. One of which was the synthesis of tetrachlorodioxopiperazine (**171**) reported in 1972,[195] from the not expected instability of disubstituted sarcosyl chloride (**169**) which led to tetraketopiperazine (**171**) as the resulting decomposition product (Scheme **29**).



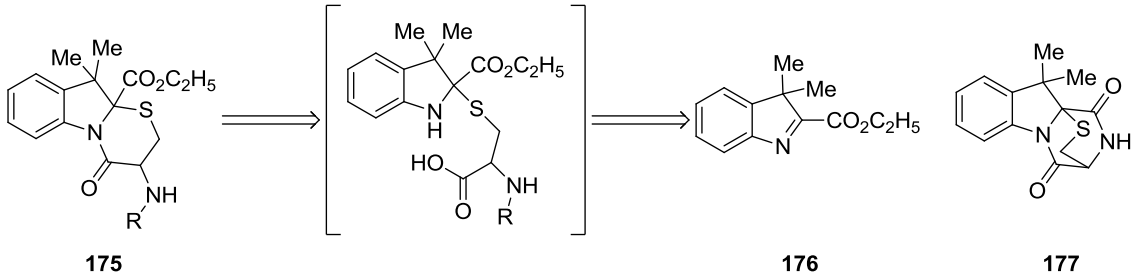
Scheme 29 – Ottenheym's approach (1)

In 1973, and following Leuchs previous studies,[193] Ottenheym attempted the nucleophilic displacement of 2-chloro substituted indolenines (**174**) by using sulfur nucleophiles. The idea was to create a sulfur adduct (**173**) that could undergo cyclization to form the dithiol-bridged diketopiperazine (**172**) (Scheme **30**).[189]



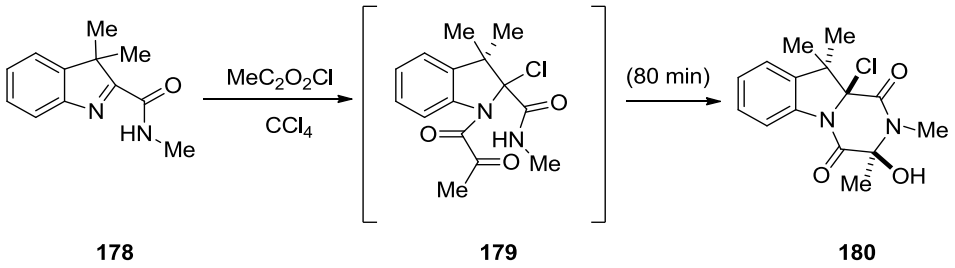
Scheme 30 – Ottenheym's approach (2)

Another model reaction reported by the author was the direct nucleophilic addition of sulfur to non-substituted Leuchs' adducts (**176**) which could cyclize to give (**175**). In way of example, a desthiomethylene analogue of anhydrogliotoxin (**177**) was synthesised by ring closure of the free amine in an intramolecular aminolysis of (**176**) (Scheme **31**).[189, 190, 191, 192]



Scheme 31 – Ottenheym's approach (3)

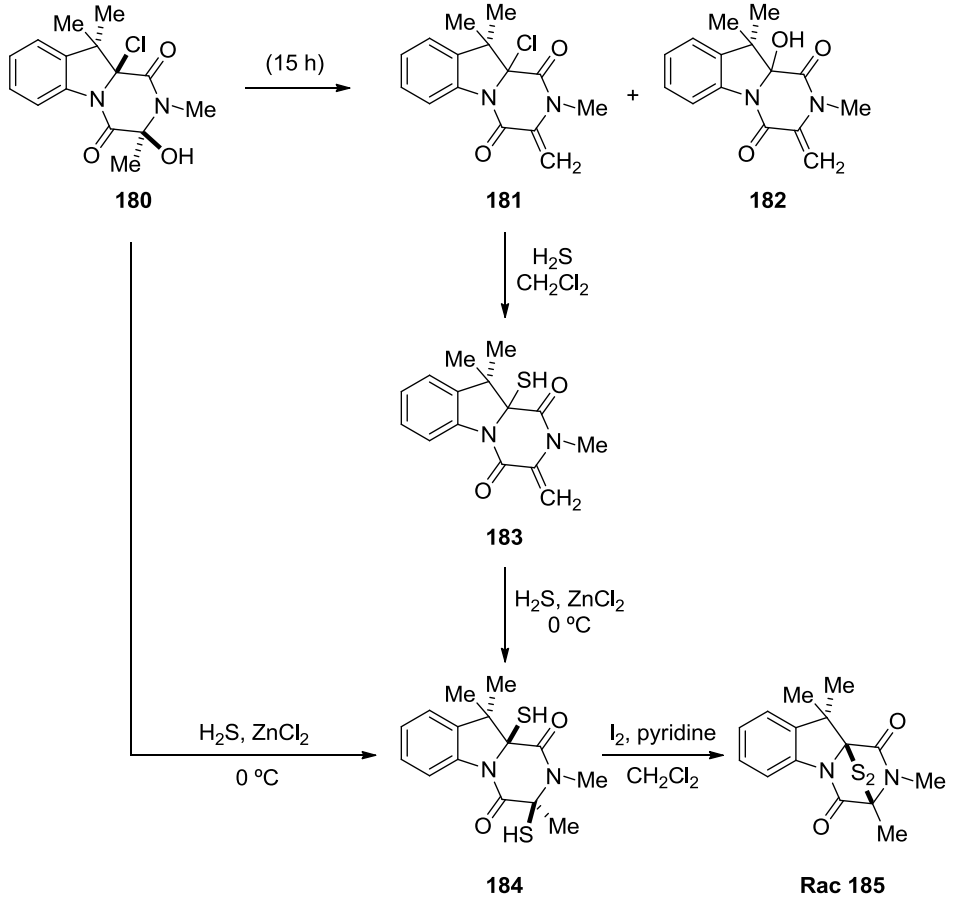
Development of this chemistry led to the synthesis of a gliotoxin analogue (**180**). Indolenine-2-carboxamide (**178**) when treated with pyruvol chloride gave (**179**). This intermediate was converted to (**180**) by spontaneous ring closing after 80 minutes of stirring (Scheme **32**).[191, 196, 197]



178 179 180

Scheme 32 – Ottenheym's approach (4)

After leaving (**180**) to stir for an extra 15 hours, products (**181**) and (**182**) were isolated. The chloroalkene (**181**) when bubbled with hydrogen sulfide yielded the mercaptoalkene (**183**), when either (**180**) or (**183**) were submitted to hydrogen sulfide and zinc chloride treatment and *cis*-dithiol (**184**) was formed. The *cis* configuration of this product was proven by disulfide formation after oxidation to yield the desired racemic ETP (**185**) (Scheme **33**).[19, 198]

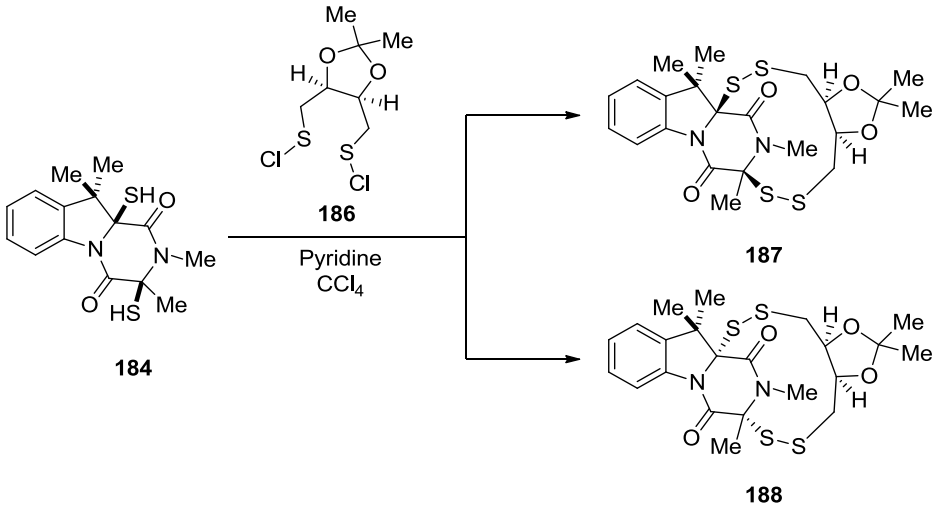


180 181 182 183 184 Rac 185

Scheme 33 – Ottenheym's approach (5)

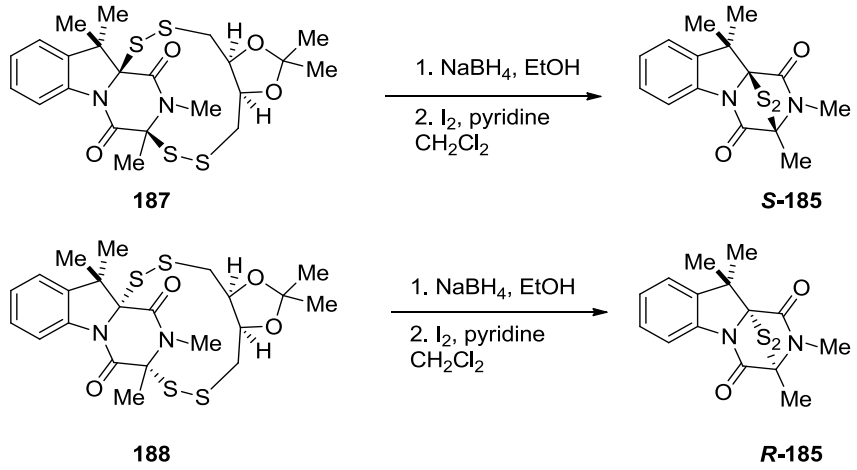
Although this synthetic pathway led to a racemic mixture of (**185**), chemical resolution was achieved by reduction of the disulfide bridge to give (**184**) which was consequently

reacted with a chiral *bis*-disulfenyl chloride (**186**) to yield diastereoisomers (**187**) and (**188**) (Scheme **34**).[198, 199]



Scheme 34 – Ottenheym's approach (6)

This diastereoisomeric mixture was separated using silica gel chromatography and the optically pure compounds (***S*-185**) and (***R*-185**) were subsequently obtained by reduction using sodium borohydride followed by the previously described oxidation process (Scheme **35**).[198]

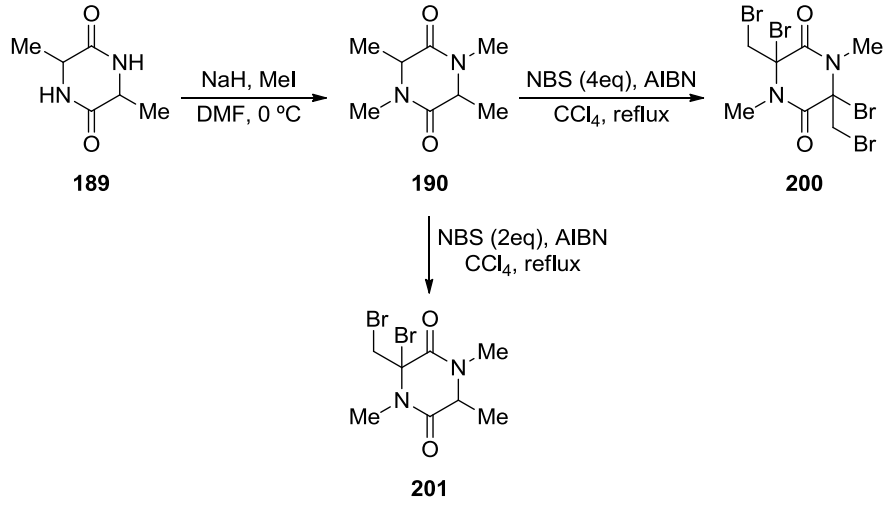


Scheme 35 – Ottenheym's approach (7)

1.2.7 Matsunari's approach

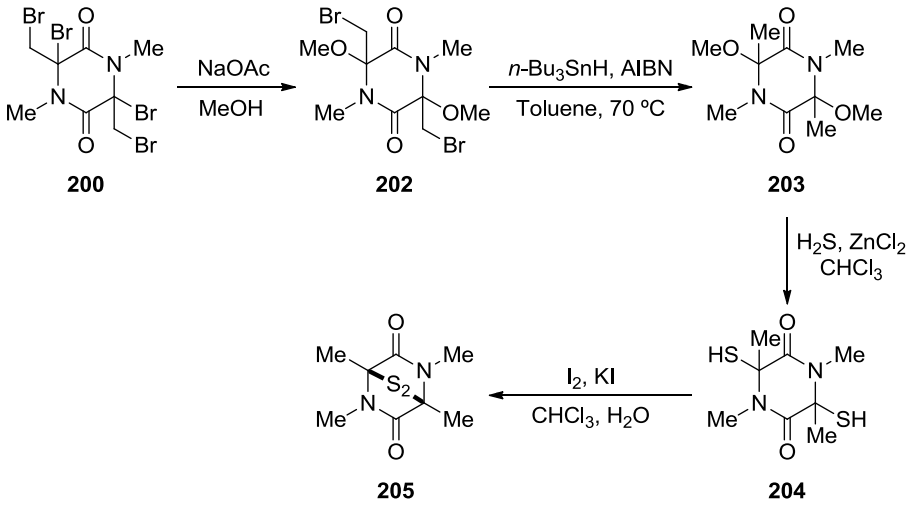
In 1973, Nakamura *et al* [200] published their work on the synthesis and substitution of 1,3,4,6-tetra-substituted-3,6-dihalogeno-2,5-diketopiperazines which served as a basis for Matsunari´s future work on the tetra-substitution of 2,5-diketopiperazines.[201] In way of example, the synthetic pathway started by developing the *N*-methylated 3,6-dialkyl-

2,5-piperazinedione precursor (**189**) using sodium hydride and methyl iodide. The resulting compound (**190**) was then treated with *N*-bromosuccinimide and 2,2'-azobisiso-butyronitrile to yield 3,3α-dibromide (**201**) and 3,3α,6,6α-tetrabromide (**200**), both generated depending on the equivalents of brominating agent used for the procedure (Scheme **36**).



Scheme 36 – Matsunari's approach (1)

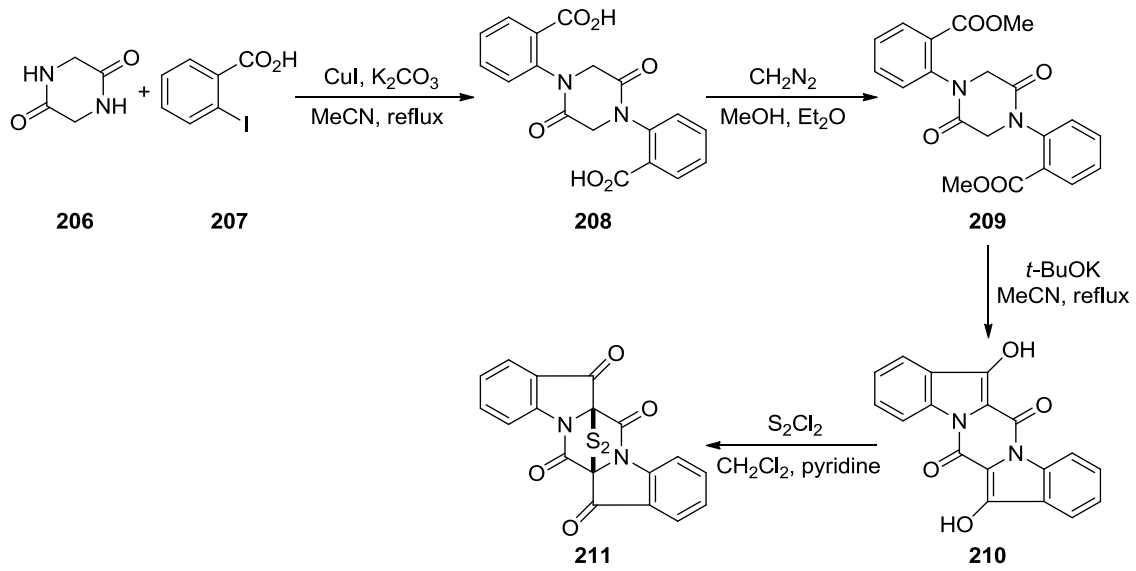
To avoid sulfenylation of both the 3*α*- and 6*α*-bromines, the 3,6-bromine atoms of compound (**200**) were converted to the correspondent dimethoxy intermediate (**202**) followed by reduction to give dimethyl compound (**203**). The resultant intermediate was then treated with hydrogen sulfide and zinc chloride yielding (**204**) which after oxidation gave the desired ETP (**205**) (Scheme **37**).[202, 203]



Scheme 37 – Matsunari's approach (2)

1.2.8 Coffen's approach

In 1977, Coffen *et al* [204] were able to create synthetic analogues of aranotin's. The synthetic process commenced with a copper-catalysed reaction to give (**208**) from glycine anhydride (**206**) and *o*-iodobenzoic acid (**207**). The diacid was then dissolved in methanol and reacted with diazomethane to yield (**209**), which after treatment with potassium *tert*-butoxide underwent cyclisation rendering the substituted indole moiety (**210**). The last step of the synthesis incorporated the disulfide bridge in the already known cyclic system by using disulfur dichloride in a basic environment to give the desired ETP (**211**) (Scheme **38**).



Scheme 38 – Coffen's approach

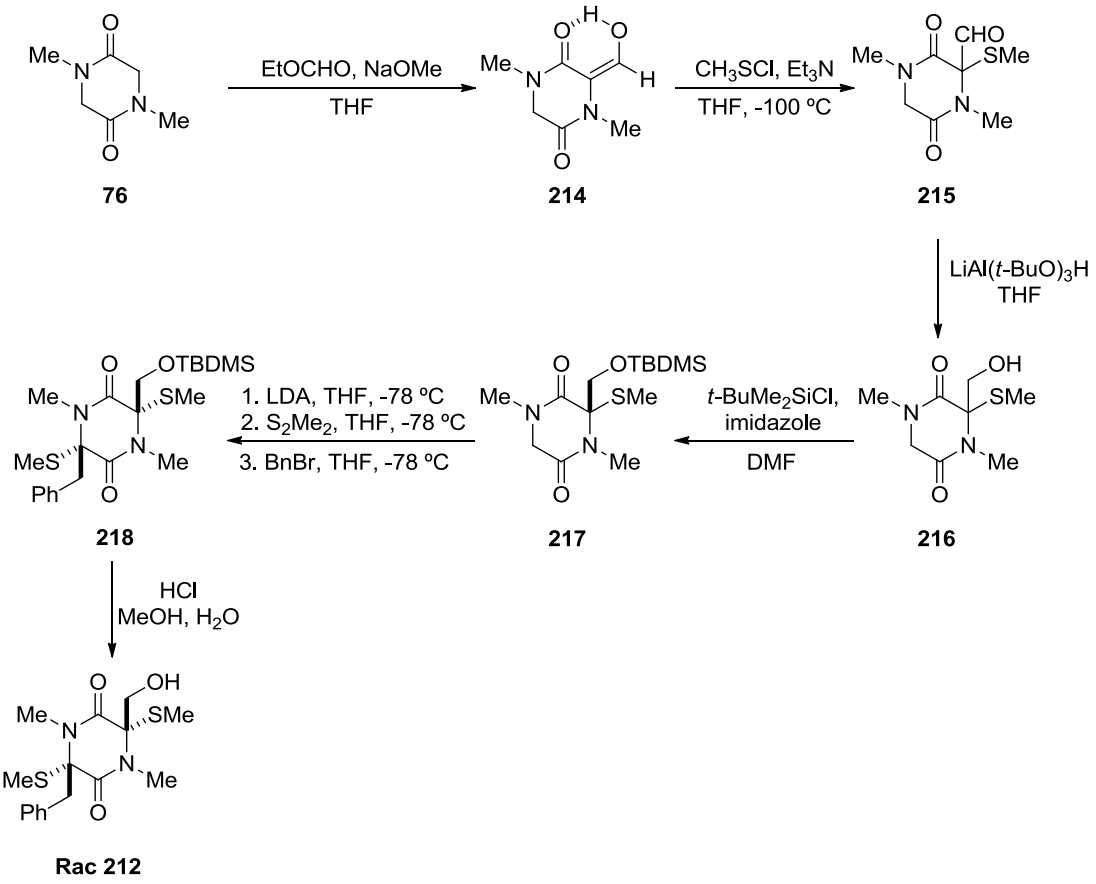
1.2.9 Rastetter and Williams' approach

The total synthesis of racemic gliovictin (**212**),[205, 157] as well as the attempt for a putative gliotoxin precursor model (**213**),[206] determined the initial steps for the ETP synthetic pathway development by Williams and Rastetter in 1979 (Figure **41**).

Figure 41 – Rastetter and Williams' approach

Gliovictin (**212**) synthesis started with monoformylation of sarcosine anhydride (**76**) to form the enolic aldehyde (**214**). Sulfenylation using methylsulfenyl chloride in a basic

environment yielded (**215**). This product was reduced to the corresponding alcohol (**216**) and thus protected as the *tert*-butyldimethylsilyl to give (**217**). The resultant compound was then treated with a strong base and alkylated using dimethyl disulfide and benzyl bromide to produce (**218**). Acid hydrolysis of the protecting silyl group yielded a racemic mixture of gliovictin (**212**) (Scheme **39**).

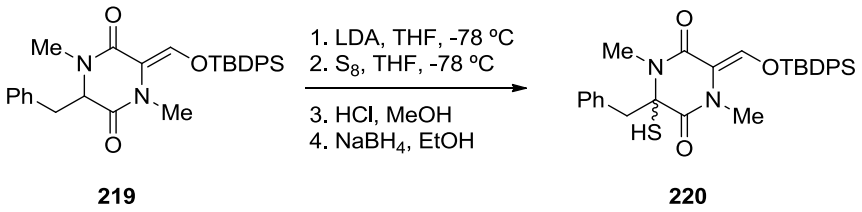


Scheme 39 – Rastetter and Williams' synthesis of gliovictin

The basis of this synthetic route was subsequently used to obtain the different sulfur containing molecules as shown below.

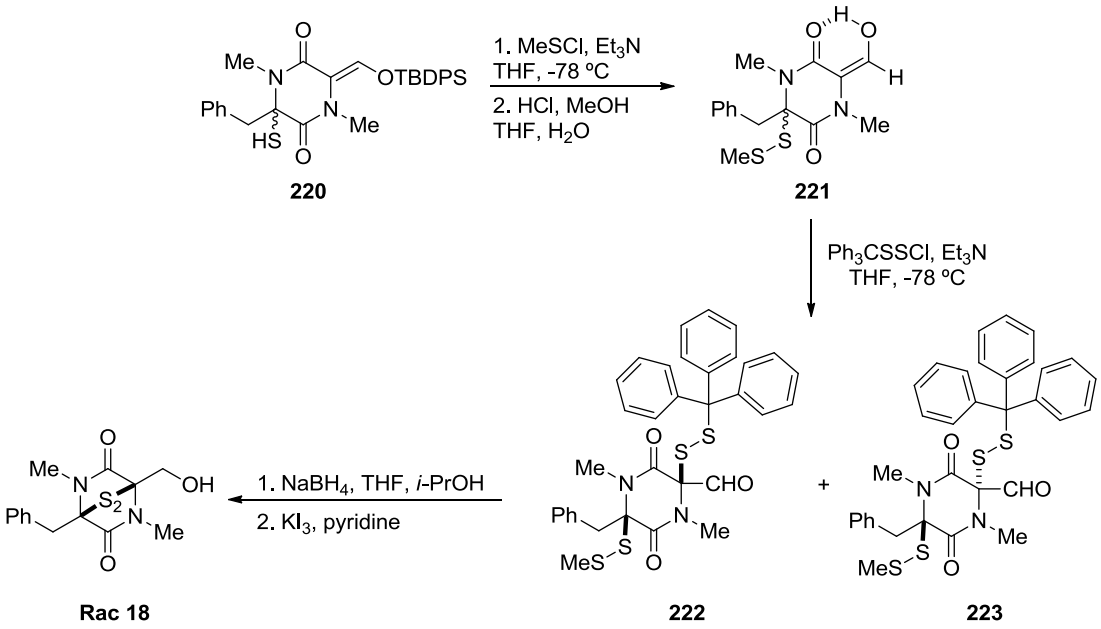
1.2.9.1 Hyalodendrin

The use of the same synthetic approach led to synthesis of hyalodendrin (**18**). The primary step involved similar intermediates from the previously discussed gliovictin synthesis in which the *tert*-butyldimethylsilyl protecting group was replaced by a *tert*-butyldiphenylsilyl substituent. Alkylation of compound (**219**) using LDA with previously treated elemental sulfur and following acidic work up with sodium borohydride treatment yielded (**220**) (Scheme **40**).[157]



Scheme 40 – Rastetter and Williams' synthesis of hyalodendrin (1)

Product (**220**) was treated with methylsulfenyl chloride followed by acid hydrolysis to give compound (**221**). Sulfenylation of the given product using triphenylmethyl chlorodissulfide in triethylamine rendered both diastereomers (**222**) and (**223**). Reduction of the desired isomer (**222**) followed by oxidation using Lugol's iodine yielded hyalodendrin (**18**) as a racemic mixture (Scheme **41**).[157]



Scheme 41 – Rastetter and Williams' synthesis of hyalodendrin (2)

Rastetter's studies continued towards developing models for ETPs biogenesis of arene oxides (**213**) and later for *sym*-oxepin oxides (**224**), initially planned to develop a partial synthetic route to the aranotin family of compounds as shown below (**9**) (Figure **42**).[207]

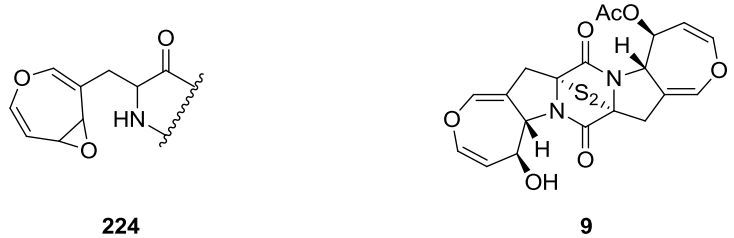


Figure 42 – Rastetter's model for ETP synthesis

Williams work led to important achievements in the generation of bridgehead carbanions of the diketopiperazine ring that would be crucial for synthesis of the ETP cogenerate bicyclomycin (**225**).[208, 209, 210] Other works reported by the author included the chiral right half synthesis of sirodesmin A (**26**) (Figure **43**).[211]

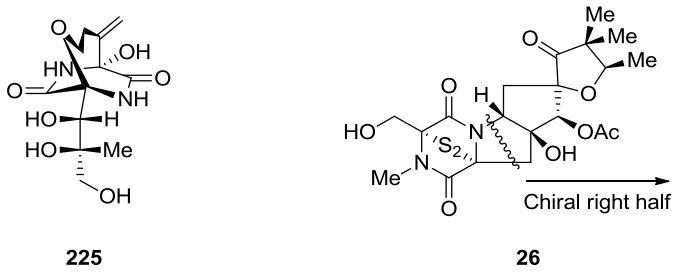
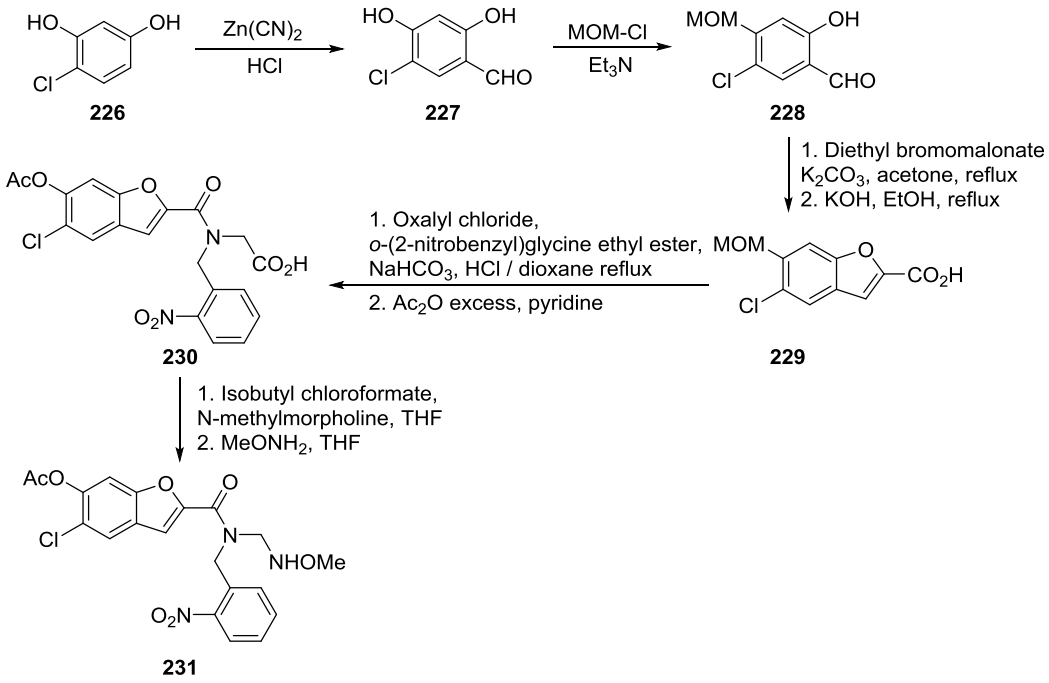


Figure 43 – Williams' model for ETP synthesis

These efforts were influential to the total synthesis of epidithiapiperazinedione – aspirochlorine (**238**). The synthetic route towards this compound is shown below.[212]

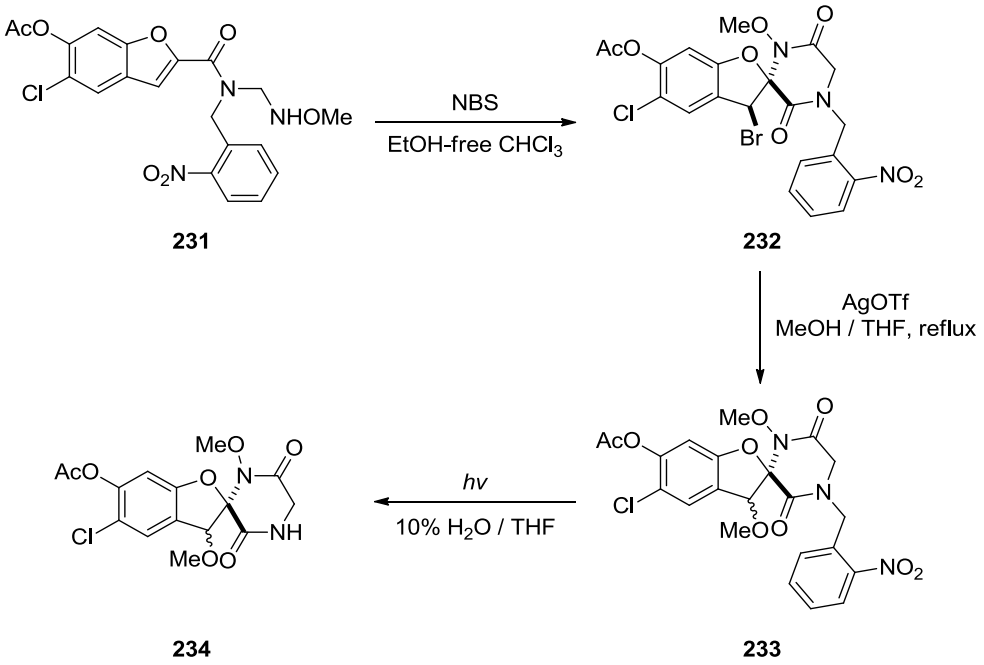
1.2.9.2 Aspirochlorine

The synthesis of this product started with formylation of 4-chloro-resorcinol (**226**) using zinc cyanide under acidic conditions to yield (**227**). The next step involved protection of the 4-hydroxyl group using chloromethyl methyl ether to give (**228**) followed by treatment with diethyl bromomalonate and potassium carbonate. Hydrolysis of the formed diester, rendered the desired coumarilic acid (**229**) (Scheme **42**).



Scheme 42 – Williams' synthesis of aspirochlorine (1)

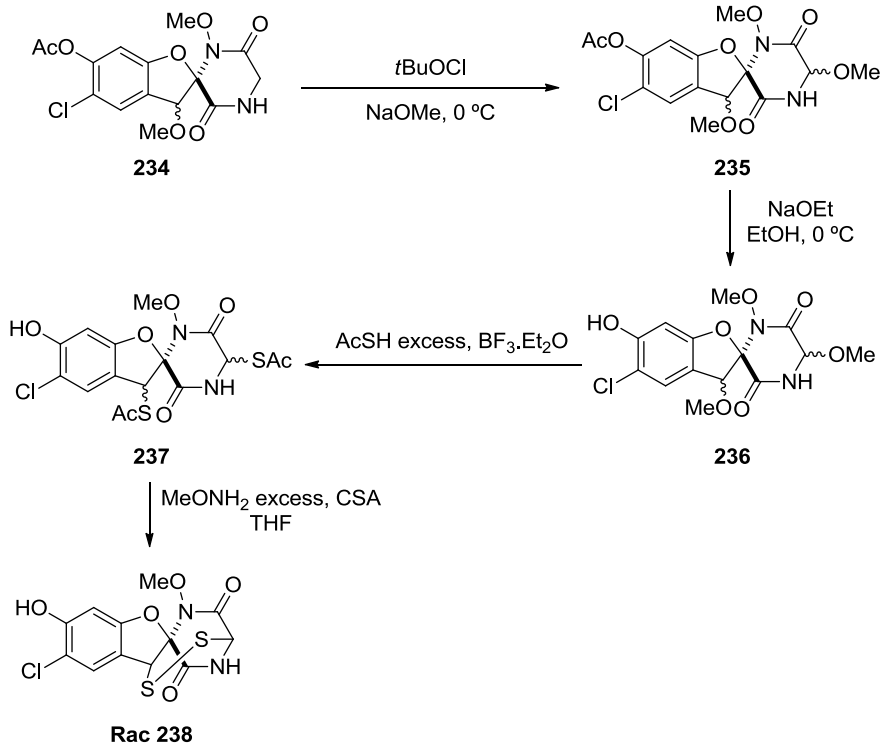
This derivative was coupled to *o*-(2-nitrobenzyl)glycine ethyl ester and the resulting intermediate, after acid hydrolysis using hydrochloric acid at high temperatures followed by acetylation, was converted to (**230**). The resulting product was treated with *N*-methylmorpholine and methoxylamine to afford (**231**) (Scheme **42**).[212, 213, 214] Compound (**231**) was then treated with *N*-bromosuccinimide to form the desired tricyclic moiety with the required *trans* stereochemistry (**232**), followed by displacement of the bromide with silver triflate in methanol to give the methyl ether (**233**) as a mixture of diastereomers. Photolytic deprotection of the *o*-nitrobenzyl group under optimal conditions (10% water in the reaction mixture; five hours photolysis) led to (**234**) (Scheme **43**).[212]



231 **232** **233** **234**

Scheme 43 – Williams' synthesis of aspirochlorine (2)

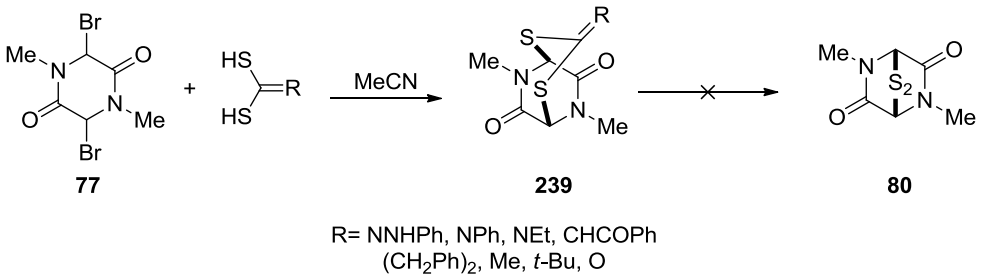
Sulfenylation was achieved in three steps: oxidation of (**234**) to the corresponding bismethyl ether (**235**); removal of the acetate protecting group to give the free phenol (**236**); and bisthioacetate formation (**237**) using thiolacetic acid and boron trifluoride etherate. The final phase of the synthesis was difficult to achieve. However, optimal condition exploration demonstrated that treatment of (**237**) with excess methoxylamine and camphor sulfonic acid (CSA) under an aerobic environment led to a racemic mixture of aspirochlorine (**238**) (Scheme **44**).[212, 213, 214]



Scheme 44 – Williams' synthesis of aspirochlorine (3)

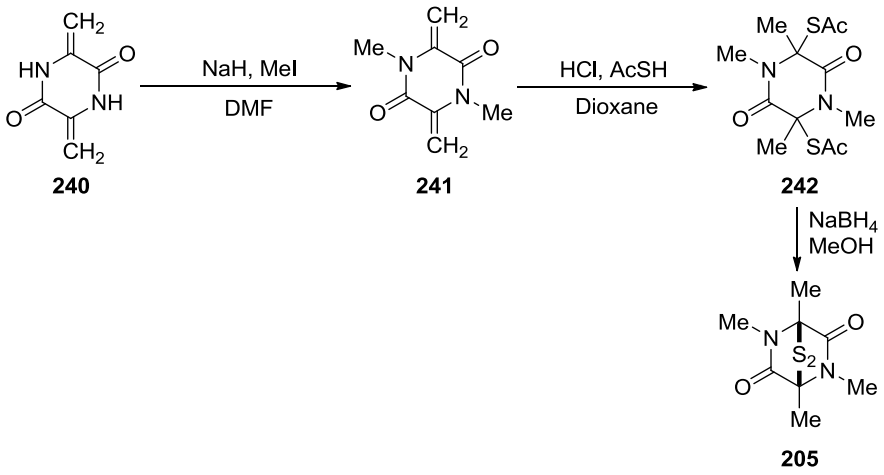
1.2.10 Olsen's approach

In 1981, Olsen *et al* [215] tried to expand a model based on Kishi's findings in which geminal dithiols were reacted with dibromo piperazinedione (**77**) to give intermediate compounds (**239**) substituted in both the *3*- and *6*- positions with the sulfides connected through a carbon bridging group. After introducing sulfide functions, attempts to remove the bridging group that after oxidation would render the desired ETPs were unsuccessful (Scheme **45**).



Scheme 45 – Olsen's approach (1)

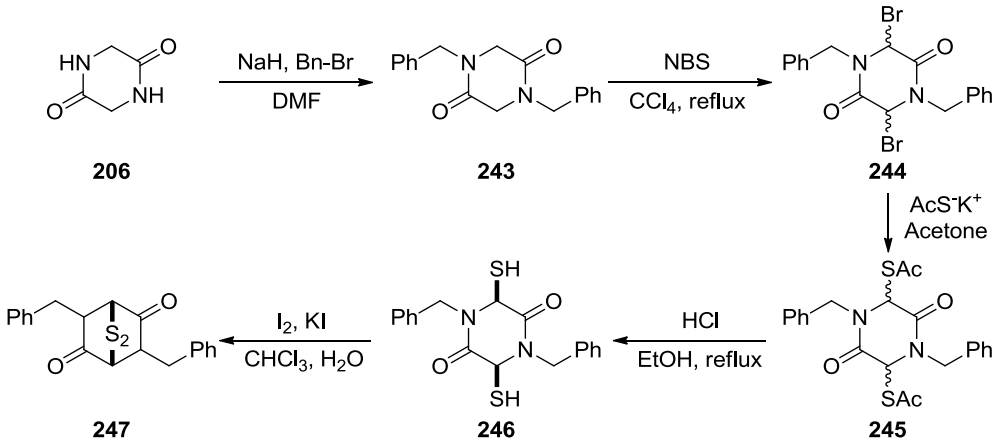
These results followed previous results that started with *bis N*-methylation of unsaturated piperazinedione (**240**) to give (**241**). After treatment with thioacetic acid under strong acidic conditions, compound (**242**) was formed and further reduction using sodium borohydride to give the desired ETP (**205**) (Scheme **46**).[215]



Scheme 46 – Olsen's approach (2)

1.2.11 Waring's approach

In 1993, Waring *et al* [216] used a very similar approach to the one developed by Trown and co-workers to obtain ETPs. The synthetic pathway, although having the same intermediates, was modified by different reaction conditions. In way of example, glycine anhydride (**206**) was *bis* alkylated in positions *1* and *4* using a strong base and benzyl bromide to give (**243**). The resulting product underwent bromination using *N*-bromosuccinimide to render the desired dibrominated intermediate (**244**). Following treatment with potassium thioacetate yielded dithioacetate (**245**). Hydrolysis of (**245**) using boiling ethanol and hydrochloric acid gave the corresponding dithiol (**246**) that after oxidation rendered the desired ETP (**247**) (Scheme **47**).

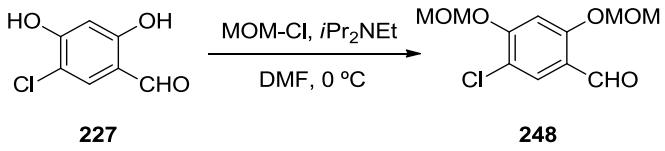


Scheme 47 – Waring's approach

1.2.12 Danishefsky's approach

In 2000, Danishefsky *et al* [213] were able to synthesise an aspirochlorine analogue (**254**) using Kishi's thioacetal (**106**) and Williams' intermediate (**227**).[179, 212] Following *bis*

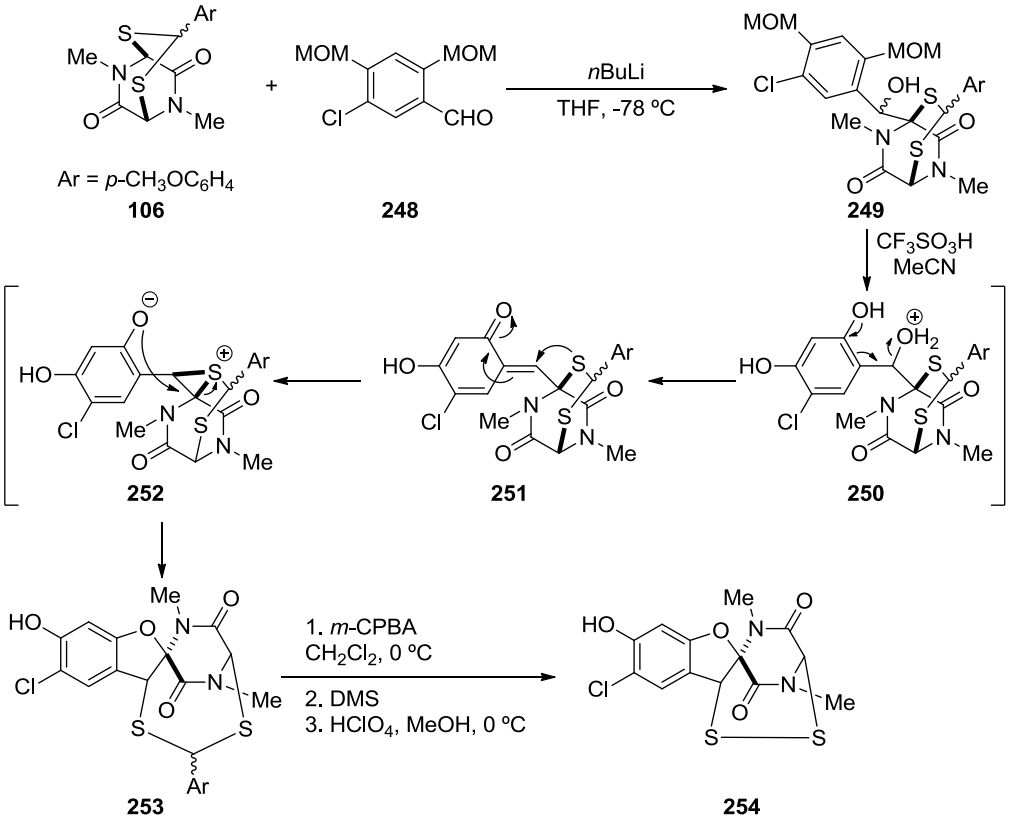
methoxymethyl ether protection of (**227**) compound (**248**) was obtained which was used as the substituent's left half of the natural product analogue precursor (Scheme **48**).



227 **248**

Scheme 48 – Danishefsky's approach (1)

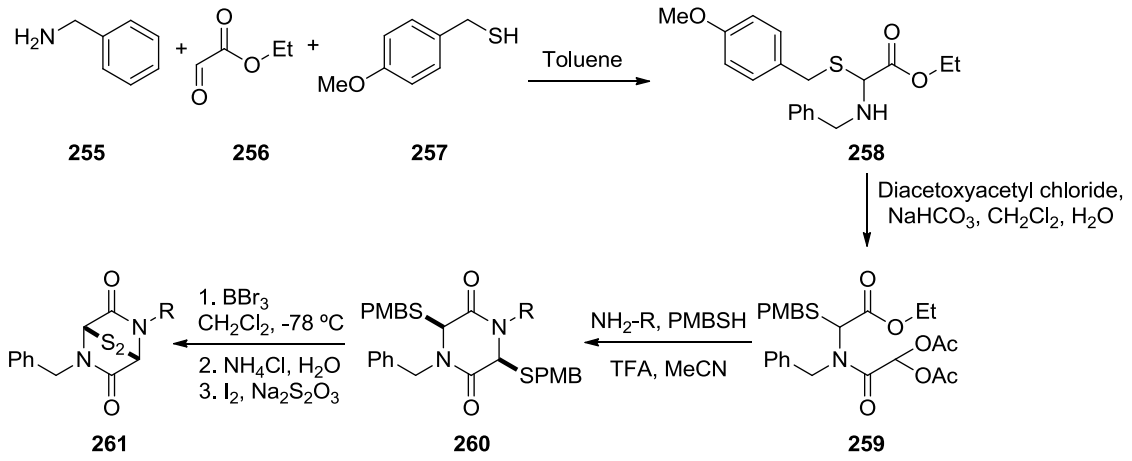
The bicyclic moiety (**106**) was reacted with the aldehyde under strong basic conditions to give (**249**). The next step of the synthetic pathway elegantly yielded (**253**) on acid catalysis. The rationalization of this noteworthy rearrangement is illustrated from intermediate (**250**) to (**252**); the chain of events starts with protonation of the hydroxy group that aided by the phenolic oxygen gives the *o*-quinomethide compound (**251**). This product underwent subsequent stereospecific sulfur migration to yield (**252**). Treatment of (**253**) with *m*-chloroperbenzoic acid and perchloric acid in the presence of dimethyl sulfide rendered the desired aspirochlorine analogue (**254**) (Scheme **49**).



Scheme 49 – Danishefsky's approach (2)

1.2.13 Hilton's approach

In 2004, Hilton *et al* [1, 217] developed a novel method to achieve the substituted diketopiperazine core by early introduction of one of the sulfur atoms necessary to form the disulfide bridge in the first reaction step. To achieve this, ethyl glyoxalate (**256**) was first premixed with benzylamine (**255**) and the resulting intermediate treated with a protected thiol substituent (**257**) to yield (**258**). The *α*-mercaptoamino acid ester was then reacted with diacetoxyacetyl chloride to give (**259**). This compound when treated with different amines and *para*-methoxybenzyl mercaptan in an acid environment elegantly rendered the protected dithiol diketopiperazines (**260**). The last stage on forming the desired ETPs consisted of a two phase one-pot process: thiol groups deprotection using a Lewis acid; followed by oxidation of the worked-up reaction using iodine to yield (**261**) (Scheme **50**).

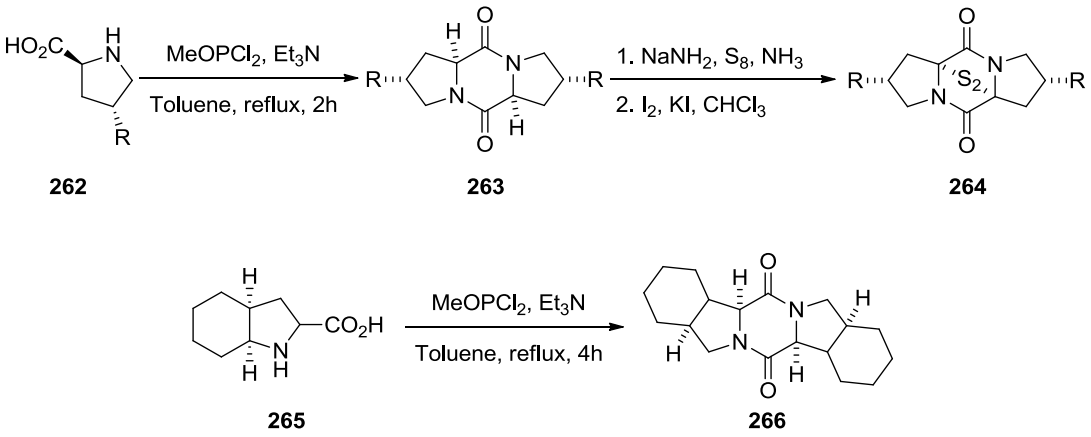


Scheme 50 – Hilton's approach

1.2.14 Bräse's approach

In 2007, Bräse *et al* [218] synthesised various examples of both symmetrical and unsymmetrical diketopiperazines that could subsequently be thiolated using Schmidt's methodology.[172] The root method was based on a modified procedure from previous studies by Ugi *et al* [219] that described the one-pot synthesis of symmetrical diketopiperazines using amino acids and proline in the presence of dichloromethoxy phosphine. The use of functionalised prolines (**262**), hexahydroindolecarboxylic acid (**265**) and other substituted indole derivatives allied to enhanced reaction conditions enabled Bräse to obtain different examples of diketopiperazines (**263**) and (**266**). Introduction of the disulfide bridge was achieved via Schmidt's synthetic pathway using

sodamide. Elemental sulfur and a final oxidation step yielded the desired ETP (**264**) (Scheme **51**).

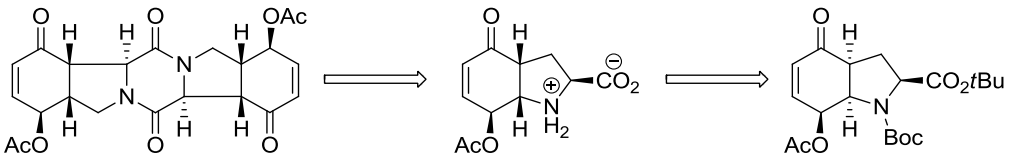


262 **263** **264**

265 **266**

Scheme 51 – Bräse's approach (1)

This unified methodology allowed Bräse and co-workers to create different building blocks that after dimerisation yielded the desired diketopiperazines. One of these was the core structure (**276**) of epicoccins A, C and D from a pre-formed *cis*-annelated cyclohexane (**269**) (Scheme **52**).[220]



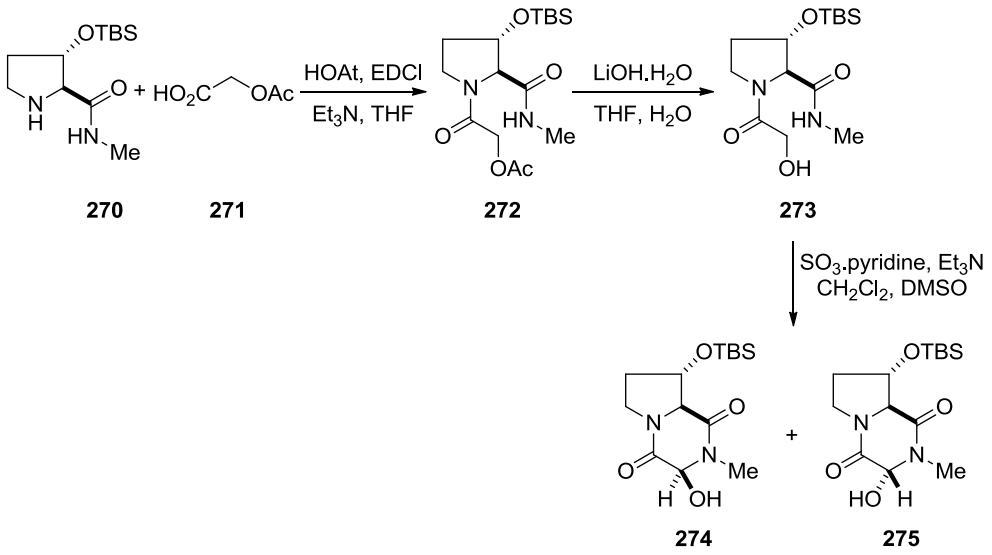
276 **268** **269**

Scheme 52 – Bräse's approach (2)

Former work by the author concerning the thiolation process of these compounds allowed a number of approaches to be considered for the enantioselective total synthesis of ETPs natural products.[221]

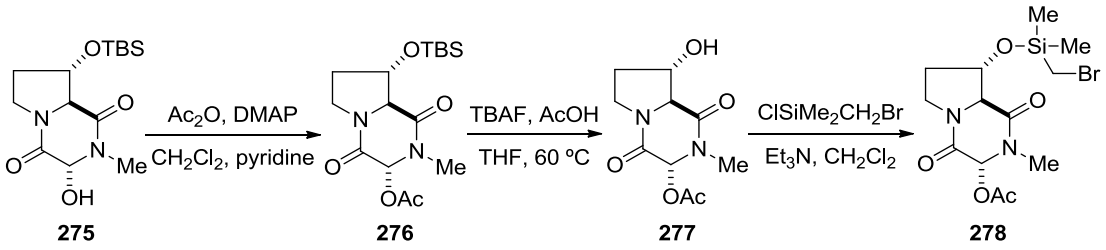
1.2.15 Overman's approach

In 2007, Overman et al [222, 223] developed a method to construct tricyclic ETPs from dioxopiperazines containing a hydroxyproline unit. The synthetic process started with coupling of 3-hydroxyproline derivative (**270**) to acetylglycolic acid (**271**) to give (**272**). The resultant acetate was then cleaved yielding (**273**) that after Parikh-Doering oxidation and resultant cyclization rendered a mixture of (**274**) and (**275**) (Scheme **53**).



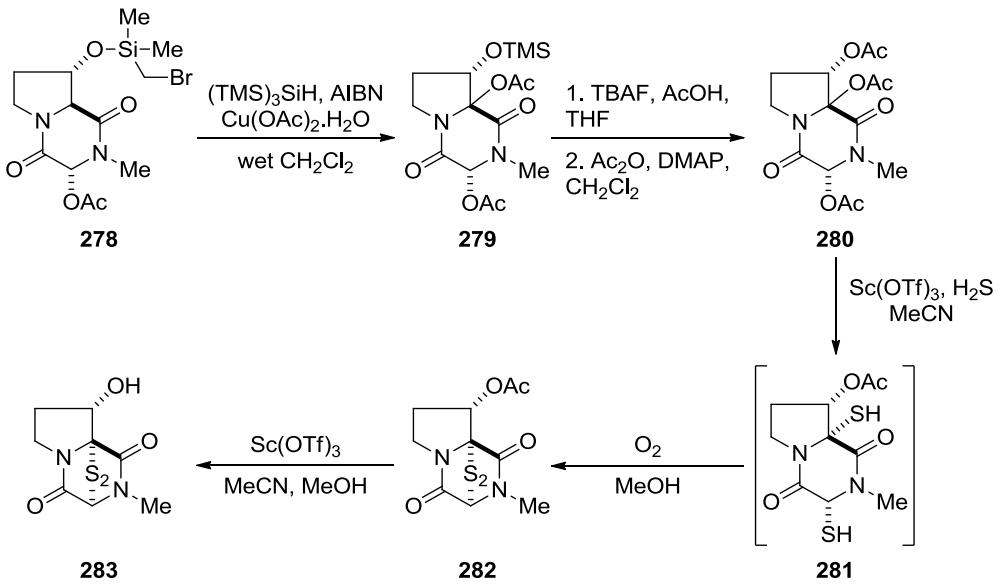
Scheme 53 – Overman's approach (1)

Compound (**275**) was acetylated to give (**276**) and the *tert*-butylsilyl group removed upon treatment with tetra-*n*-butylammonium fluoride in acetic acid to render the corresponding alcohol (**277**). This secondary alcohol was then transformed into the (bromomethyl)dimethylsiloxy ether (**278**) derivative (Scheme **54**).[222]



Scheme 54 – Overman's approach (2)

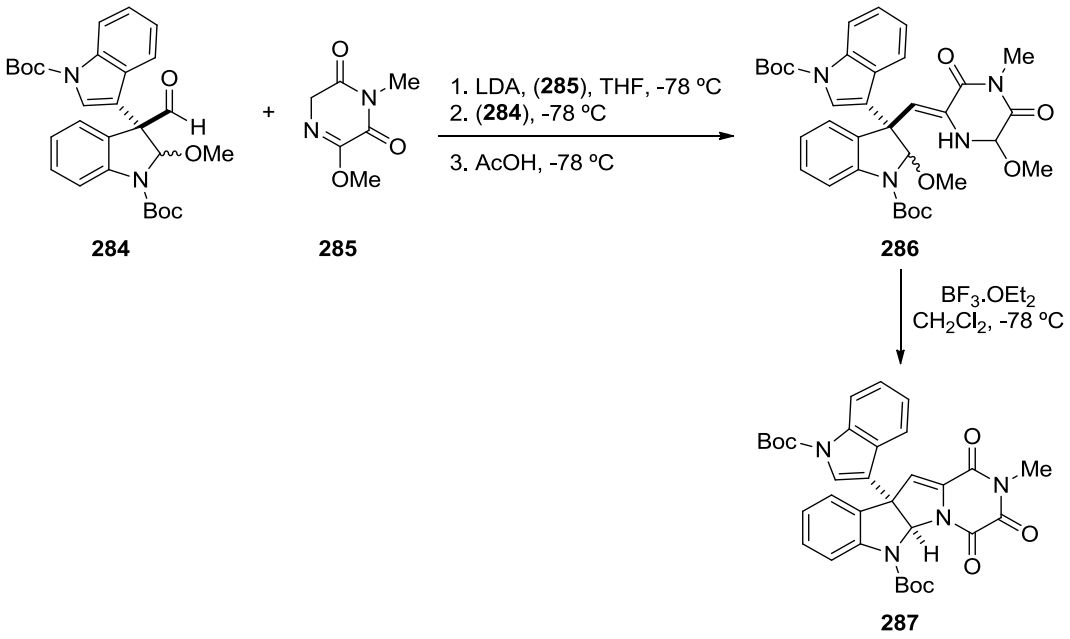
Radical-promoted oxidation of (**278**) yielded the acetate species (**279**) that was next deprotected and acetylated to give (**280**). The resulting triacetate was treated with excess hydrogen sulfide and a catalytic amount of scandium triflate rendering the dithiol (**281**) that after exposure to oxygen, yielded ETP (**282**). The authors transformed the previous acetylated ETP to the given alcohol (**283**) (Scheme **55**).[222]



Scheme 55 – Overman's approach (3)

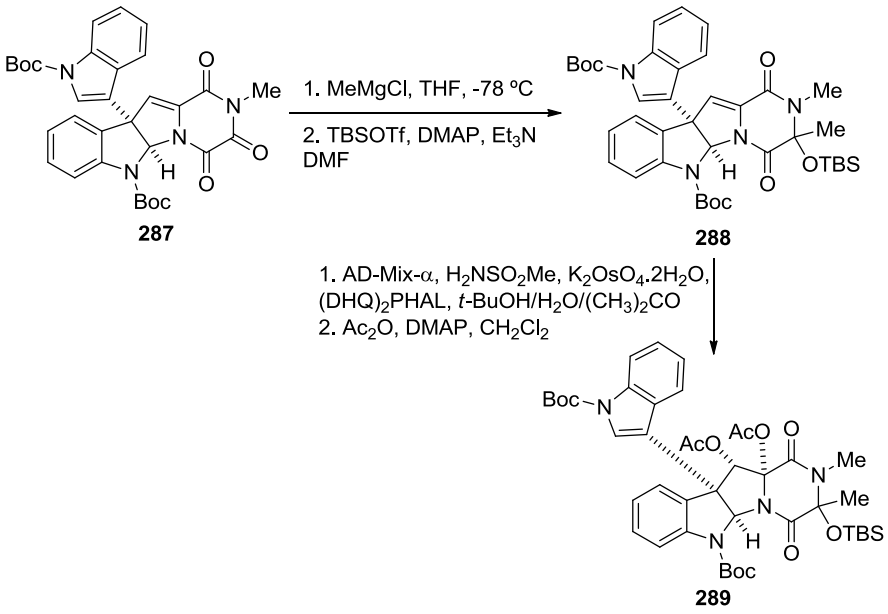
1.2.15.1 (+)-Gliocladine C

Using a different approach, Overman and co-workers developed a convergent method for constructing cyclotryptamine-fused polyoxopiperazines which led to the synthesis of gliocladine C (**72**).[149, 223] Pre-constructed indole aldehyde (**284**) was coupled with trioxopiperazine (**285**) in the presence of a strong base followed by acid quenching that rendered (**286**). This compound, after treatment with boron trifluoride etharate, yielded cyclized intermediate (**287**) (Scheme **56**).



Scheme 56 – Overman's synthesis of (+)-gliocladine C (1)

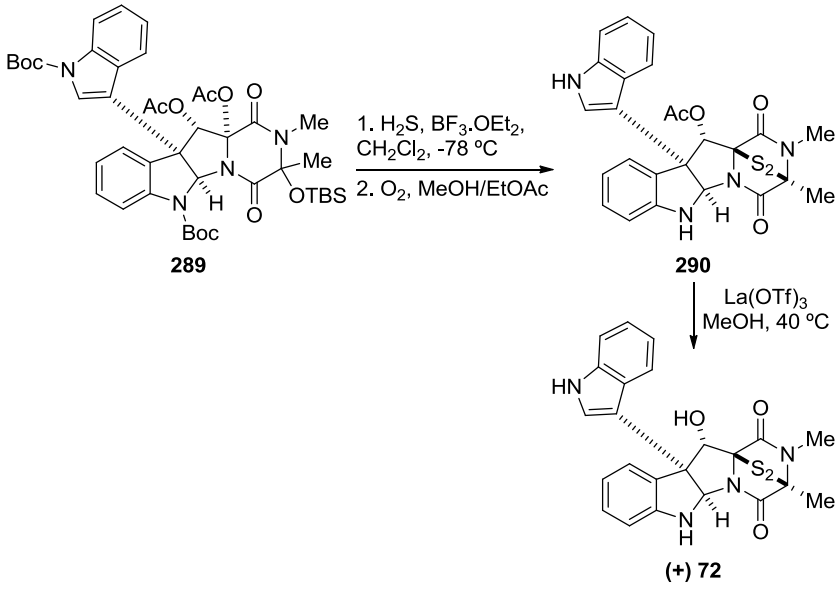
Trioxopiperazine (**287**) was treated with methylmagnesium chloride to give the corresponding tertiary alcohol that after protection rendered a siloxy epimeric mixture of (**288**). This mixture was then dihydroxylated and the resultant diol was acetylated yielding diacetate (**289**) (Scheme **57**).[223]



287 **288** **289**

Scheme 57 – Overman's synthesis of (+)-gliocladine C (2)

Diacetate (**289**) was treated with hydrogen sulfide and boron trifluoride etherate followed by oxidation to give (**290**). This compound was heated in a methanolic solution of lanthanum(III) trifluoromethanesulfonate that rendered (+)-Gliocladine C (**72**) (Scheme **58**).[223]



289 **290** **(+) 72**

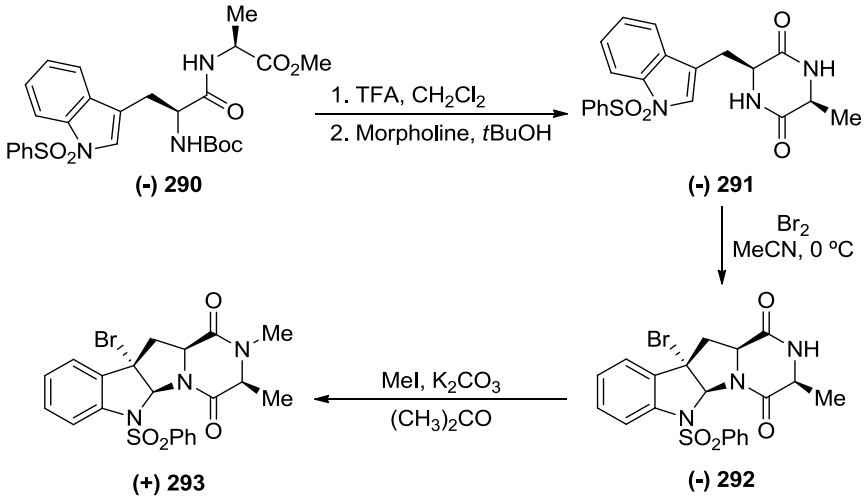
Scheme 58 – Overman's synthesis of (+)-gliocladine C (3)

1.2.16 Movassaghi's approach

In 2009 Movassaghi *et al* [156, 224] were able to very elegantly synthesise the dimeric ETP (+)-11,11-dideoxyverticillin A (**65**). The strategic synthetic pathway was developed to introduce the disulfide bridge at a late stage into the molecule core as shown below.

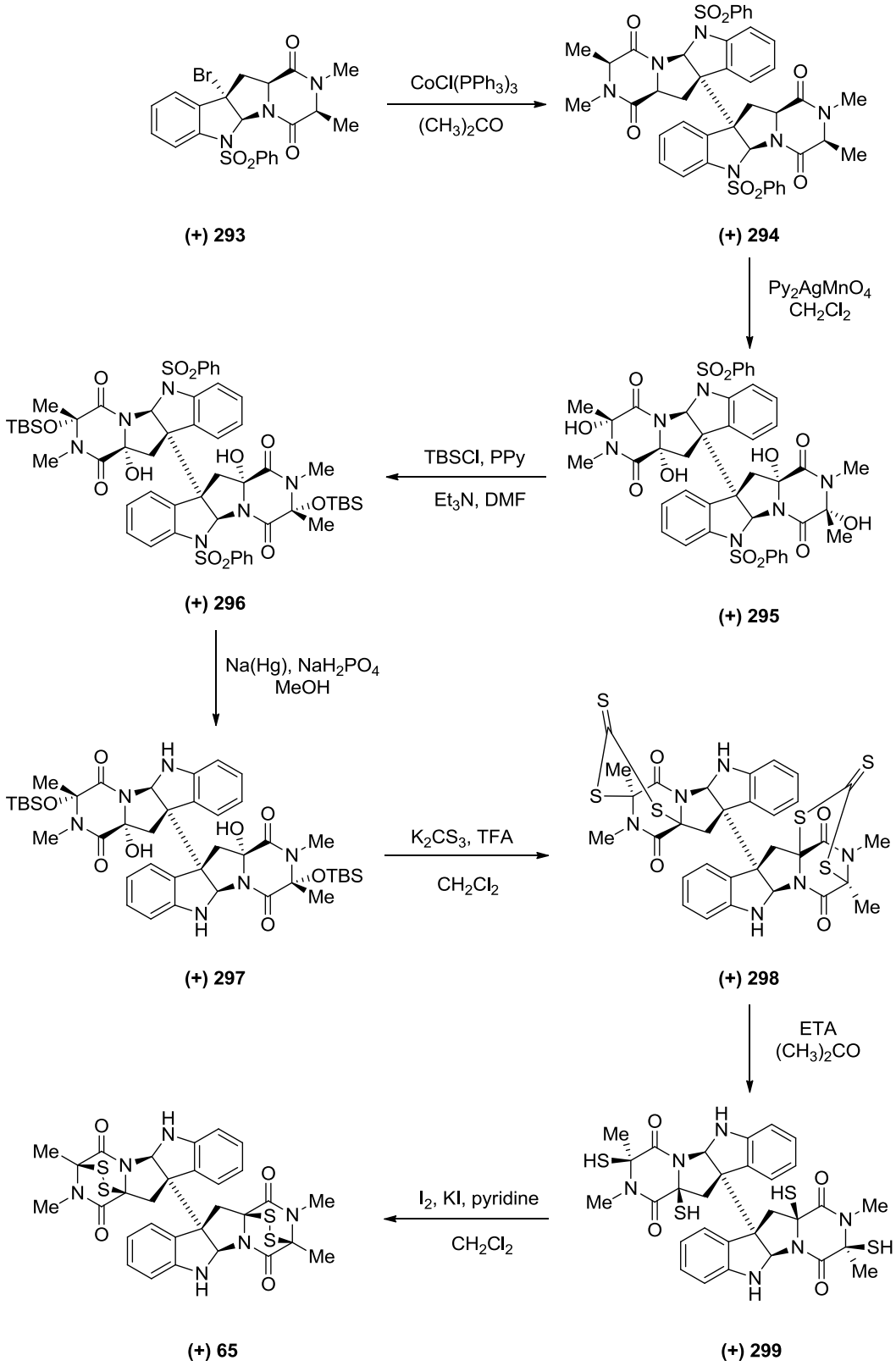
1.2.16.1 (+)-11,11-Dideoxyverticillin A

The synthesis started with *cis*-diketopiperazine (**291**) formation, with deprotection of amide (**290**) and morpholine treatment to give the diketopiperazine moiety. The resultant compound was treated with bromine in acetonitrile at 0 °C to give the desired monomeric tetracyclic bromide (**292**) that after reaction with methyl iodide and potassium carbonate yielded the tertiary benzylic bromide (**293**) (Scheme **59**).[156]



Scheme 59 – Movassaghi's synthesis of (+)-11,11-dideoxyverticillin A (1)

Compound (**293**) when treated with tris(triphenylphosphine)cobalt(I) chloride rendered the dimeric octacyclic intermediate (**294**) which was followed by oxidation using bis(pyridine)-silver(I) permanganate yielded the desired dimeric octacyclic tetraol (**295**) as a single diastereoisomer. This procedure avoided competing epimerisations that would result in complex mixtures. After alcohol protection, treatment of (**296**) with monobasic sodium phosphate with sodium amalgam provided the dimeric intermediate (**297**). This was reacted with trithiocarbonate under acid conditions to give the bisdithiepanethione (**298**). Ethanolamine was then added rendering the diaminotetrathiol (**299**) that after mild oxidation yielded (+)-11,11-dideoxyverticillin A (**65**) (Scheme **60**).[156]



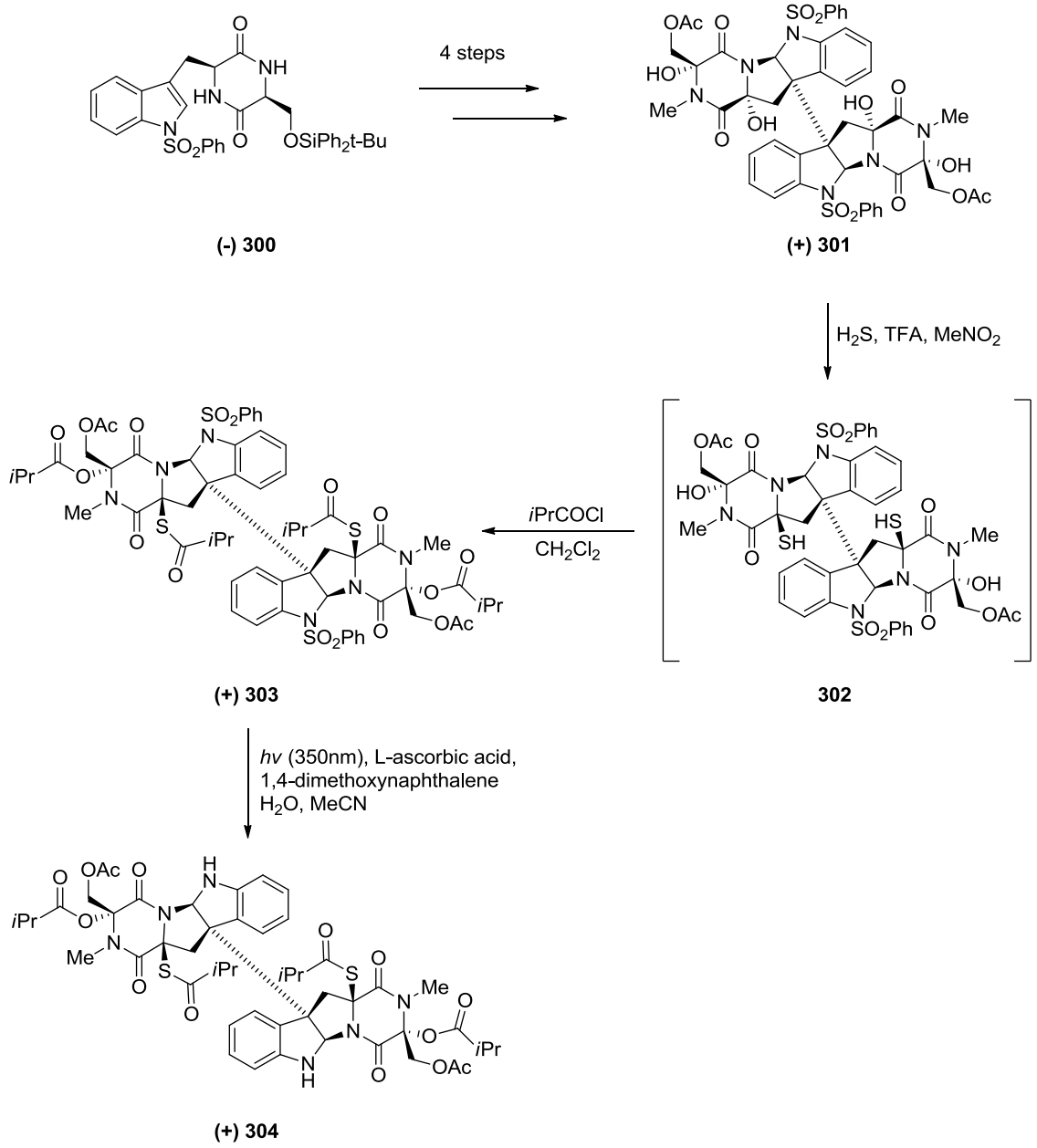
Scheme 60 – Movassaghi's synthesis of (+)-11,11-dideoxyverticillin A (2)

In 2010, Movassaghi and co-workers [225] were able to synthesise (+)-chaetocin A (**42**) by undertaking a similar strategy as reported for (+)-11,11-dideoxyverticillin A (**65**) which

enabled the synthesis of a modified intermediate of (**291**), proven to be crucial for the synthetic pathway.

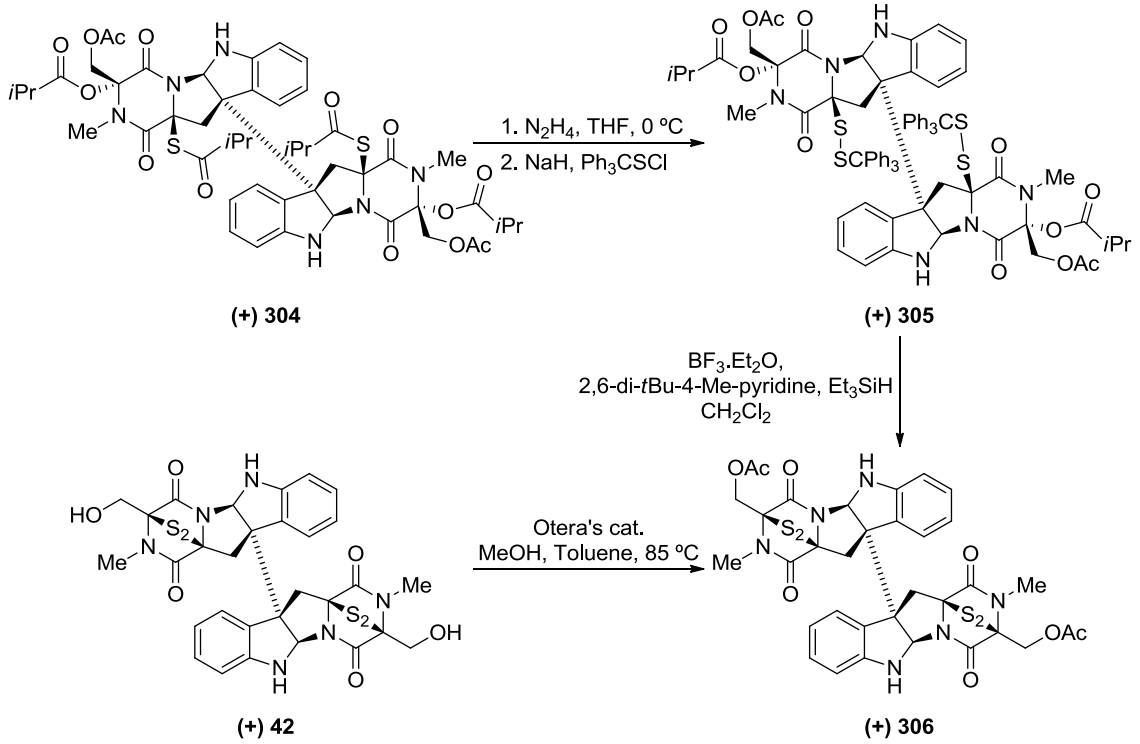
1.2.16.2 (+)-Chaetocin A

Movassaghi *et al* used diketopiperazine construct (**300**) as the basis to form tetraol (**301**) that after exposal to hydrogen sulfide under acidic conditions formed bisthiohemiaminal (**302**). Addition of isobutyryl chloride gave (**303**) that after irradiation with a black-light phosphor-coated lamp with photosensitizer 1,4-dimethoxynaphthalene and L-ascorbic acid rendered (**304**) (Scheme **61**).[225]



Scheme 61 – Movassaghi's synthesis of chaetocin A (1)

Hydrazinolysis of the thioesters yielded the dithiol (**304**) that was treated under basic conditions with trityl hypochlorothioite to give (**305**). Ionisation of the isobutyrates and cyclisation with triphenylmethyl cation loss rendered diacetate (**306**) that after mild methanolysis gave (+)-chaetocin A (**42**) (Scheme **62**).[225]



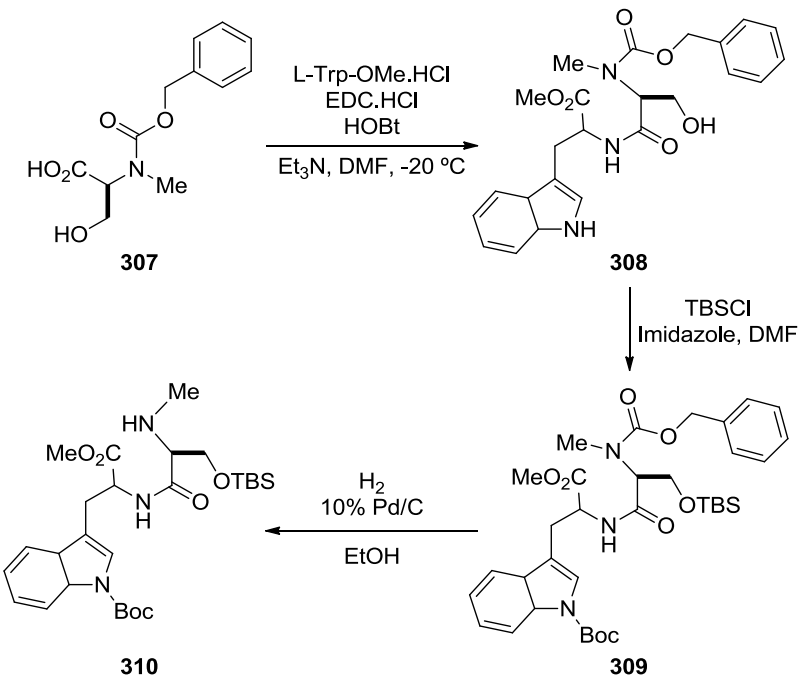
Scheme 62 – Movassaghi's synthesis of chaetocin A (2)

1.2.17 Iwasa's approach

In 2011, Iwasa *et al* [115, 226, 227, 228] developed an elegant synthetic route to achieve chaetocin A (**42**) as well as its corresponding enantiomer (*ent*-**42**).

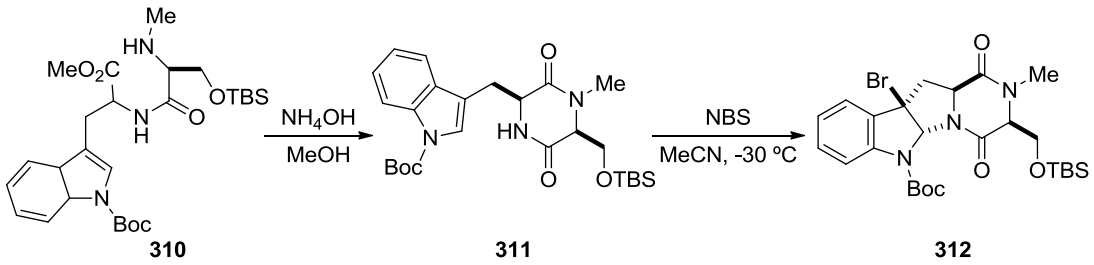
1.2.17.1 Chaetocin A

The synthetic process to achieve chaetocin A (**42**) started by condensation of previously formed *N*-methyl-L-serine (**307**) and L-tryptophan methyl ester that when treated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and 1-hydroxybenzotriazole monohydrate rendered dipeptide (**308**). The hydroxyl group and the nitrogen atom from the indole moiety were protected using *tert*-butyldimethylchlorosilane and di*tert*-butyl dicarbonate respectively yielding (**309**). Reduction of the previously formed protected dipeptide gave (**310**) (Scheme **63**).[226, 227]



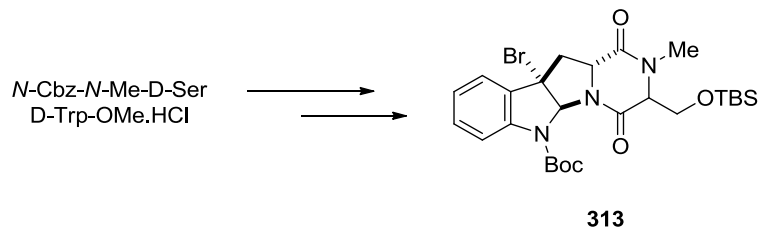
Scheme 63 – Iwasa's synthesis of chaetocin A (1)

Compound (**310**) was reacted with ammonium hydroxide to afford cyclised product (**311**); that upon treatment with *N*-bromosuccinimide under mild conditions yielded the tetracyclic bromo compound (**312**) (Scheme **64**).[226, 227]



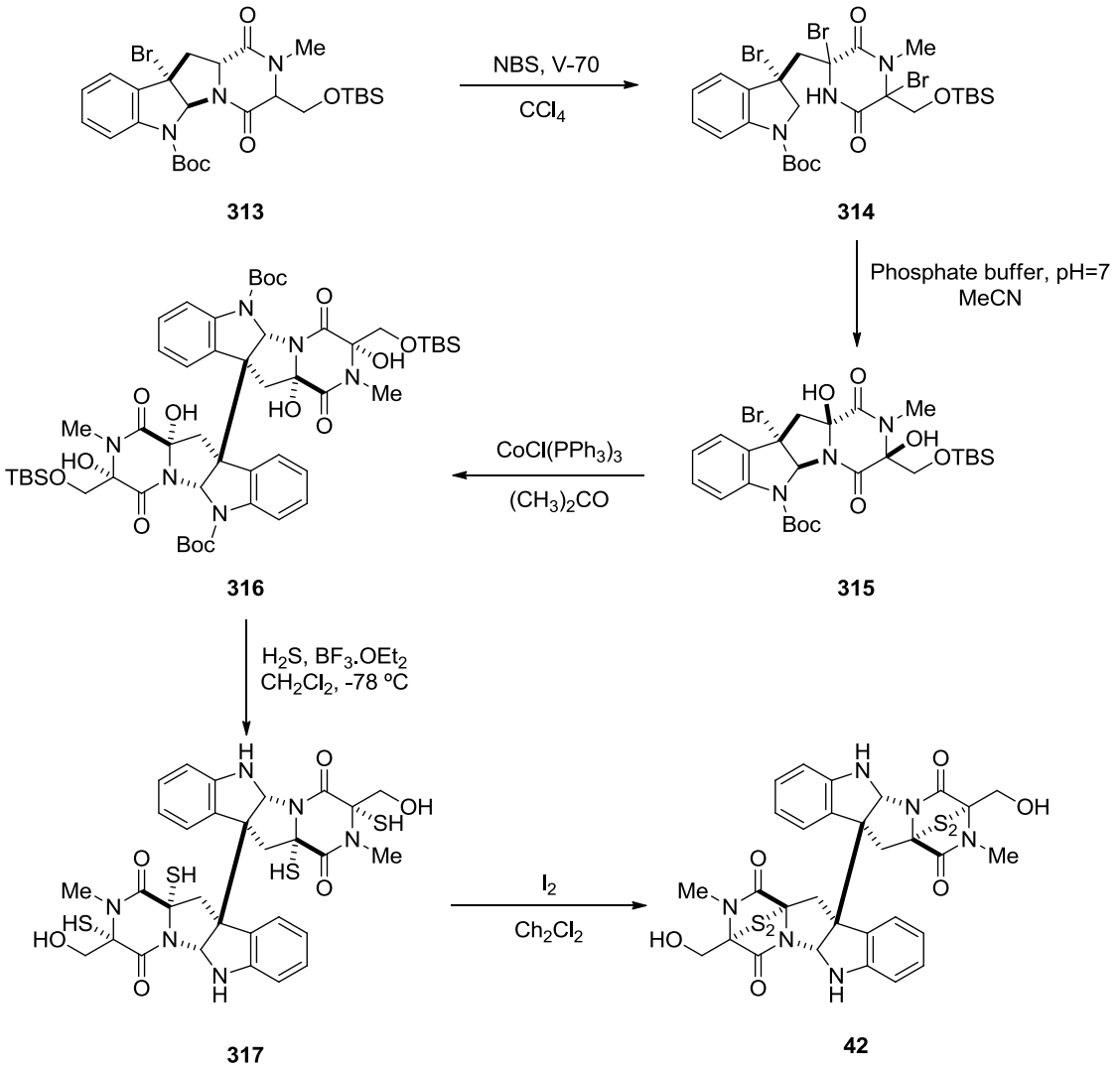
Scheme 64 – Iwasa's synthesis of chaetocin A (2)

In light of these results, the enantiomer of (**312**) was formed in order to continue the synthesis. This was achieved by using the D-amino acid derivative as starting material rendering *ent*-(**313**) (Scheme **65**).[226, 227]



Scheme 65 – Iwasa's synthesis of chaetocin A (3)

Compound (**313**) was α-brominated to give (**314**) using 2,2'-azobis(4-methoxy-2,4-dimethylvaleronitrile) as a radical initiator and *N*-bromosuccinimide as the halogenating agent. The use of a radical initiator was shown to be very important in this synthetic route since it supressed β-elimination of the alcohol protecting group. The next step formed diol (**315**) upon treatment with phosphate buffer which by using Movassaghi's reductive coupling reaction yielded (**316**). This intermediate was then treated with hydrogen sulfide and boron trifluoride etharate to give (**317**) followed by oxidation to render chaetocin A (**42**). This established pathway enabled the synthesis of *ent*-(**42**) from L-amino acid derivatives when used as starting materials (Scheme **66**).[226, 227]

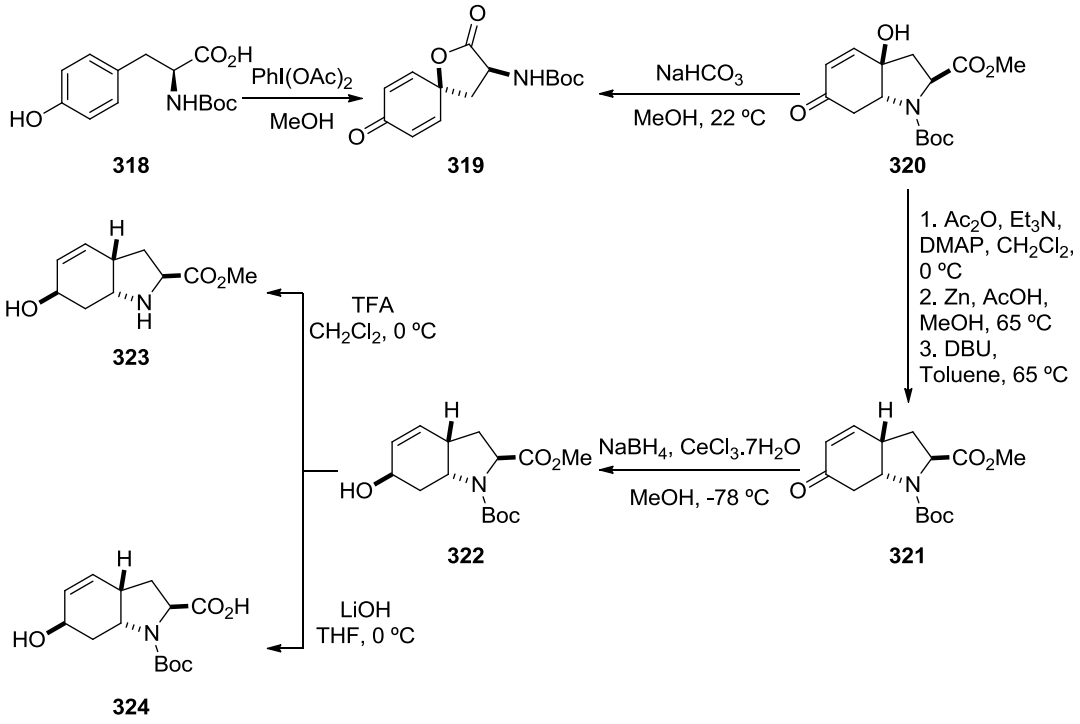


Scheme 66 – Iwasa's synthesis of chaetocin A (4)

1.2.18 Nicolaou's approach

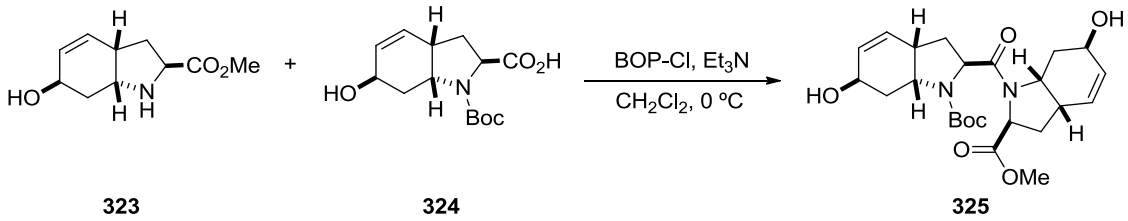
In 2011, Nicolaou *et al* [229, 230] developed their own methodology to achieve the ETP core. The synthetic pathway started with the use of *N*-protected tyrosine (**318**) that after

proper cyclisation using excess iodobenzene diacetate rendered spirolactone (**319**).[231] This compound was then treated with methanol and sodium bicarbonate to yield (**320**). Formation of enone (**321**) was achieved by acetylation, zinc reduction and isomerization using 1,8-diazabicycloundec-7-ene. Reduction of the formed compound using cerium(III) chloride and sodium borohydride led to (**322**). Different treatment of the given product led to two dissimilar compounds; when exposed to trifluoroacetic acid, (**322**) was transformed into (**323**) whereas basic treatment of (**322**) gave (**324**) (Scheme **67**).[229]



Scheme 67 – Nicolaou's approach (1)

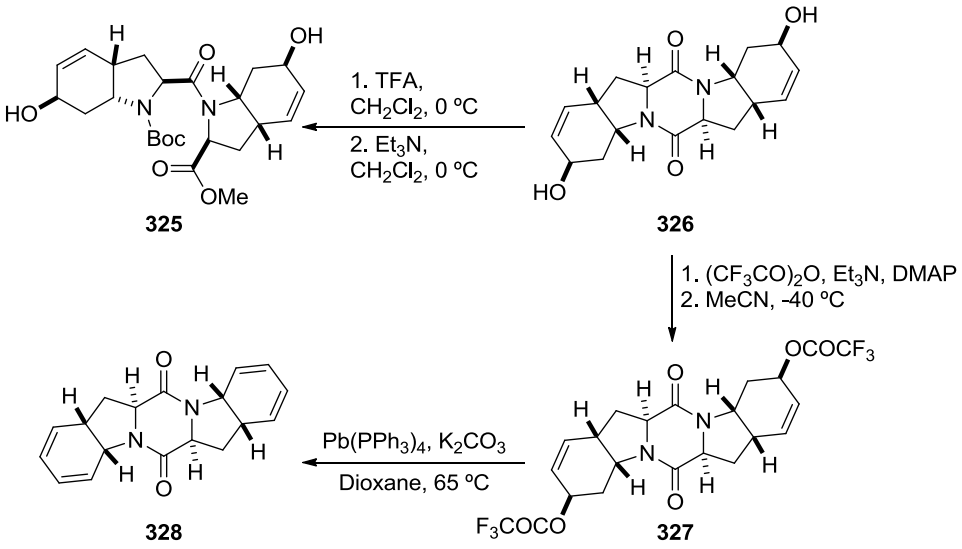
Intermediates (**323**) and (**324**) were coupled using phosphoric acid bis(2-oxooxazolidide) chloride affording *N*-Boc methyl ester amide (**325**) (Scheme **68**).[229]



Scheme 68 – Nicolaou's approach (2)

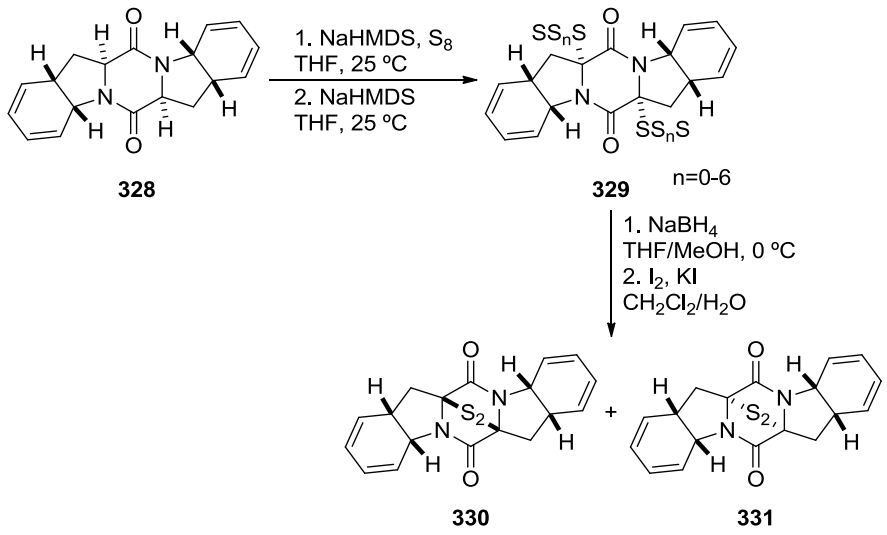
Amine deprotection of (**325**) followed by amine induced cyclisation gave (**326**). Diketopiperazine (**328**) was formed after treatment of (**326**) with trifluoroacetic

anhydride yielding (**327**) followed by elimination to render the desired product (Scheme **69**).[229]



Scheme 69 – Nicolaou's approach (3)

Diketopiperazine sulfenylation step was well studied by Nicolaou and co-workers [230] who concluded that optimal conditions required pre-treatment of elemental sulfur with three equivalents of sodium bis(trimethylsilyl) amide followed by addition of this to (**328**) with an extra two equivalents of the previously used base yielding (**329**). Reduction of (**329**) using sodium borohydride followed by oxidation gave ETP (**331**) and the corresponding 2,2'-epimer (**330**) (Scheme **70**).[230]

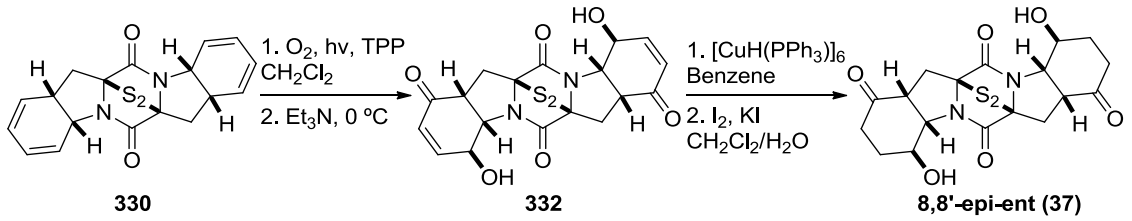


Scheme 70 – Nicolaou's approach (4)

Having achieved a route to the ETP core, Nicolaou used previously formed ETP (**330**) to synthesise 8,8'-*epi*-*ent*-rostratin B (**37**).[229]

1.2.18.1 8,8'-Epi-ent-rostratin B

Photooxygenation of (**330**) followed by treatment with triethylamine gave (**332**) that after reduction of the olefinic bonds using cuprous hydride triphenylphosphine hexamer and subsequent oxidation yielded 8,8'-epi-ent-rostratin B (**37**) (Scheme **71**).[229]



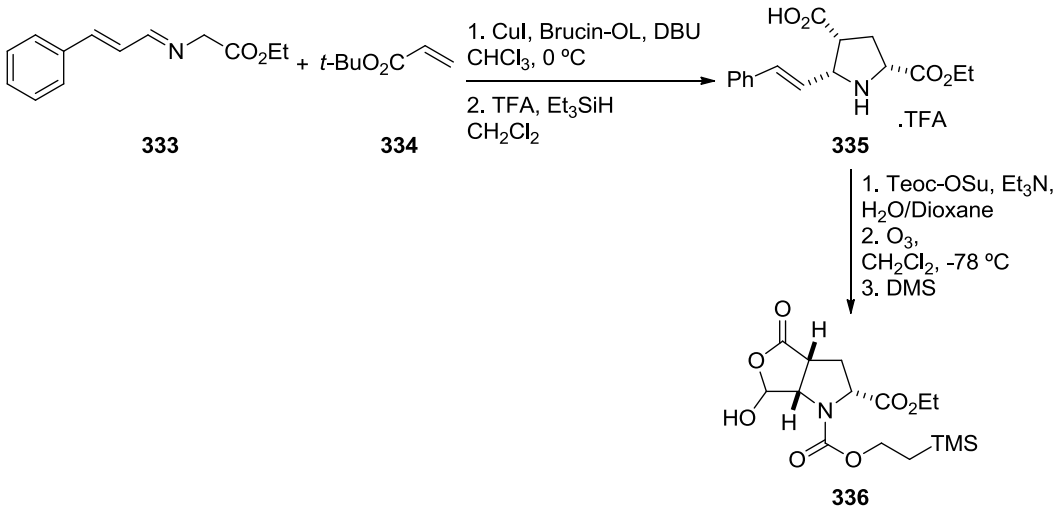
Scheme 71 – Nicolaou's synthesis of 8,8'-epi-ent-rostratin B

1.2.19 Reisman's approach

In 2012, Reisman *et al* [232] were able to synthesise (-)-acetylaranotin (**8**), developing their own methodology to achieve ETP based natural products.

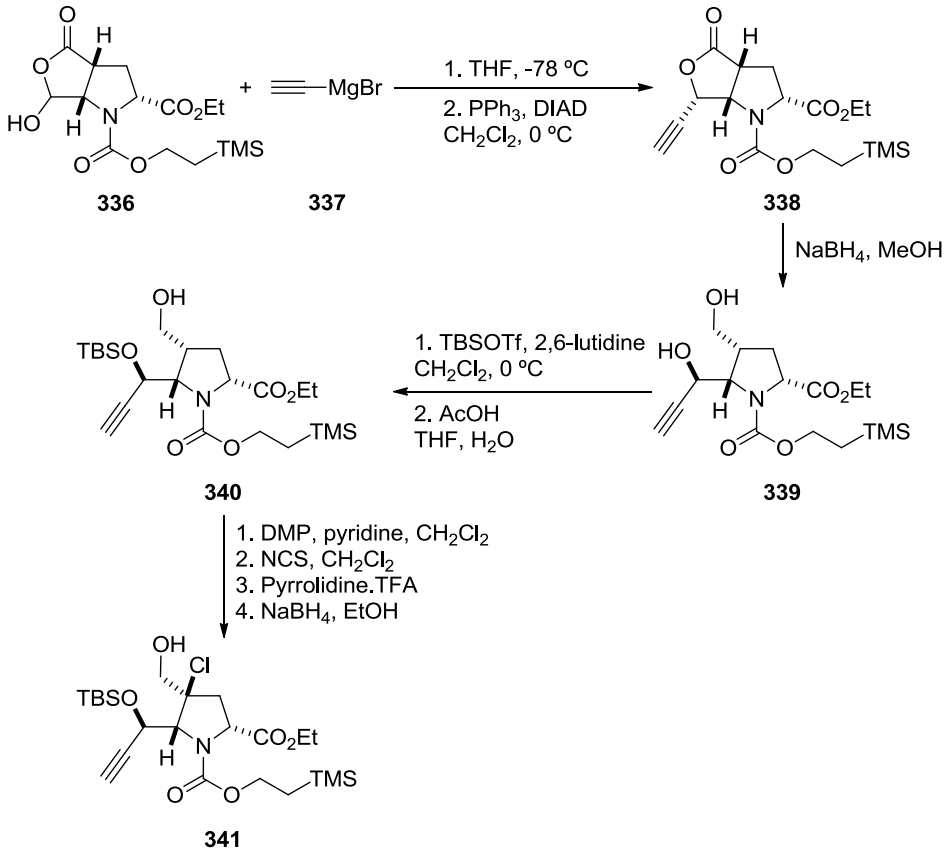
1.2.19.1 (-)-Acetylaranotin

The synthetic route started by reacting cinnamaldimine (**333**) with *tert*-butyl acrylate (**334**) using a catalytic amount of copper iodide and brucin-OL to give the *endo*-pyrrolidine which after treatment with trifluoroacetic acid rendered (**335**). The next stage involved amine protection using trimethylsilyl carbamate and ozonolysis of the alkene to give the hydroxylactone (**336**) (Scheme **72**).[232]



Scheme 72 – Reisman's synthesis of (-)-acetylaranotin (1)

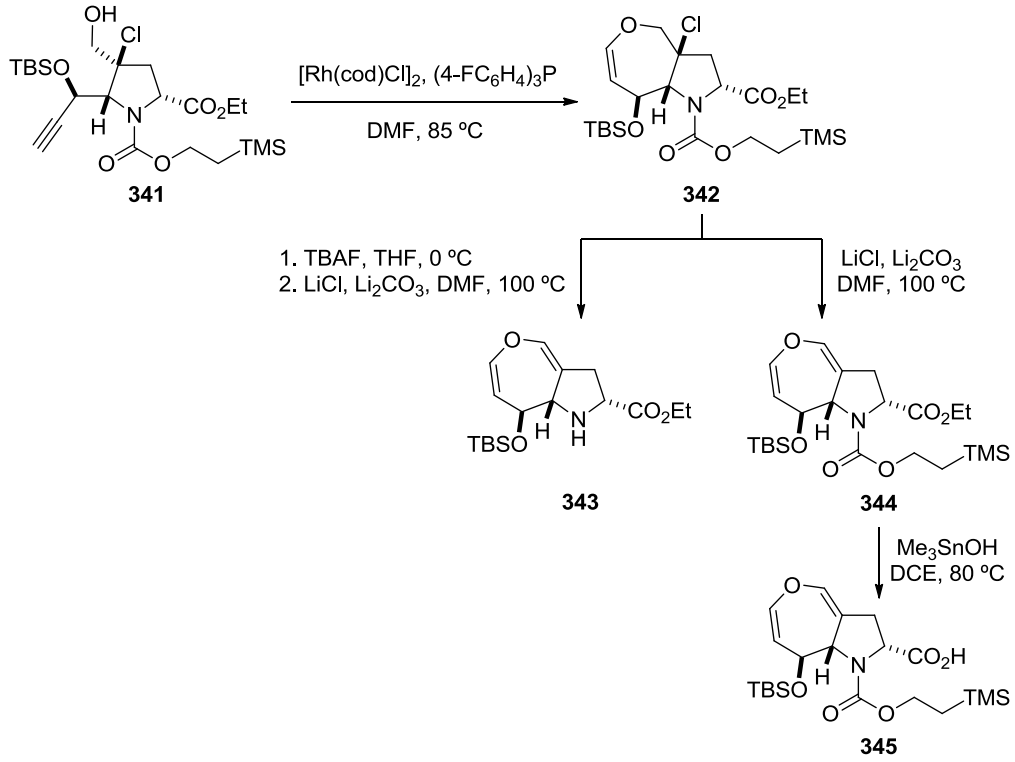
Intermediate (**336**) was reacted with ethynylmagnesium bromide (**337**) that after acid work-up, triphenylphosphine and diisopropylazodicarboxylate treatment yielded lactone (**338**). Diol (**339**) formation was achieved by reduction using sodium borohydride followed by bis-silylation and selective cleavage of the primary silyl ether to give alcohol (**340**). Oxidation of the formed alcohol by Dess-Martin periodinane followed by treatment with *N*-chlorosuccinimide and pyrrolidine-trifluoroacetic acid and sodium borohydride reduction gave the desired alkynol (**341**) (Scheme **73**).[232]



336 **337** **338** **339** **340** **341**

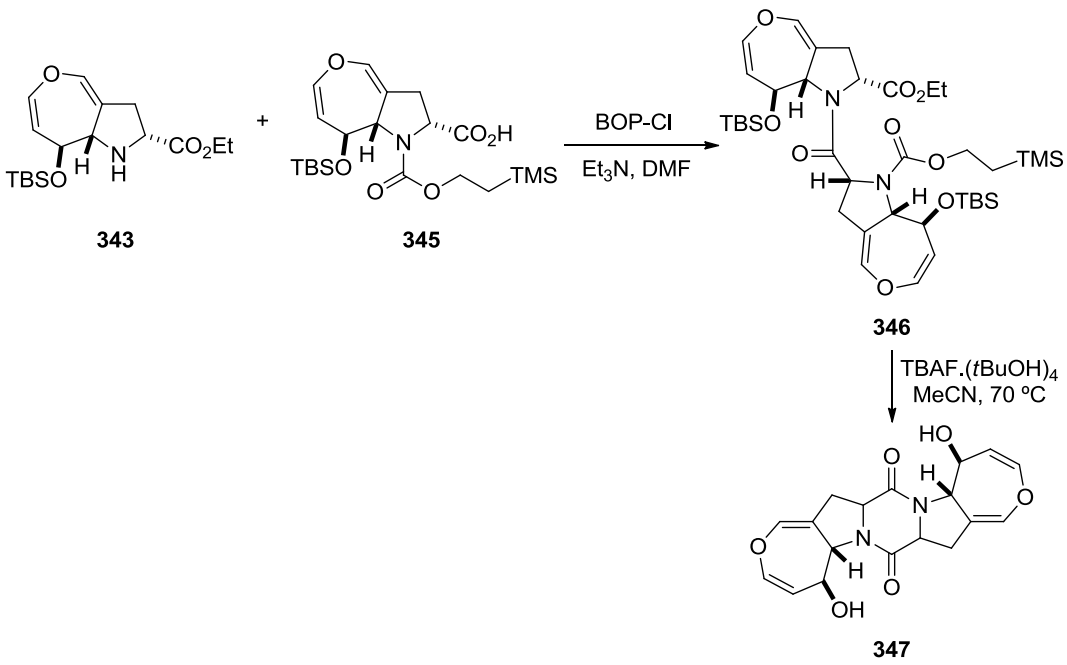
Scheme 73 – Reisman's synthesis of (-)-acetylaranotin (2)

Compound (**341**) when reacted with cyclooctadiene rhodium chloride dimer and tris(4-fluorophenyl)phosphine yielded (**342**). Treatment with tetrabutylammomium fluoride provided the free amine that when exposed to lithium chloride and lithium carbonate rendered dihydrooxepine (**343**). Using the same type of chloride elimination, compound (**342**) was converted to (**344**), which when treated with trimethyltin hydroxide gave (**345**) (Scheme **74**).[232]



Scheme 74 – Reisman's synthesis of (-)-acetylaranotin (3)

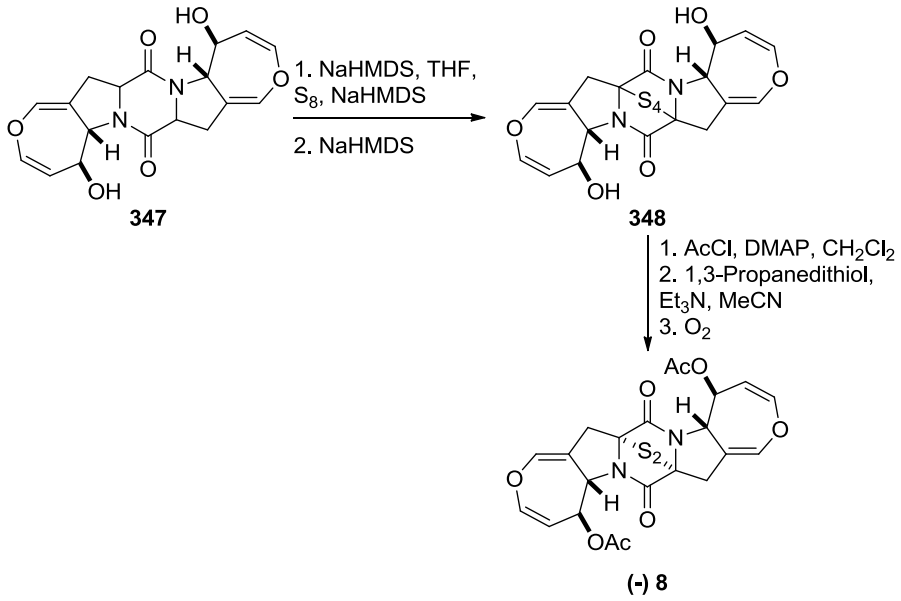
Coupling of (**343**) and (**345**) under basic conditions yielded compound (**346**). Desilylation/cyclisation followed, to give diketopiperazine (**347**) (Scheme **75**).[232]



Scheme 75 – Reisman's synthesis of (-)-acetylaranotin (4)

Introduction of the tetrasulfide bridge was achieved using the previously reported method by Nicolaou,[230] which used a pre-mixture of sodium bis(trimethylsilyl)amide

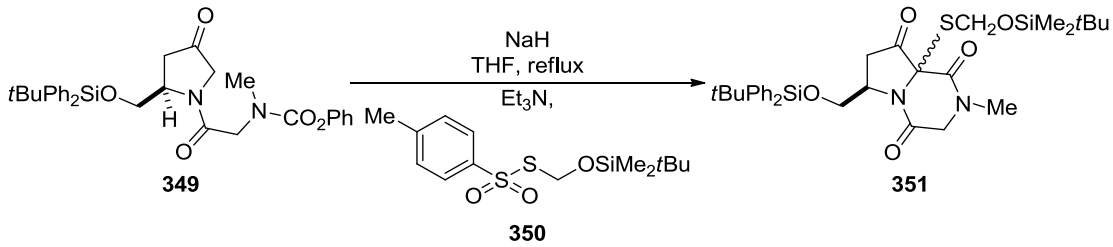
and elemental sulfur that was added to a solution of (**347**) in the same strong basic conditions to give (**348**). The formed compound was bisacetylated using acetyl chloride. The tetrasulfide bridge was further reduced using propanedithiol in a triethylamine mixture that was oxidized yielding (-)-acetylaranotin (**8**) (Scheme **76**).[232]



Scheme 76 – Reisman's synthesis of (-)-acetylaranotin (5)

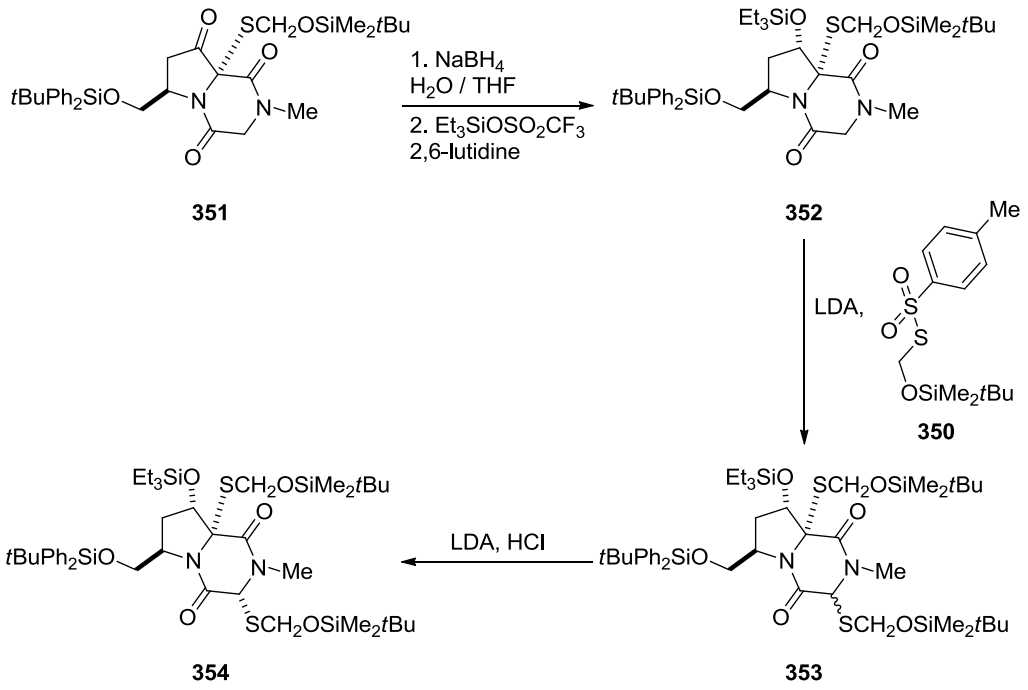
1.2.20 Clive's approach

In 2012, Clive *et al* [99, 233] generated their own model to achieve ETPs. Studies regarding MPC1001 (**33**) led the author to examine several methods to introduce sulfur in different stages of the synthesis. For the purpose, reagent (**350**) was developed which enabled the conversion of (**349**) into a diastereomeric mixture of (**351**).



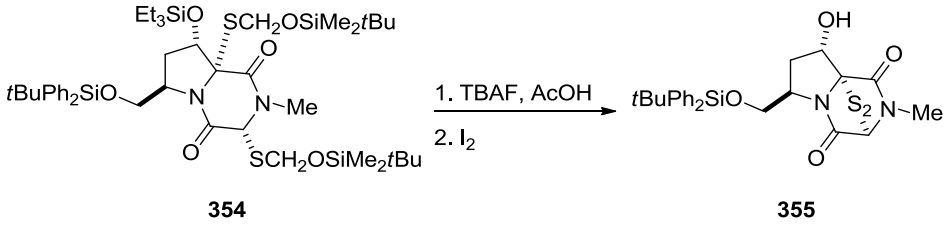
Scheme 77 – Clive's approach (1)

Compound (**351**) was then reduced to the corresponding alcohol followed by silyl protection to give (**352**). The second sulfur atom was introduced after deprotonation and using the previously referenced reagent (**350**) to give compound (**353**). Treatment with sodium bis(trimethylsilyl)amide and hydrochloric acid yielded the single isomer (**354**) (Scheme **78**).[99]



Scheme 78 – Clive's approach (2)

Having both sulfur atoms attached to the diketopiperazine moiety took Clive and co-workers to the next stage of the synthetic pathway of sulfur deprotection and oxidation. This was achieved using tetra-*n*-butylammonium fluoride in a mixture of (**354**) in acetic acid followed by iodine treatment to give the desired ETP (**355**) (Scheme **79**).[99]



Scheme 79 – Clive's approach (3)

1.2.21 Overview and outlook on the synthetic approaches towards the epi-3,6-dithio-2,5-diketopiperazine core and related natural products

The increasing innovation in the field of synthetic chemistry allied to the quest for better pharmacological and medicinal outcomes led scientists to modernize and improve synthetic pathways to ETPs. Since the discovery of the first ETP based natural products much has been achieved in the synthetic chemistry field following Trown's seminary approach that stimulated scientists like Svokos and Waring to the different pathways distinguished by Schmidt that led to amazing breakthroughs by other authors such as Bräse.[155, 164, 167, 216, 218]

1.3 Epi-3,6-dithio-2,5-diketopiperazines disulfide bridge and mechanism of action

Following, it is clear that the development of a single and straightforward way to achieve the ETP core proved to be an exciting challenge to many authors. Understanding how these molecules behave and translating their structural components into biological outcomes led researchers to accept the importance of the disulfide bridge since depletion of it resulted in a loss of activity.[234] In order to determine the characteristics behind the ETPs sulfur-sulfur bond, a number of physical techniques were used over the years that differentiated this connection from their simpler disulfide congeners.

1.3.1 X-Ray studies

X-Ray studies have demonstrated that the preferred conformation of the disulfide bridge is a helical twist where sulfur atoms are closer to the carbon atoms from the carbonyl groups than to the nitrogen atoms of the diketopiperazine ring.[235] Bridged dithiodiketopiperazines also showed greater torsional strain compared to acyclic sulfur containing compounds.[236] Dihedral angles (CSSC) vary between different ETPs and can go from 8° to 18°.[237] When compared to simpler examples of acyclic disulfide bond containing molecules, i. e. HSSH and H_3CSSCH_3 the difference is noticeable since dihedral angles are shown to be approximately 90°.[238] The conformation behind the disulfide bridge can be viewed as a repulsion of sulfur and nitrogen atoms lone pairs which can then involve the interaction in-between sulfur's lone-pair electrons and the corresponding carbon atom from the amide bond (Figure **44**).[239]

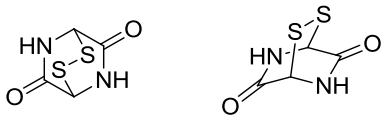


Figure 44 – Schematic of the dissulfide bridge above the diketopiperazine ring

1.3.2 Infra-red and Raman spectra studies

Studies involving sporidesmin A (**3**) and its corresponding reduced form (**356**) showed relaxation of the disulfide bridge strain imposed on the carbonyls of the diketopiperazine core. Amide carbonyl stretching frequencies for sporidesmin A (**3**) indicated values of 1655 and 1680 cm^{-1}, whereas the methylated reduced form of this

same molecule, sporidesmin D (**356**), presented frequencies of 1675 and 1700cm^{-1} (Figure **45**).[38, 240]

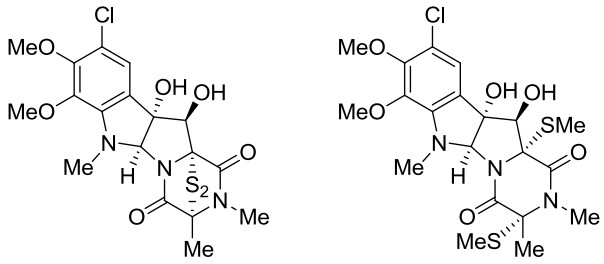


Figure 45 – Sporidesmin A and Sporidesmin A disulfide reduced form

Raman spectral studies performed by Scheraga and co-workers [240] showed similar conceptual results which confirmed the strained environment around the disulfide bridge. Sulfur-sulfur bonds for different natural products revealed stretching frequencies between 486 and 510cm^{-1} while simpler aliphatic disulfide compounds (ex. 1,2-dithiane) showed values between 509 and 532cm^{-1}.

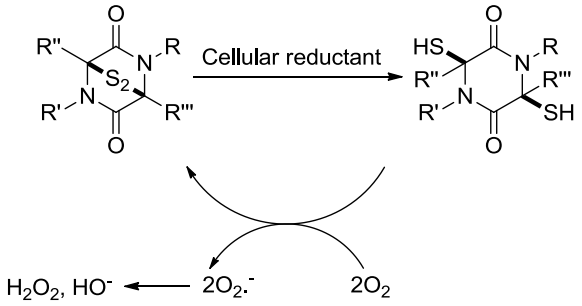
1.3.3 NMR studies

The same examples previously used by Scheraga,[240] sporidesmin A (**3**) and D (**356**), were used by Ronaldson [36, 37] in 1975 as NMR testing examples. Proton NMR studies showed a 0.16ppm (in $CDCl_3$) downfield shift for the 3-methyl group of sporidesmin A (**3**) when compared to its reduced analogue. Carbon 13 NMR studies demonstrated again a downfield shift of 7.3ppm for the 3-methyl substituent as well as an upfield shift for the quaternary carbon atoms linked to sulfur of 4.6 – 4.8ppm now for sporidesmin D (**356**) when compared to sporidesmin A (**3**) (Figure **45**).

1.3.4 Epi-3,6-dithio-2,5-diketopiperazines mechanism of action

The biological activity of ETPs is focused on the disulfide bridge moiety as previously discussed. The existing ideology is that one of the sulfur atoms can be involved in covalent modification through thiol disulfide interchange whilst the second thiol can be available for radical generation. In proteins, this effect results in conformational changes and altered activity when basic amino acids residues were coupled with thiol.[2, 30] Proof of the importance of the disulfide bridge came from the use of reducing agents, *e.g.* Cleland's reagent, which resulted in loss of biological activity due to reduction of the disulfide bridge.[241]

From a different perspective, the reactive disulfide bridge of ETPs has been reported to cause DNA strand breakages in anaerobic conditions of oxidative stress by the formation of reactive sulfur species (Scheme **80**). [112, 113, 114, 241]



Scheme 80 – Example of biological reduction of the ETPs dissulfide bridge

Results demonstrated the importance of disulfide bridged compounds when compared to dithiol containing molecules for different biological outcomes. With former and current research, developments have led to the applicability of the knowledge extracted from the mode of action of ETPs to a wide range of biological areas. These areas include cancer, bacterial and viral drug discovery.[18, 141, 228]

1.4 Cancer, hypoxia and other targets

Cancer is a disease caused by the uncontrolled growth of changed cells that result in the formation of a tumour. The spread of these masses to different sites throughout the body can cause death by vessel and organ obstruction, making surgical intervention impossible. In early stages of tumour growth, cancer cells usually resemble the original cells from which they derive; however, they can lose the appearance and function of their origin at a later stage.[242, 243]

Every year, more than 285,000 people are diagnosed with cancer in the United Kingdom and the current estimate is that more than one in three people will develop a form of cancer at some point in their lifetime. While numerous types of cancer are known, breast, lung, colorectal and prostate cancer, constitute over half of new cases diagnosed as seen in the chart below. In males, prostate cancer is the main cancer type with 36,000 new cases diagnosed in 2007, followed by lung cancer, ~22,000, and colorectal, ~21,000 new cases *per* year. In females, breast cancer continues to be the most common tumour type, accounting for nearly one in three female cancers, with over 45,000 new cases diagnosed in 2007 (Chart **1**).[242]

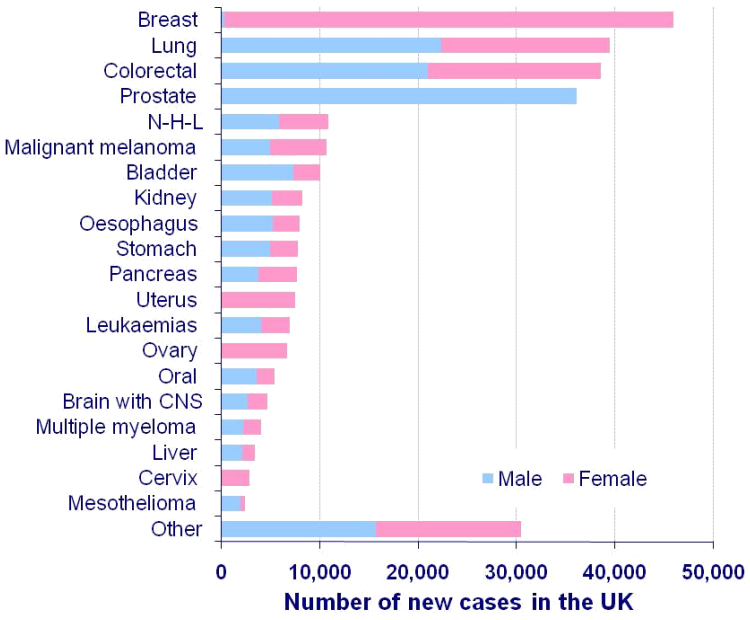


Chart 1 – The 20 most commonly diagnosed cancers (excluding non-melanoma skin cancer), UK, 2007

Tumour hypoxia, or tumour low oxygen tension, is frequently observed in solid tumours. This occurs when oxygenation levels decrease in malignant tissues forcing cell

death. Hypoxia is defined as a value of oxygenation below 2% in mammalian tissues with an average being in-between 2 and 9%. Its role in cancer treatment instigated studies that revealed a number of other crucial effects of hypoxia on tumour progression such as angiogenesis, increased metastatic potential, DNA replication and reduced protein synthesis.[244, 245]

1.4.1 Hypoxia Inducible Factor

Hypoxia Inducible Factor-1 (HIF-1) is a transcription factor that regulates the expression of a large number of genes, including those responsible for cell survival. The HIF complex is formed from one α and β subunit. Under normal oxygen conditions, levels of the hypoxia inducible factor-1α (HIF-1α) are tightly regulated, but in the deficient oxygen environment found in many tumours, HIF-1α accumulates causing transcription activation and hence tumour growth leading to poor patient prognosis. The role of HIF in cancer has become increasingly clear due to immunohistochemical techniques that have demonstrated the overexpression of HIF-1α in a broad range of human malignancies like early-stage cervical cancer, breast cancer, oligodendroglioma, ovarian cancer, endometrial cancer and oropharyngeal squamous cell carcinoma.[244, 246, 247]

As referred before, normoxia down-regulates the occurrence of HIF-1α by proteosomal degradation. This occurs by hydroxylation of two prolines on HIF-1α that enables the binding to the von Hippel-Lindau protein (pVHP) and consequently leads to ubiquitination. In a different but equally important pathway, hydroxylation of one asparagine aminoacid residue in HIF-1α disrupts the interaction between this protein and the p300 co-activator, crucial for HIF transcriptional activity. When oxygen levels decrease, the hypoxic-response is activated, leading to increased protein levels due to lack of HIF-1α hydroxylation. This enables the dimerisation of HIF-1α / HIF-1β (also known as aryl hydrocarbon receptor nuclear translocator, (ARNT) and the important p300 co-activator. This active transcriptional complex binds to the hypoxia response element (HRE) leading to the activation of hypoxic response proteins responsible for tumour survival and progression, as shown below (Figure **46**).[247, 248, 249, 250]

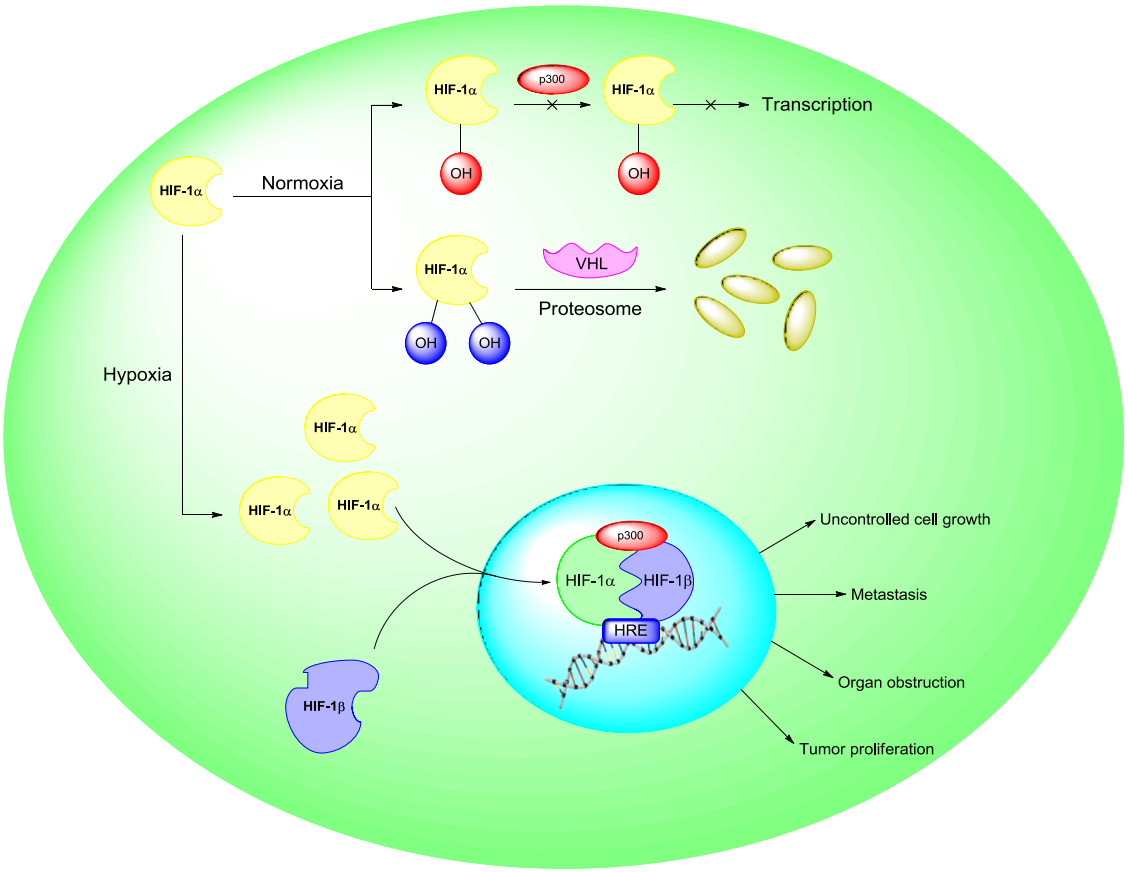


Figure 46 – Illustration of a normoxia and hypoxia state where HIF-1α is regulated. In normoxia, hydroxylation of one asparagine aminoacid residue in HIF-1α disrupts the interaction p300 leading to proteosomal degradation. Hydroxylation of two prolines on HIF-1α enables the binding to pVHP and ubiquitination. In hypoxia, dimerisation of HIF-1α / HIF-1β and p300 leads to the activation of this transcriptional complex which binds to the HRE leading to the activation of hypoxic response proteins.

1.4.2 Epi-3,6-dithio-2,5-diketopiperazines as anticancer agents

Although the role of the disulfide bridge in ETP activity has been known for some time, the mechanism by which the bridge confers anticancer activity was until recently unknown. It is now believed that the disulfide bridge disrupts the interaction between the HIF-1α C-terminal activation domain (C-TAD) and the cysteine-histidine rich 1 (CH1) domain of the p300 co-activator. The CH1 region of p300 is formed by three main α-helices motifs which are zinc dependent to maintain their proper fold and, therefore, functionality (Figure **47**).[251, 252]

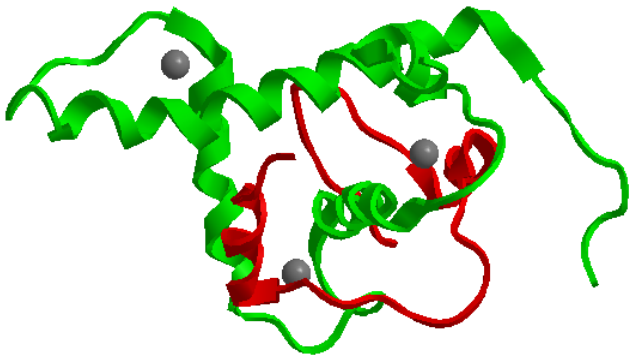
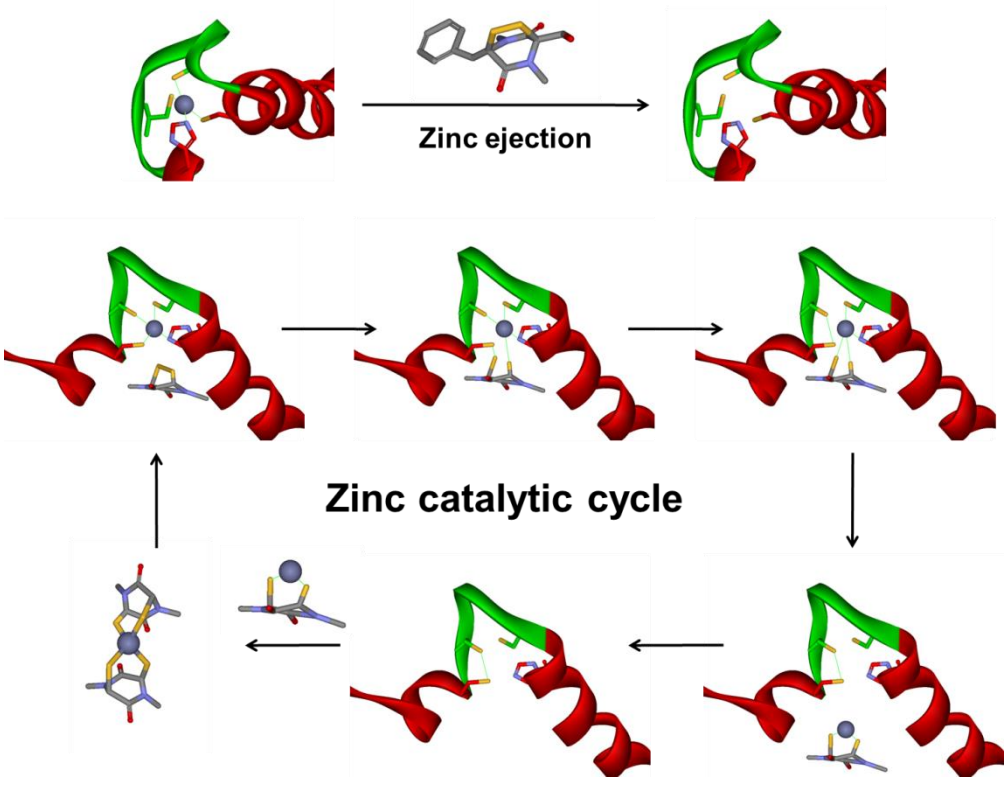


Figure 47 – CH1 region of p300 formed by three main α-helices (green) and zincs (grey)

ETPs are believed to cause zinc ejection from CH1 in a catalytic manner.[253] This interaction alters p300 conformation hence leading to protein unfold and activity reduction (Scheme **81**). [251, 242]



Scheme 81 – Schematic that illustrates the ETPs mode of action by zinc ejection from the p300 CH1 domain

Studies demonstrated that the ETP core itself is both necessary and sufficient for biological activity as equally natural and synthetic ETPs cause zinc ion ejection from its natural site.[254, 255, 256, 257, 258, 259] Cell based models furnished important information since addition of zinc to the culture environment eliminated the anti-proliferative effect of ETPs and drastically increased the percentage of viable cells.[253] By way of example, the

addition of zinc restored the cell's production of proteins responsible for neovascularization such as vascular endothelial growth factor (VEGF) during hypoxia. *In vitro* testing also confirmed that both natural and synthetic (mono and dimeric) ETPs inhibited the proliferation of cancer cell lines, proving their value for oncogenic treatment.[246, 248, 260, 261, 262]

1.4.3 Epi-3,6-dithio-2,5-diketopiperazines and other biological targets

Although it is probable that ETPs operate by more than one mechanism in cells, it is clear that proposed targets are either known, or associated, with zinc binding proteins. This suggested that the disruption of zinc binding may be one of the potential generic mechanisms of ETP action and, in part, their toxicity. Based on these findings other targets were defined as possible marks for ETP activity: matrix metalloproteinases and retroviruses.

1.4.3.1 Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are a family of zinc depending proteins that play an important role in the physiological and pathological turnover of matrix components, tissue degradation and repair.[263] They have been linked to angiogenesis in colorectal carcinomas, breast cancer and non-small-cell lung cancer.[264] One of the most well known member of this group is the matrix metalloproteinase 9 (MMP-9) (Figure **48**);[265] a VEGF modulator. Studies showed that MMP-9 plays an important role in the degradation and remodelling of damaged tissues and that its upregulation is induced by the HIF-1α/VEGF cascade of events.[251, 263, 264]

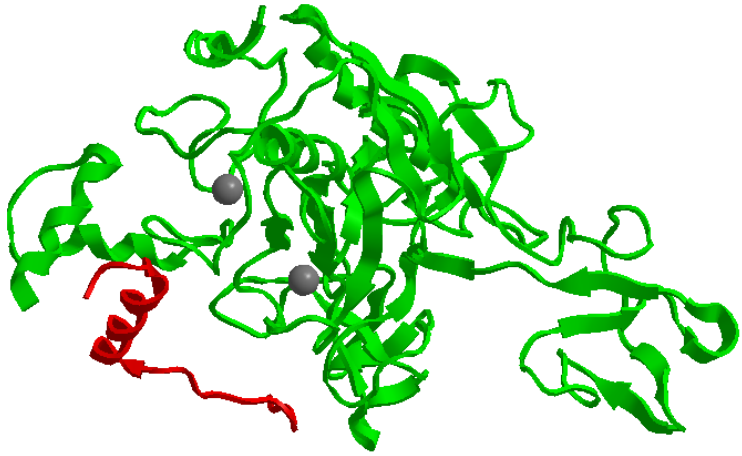


Figure 48 – MMP9

1.4.3.2 Retroviruses

ETP antiviral activity is related to inhibition of enzyme activity and viral RNA replication.[3] Retroviruses belong to one of the viral groups where ETPs can biologically act, since one of its main constituents is the nucleocapsid protein. The nucleocapsid is the protection of the viral genome. Virus genomes removed from their capsids are more susceptible to inactivation. A secondary major function of the capsid is to recognize and attach to a host cell in which the virus can be replicated. Although the nucleocapsid must be stable enough to survive in the extracellular environment, it must also have the ability to alter its conformation so that, at the appropriate time, it can release its genome into the host cell.[266] The nucleocapsid protein from human immunodeficiency virus type-1 (HIV-1) is highly basic (29 *per cent* of the amino acid residues are basic) and contains two zinc fingers associated (Figure **49**).[266, 267, 268]

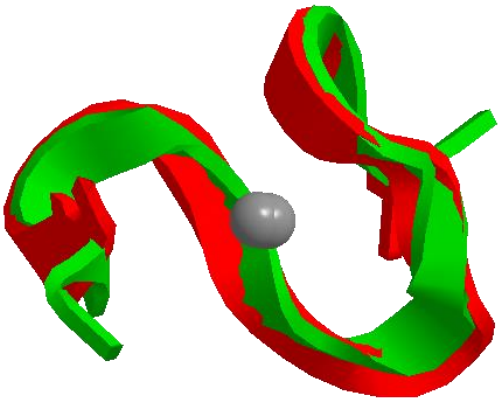


Figure 49 – HIV-1 nucleocapsid (red and green) with zincs (grey)

ETPs display anti-viral activity by zinc ejection which can render the protein inactive. Other sulfur containing molecules showed *in vitro* anti-retroviral activity against resistant strains of the nucleocapsid protein of the human immunodeficiency virus HIV-1 NCp7 using similar intrinsic zinc ejection.[264]

1.5 Dimeric natural and synthetic molecules for diseases treatment

As observed with monomeric and dimeric ETP natural products, the corresponding dimeric versions often display more potent biological activity when compared to their single entity congeners. As such, a brief discussion of other dimeric compounds will be described.[251] It was Monod *et al* [269] that emphasized the argument that isologous associations usually result in a "closed structure" that maintains intrinsic symmetry and enhanced stability. In addition to this, Monod and co-workers [269, 270] suggested that a rapid organization of the monomeric units into an oligomeric species could potentially prevent the random association of its subunits with other cellular artifacts therefore increasing specificity of a compound.[271] Genetic saving, functional gaining and structural advantages are some of the potential advantages in the dimerization of drugs such as artemisinin (**357**) (Figure **50**), a clinically efficacious antimalarial agent isolated from *Artemisia annua* L. (Asteraceae).[272, 273]

1.5.1 Dimeric artemisinin

Artemisinin (**357**) sesquiterpene lactone contains a unique endoperoxide bridge system that is crucial for its activity.[272]

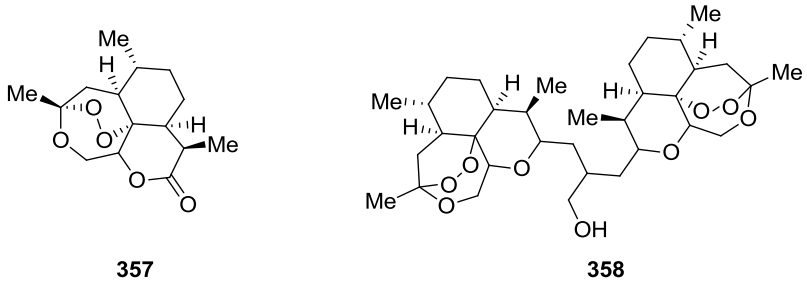


Figure 50 – Artemisinin and dimeric analogue

A synthetic campaign designed to improve the chemical stability of the parent natural product resulted in the identification of dimeric compounds that exhibited potent anti-tumor activity. In way of example, three-carbon atom linked trioxane dimer alcohol (**358**) (Figure **50**) acts as growth inhibitory towards aggressive prostate cancer cells (CH2) as the clinically used anticancer agent gemcitabine.[273] More recent advances in the development of dimeric artemisinin analogues led to a series of hydrolytically stable derivates linked through a series of phthalate acids. Several of these compounds were found to be more active against malaria than its monomeric precursor since IC_{50} values

for (**359**), (**360**) and (**361**) (Figure **51**) are correspondingly 2.9 nM, 1.6 nM and 0.77 nM compared with IC_{50} = 6.6 nM for artemisinin (**357**).[272]

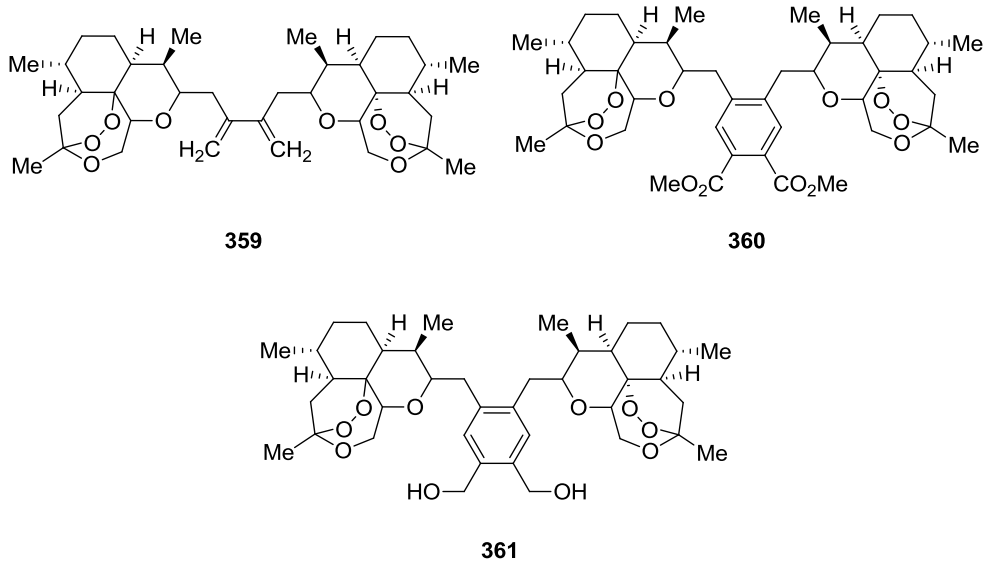


Figure 51 – Dimeric analogues of artemisinin

Compound (**360**) proved to be not only active against its primary target but potent and selective against non-small cell lung carcinoma HOP-92 cells, melanoma SK-MEL-5 cells and breast cancer BT-549 cells. In addition, dimer (**360**) exhibited potent activity against the human cervical cancer cell line, HeLa, while being nontoxic towards normal cervical cells.[269, 272]

1.5.2 Protoberberine alkaloids

Another example of the synergy behind monomeric coupling of identical molecules is represented by berberine (**362**) and the dimeric protoberberine alkaloids (**363**) (Figure 52).[274]

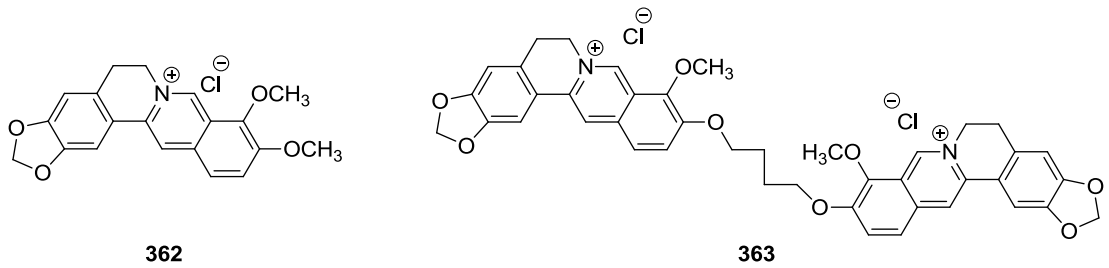


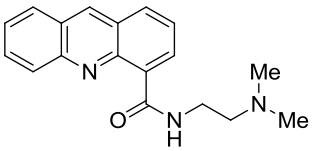
Figure 52 – Berberine and dimeric analogue

Studies showed that berberine (**362**), as well as synthetic analogues of this product, exhibited minor inhibitory activity towards DNA Topoisomerase-I.[274] DNA

topoisomerases are crucial for DNA replication, transcription and recombination. A distinction between the mono and dimeric forms of these alkaloids was seen clearly when compound (**363**) reported high active results in DNA Topoisomerase-I inhibition when compared to the corresponding monomeric species.[274]

1.5.3 Bis(DACA)

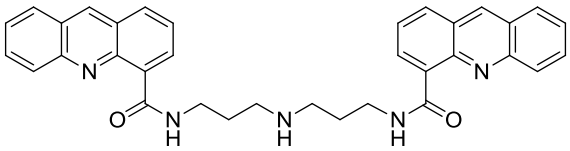
N-[(2-Dimethylamino)ethyl]acridine-4-carbomaxide (**364**) (Figure **53**), also known as DACA, is a acridinecarboxamide derivative. DACA (**364**) proved to be biologically active on inhibiting DNA topoisomerase mutants showing excellent activity in experimental solid tumour models.[275]



364

Figure 53 – DACA

Studies by Denny *et al* [276] demonstrated that dimeric tricyclic aromatic carboxamides, bis(DACA) analogues such as compound (**365**) (Figure **54**) showed an increase in cytotoxicity towards the same tumour type models. These results highlight the superior potency of dimeric conjugates towards the same target when compared with monomeric species.

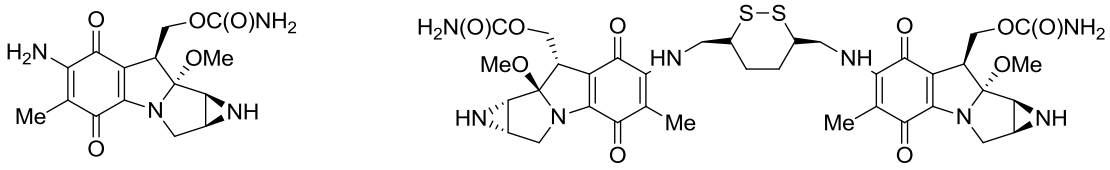


365

Figure 54 – bis(DACA)

1.5.4 Bis(mitomycin C)

A dimeric version of mitomycin C (**366**), the 7-*N*,7'-N'-(1'',2''-dithianyl-3''-6''-dimethylenyl)bismitomycin C (**367**) (Figure **55**), was developed by Kohn and Lee in 2005.[277] This new dimeric product was the result of bridging two mitomycin C (**366**) units by a dithiane linker.



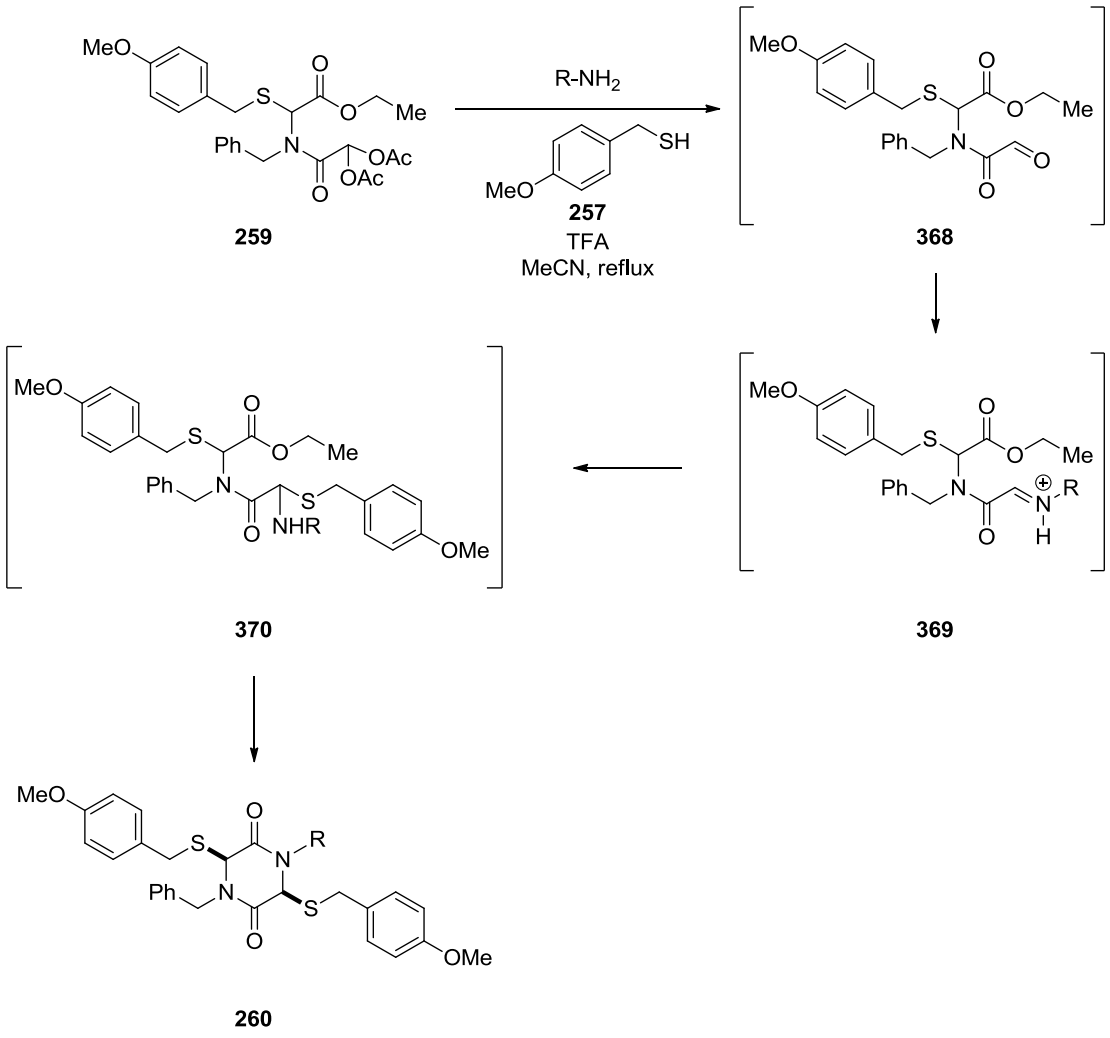
366 **367**

Figure 55 – Mitomycin and dimeric analogue

When compared to its monomeric predecessor, bis(mitomycin C) showed higher levels of DNA interstrand cross-links which demonstrated the increased potential of this new dimeric conjugate towards cancer treatment.[277]

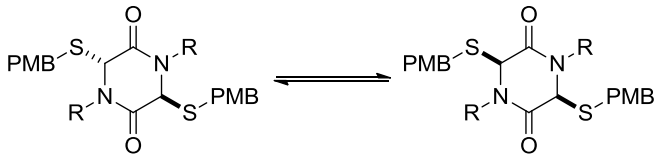
2. Aims & Objectives

As a result of the potent biological activity and structural complexities associated with the ETP family of compounds, there have been numerous efforts directed towards the obtention of both natural and synthetic congeners and examination of their biological efficacy. However, the need and difficulty associated with transferrring these products into therapeutically active molecules underlines the challenge associated with the ETP family of compounds. One of our key medicinal chemistry aims was therefore the development of a common precursor approach towards the ETP core structure. This would enable us to develop a full range of compounds, which after biological screening would enable us to explore their molecular prospects. Our proposed method was based on studies described by Hilton and co-workers.[1, 217] Whilst current synthetic methodology developed by this author has led to efficient formation of the ETP core (**260**) from the diacetate (**259**), the final ring closure multi-component reaction requires the use of TFA, which is incompatible with a number of functional groups (Scheme **82**).



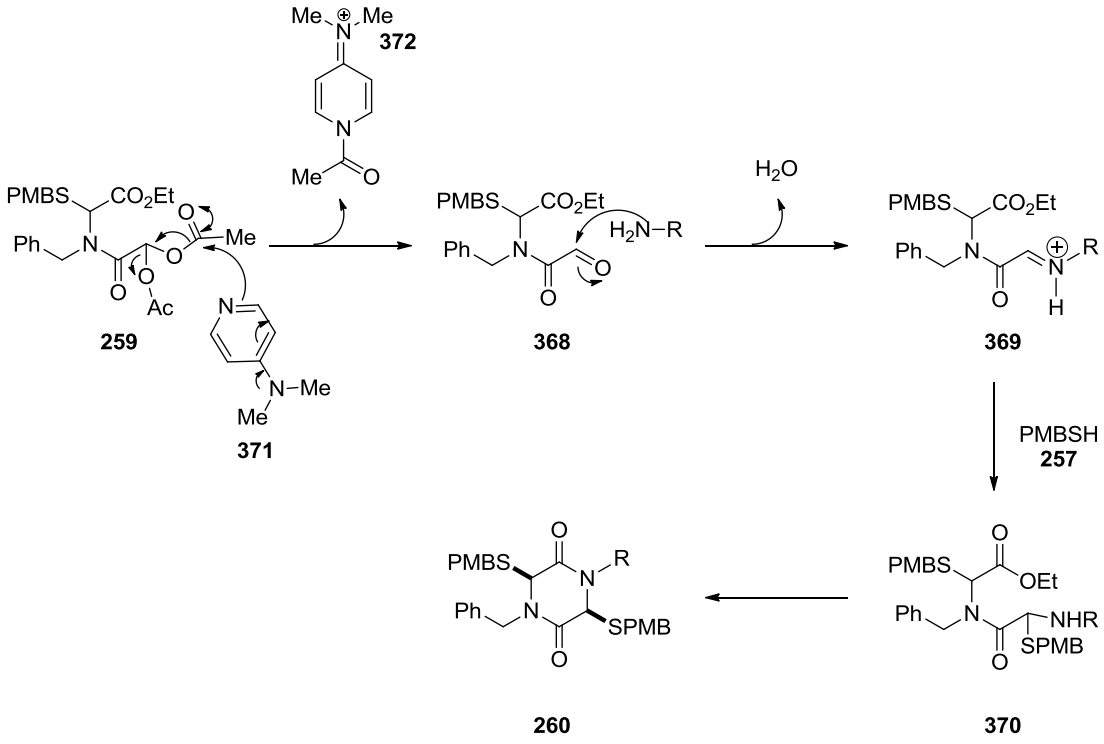
Scheme 82 – Methodology described by Hilton for the synthesis of the ETP core

As such, an additional aim of this project was to develop new methodology for the synthesis of the ETP core that would obviate the use of TFA. In addition, the current cyclisation / isomerisation protocol between the *cis* and *trans*-ETP requires long reaction times (average of 16 hours) and we therefore wanted to explore the effects of microwave acceleration of this step to reduce reaction times (Scheme **83**).



Scheme 83 – *Trans* and *cis* isomers of the protected ETP core

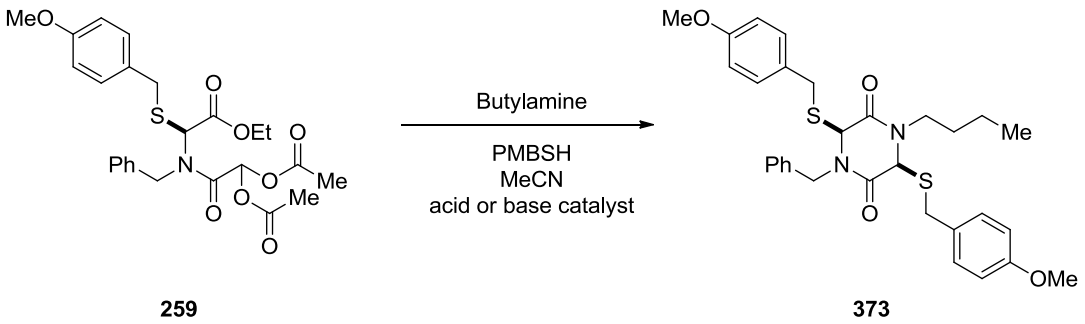
We also could investigate the possibility of an *in-situ* base-catalysed deprotection of the diacetate (**259**) to the aldehyde (**368**). Using Hilton's defined approach, we predicted that (**259**) should also react with 4-*N*,*N*-dimethylaminopyridine (DMAP) (**371**). This would unmask the aldehyde from (**259**) to form (**368**), which could then undergo a three component reaction with an amine, and *para*-methoxybenzyl mercaptan (**257**), followed by cyclisation to the diketopiperazine (**260**) (Scheme **84**).



Scheme 84 – Diacetate cyclisation procedure using DMAP

One key aspect that would be investigated in detail was the *cis/trans* ratio of the ETP core formation (**260**). To carry this out, we would follow the reaction using HPLC and

NMR to determine the ratio for both *cis/trans* products. In the first instance, we could focus on the reaction between the diacetate (**259**) and *n*-butylamine to give the protected ETP (**373**) (Scheme **85**).



259 **373**

Scheme 85 – Diacetate cyclisation using *n*-butylamine

After completing this step, we would next investigate which optimal conditions permit the introduction of different amine nucleophiles (Figure **56**). These new groups would increase diversity when exploring the biological effects of the ETPs.[256]

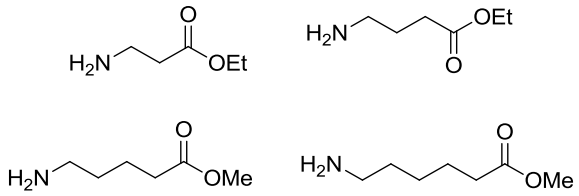
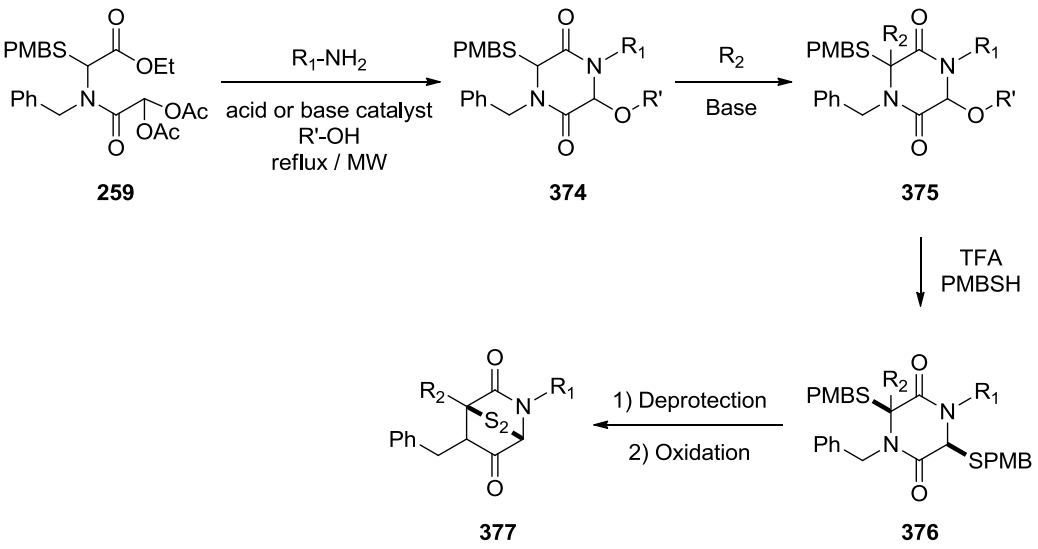


Figure 56 – Examples of different amino esters for diacetate cyclisation

In conjunction with this phase of the research project, we would also explore the use of microwave acceleration of the reaction to try to optimise the ratio of the *cis/trans* products as well as reduce the reaction times that are currently used.[278, 279, 280]

Another associated aim of this project would be the synthesis of tri-substituted ETPs. Using Hilton's diacetate (**259**) we intended to develop a cyclisation pathway that would enable the later introduction of a tertiary substituent to the ETP core as shown below (Scheme **86**).



Scheme 86 – Methodology for the synthesis of trisubstituted ETPs

The next stage of the research programme would involve different approaches towards dimeric ETPs using our optimised conditions determined from the early investigational stages. As outlined before, dimeric ETPs often display more potent biological activities than their monomeric counterparts. We therefore intended to adapt our approach towards simplified dimeric ETP systems (Figure **57**).

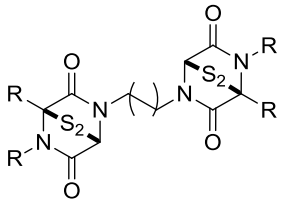
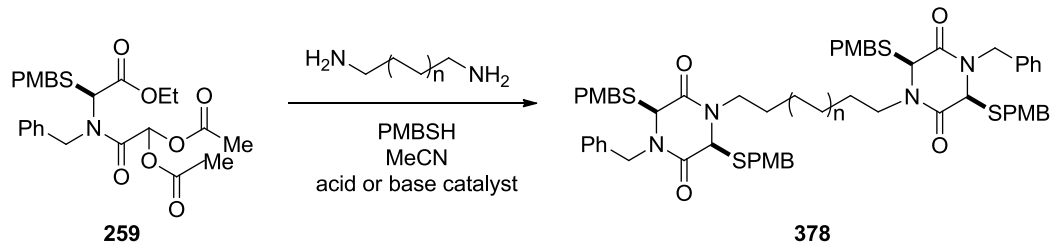


Figure 57 – Disubstituted dimeric ETP

Our proposed mechanistic approaches towards dimeric ETPs are outlined below. In the first instance we anticipate that reaction of one diamine linker with two equivalents of Hilton's diacetate (**259**) and two equivalents of PMBSH (**257**) should facilitate the formation of simple dimeric ETPs (**378**) (Scheme **87**).



Scheme 87 – Synthesis of dimeric ETP using a diamine linker

In our second synthetic pathway we intended to use vinyl integrated ETP derivatives and form the dimeric molecules by olefin metathesis using Grubbs type catalysts (Figure **58**).[281, 282, 283, 284]

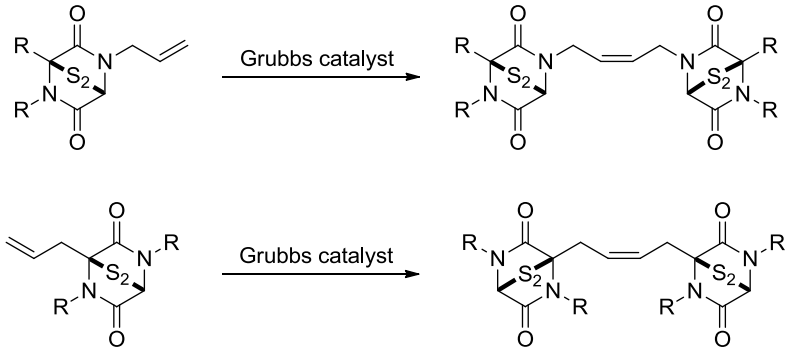


Figure 58 – Synthesis of disubstitued dimeric ETPs using Grubbs catalyst

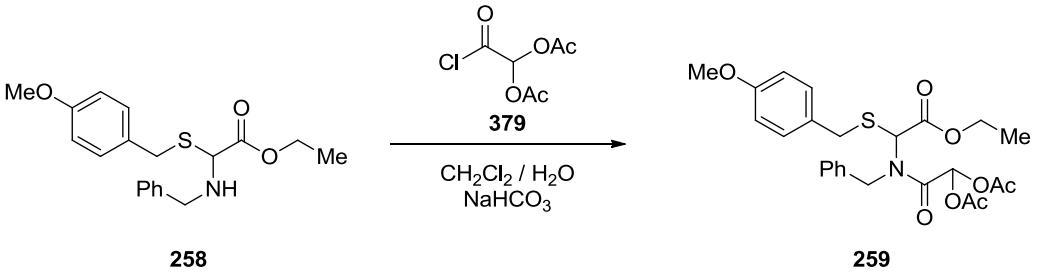
Following this research stage, we should be able to generate a library of ETPs and test them to see which are more biologically active against cancer cell lines and other targets; increasing their specificity and minimizing toxicity.

To summarize, our objectives are as follows:

- Cyclisation / isomerisation of the diacetate ETP precursor (**259**) using base-catalysed deprotection.
- Explore the effect of microwave acceleration to reduce reaction times for the formation of the protected ETP core.
- Investigate the *cis/trans* ratio of the ETP core formation.
- Introduce new amine nucleophiles to the ETP core.
- Develop a modified route using Hilton's diacetate (**259**) precursor to construct tri-substituted ETPs.
- Synthesise dimeric ETPs using different approaches based on the initial studies towards their monomeric counterparts.
- Produce a library of ETP based compounds and test their potency for different biological targets.

3. Results & Discussion

As outlined in the project aims, one of the main objectives was to synthesise the ETP core using our approach. The crucial step in our synthetic pathway is the formation of the diacetate intermediate (**259**). To achieve this we needed to synthesise diacetoxyacetyl chloride (**379**) in sufficient amounts in order to make a range of analogues (Scheme **88**).

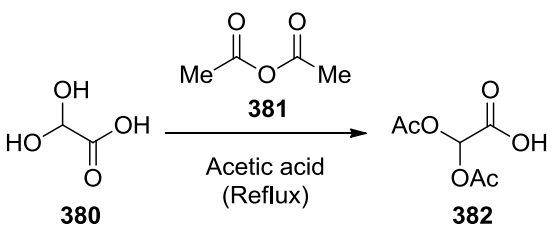


Scheme 88 – 2-(Benzyl(2-ethoxy-1-(4-methoxybenzylthio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate synthesis

However, when we attempted to synthesise diacetoxyacetyl chloride (**379**) on a large scale, the route used to prepare it on a multi-gramme scale often proved to be low yielding.[285] To overcome this, our first step consisted of optimising the synthesis and purification of diacetoxyacetyl chloride (**379**) to avoid repeating the process multiple times, as this compound was an essential part of our synthetic strategy.

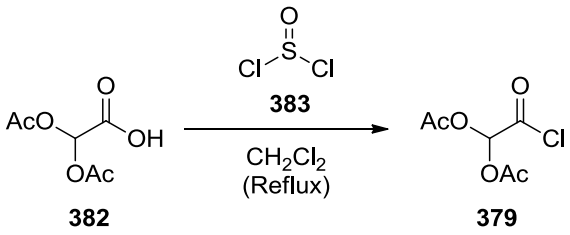
3.1 Diacetoxyacetyl chloride synthesis

The reported patented synthetic route to produce diacetoxyacetyl chloride (**379**) [285] involves mixing acetic anhydride (**381**), acetic acid and glyoxylic acid monohydrate (**380**) and heating the reaction at 150 °C (Scheme **89**) to give intermediate (**382**). This initial step was maintained in our synthetic protocol since we had found it to be straightforward and produced the intermediate (**382**) without any side products.



Scheme 89 – Diacetoxyacetyl chloride synthesis (1)

The second step in the formation of diacetoxyacetyl chloride (**379**) required addition of thionyl chloride (**383**) to crude compound (**382**) in dichloromethane followed by heating the reaction under reflux conditions using an oil bath, followed by distillation (Scheme **90**).



Scheme 90 – Diacetoxyacetyl chloride synthesis (2)

However, on carrying out the same protocol in our laboratory, compound (**379**) could only be obtained in low yield (50%) and analysis of the crude material prior to distillation showed that considerable amounts of intermediate (**382**) could still be found. ^{1}H NMR spectroscopy clarified the presence of the starting material (**382**) hydroxy group at 10.7 ppm (Figure **59**) clearly indicating that we needed to optimise this reaction if we are to use this synthetic route.

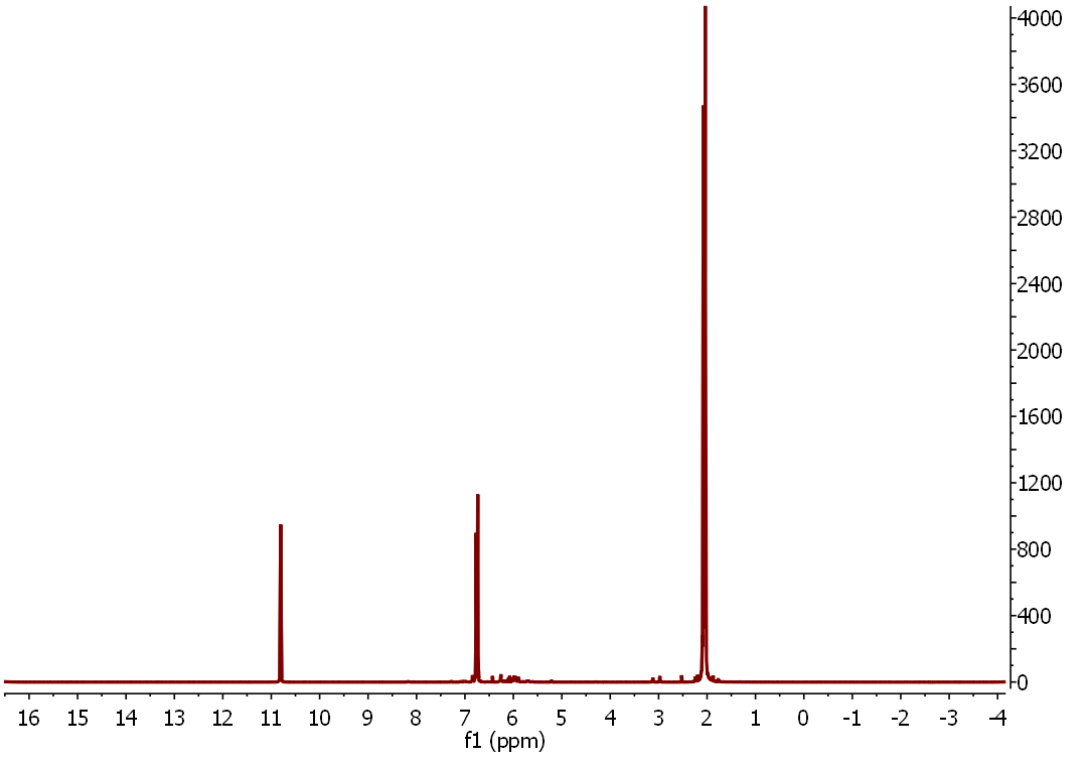
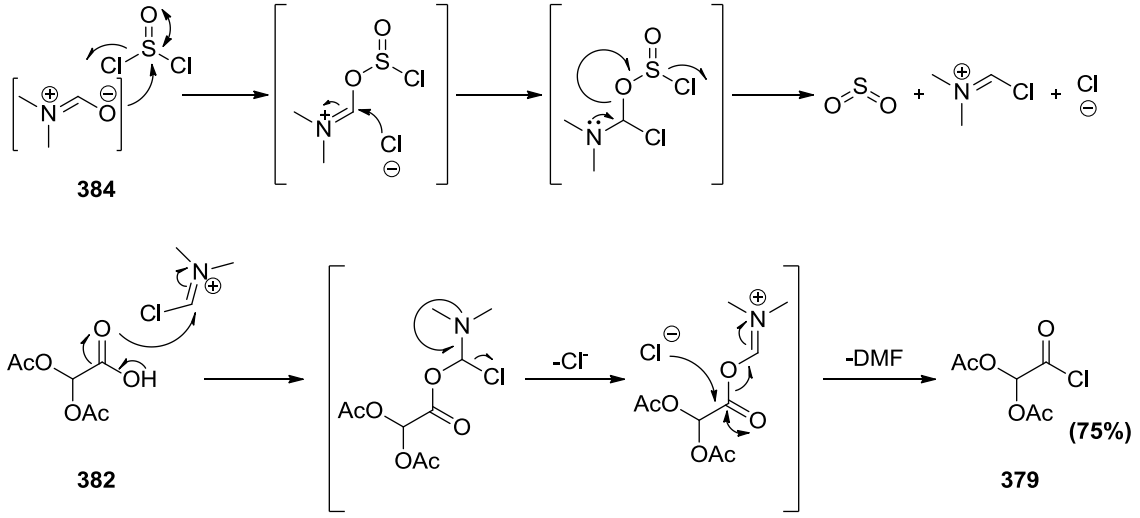


Figure 59 – ^{1}H NMR showing the incomplition of diacetoxyacetyl chloride synthesis

In our laboratory, we had moved from oil bath heating used in previous laboratories to drysyn heating blocks to accomplish this reaction. However, following the previously reported protocol, only small amounts of diacetoxyacetyl chloride (**379**) were observed. We found this to be anomalous to the formerly described synthesis and thus decided to investigate it in more detail since diacetoxyacetyl chloride (**379**) was a key starting material for our work. Whilst it was clear that these differences are between the efficiency of an oil bath and drysyn heating, we considered it worthwhile to employ different conditions for the synthesis. This led us to use dimethylformamide (**384**) as a reaction catalyst which could promote chlorination and optimistically push the synthetic pathway towards completion (Scheme **91**).



Scheme 91 – Diacetoxyacetyl chloride synthesis (3)

Whilst using dimethylformamide (**384**) we elected to follow the synthesis by NMR in which aliquouts of the mixture were removed at different time intervals. Throughout the experiment, three time periods (1.5, 3, and 5 hours) were studied in order to evaluate the conversion of the hydroxy group from compound (**382**) to the acid chloride (**379**).

Using the previously described protocol in which oil bath heating conditions were used coupled with dimethylformamide (**384**), it can clearly be seen that after 1.5 hours (Figure **60**, entry 1) the ^{1}H NMR spectrum still showed the presence of the hydrogen of the carboxylic acid (10.7 ppm), leading to evaluation of the next 2 periods: 3 and 5 hours. After 3 hours (Figure **60**, entry 2), the presence of the carboxylic acid *OH* was still present in a 1:3 ratio to the desired acid chloride (**379**) product. Analysis of the 5 hour aliquot showed complete conversion of the acid to the desired diacetoxyacetyl chloride (**379**) (Figure **60**, entry 3).

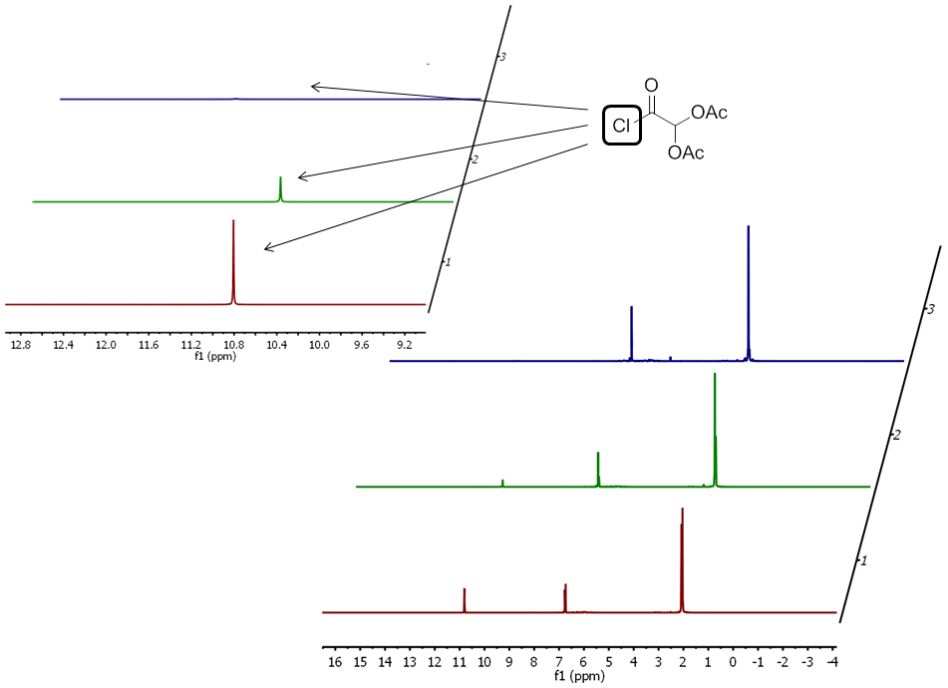


Figure 60 – ^{1}H NMRs of three time points collected during the synthesis of diacetoxyacetyl chloride: 1.5 hours (entry 1), 3 hours (entry 2) and 5 hours (entry 3).

Distillation of the crude reaction mixture led to the desired conversion of the starting material to the required diacetoxyacetyl chloride (**379**) in a much improved yield of 75% (Figure **61**). Allied to this yield increasing was the large amount of material (> 30.0 g) obtained, which was ideal for our planned route to the ETP core.

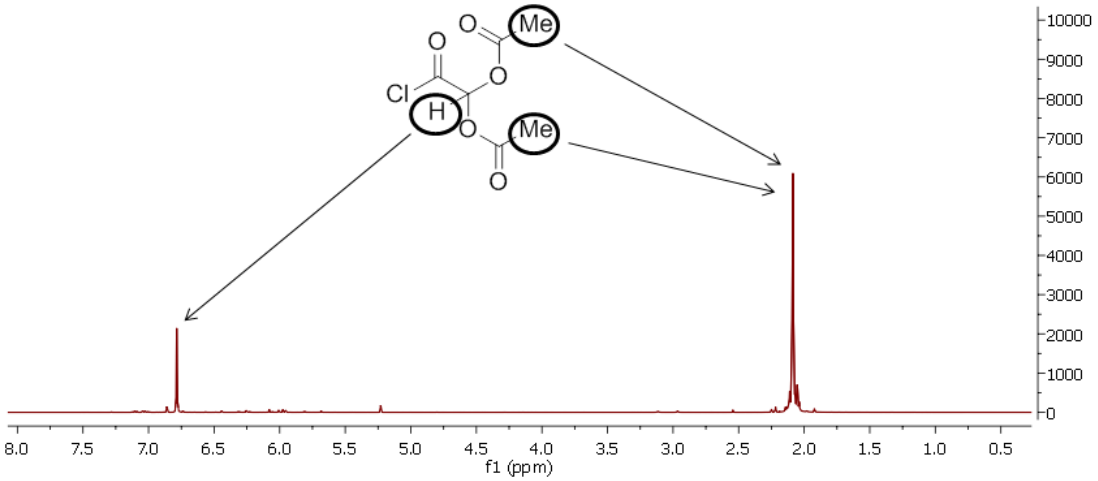
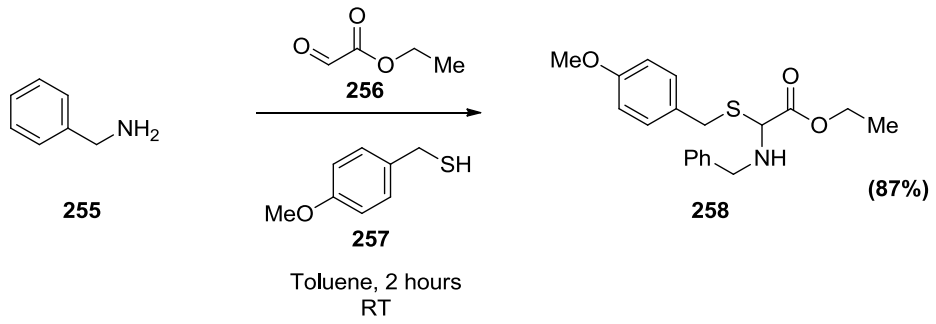


Figure 61 – ^{1}H NMR of diacetoxyacetyl chloride synthesis

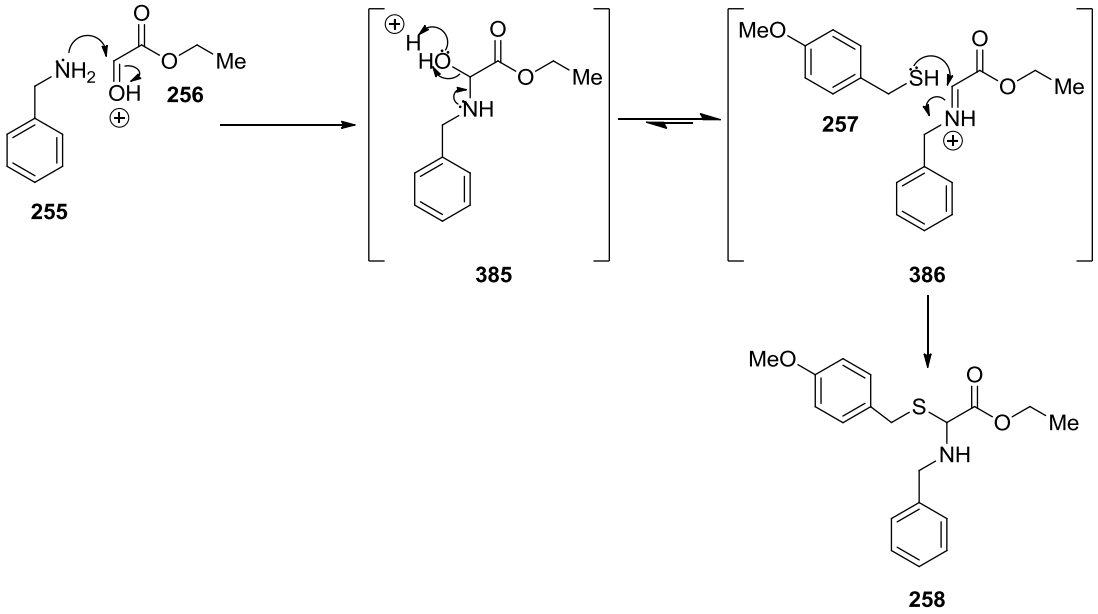
3.2 ETP diacetate formation

With the essential diacetoxyacetyl chloride (**379**) reagent available in multi-gramme amounts using our adapted methodology, we next moved to the synthesis of ETPs. The first step in the synthetic pathway is the production of the diacetate precursor (**259**) following the previous synthetic pathway developed by ourselves.[1] In order to synthesise diacetate precursor (**259**), benzylamine (**255**) was first reacted with ethyl glyoxalate (**256**) and *para*-methoxybenzyl mercaptan (**257**), in a three component reaction, to give amine (**258**) (Scheme **92**).



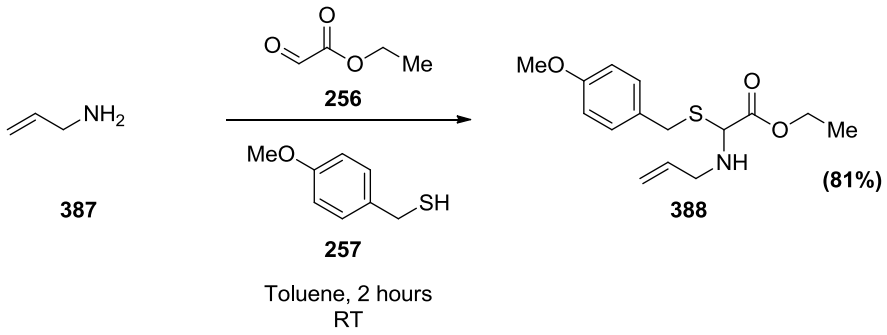
Scheme 92 – Ethyl-2-(benzylamino)-2-((4-methoxybenzyl)thio)acetate synthesis (1)

As shown below, this reaction proceeds with imine formation (**386**) and loss of water prior to addition of *para*-methoxybenzyl mercaptan (**257**) leading to the amine product (**258**) formation. Following this three-component reaction we obtained amine (**258**) as a colourless oil in 87% yield as well as > 7.00 g amounts (Scheme **93**).



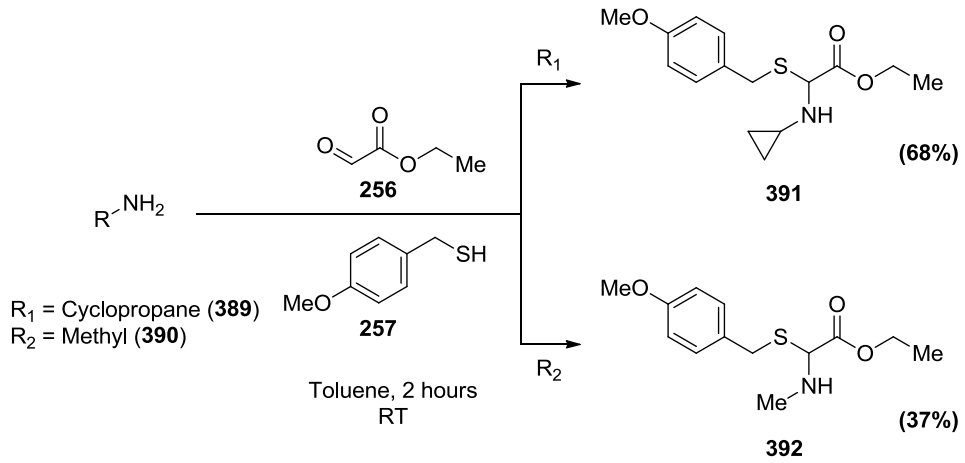
Scheme 93 – Ethyl-2-(benzylamino)-2-((4-methoxybenzyl)thio)acetate synthesis (2)

Analogues of diacetate precursor (**258**) were also synthesised by the use of a number of different amines in the reaction, clearly demonstrating the procedure's reproducibility. Using this same principle, we were able to synthesise allyl (**388**), cyclopropyl (**391**) and methyl (**392**) cyclisation precursors with the use of allylamine (**387**), cyclopropylamine (**389**) and methylamine (**390**) respectively. Hence, for example, reaction of ethyl glyoxalate (**256**) with allylamine (**387**) and *para*-methoxybenzyl mercaptan (**257**) gave the allyl substituted amine product (**388**) in a yield of 81% as a colourless oil (Scheme **94**).



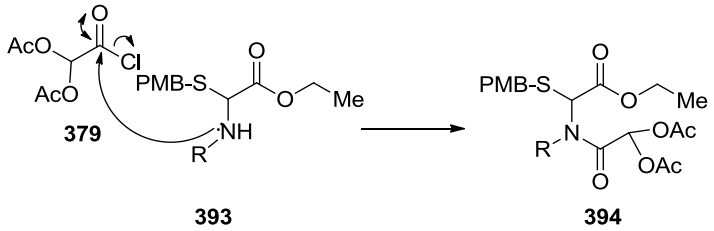
Scheme 94 – Ethyl-2-(allylamino)-2-(4-methoxybenzylthio)acetate synthesis

The same type three component reaction using cyclopropylamine (**389**) and methylamine (**390**) gave the desired cyclopropane and methyl substituted amine products (**391**) and (**392**), as colourless oils in 68% and 37% yield, respectively (Scheme **95**). We attributed the lower yield for the synthesis of intermediate (**392**) to both volatility of methylamine and the form by which this reagent is supplied as a tetrahydrofuran solution making water removal more difficult.



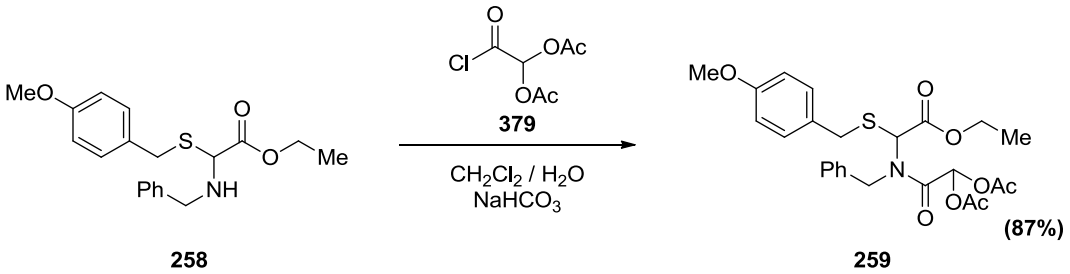
Scheme 95 – Synthesis of different diacetate precursors

With amines (**258**), (**388**), (**391**) and (**292**) in hand, we next explored the formation of the corresponding diacetate cyclisation precursors (Scheme **96**) using our previously synthesised diacetoxyacetyl chloride (**379**).



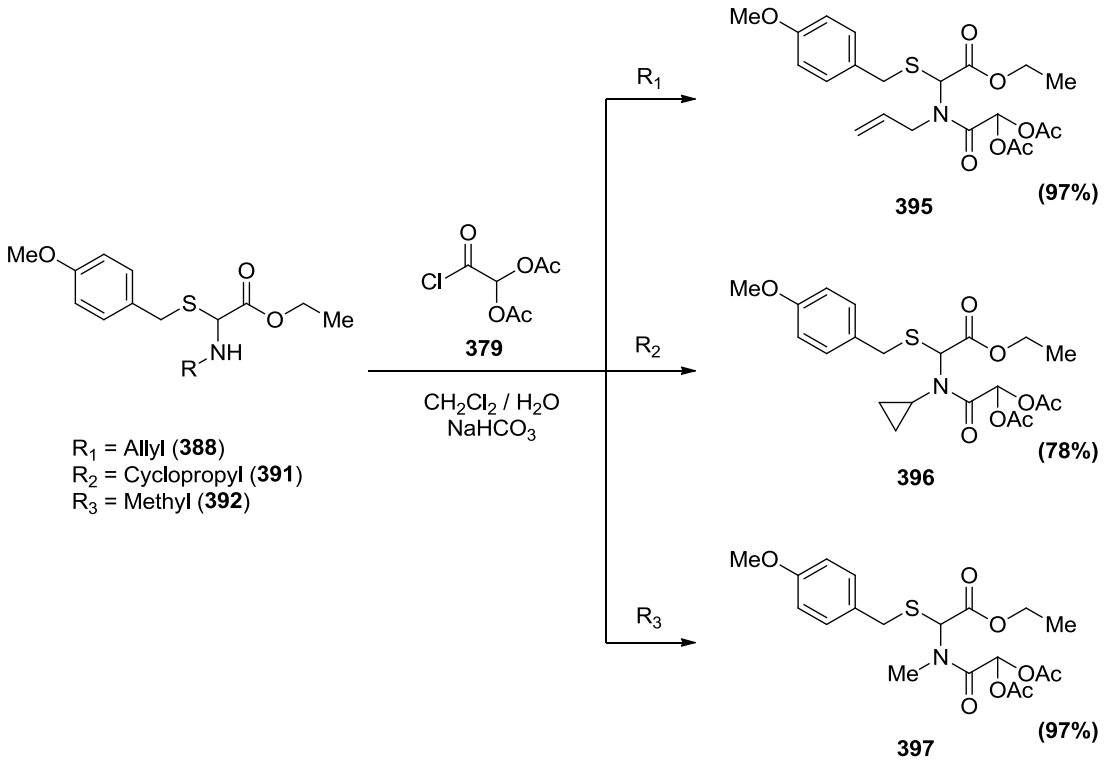
Scheme 96 – Molecular mechanism for diacetate formation using diacetoxyacetyl chloride

Amine (**258**) was reacted with diacetoxyacetyl chloride (**379**) under Schotten-Baümann conditions to give the diacetate cyclisation precursor (**259**) in 95% yield (Scheme **97**).



Scheme 97 – 2-(Benzyl(2-ethoxy-1-(4-methoxybenzylthio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate synthesis

TLC analysis of the reaction mixture showed the presence of only the desired product (**259**) in amounts over 10.0 g with complete consumption of the starting amine (**258**). Analysis of the crude ^{1}H NMR spectrum indicated that purification by flash column chromatography was not required, showing that this was a very efficient and clean reaction. Similarly, allyl (**388**), cyclopropyl (**391**) and methyl (**392**) containing amines were reacted in an analogous manner to provide cyclisation precursors (**395**), (**396**) and (**397**) in 97%, 78% and 97% yield, respectively (Scheme **98**).

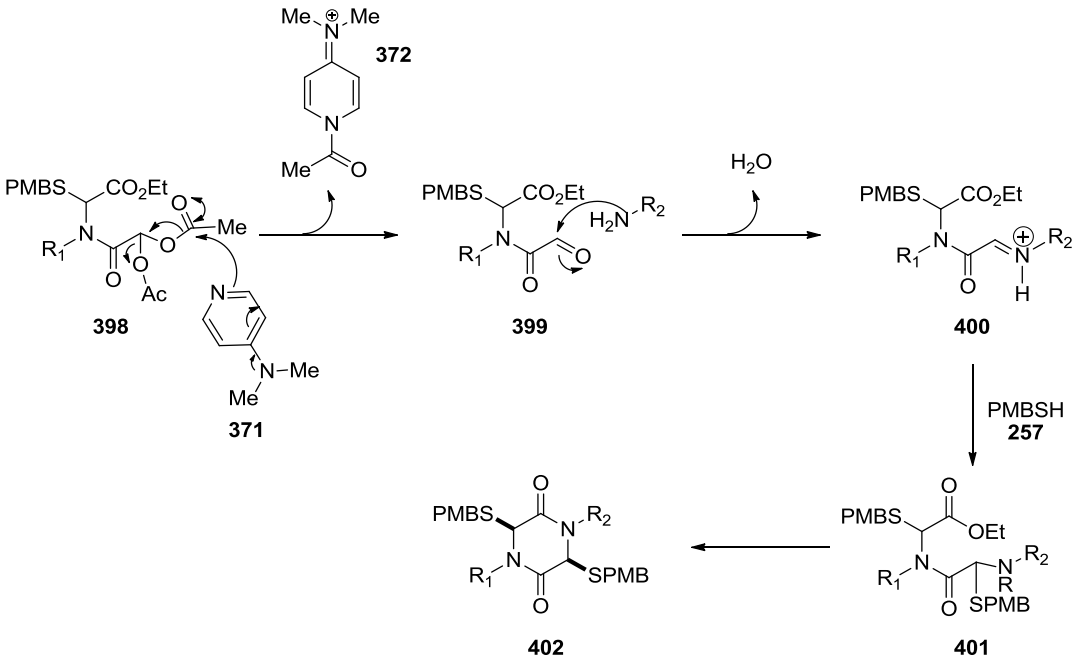


Scheme 98 – Synthesis of different diacetates

3.2.1 Diacetate cyclisation – Base catalyse synthesis

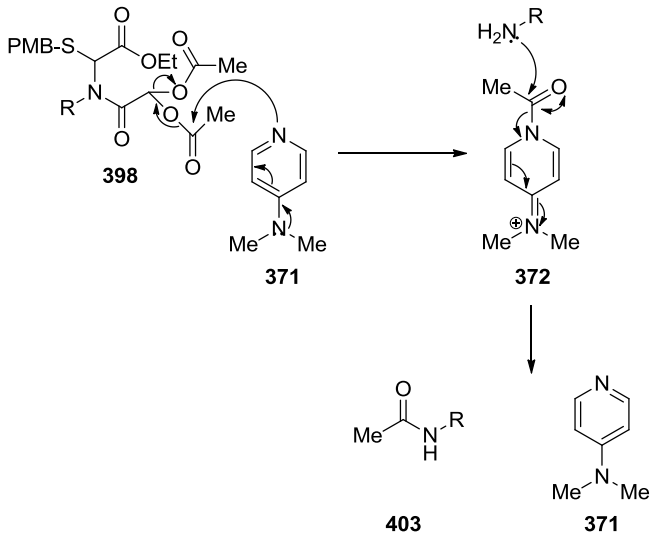
According to previous work developed by ourselves, cyclisation of the diacetate precursor to the protected ETP core involved the use of an acid catalyst (TFA) which has a number of drawbacks associated with its use. TFA is a strong acid that is unpleasant to use and incompatible with some functional groups such as esters and protected amines. Therefore we looked at the ability of a base (DMAP) to catalyse the cyclisation of the diacetate (**398**) to the protected dithiodiketopiperazine for the development of a new synthetic strategy for ETP production.

DMAP (**371**) is a very good acylating agent for activation of anhydrides and carbamates and for group transfer reactions. For our ETP synthesis, we anticipated that DMAP could act by forming reactive acylating groups. We envisioned that it could unmask the aldehyde (**399**) by nucleophilic attack at the carbonyl group of one of the acetate groups of the diacetate (**398**), resulting in concommitant removal of the second acetate group and formation of the aldehyde. This would facilitate the formation of a site for the nucleophilic amine to attack and lead to formation of an iminium ion (**400**) where the nucleophilic sulfur of *para*-methoxybenzyl mercaptan can react, followed by cyclisation to produce the ETP precursor (**402**) (Scheme **99**).



Scheme 99 – Synthesis of disubstituted protected ETPs using DMAP

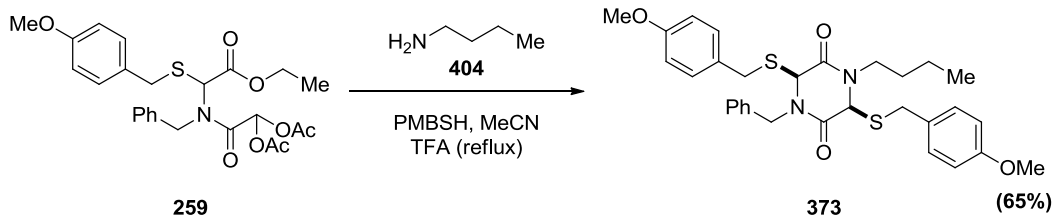
This reaction clearly required at least two equivalents of amine since after deprotection of the diacetate (**398**), DMAP (**371**) formed an inactive acetylpyridinium cation (**372**) due to donation of the acyl group from the diacetate (**398**) to DMAP, therefore disabling its purpose as a catalyst for the reaction. However, reaction of the acetylpyridinium cation (**372**) with another amine molecule would regenerate the active form of DMAP as a catalyst. DMAP could then undergo another reaction with the generation of a new amide by-product (**403**). For this reason, we first decided to use 0.5 equivalents of DMAP to catalyse the reaction (Scheme **100**).



Scheme 100 – By-products of DMAP catalysed cyclisation (1)

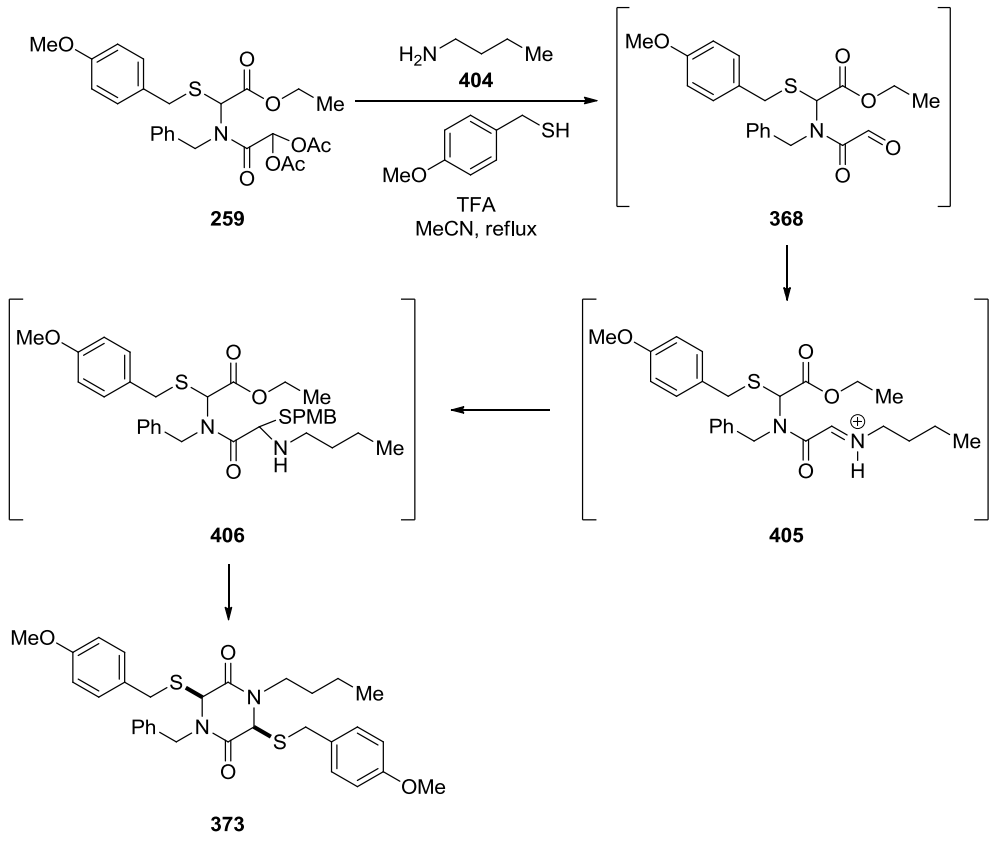
However, in order to determine the requirements for optimisation of our already published TFA mediated cyclisation procedure, we first needed to repeat classical reaction conditions (using acid catalysis) to provide a reference point for further investigations.

Therefore, diacetate (**259**) was reacted with 1.5 equivalents of PMBSH, 3 equivalents of *n*-butylamine (**404**) and 1 equivalent of TFA in acetonitrile and the resulting mixture heated at 51 °C reflux for 16 hours (Scheme **101**).



Scheme 101 – (3S,6S)-1-benzyl-4-butyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione synthesis (1)

TFA was used to catalyse the formation of the ETP precursor (**260**) by the formation of the aldehyde intermediate (**368**) which after nucleophilic attack by the amine resulted in the reactive iminium cation (**405**) (Scheme **102**).



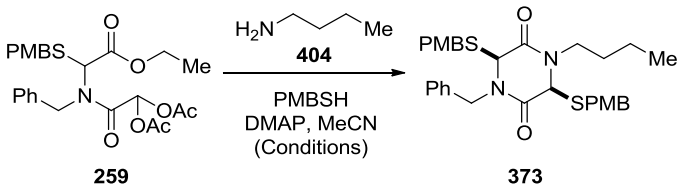
Scheme 102 – (3S,6S)-1-benzyl-4-butyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione synthesis (2)

Following completion of the reaction, analysis of the crude NMR spectrum indicated complete conversion to the *cis* protected ETP following heating of the reaction overnight at reflux. Purification by flash column chromatography gave (**273**) as a colourless solid in 65% yield.

With the pure *cis* protected compound in hand using TFA as a catalyst for the reaction, we next directed our attention towards optimising the procedure. For this purpose, we elected to use a base catalysed methodology combined with microwave assisted synthesis to try to reduce reactions times and increase product formation.

3.2.2 Optimisation of Hilton's diacetate cyclisation conditions

In order to promote the cyclisation of the diacetate using the new developed conditions, a series of reactions were carried out towards the synthesis of the *n*-butyl ETP precursor (**373**). The results from our studies are shown below (Table **1**).



Method	Entry	Temperature (°C)	Time	Yield (%)
Reflux	1	51	16h	81
Microwave	2	130	15min	48
	3	130	20min	50
	4	130	30min	47
	5	160	30min	23

Table 1 – Conditions for (3S,6S)-1-benzyl-4-butyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione synthesis

Conventional reaction methodology using DMAP as a catalyst with stirring for 16 hours under reflux conditions (entry 1, Table **1**) gave the best yield for the reaction, with 81% of a *cis/trans* mixture of the product.

The next step involved the use of a microwave reactor to accelerate the reaction. A Biotage™ microwave reactor was used in an attempt to accelerate the reaction rate so to reduce the time the synthesis took. Uniform heating as well as efficient heat transfer can

be obtained by this method leading to high reproducibility of results with both pressure and temperature control. In addition, the use of microwave heating allows experimental simplification and rapid optimisation of reaction conditions.[280] Heating at 160 °C for 30 minutes using the microwave (entry 5, Table **1**) resulted in a reduced yield of 23% whilst heating at 130 °C for 20 minutes produced the best outcome of a 50% yield (entry 3, Table **1**).

The results clearly showed a greater yield was obtained using the conventional reflux method when compared to microwave assisted reactions. The optimum conditions for microwave usage were obtained for the formation of the *n*-butyl ETP precursor (**373**) using DMAP after 15 minutes pre-stirring and heating at 130 °C for 20 minutes. Possibly this was due to product degradation as a result of the increased reaction temperature. However, DMAP proved to be an effective catalyst for deprotection of diacetate (**259**) and formation of the ETP core using conventional heating.

To determine the most advantageous reaction conditions for the synthesis of the protected ETP, *cis/trans* ratios of the reactions' final products, using either TFA or DMAP as catalysts, were analysed by HPLC. HPLC is a useful analytical tool, providing rapid analysis and quantitative data directly from aliquots of the reaction mixture. However, after much analysis and effort we abandoned this method for determination of the selectivity of the reaction due to poor separation of the *cis*/*trans*-isomers as shown below (Figure **62**).

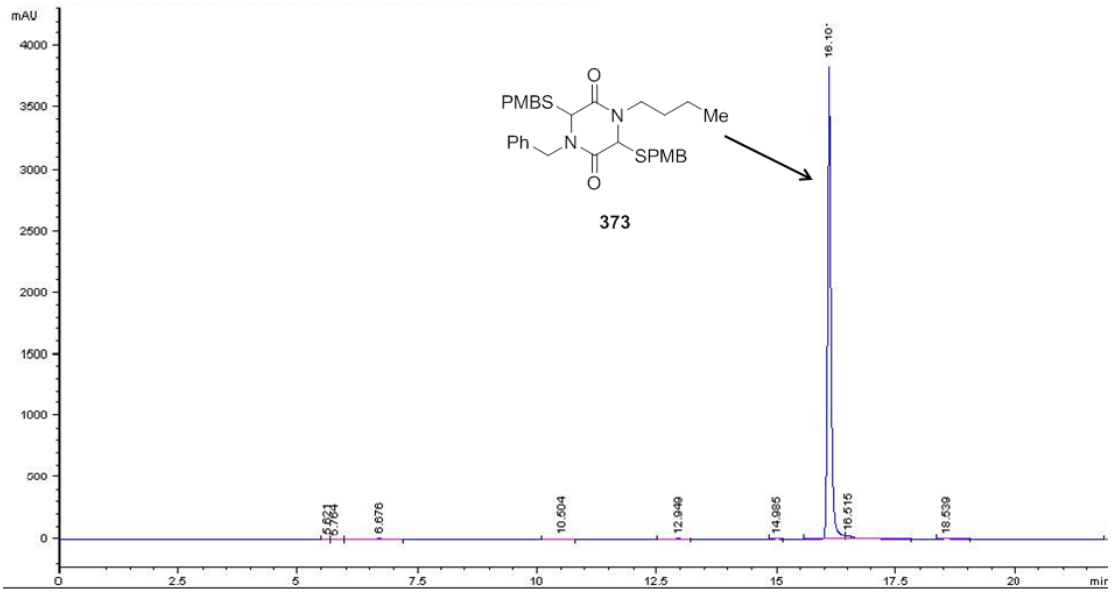


Figure 62 – Separation of (3,6)-1-benzyl-4-butyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione isomers using HPLC

Although the HPLC chromatograph clearly showed two peaks, one representing the *cis* and the other the *trans* compound, these peaks overlapped and separation of the isomers proved to be too difficult due to the close similarity in both physical and chemical properties. TLC analysis was also used to study these reactions and to monitor their progress. The TLC showed reduction in the amount of starting material and an increase in the synthesis of the diketopiperazine product with increased heating time. Therefore we moved to ^{1}H NMR spectrum analysis to follow the reaction and to determine the most favourable conditions using microwave assisted ETP synthesis, when compared to conventional synthesis (Figure **63**).

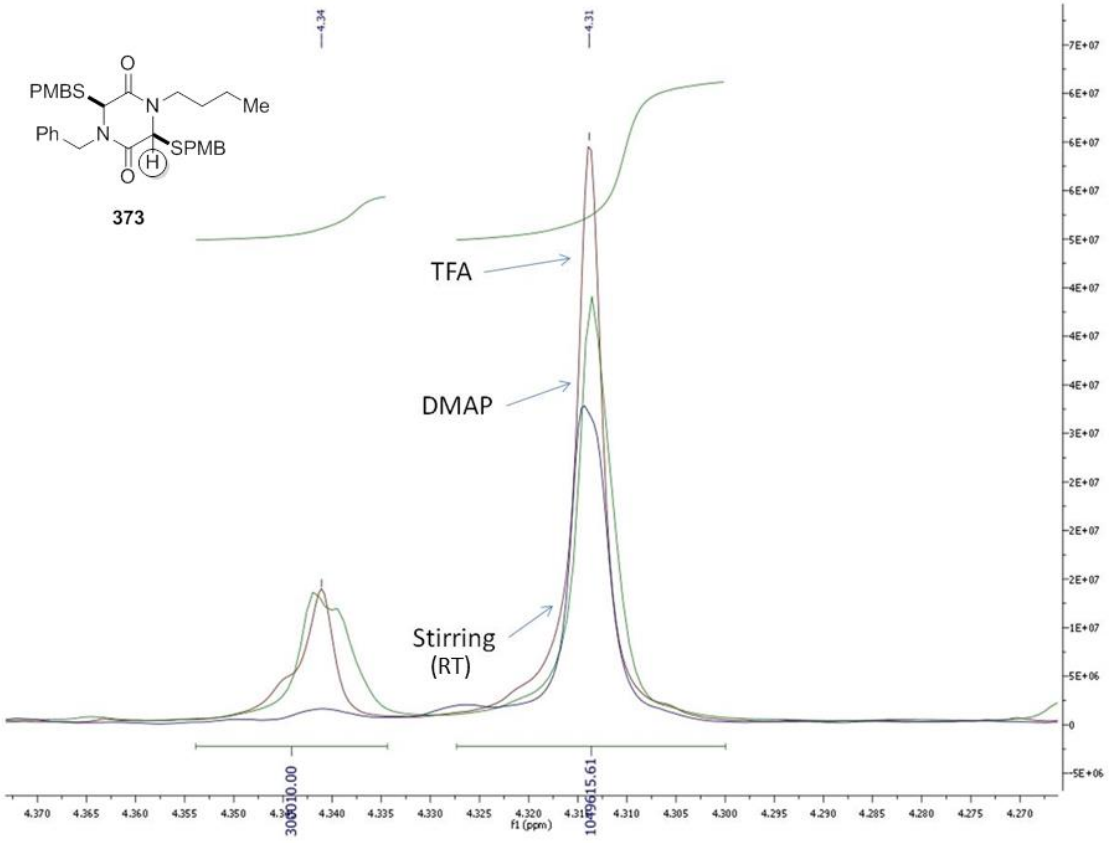
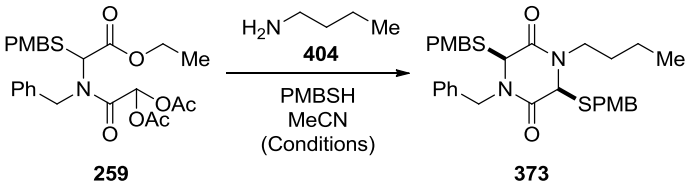


Figure 63 – ^{1}H NMR ratio of the isomeric forms of (3,6)-1-benzyl-4-butyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione using different catalysts for diacetate cyclisation

The ^{1}H NMR spectrum of the product clearly showed a *cis/trans* ratio of 94:6 for TFA catalysed reaction (entry 4, Table **2**) against 57:43 when DMAP was used (entry 3, Table **2**). From these results we found that in the presence of TFA, there was virtually complete conversion to the *cis n*-butyl compound at 130 °C after 30 minutes indicating that these are the optimum conditions for its synthesis. This may be due to the presence of protons from TFA, which results in the conversion of the *trans* to *cis* isomer which is more thermodynamically stable. By using the Biotage™ microwave, the reaction rate

was accelerated resulting in more rapid conversion to the *cis*-butyl ETP precursor in reasonable yield.

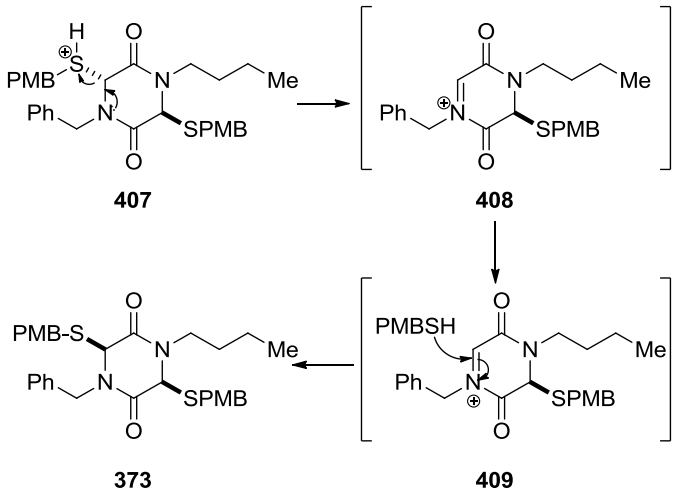


259 **373**

Method	Entry	Temperature (°C)	Time (hours)	Catalyst	Ratio (*cis/trans*)
Conventional	1	51	16	DMAP	N/D
	2	51	16	TFA	N/D
Microwave	3	130	0.5	DMAP	57/43
	4	130	0.5	TFA	94/6

Table 2 – Ratio of the isomeric forms of (3,6)-1-benzyl-4-butyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione using different catalysts for diacetate cyclisation

It is known that this reaction initially occurs under kinetic control resulting in the most easily formed but least stable product.[1] As it proceeds, the most stable thermodynamic isomer is formed as the major product. The most thermodynamically stable stereoisomer is the *cis*-ETP precursor.[1] However, the *cis/trans* stereoisomers can only be converted to one another by the process of breaking and reforming of the carbon sulfur bond. TFA facilitates this conversion of the *trans* to *cis* compound apparently by two reasons: the loss of the mercaptan group from the ETP precursor (**407**) resulting in the formation of acyliminium cation (**408**) or by the inference of the thiocarbenium ion and cleavage of the diketopiperazine ring as proposed by Williams.[157] Re-addition of nucleophilic sulfur to the intermediate (**408**) results in formation of the *cis* compound (**373**) (Scheme **103**).[1]



Scheme 103 – (3S,6S)-1-benzyl-4-butyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione synthesis using an acid catalysed reaction

However, this conversion from the *trans* to *cis* isomer requires the presence of protons which cannot be provided by DMAP. This may explain the lower yield compared to its analogous catalyst TFA. Nevertheless, a good yield was still achieved for *cis*-diketopiperazines synthesis with the use of DMAP with purification being achievable by recrystallisation of the *cis/trans* mixture. This method is therefore useful for the synthesis of compounds that contain acid sensitive functional groups.

3.3 Monomeric ETP formation

The next phase of the research project involved determining the optimum reaction conditions for the introduction of a number of amines into the ETP core. In order to synthesise these protected ETP precursors, changes were made in three areas to obtain the desired products:

- Variation of the diacetate precursor.
- Catalyst selection (TFA and DMAP).
- Use of reflux and microwave heating conditions.

Combinations of the above parameters facilitated the synthesis of a small library of monomeric compounds as described below.

3.3.1 Monomeric disubstituted ETPs

The first series used diacetate (**259**) to give the following protected ETPs (Table **3**). It was noticeable that the use of reflux conditions to achieve protected ETP formation surpasses the use of microwave accelerated reactions. Only compound (**416**) gave a better reaction yield when microwave conditions were used. Base catalysed reactions in the example of protect 3-chlorobenzylamine substituted ETP (**410**) clearly highlighted the utility of this alternative method, since yields for both conditions (i. e. reflux and microwave) showed an increase in product formation. When comparing product formation under reflux conditions, compound (**410**) showed a 15% yield increase when a base catalyst was used compared to the analogous acid catalysed reaction. The same type of correlation was obtained using microwave accelerated conditions with a 17% difference in yields between both base and acid catalysed reactions.

The production of compounds (**411**), (**412**), (**413**) and (**414**) demonstrated the importance of DMAP as an alternative catalyst for the cyclisation of ETP intermediates. Using this approach, we were able to introduce different carbon length ester groups in the ETP core avoiding acid hydrolysis. Reaction outcomes reflected the results and yields obtained with the use of reflux conditions clearly surpassing microwave usage. The optimum reaction conditions for ethyl 4-aminobutanoate (**411**) and aminocaproic methyl ester (**412**) protected ETP derivatives were to use reflux methodology which gave better yields in both cases.

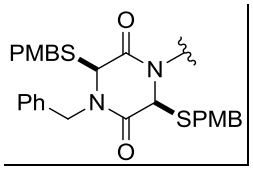
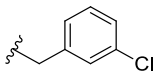
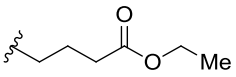
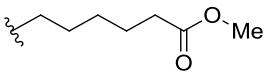
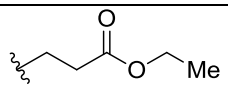
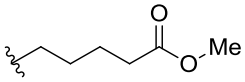
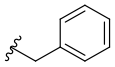
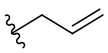
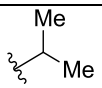
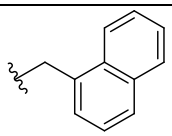
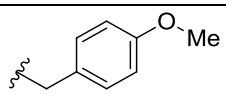
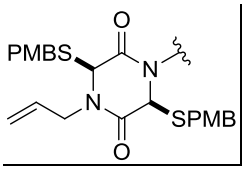
 R Group	Compound	Catalyst	Conditions		Yield (%)
			Temperature (°C)	Time	
	**410**	TFA	51 (Reflux)	16h	60
		DMAP	51 (Reflux)	16h	75
		TFA	150 (MW)	5min	51
		DMAP	150 (MW)	5min	68
	**411**	DMAP	51 (Reflux)	16h	70
		DMAP	130 (MW)	35min	28
	**412**	DMAP	51 (Reflux)	16h	76
		DMAP	130 (MW)	25min	46
	**413**	DMAP	160 (MW)	16h	61
	**414**	DMAP	51 (Reflux)	16h	78
	**415**	TFA	51 (Reflux)	16h	64
		TFA	150 (MW)	5min	60
	**416**	TFA	51 (Reflux)	16h	54
		DMAP	51 (Reflux)	16h	53
		DMAP	140 (MW)	30min	76
	**417**	TFA	51 (Reflux)	16h	64
	**418**	TFA	51 (Reflux)	16h	33
	**419**	TFA	51 (Reflux)	16h	58
	**420**	TFA	51 (Reflux)	16h	84

Table 3 – Conditions for the synthesis of different protected disubstituted ETPs (1)

Diacetate (**395**) was also used to produce different protected ETP intermediates (Table **4**). These two protected ETP examples share similar conditions as previously described

(*i. e.* reflux and microwave). However, unlike the previous examples, the best yield achieved in the synthesis of the allyl protected ETP precursor (**421**) was attained using microwave conditions. This clearly correlates with the previous result reported for this substituent to its *N*-benzyl analogue (**416**) which differed from the other results with an increased yield when compared to similar synthetic compounds using reflux conditions.

Using the same base catalyse reaction methodology, benzyl protected ETP precursor (**416**) was synthesised under reflux conditions with a 72% yield.



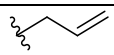
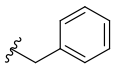
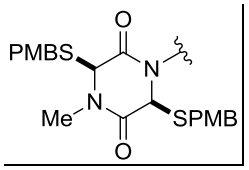
R Group	Compound	Catalyst	Conditions		Yield (%)
			Temperature (°C)	Time	
	**421**	DMAP	140 (MW)	30min	72
	**416**	TFA	51 (Reflux)	16h	80
		DMAP	51 (Reflux)	16h	72

Table 4 – Conditions for the synthesis of different protected disubstituted ETPs (2)

Diacetate (**397**) containing the *N*-methyl substituent enabled the synthesis of compound (**423**) (Table **5**). The use of our approach to achieve cyclisation of the bis-*N*-methyl protected ETP (**423**) via acid catalysis with reflux conditions was the preferred method to attain the highest reaction yield. Dean and Stark conditions were also used for the synthesis of intermediate (**423**) as before for compound (**392**) to facilitate water removal from the reaction.

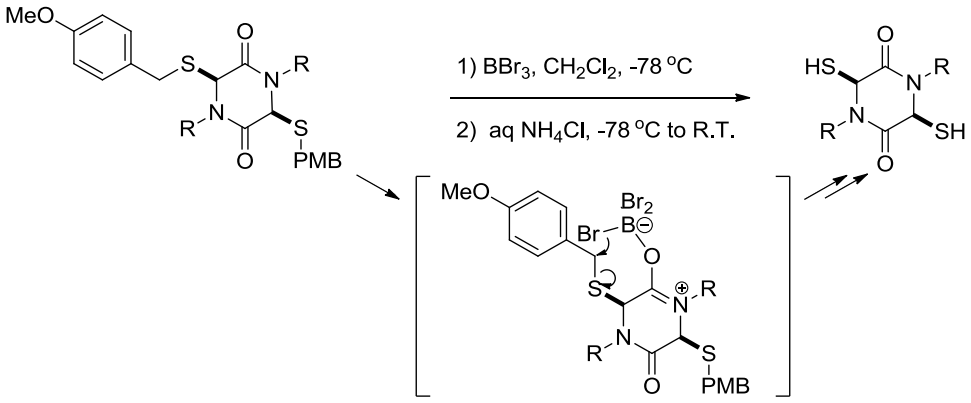


R Group	Compound	Catalyst	Conditions		Yield (%)
			Temperature (°C)	Time	
	**423**	TFA	51 (Reflux)	24h	62

Table 5 – Conditions for the synthesis of different protected disubstituted ETPs (3)

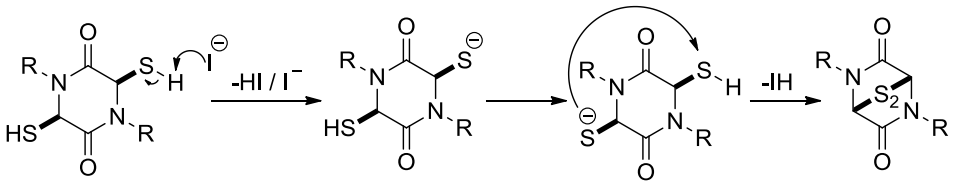
Following the obtention of the ETP core, the last step for ETP synthesis was formation of the disulfide bridge. Conversion to the ETP core was achieved by the use of neat boron tribromide which as a Lewis acid, accepting a pair of electrons from the oxygen atom from the diketopiperazine core, which results in the loss of the PMB protecting

group from the ETP precursor via nucleophilic attack of the bromide anion as outlined below (Scheme **104**).



Scheme 104 – Molecular mechanism of ETP intermediate sulfur deprotection

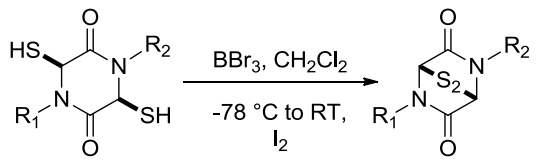
Protonation of the sulfur anion forming the dithiol intermediate followed by the addition of iodine, resulted in the concomminat oxidation and formation of the disulfide bridge (Scheme **105**).[217]



Scheme 105 – Molecular mechanism of ETP dissulfide bridge formation

As part of the work-up procedure, sodium thiosulfate was added to the mixture to quench any excess iodine that might be present. Consequently, this reaction results in a one pot deprotection and oxidation of the ETP precursor leading to the final compound containing the disulfide bridge.

However, variable yields were reported in the formation of the disulfide bridge showing that this reaction clearly requires optimisation regarding the number of steps involved in this transformation. We therefore decided to use different conditions during the reaction procedure to overcome previous difficulties encountered. In the dithiol deprotection step, changes were made by the use of either sodium hydrogen carbonate or hydrochloric acid instead of ammonium chloride as previously described by Hilton.[1] However, the use of iodine was maintained for the oxidation step. Compounds that were synthesised along with appropriate optimised conditions are shown below (Table **6**).



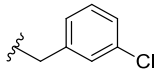
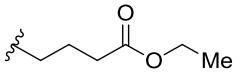
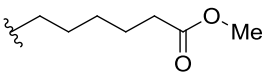
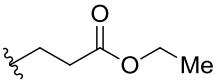
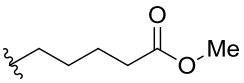
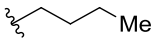
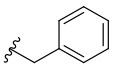
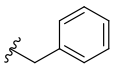
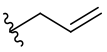
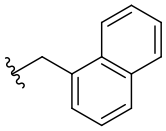
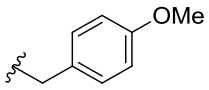
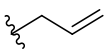
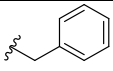
R_1 Group	R_2 Group	Compound	H^+ Source	Yield (%)
		**424**	NH_4Cl	32
		**425**	$NaHCO_3$	64
		**426**	$NaHCO_3$	75
		**427**	$NaHCO_3$	51
		**428**	$NaHCO_3$	29
		**429**	HCl	54
		**430**	NH_4Cl	85
		**431**	NH_4Cl	60
		**432**	HCl	65
		**433**	NH_4Cl	65
		**434**	NH_4Cl	85
		**435**	NH_4Cl	48
		**436**	NH_4Cl	67
		**437**	NH_4Cl	68

Table 6 – Conditions for the ETPs dissulfide bridge formation

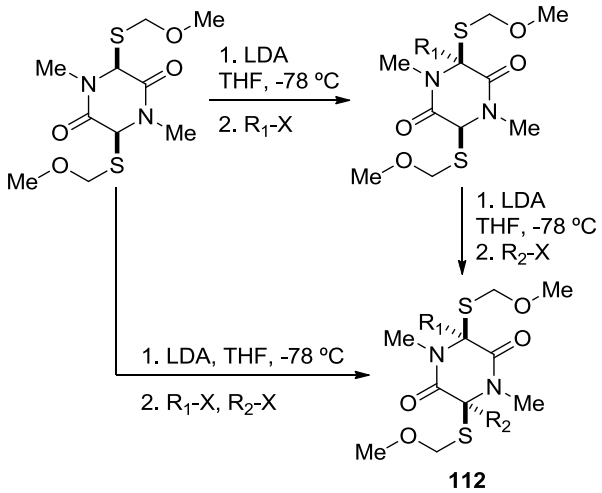
The yields obtained from the deprotection / oxidation step leading to the desired ETPs vary between the substituted ETP examples that were used. Despite the variations, only three examples gave final yields below 50%: 3-chlorobenzyl (**424**), valeric methyl ester

(**428**) and *para*-methoxybenzyl substituted (**435**) ETPs. All other compounds gave yields that were consistently above 50% when the optimised conditions were used.

3.3.2 Monomeric tri-substituted ETPs

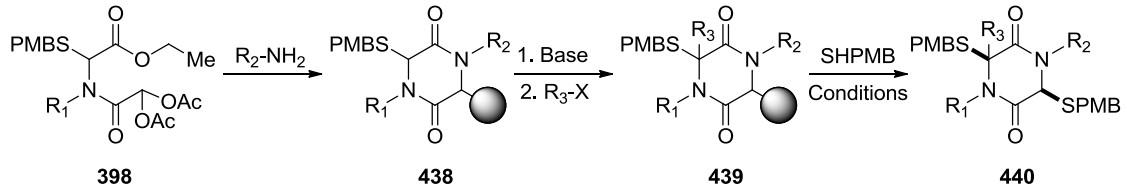
In order to extend the diversity of monomeric compounds developed, we decided to instigate the production of tri-substituted ETPs. With this objective in hand, a new approach for our diacetate precursor was developed.

The idea behind the need to synthesise tri-functionalised ETPs arose from the need to produce a wider and more diversified library of ETP compounds than had previously been achievable with our methodology. Solubility of the final monomeric ETP molecules was another argument that compelled this work. This is a key molecular property that is crucial for both biological testing and drug formulation. The solution that we expected to overcome solubility difficulties rested on functionalising the available *3*- and *6*- positions that surround the ETP core. Hence, deprotonation / alkylation of the carbons adjacent to the sulfur atoms would give the desired compounds. This approach previously used by Kishi encountered regioselectivity problems, since both hydrogens present in the *3*- and *6*- positions of the diketopiperazine ring could easily be deprotonated in the same reaction step (Scheme **106**).[179,182] The equal acidity of both these hydrogens coupled with molecular symmetry directed the alkylation of both positions in a nonspecific manner. The use of different alkylating groups with dissimilar reactivity also led to mixtures of tri and tetra-substituted ETP precursors.[182]



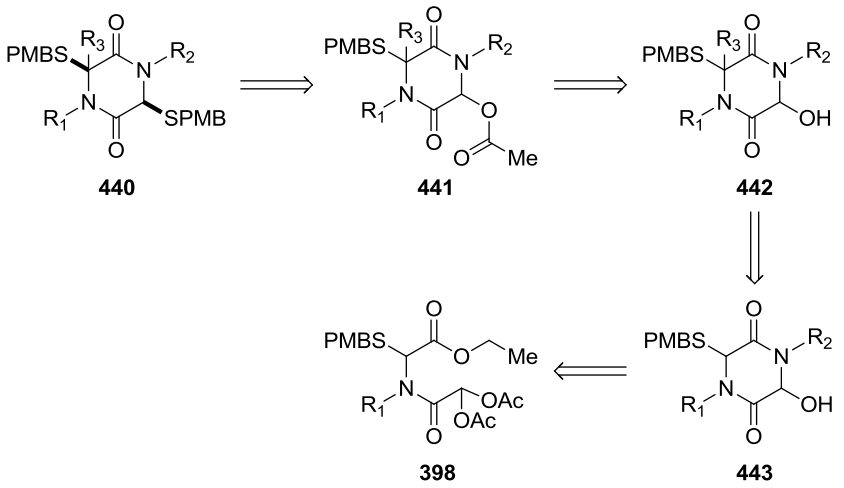
Scheme 106 – Kishi's trisubstituted ETP synthesis

To overcome this, we decided to design a new cyclisation strategy for our diacetate with a different and easy exchangeable group introduced instead of the protected thiol as reported previously, leading to selective deprotonation / alkylation on the nonadjacent carbon. Alkylation should be more specific using this approach with different acidities being displayed by hydrogens in both the *3*- and *6*- positions. Following this, the new substitutent groups should be readily converted into the *para*-methoxybenzyl mercaptan group, leading to the desired protected ETP (Scheme **107**).



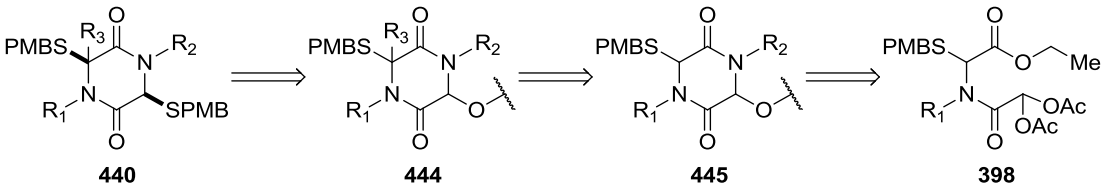
Scheme 107 – Protected trisubstituted ETP synthesis

As described previously, the first step involved modifying our diacetate cyclisation procedure, by introducing a different substituent on the *3*- position of the ETP precursor core to allow selective mono- alkylation. For this, different approaches were envisaged (Scheme **108**) where cyclisation of our diacetate (**398**) would be altered by using one of the reactions by-products – water, as a nucleophile. This would give rise to a hydroxy intermediate (**443**) with differing acidities on both the *3*- and *6*- positions hydrogens of the diketopiperazine ring. Since the hydrogen closer to the sulfur protected substituent would be more readily removable, the synthesis of such an ETP precursor would enable alkylation on the *6*- position of the diketopiperazine ring. However, substitution of the hydroxy group for PMBSH could prove to be extremely difficult as a strong acidic environment would be needed. We then intended to convert this newly tri-substituted intermediate (**442**) with acetic anhydride to form the correspondent acetate (**441**). Subsequent acid treatment followed by reaction with PMBSH would render the desired protected ETP core (**440**).



Scheme 108 – Retrosynthetic approach for the synthesis of protected trisubstituted ETPs (1)

Although this new methodology could provide good overall results, it would also increase the number of steps. We therefore first decided to investigate the use of different nucleophiles for the initial diacetate cyclisation step. The idea was based on avoiding extra unnecessary steps which would reduce the efficiency of the synthetic pathway and therefore the overall yield. One of the reported steps which involved acetate (**441**) formation on diketopiperazine *6*- position could be avoided by introducing a simpler removable group in the initial part of the synthesis (Scheme **109**). Thus, the use of different reaction solvents that could also act as nucleophiles and change the reactivity of the two equidistant hydrogens were used for our diacetate cyclisation process.



Scheme 109 – Retrosynthetic approach for the synthesis of protected trisubstituted ETPs (2)

Experiments were carried out with a number of different alcohols as solvents. Imine formation followed and nucleophilic attack by the new substitutent group allowed for the formation of the new monothiol-protected products. As shown below (Figure **64**), three compounds were synthesised by the use of methanol (Entry 1), ethanol (Entry 2) and isopropanol (Entry 3) as reaction solvents. All new compounds shared ^{1}H NMR spectrum similarities with the dibenzyl protected ETP precursor (**415**) since the methyl group belonging to the *para*-methoxybenzyl mercaptan substituent was present in the

3.80ppm region. However, different shifts due to the introduction of dissimilar substituents could be seen in between the 1.05 and 1.30ppm region.

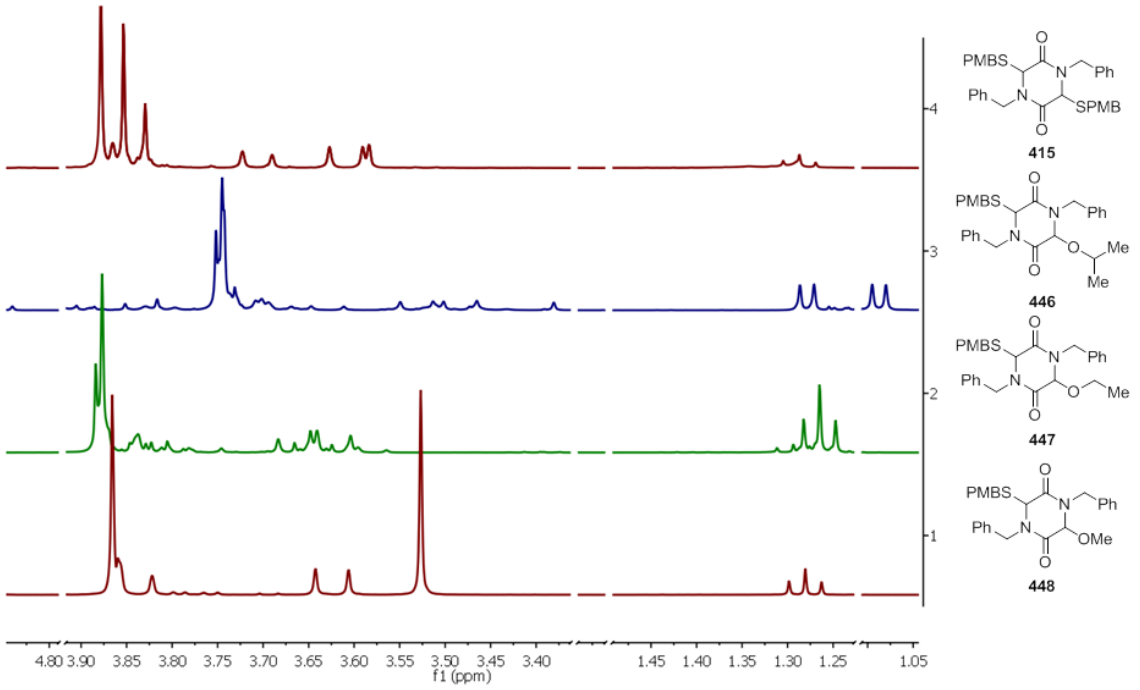


Figure 64 – ^{1}H NMR showing diacetate cyclisation using different solvents: methanol (entry 1), ethanol (entry 2), isopropanol (entry 3) and acetonitrile with *para*-methoxybenzyl mercaptan (entry 4)

Though *tert*-butanol was also used as a nucleophile for the described reaction, no molecule that contained a *tert*-butoxy group was found. However, NMR analysis (Figure **65**) showed the presence of a hydroxy product (**450**) as already described. This may have occurred due to the weak nucleophilicity of *tert*-butanol allowed the reaction of another nucleophile present in the reaction – water. Since one molecule of water is generated after the protonation of the aldehyde intermediate and with poor solubility of the reactions solvent, a nucleophilic attack by the formed water molecule might be the reason for the synthesis of this compound (**450**).

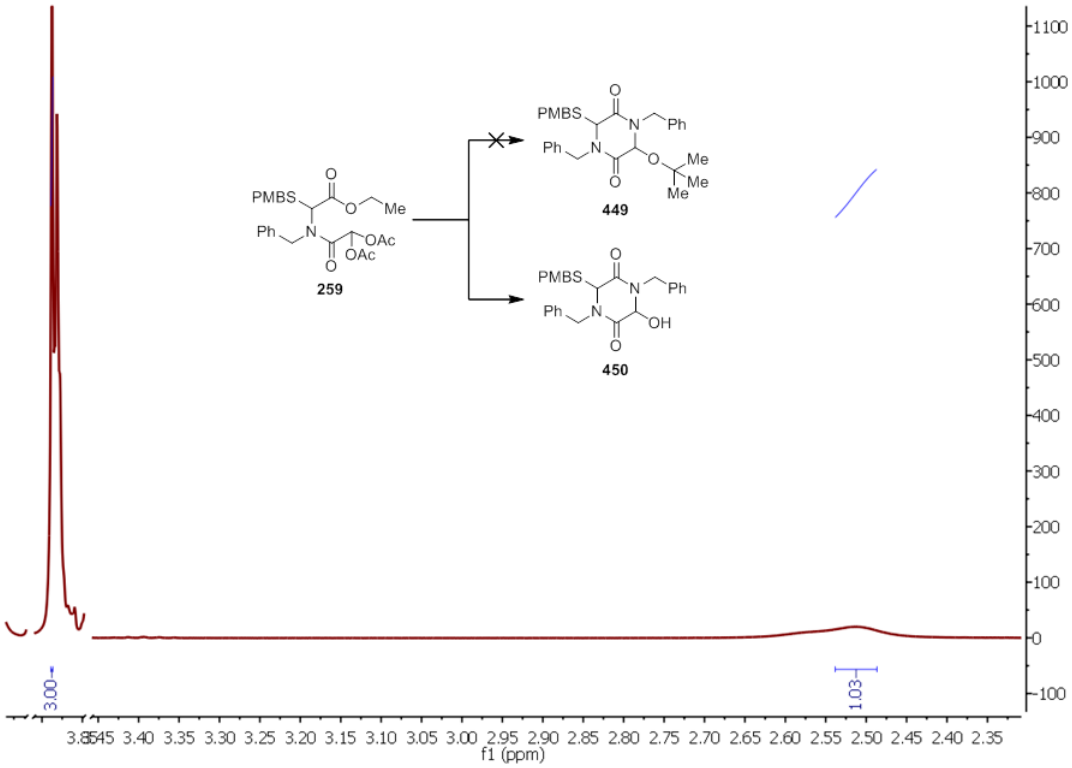
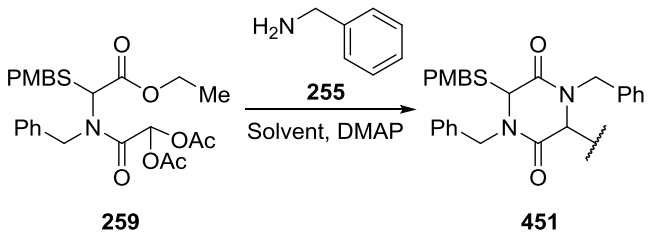


Figure 65 – ^{1}H NMR of diacetate cyclisatio using *tert*-butanol

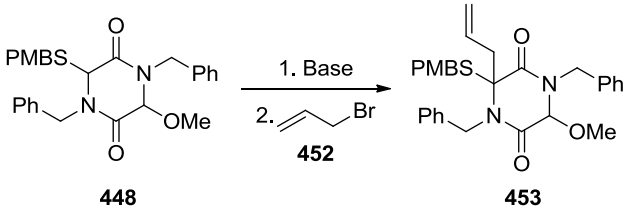
After purification and analysis of the reaction yields, we decided to take the simplest product which was the methoxy intermediate (**448**) since it gave consistently higher cyclisation yields than other precursors (i. e. ethoxyde, isopropyl and hydroxide derivatives) as shown below (Table **7**).



Substituent	Solvent	Compound	Yield (%)
OMe	Methanol	**448**	79
O Me	Ethanol	**447**	74
Me O Me	*iso*-Propanol	**446**	60
OH	*tert*-Butanol	**450**	59

Table 7 – Diacetate cyclisation using different solvents

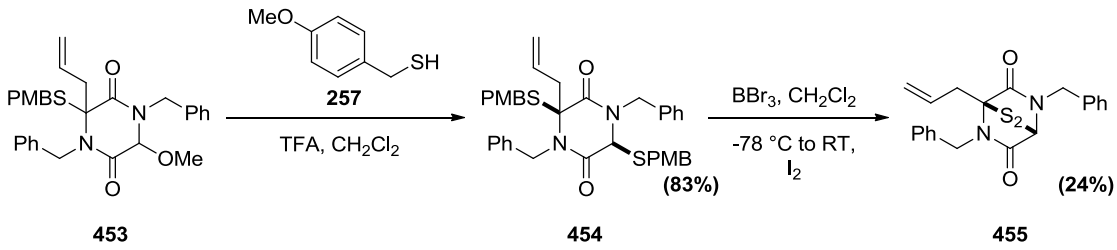
Deprotonation of (**448**) was attempted using a number of basic conditions (Table **8**) for the introduction of the vinyl halide.[210] From these, lithium bis(trimethylsilyl)amide (LiHMDS) (Entry 1) was the base of choice to proceed with the synthetic pathway with a 79% yield. Other bases included lithium diisopropylamide (Entry 2), sodium hydride (Entry 3) and two different forms of butyllithium (Entries 4, 5) but all gave lower yields of alkylated product (**453**).



Base	Entry	Yield (%)
LiHMDS	1	79
LDA	2	32
NaH	3	22
*n*BuLi	4	0
*sec*BuLi	5	0

Table 8 – Conditions for alkylation of the methoxy trisubstituted ETP intermediate

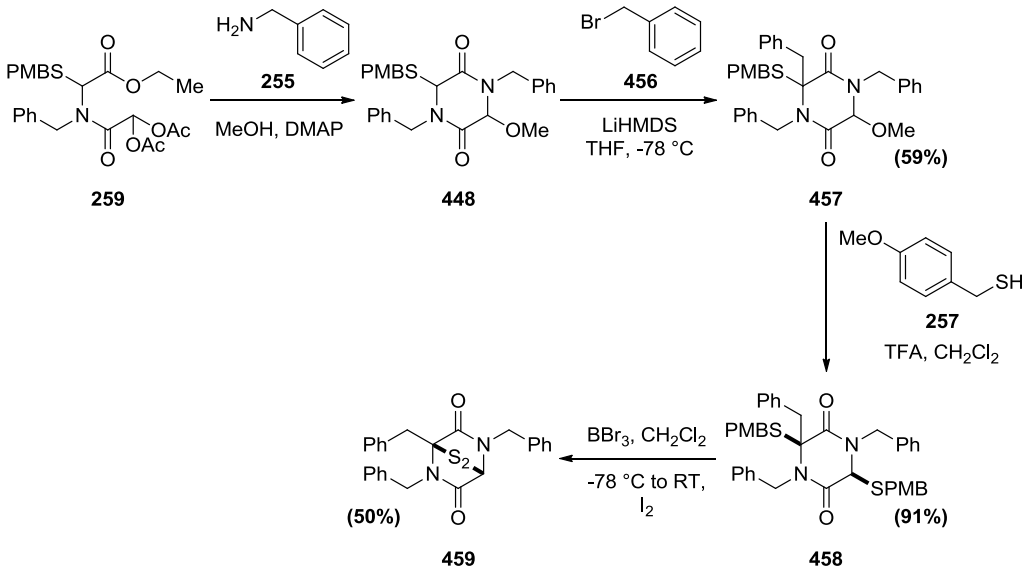
Substitution of the methoxy group for the desired *para*-methoxybenzyl mercaptan group was achieved using trifluoroacetic acid with an 83% yield after purification. The final step for ETP formation underwent the same procedure as described before giving compound (**455**) in 24% yield (Scheme **110**).



Scheme 110 – (±)-(1S,4S)-1-allyl-2,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide synthesis

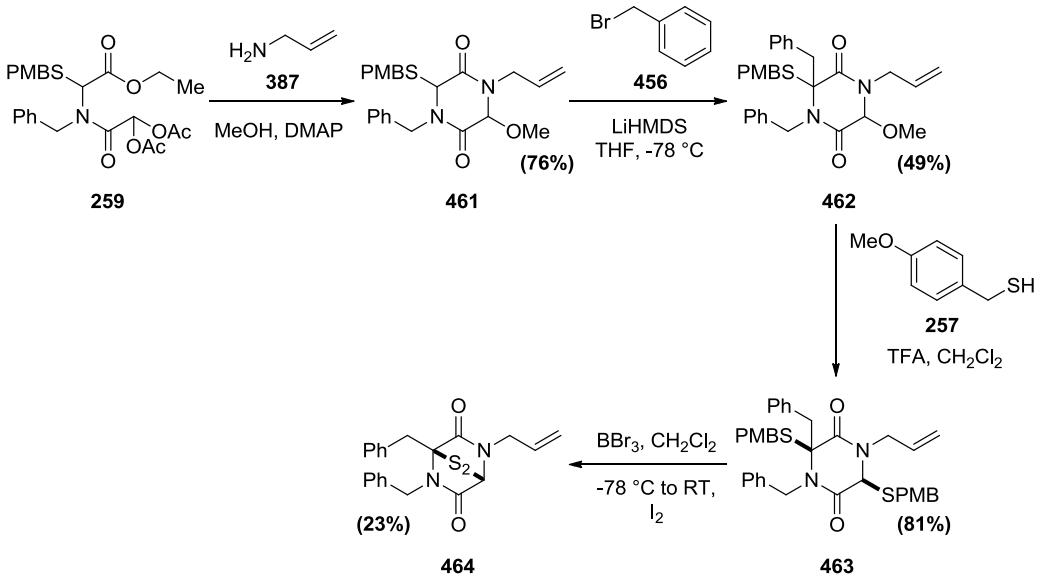
The same methodology was used to obtain compound (**459**). Substituting the previously used allyl bromide for benzyl bromide gave intermediate (**457**) in 59% yield. The next

two synthetic steps involved *para*-methoxybenzyl mercaptan introduction (91% yield) and thiol deprotection / oxidation to form the desired ETP (**459**) in a 50% yield as shown below (Scheme **111**).



Scheme 111 - (±)-(1S,4S)-1,2,5-tribenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide synthesis

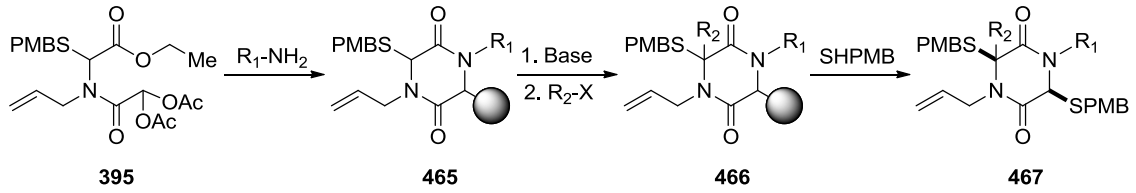
Cyclisation of diacetate (**259**) using allyl amine and DMAP in a methanolic mixture gave compound (**461**) in a 76% yield. Benzyl bromide (**456**) was then reacted with the methoxy derivative (**461**) in the presence of LiHMDS to give (**462**) in a reduced 49% yield. Following the previously described procedure to introduce the protected thiol group gave intermediate (**463**) in 81% yield that after disulfide formation gave ETP (**464**) in 23% yield as shown below (Scheme **112**).



Scheme 112 - (±)-(1S,4S)-5-allyl-1,2-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide synthesis

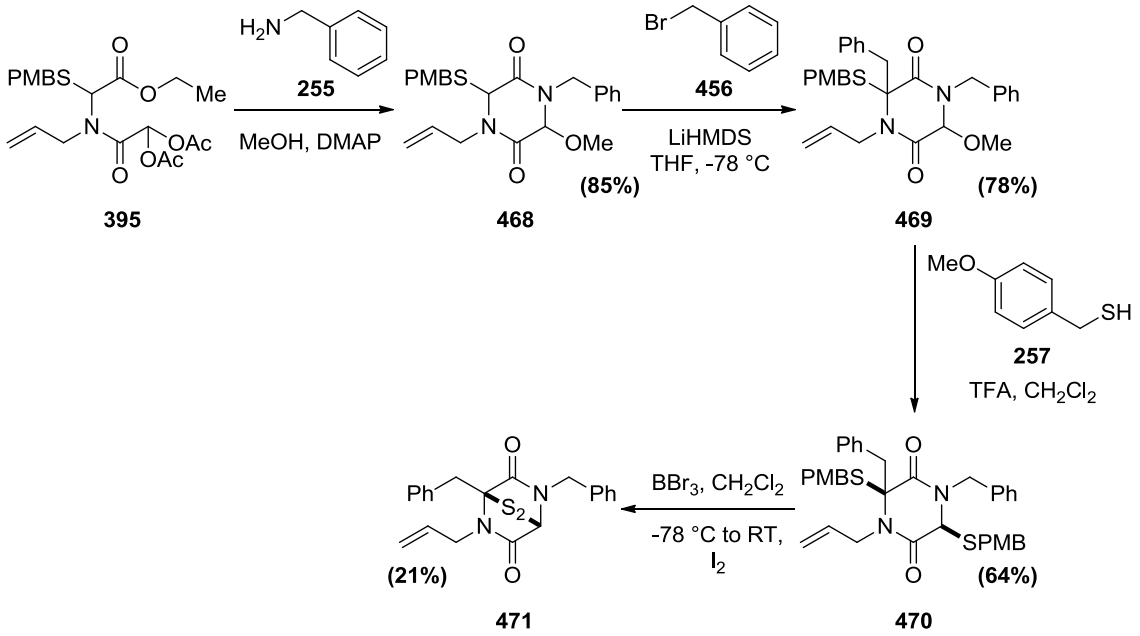
Both allyl tri-substituted ETP derivatives (i. e. compounds (**455**) and (**464**)) gave poor yields in the deprotection / oxidation synthetic steps when compared to the tri-benzyl substituted ETP (**459**). This may occur due to the increased lipophilicity of compound (**459**) which makes it more sterically protected facilitating the formation of the disulfide bridge.

Our synthesis was then directed to the missing ETP analogue that would include two benzyl as well as one allyl substituents, compound (**467**). The uses of diacetate (**395**) facilitated the formation of desired precursor (**465**) and in addition enabled us to test the procedure reproducibility when using a different diacetate as a building block. We then decided to use different diacetate ETP intermediates to synthesise other molecules and prove the reproductibility of this new approach (Scheme **113**).



Scheme 113 – 2-(Allyl(2-ethoxy-1-(4-methoxybenzylthio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate cyclisation for the synthesis of trisubstituted ETPs

The formation of compound (**471**) started with cyclisation of diacetate (**395**) using the above described conditions: methanol, DMAP and amine (**255**); with a 85% yield. Introduction of the benzyl group on the *3*- position followed by methoxy substitution for *para*-methoxybenzyl mercaptan gave intermediate (**470**) in 64% yield. Thiol deprotection and disulfide bridge formation gave ETP (**471**) in a reduced 21% yield (Scheme **114**) with similar yields achieved for the final synthetic step for ETPs (**455**) and (**464**) (Schemes **110** and **112**).

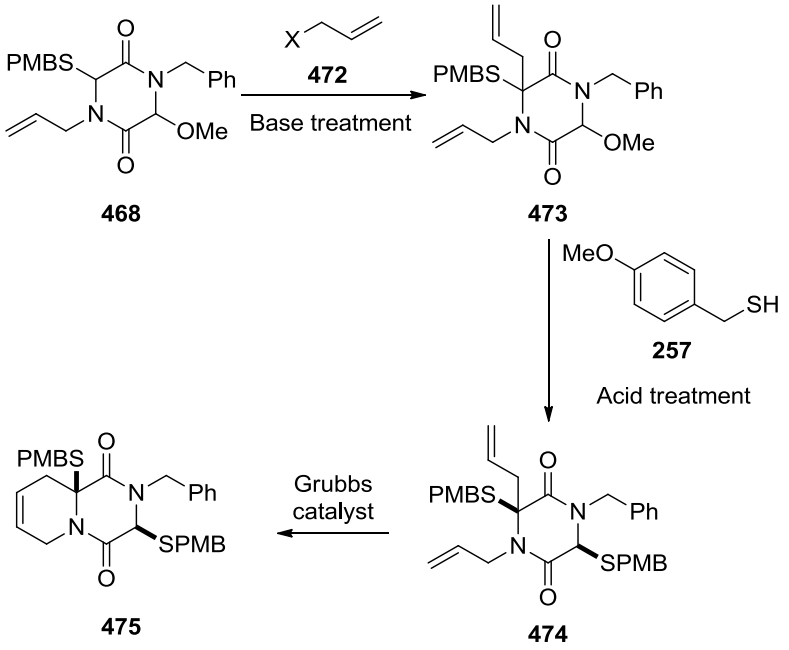


Scheme 114 – (±)-(1S,4S)-2-allyl-1,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide synthesis

This approach that enabled selective alkylation of the ETP core directed our synthesis to different structures with the introduction of new cyclic systems.

3.3.2.1 Bicyclic monomeric ETP

Following the synthesis of a precursor that was readily alkylated on the ETP's *3*-position, we decided to attempt a new approach towards bicyclic ETPs, in order to synthesise more complex systems. As a result of the incorporation of an alkyl group on the ETP core, olefin metathesis seemed to be an ideal opportunity to achieve this. Olefin metathesis includes multiple features, from ring-closing metathesis (RCM) to cross metathesis (CM), and has become one of the most powerful and useful tools in organic synthesis for the formation of carbon-carbon double bonds.[286, 287] Our primary objective was to make a terminal diolefin intermediate using our previously synthesised ETP precursor (**468**) in the presence of a strong base and allyl halide (**472**) addition. Introduction of the protected thiol group (**474**) followed by RCM using Grubbs catalyst would therefore give product (**475**) (Scheme **115**).



Scheme 115 – Synthetic approach for the synthesis of 2-benzyl-3,9a-bis((4-methoxybenzyl)thio)-2,3,9,9a-tetrahydro-1H-pyrido[1,2-a]pyrazine-1,4(6H)-dione

RCM is a well known reaction that can be achieved in many cases under ambient conditions, using different types of Grubbs catalysts as shown below (Figure **66**).[281]

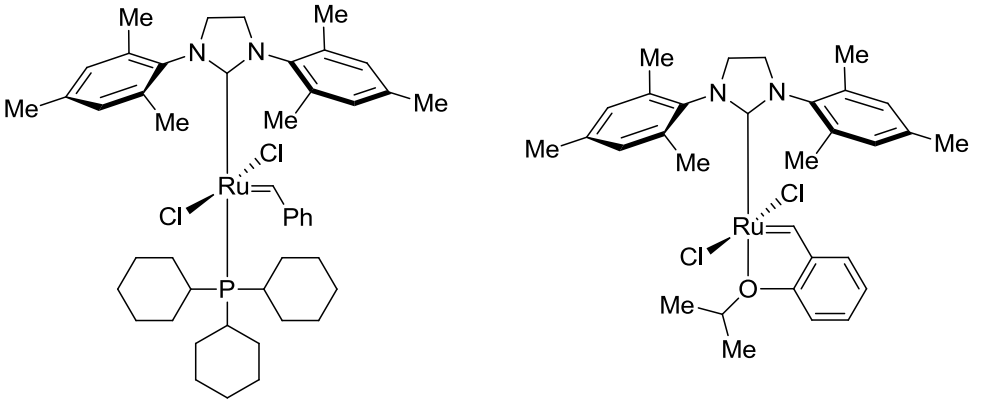
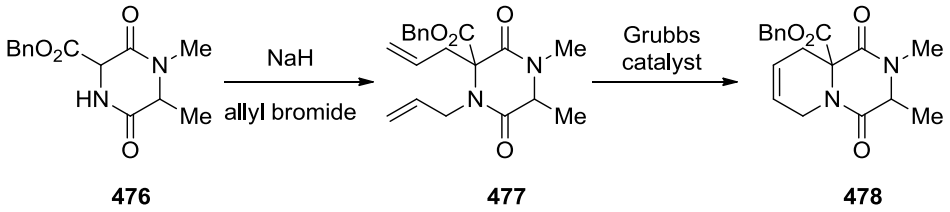


Figure 66 – Grubbs 2nd generation catalyst and Grubbs-Hoveyda 2nd generation catalyst

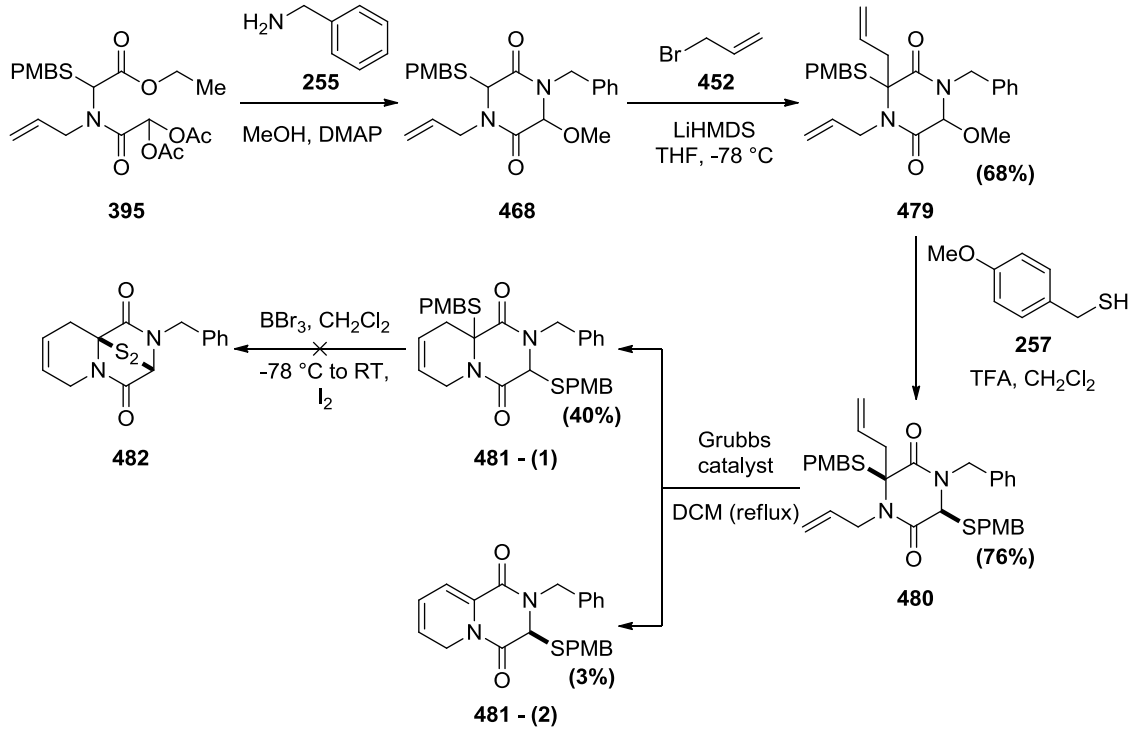
Recently, it has been shown that RCM represents an important advance in the construction of functionalized heterocycles.[288] Studies by Chai *et al* [289] demonstrated successful functionalisation of the piperazine-2,5-dione motif using this methodology (Scheme **116**). Mono and dual functionalization was achieved using piperazinedione (**476**) chemoselective *N*- and *C*-alkylation; the diallylated product (**477**) was then

submitted for ring-closing metathesis using a first generation Grubbs catalyst to give the cyclic alkene (**478**) in 82% yield.



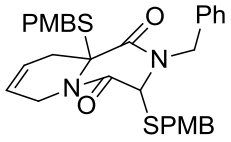
Scheme 116 – Chai's synthetic functionalisation of the piperazine-2,5-dione core

Our synthetic pathway started with formation of the previously described methoxy intermediate (**468**) followed by deprotonation and subsequent alkylation using allyl bromide to give compound (**479**) in a 68% yield. After acid treatment and *para*-methoxybenzyl mercaptan addition, protected dithiol ETP precursor (**480**) was then reacted with Grubbs second generation catalyst under reflux conditions to give the bicyclic products (**481 – (1)**) and (**481 – (2)**) in 40% and 3% yields, respectively. Unfortunately, compound (**482**) disulfide bridge formation was inaccessible (Scheme **117**).



Scheme 117 – (±)-(3S,9aS)-2-benzyl-2,3-dihydro-3,9a-epithiopyrido[1,2-a]pyrazine-1,4(6H,9H)-dione 10-sulfide synthesis

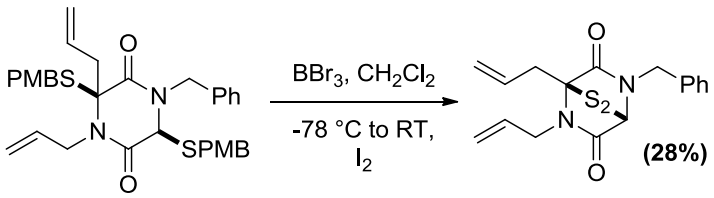
Our evaluation of the failure of this approach led us to consider whether the compound possessed a *trans* conformation (**481 – (1)**) (Figure **67**). This conformer would not be able to form the desired ETP. The solution found to surpass this problem was to re-arrange the synthetic route i. e. promote first, disulfide bridge formation, and second, RCM of the resulting diallyl ETP.



481

Figure 67 – Protected dithiol probable conformation in the synthesis of 2-benzyl-3,9a-bis((4-methoxybenzyl)thio)-2,3,9,9a-tetrahydro-1H-pyrido[1,2-a]pyrazine-1,4(6H)-dione

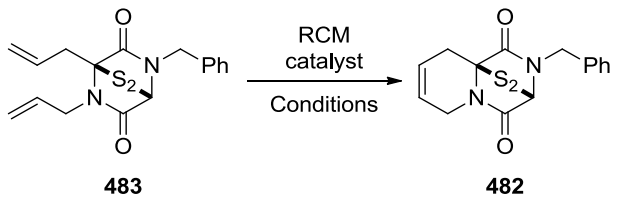
Similar synthetic steps, as described before, were used to obtain intermediate (**480**). This was submitted to thiol deprotection and subsequent oxidation gave ETP (**483**) in a 28% yield (Scheme **118**).



480 **483**

Scheme 118 – (±)-(1S,4S)-1,2-diallyl-5-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide synthesis

Ring closing metathesis of ETP (**483**) required the study of different conditions since bicyclic ETP (**482**) formation was inaccessible using the same experimental methodology as for intermediate (**481**). As shown below (Table **9**), the conversion of diallyl ETP (**483**) to the desired product (**482**) was achieved with the use of microwave accelerated reaction. Comparing both catalysing agents used, Grubbs-Hoveyda second-generation catalyst showed the best result with a 40% yield (Entry 4).



483 **482**

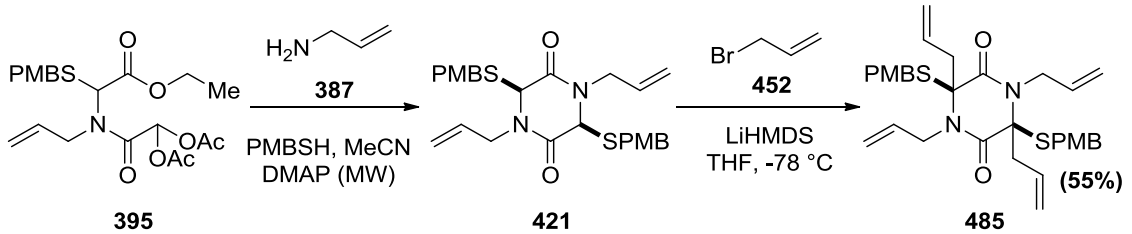
		Conditions		
RCM catalyst	**Entry**	**Temperature (°C)**	**Time**	**Yield (%)**
Grubbs second generation	1	160 (Reflux)	24h	0
Grubbs second generation	2	80 (MW)	30min	8
Grubbs-Hoveyda second-generation	3	160 (Reflux)	24h	0
Grubbs-Hoveyda second-generation	4	80 (MW)	30min	40

Table 9 – Conditions for the synthesis of (±)-(3S,9aS)-2-benzyl-2,3-dihydro-3,9a-epithiopyrido[1,2-a]pyrazine-1,4(6H,9H)-dione 10-sulfide

The synthesis of tri-substituted ETPs enabled us to not only increase the library of compounds produced, but to diversify the synthetic methodology behind cyclisation of our diacetate ETP precursors. Success in the synthesis of a bicyclic ETP molecule using a common building block allowed us to extend our research to the next objective where we elected to design a tri-cyclic ETP using a tetra-substituted ETP approach.

3.3.3 Monomeric tetra-substituted ETPs

In order to form a tri-cyclic ETP derivative we first needed to synthesise a tetra-substituted symmetric ETP by adding two allyl groups on both the *3*- and *6*- positions. Formation of compound (**485**) commenced with the already described base induced cyclisation procedure of our diacetate (**395**), to give (**421**) in a 72% yield. Deprotonation / alkylation of (**421**) using allyl bromide in the presence of LiHMDS gave the protected tetra-substituted ETP (**485**) in 55% yield (Scheme **119**).

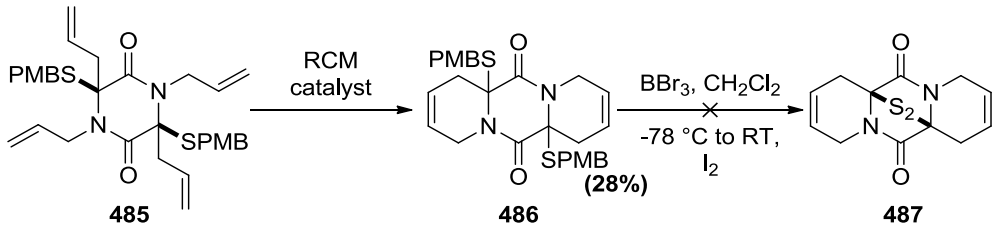


395 **421** **485**

Scheme 119 – (3S,6S)-1,3,4,6-tetraallyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione synthesis

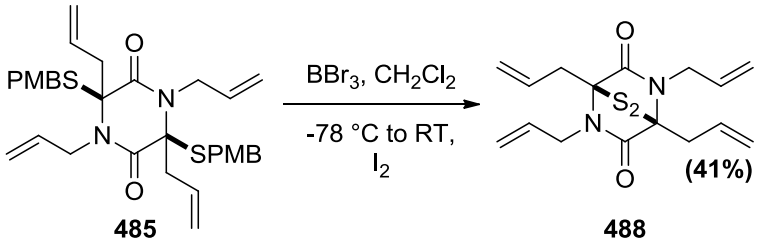
3.3.3.1 Tricyclic monomeric ETP

Ring-closing of the previously synthesised tetra-vinyl protected ETP (**485**) using Grubbs second generation catalyst led to intermediate (**486**) in 28% yield. Deprotection / oxidation of (**486**) did not however give rise to the desired tri-cyclic ETP (**487**) (Scheme **120**). This result clearly highlighted the already demonstrated problem of synthesising an extra ring before disulfide bridge formation.



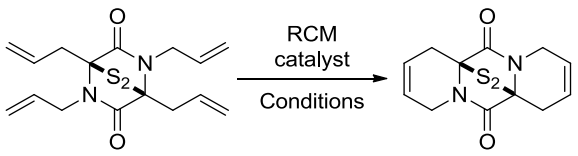
Scheme 120 – Inital planned synthetic route for (±)-(6aS,12aS)-7,10-dihydro-6a,12a-epithiodipyrido[1,2-a:1',2'-d] pyrazine-6,12(1H,4H)-dione 13-sulfide synthesis

We therefore decided to synthesise a tetra-substituted ETP which could then be submitted to RCM conditions. To this end, compound (**485**) was reacted with boron tribromide and oxidised using iodine to give (**488**) in 41% yield (Scheme **121**).



Scheme 121 – (±)-(1S,4S)-1,2,4,5-tetraallyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide synthesis

The results shown below (Table **10**) demonstrate that ring closing metathesis of ETP (**488**) using Grubbs-Hoveyda second generation catalyst under microwave conditions gave the best result to achieve the desired tri-cyclic ETP system (**487**) with a 39% yield (Entry 4).



488 **487**

		Conditions		
RCM catalyst	**Entry**	**Temperature (°C)**	**Time**	**Yield (%)**
Grubbs second generation	1	160 (Reflux)	24h	0
Grubbs second generation	2	80 (MW)	30min	7
Grubbs-Hoveyda second-generation	3	160 (Reflux)	24h	0
Grubbs-Hoveyda second-generation	4	80 (MW)	30min	39

Table 10 – Conditions for the synthesis of (±)-(6aS,12aS)-7,10-dihydro-6a,12a-epithiodipyrido[1,2-a:1',2'-d] pyrazine-6,12(1H,4H)-dione 13-sulfide

The formation of the tricyclic ETP (**487**) clearly demonstrated the diversity of compounds which can be created using different diacetate ETP precursors. Based on these results, our synthesis turned to the development of molecules that would incorporate two disulfide bridged cores, namely that of dimeric ETPs.

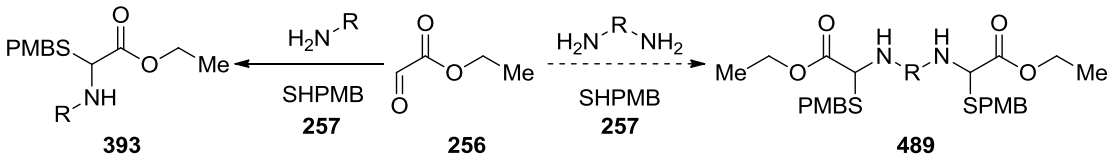
3.4 Dimeric ETP formation

The dimeric ETPs that occur in Nature often display far more potent biological activity than those of their monomeric ETP counterparts. However, due to the greater complexity, these structures are often more difficult to synthesise. As a result of this, only a few dimeric ETP syntheses have been reported in the literature to date.[156, 225, 227] In way of example, Movassaghi's elegant enantioselective synthesis of (+)-11,11-dideoxyverticillin A (**65**) involved a number of steps that cannot be applicable towards the synthesis of a library of medicinally useful analogues.[156]

We therefore decided to design different methods that could simplify the approach towards these dimeric constructs. By adapting the synthetic pathways described to build monomeric ETPs and extending this methodology towards their corresponding dimeric derivatives, we could vary our library of compounds.

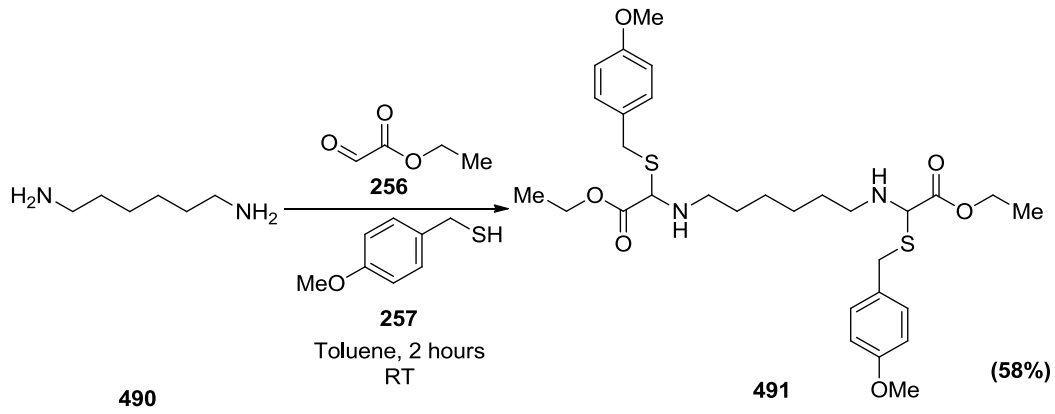
3.4.1 Diamino-tetra-acetate intermediate formation

Our first approach to develop a simple method that could facilitate the formation of two ETP cores in the same molecule was to introduce a linker unit in the first step of the ETP synthesis (Scheme **122**). This linker would include two amine motifs that could undergo a corresponding five component reaction, analogous to the previously described formation of the diacetate, in the presence of ethyl glyoxalate (**256**) and *para*-methoxybenzyl mercaptan (**257**) (Scheme **122**).[1]



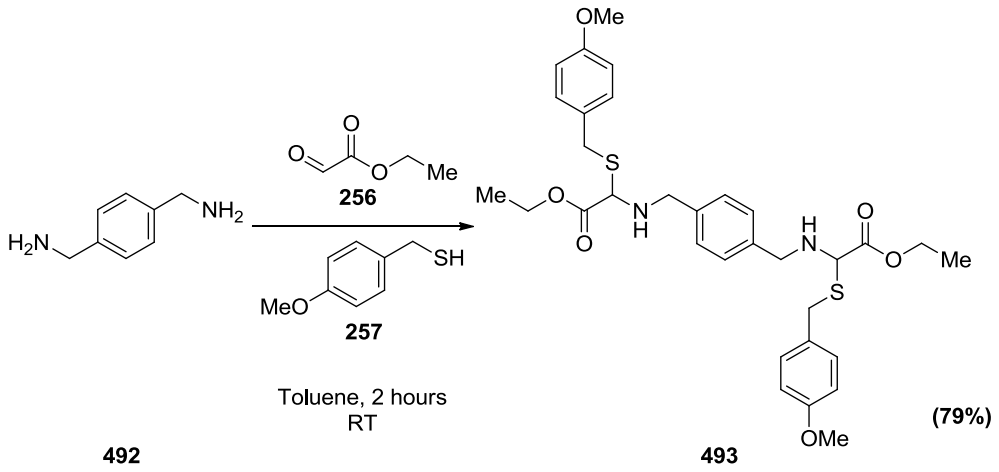
Scheme 122 – Model proposed for the synthesis of the diamino ETP intermediate based on a singular amine approach

Using this methodology, diamine (**490**) was reacted with two equivalents of both ethyl glyoxalate (**256**) and PMBSH (**257**) to give intermediate (**491**) in 58% yield (Scheme **123**).



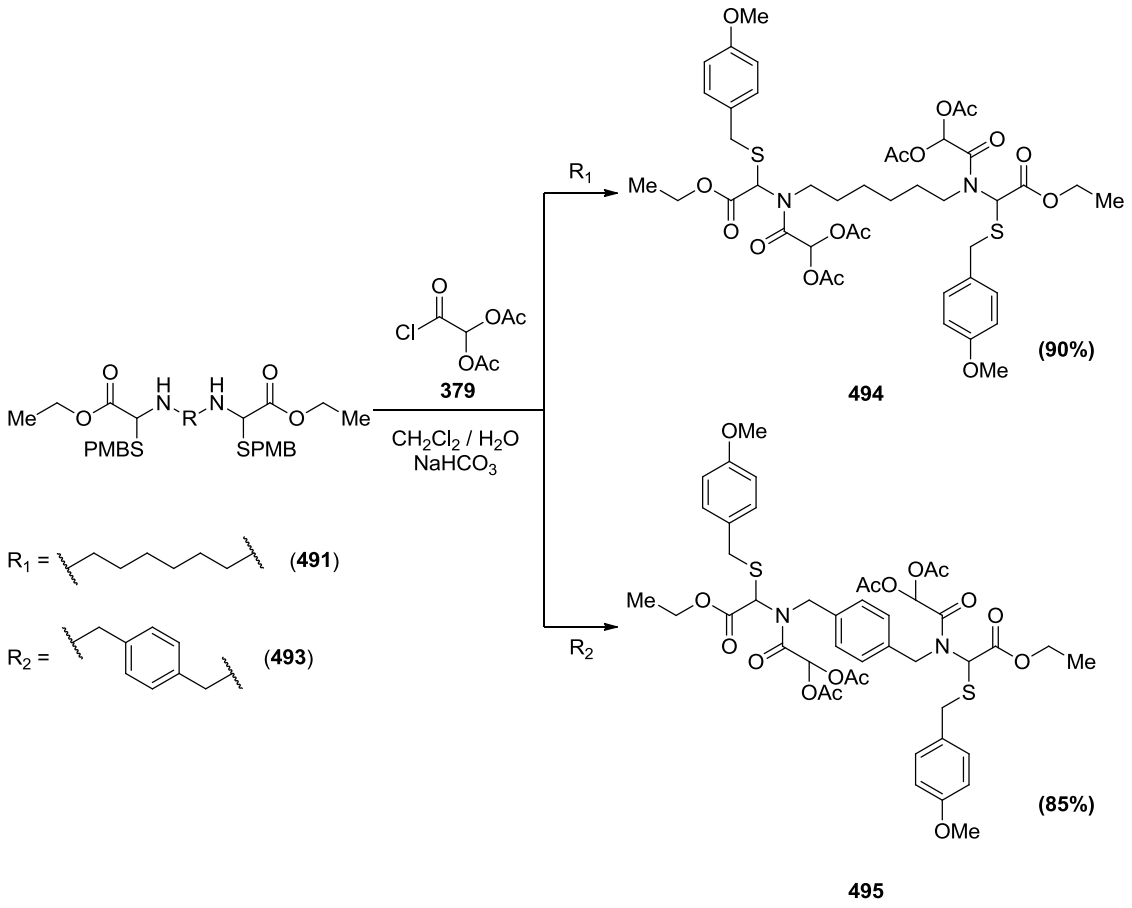
Scheme 123 – Ethyl 2-((6-((2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino) hexyl)amino)-2-((4-methoxy benzyl)thio)acetate synthesis

Following the same approach, intermediate (**493**) was also produced in 79% yield. This was achieved using *p*-xylylenediamine (**492**) as a linker unit between the two esters elements as well as ethyl glyoxalate (**256**) and PMBSH (**257**) (Scheme **124**).



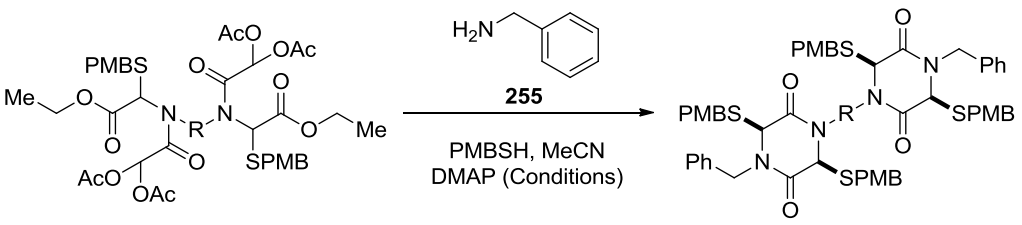
Scheme 124 – Diethyl 2,2'-((1,4-phenylenebis(methylene))bis(azanediyl))bis(2-((4-methoxybenzyl)thio)acetate) synthesis

The second step towards the synthesis of a modified dimeric diacetate compound involved reaction of intermediates (**491**) and (**493**) with diacetoxyacetyl chloride (**379**) under double Schotten-Baümann conditions as before. This led to the formation of both diacetate cyclisation precursors (**494**) and (**495**) in 90 and 85% yield respectively (Scheme **125**).



Scheme 125 – Synthesis of different diamino diacetate ETP intermediates

The synthesis of these two diamino-didiacetate compounds led to the previously reported reaction model of ring closing using an amine, a protected thiol group and a catalyst. For this purpose, diacetates (**494**) and (**495**) were reacted with benzylamine (**255**) and *para*-methoxybenzyl mercaptan using our base catalysed reaction. Different conditions were used in order to achieve the protected tetra-thiol intermediates (**496**) and (**497**). Results showed no significant prevalence in yield for either reflux or microwave conditions (Table **11**).



Conditions

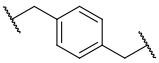
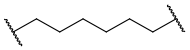
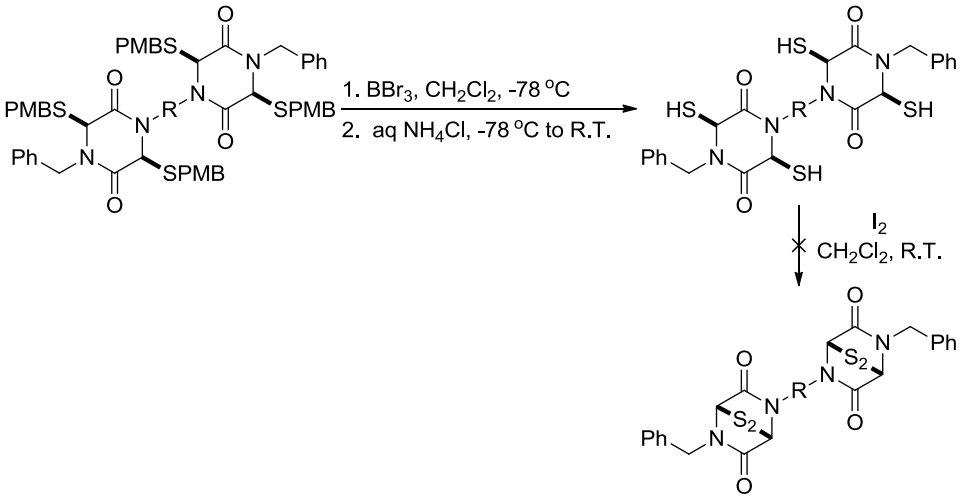
R group	Entry	Temperature (°C)	Time	Yield (%)	Compound
	1	51 (Reflux)	16h	43	**496**
	2	130 (MW)	30min	48	
	3	51 (Reflux)	16h	50	**497**
	4	130 (MW)	30min	47	

Table 11 – Conditions for the cyclisation of different diamino diacetate ETP intermediates

Unfortunately, the subsequent synthetic step which included deprotection / oxidation of the previously obtained protected thiol intermediates was found to be extremely difficult to achieve. This was due to the very low solubility of compounds containing four thiol deprotected groups (Scheme **126**) as well as different by-products originating from the reaction.

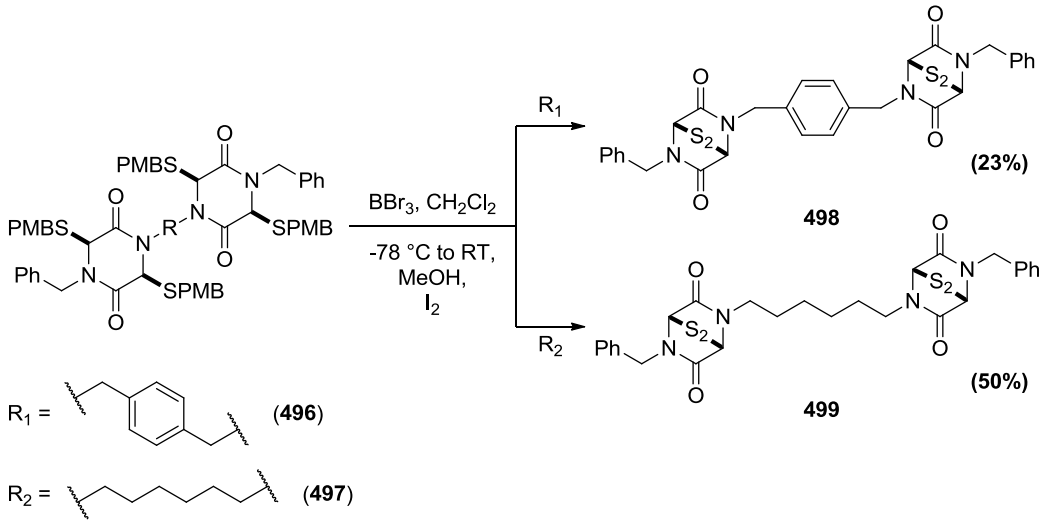


Scheme 126 – Inital planned synthetic route for dimeric ETP thiol deprotection / oxidation

To overcome this problem we decided to the use a 1M solution of boron tribromide instead of the neat form of this reagent, since dilution of this Lewis acid could lead to better solubility and hopefully a better outcome. In addition, we decided to incorporate methanol after reaction of boron tribromide. We envisioned that methanol would

increase the solubility of the suspension by dissolving the reaction products, by-products and existing excess reagent. Treatment with sodium hydrogen carbonate and iodine would then enable the desired tetra-thiol compounds to be obtained.

Using this new methodology, dimeric protected ETPs were deprotected using a 1M solution of boron tribromide in dichloromethane. Solubility difficulties were dissipated with the addition of methanol before treatment with aqueous sodium hydrogen carbonate. Oxidation using iodine to form the required disulfide bridge followed. Automatic column purification and HPLC separation of the reaction products rendered compounds (**498**) and (**499**) in 23 and 50% yields, respectively (Scheme **127**).

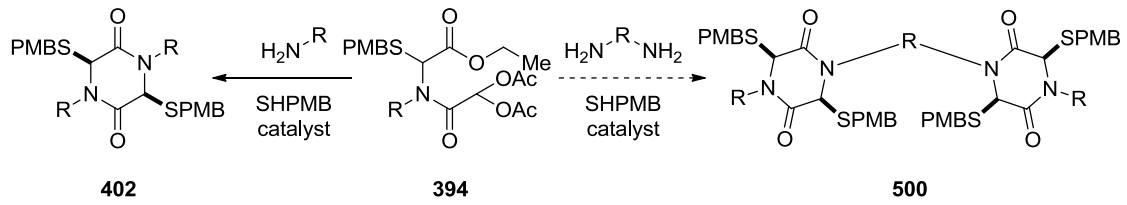


Scheme 127 – Different dimeric ETPs thiol deprotection / oxidation

Using similar methodology as described previously for monomeric ETPs enabled the formation of molecules that included two ETP cores in the same structure. However, to simplify the approach towards dimeric compounds we decided to use our diacetate ETP precursors and link them by using one diamine as described below.

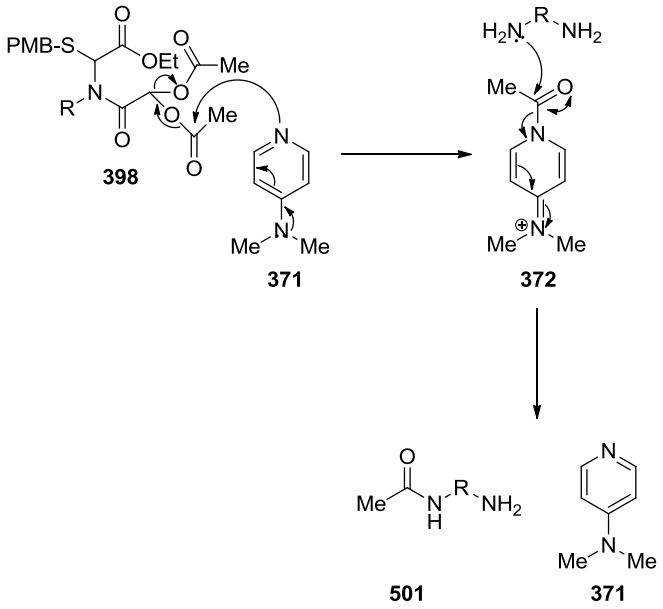
3.4.2 Diacetate cyclisation using a diamino linker unit

In order to synthesise dimeric ETPs, one possibility arose where one could potentially adapt the methodology for our monomeric ETPs. In our current protocol, primary amines were reacted with our diacetate (**394**) to furnish the required ETP core (**402**). If different diamines were to be used in its place, we could potentially access dimeric ETPs (**500**) via reaction of each amine with a diacetate precursor (Scheme **128**).



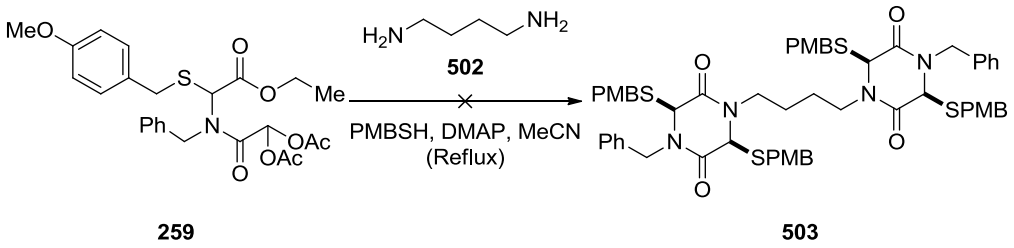
Scheme 128 – Model proposed for the synthesis of different protected dimeric ETP intermediates based on a diamine linker unit approach

The restructured methods developed to create these dimeric constructs require two diacetate molecules for every diamine linker, as shown above (Scheme **128**). However, the use of a base catalysed reaction would obviously require the presence of another diamine molecule due to by-products formed during the catalyst's regeneration (Scheme **129**). In addition, two *para*-methoxybenzyl mercaptan molecules were also required for the formation of the dimeric ETP molecule. This model defined the reaction's ratio of 2:2:2 diacetate, diamine and *para*-methoxybenzyl mercaptan in our initial attempts to form protected dimeric ETPs.



Scheme 129 – By-products of DMAP catalysed cyclisation (2)

Following the outlined protocol, different amine linkers were used to attempt cyclisation. 1,4-Diaminobutane (**502**) was the first diamine that was used for this purpose. This diamine nucleophile would potentially provide access to protected ETPs linked via four carbon atoms (Scheme **130**).



Scheme 130 – Model for the synthetic route of (3S,3'S,6S,6'S)-4,4'-(butane-1,4-diyl)bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione)

Reaction of the diamine (**502**) with diacetate (**259**), *para*-methoxybenzyl mercaptan and DMAP under reflux conditions clearly showed the presence of two spots with low Rf values on the TLC plate, indicating the presence of extremely polar compounds. This was unexpected since we anticipated that the Rf of the desired compound (**503**) would be closer to that of the starting material. Following purification by flash column chromatography, two products (**504**) and (**505**) were isolated. The structures for these two compounds were established by ^{1}H NMR and ^{13}C NMR spectrum as shown below (Figure **68**).

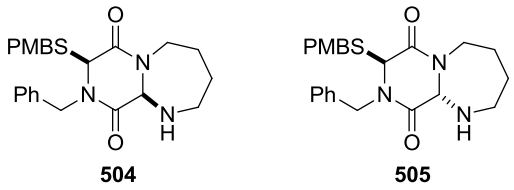
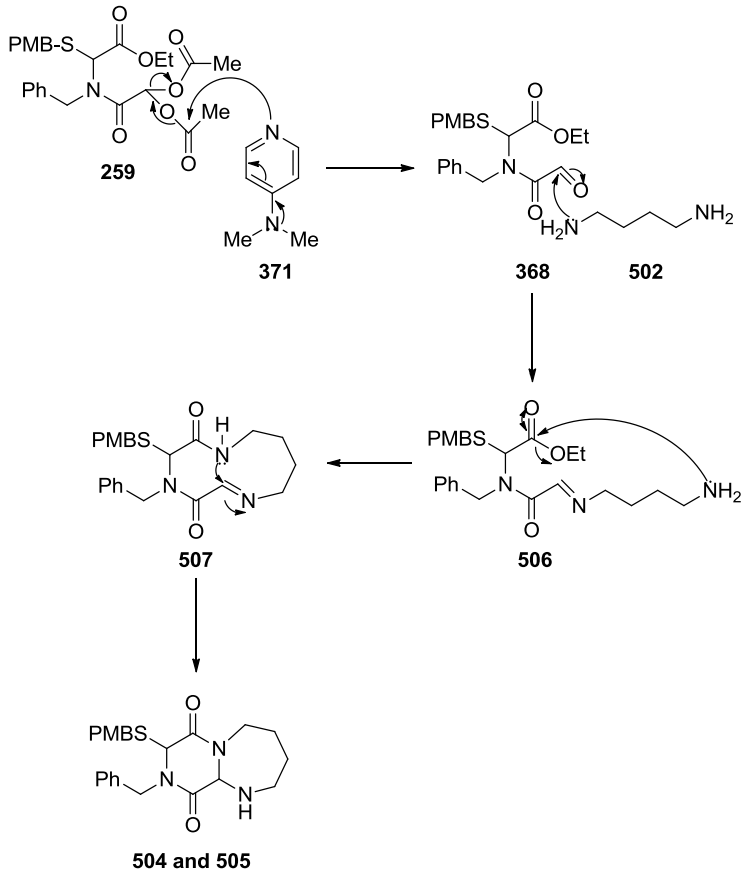


Figure 68 – Isomeric forms of (8S,10a)-9-benzyl-8-((4-methoxybenzyl)thio) octahydropyrazino[1,2-a][1,3] diazepine-7,10-dione

Following purification, 36% of the *cis* cyclised product (**504**) was obtained along with 12% of the *trans* isomer (**505**), indicating that the *cis* compound was more favourable as a result of its higher stability compared to the *trans* form. NMR spectrum analysis of both cyclised products showed only one *CH* group next to the sulfur of the PMB group at 4.43 ppm (**504**) and 4.38 ppm (**505**), whereas two *CH* groups are normally found. The ^{13}C NMR spectrum showed a shift in the position of one of the peaks representative of a *CH* group; this indicated that the *CH* group was next to the nitrogen atom rather than the sulfur as expected from the diketopiperazine synthesis. Therefore, the NMR spectrum showed two peaks representing the *CH* groups but with different shifts of 72.06 (**504**), 72.48 (**505**) ppm and 58.08 (**504**), 57.02 (**505**) ppm representing the *CH*s next to nitrogen and sulfur respectively. Formation of these products was also confirmed with mass spectrometry data. The use of a short length carbon chain (four

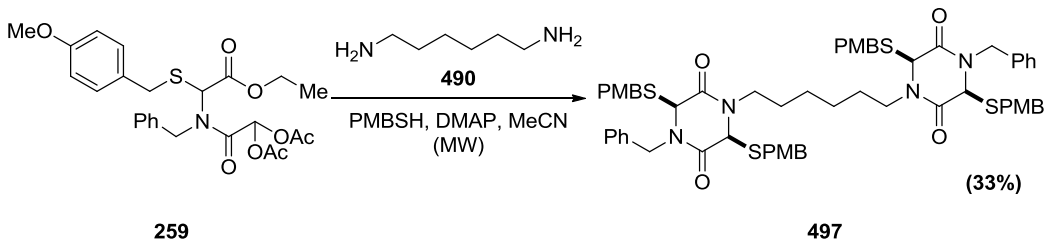
carbon chain) resulted in an intramolecular cyclisation and consequently the protected dimeric ETP (**503**) synthesis was not successful. This may had arisen since cyclisation of the four carbon diamine with one diacetate intermediate (**259**) results in the formation of a seven membered ring which was perhaps more favourable than the synthesis of the dimeric prospect molecule (**503**). Scheme **131** below illustrates the proposed synthetic pathway for the formation of the intramolecular cyclised products.



Scheme 131 – Molecular mechanism for the synthesis of (8S,10a)-9-benzyl-8-((4-methoxybenzyl)thio) octahydropyrazino[1,2-a][1,3] diazepine-7,10-dione

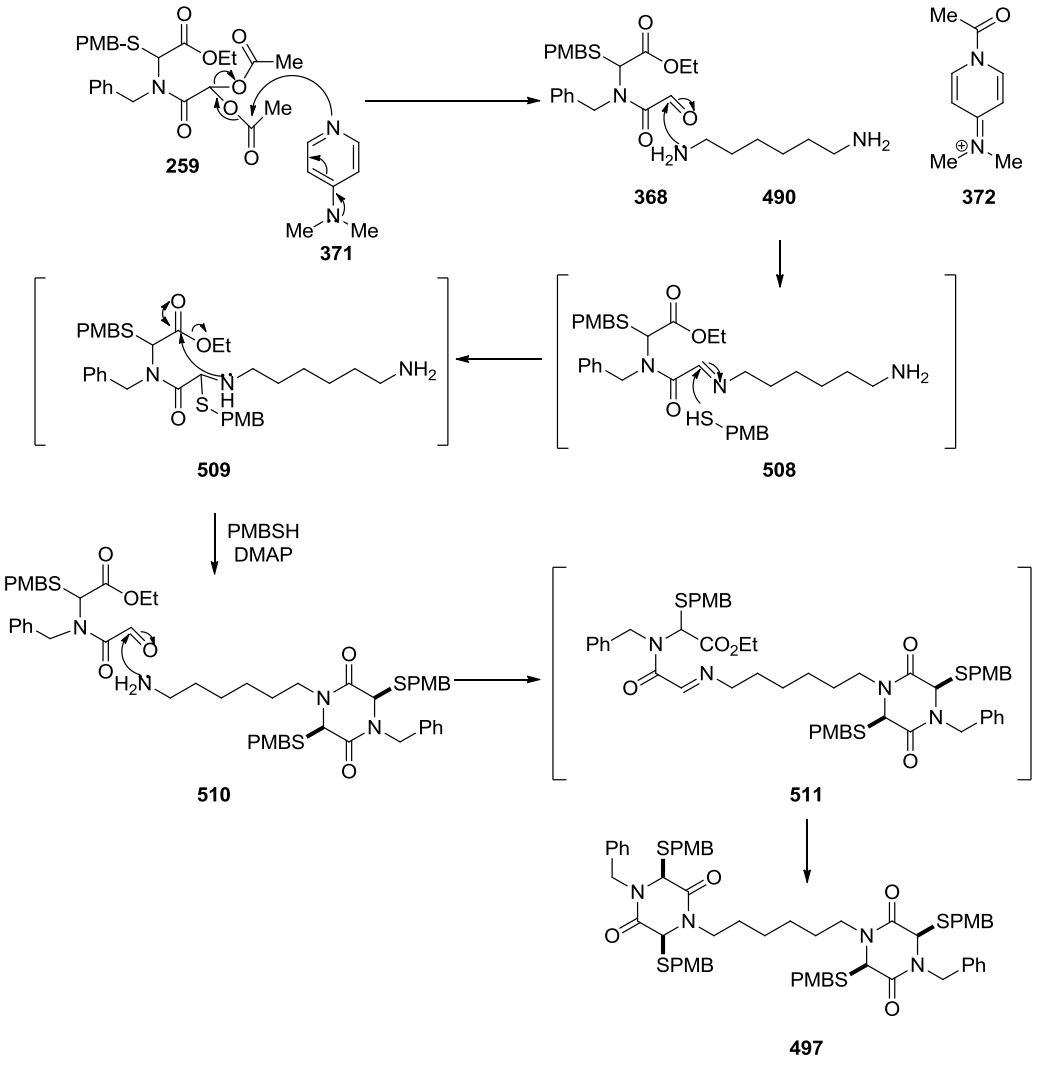
Following this, we decided to use 1,6-diaminohexane (**490**) as the nucleophile to link two ETP cores. The reason behind the use of longer chain diamine comes from the reduced probability of an intramolecular cyclisation that would produce a nine membered ring, which is disfavoured when compared to the formation of desired protected dimeric ETP.

This outlook led to the reaction of diacetate (**259**) with 1,6-diaminohexane (**490**) and *para*-methoxybenzyl mercaptan in the presence of DMAP. The reaction mixture was stirred at room temperature for 10 min and after heated at 130 °C for 30 minutes under microwave conditions (Scheme **132**).



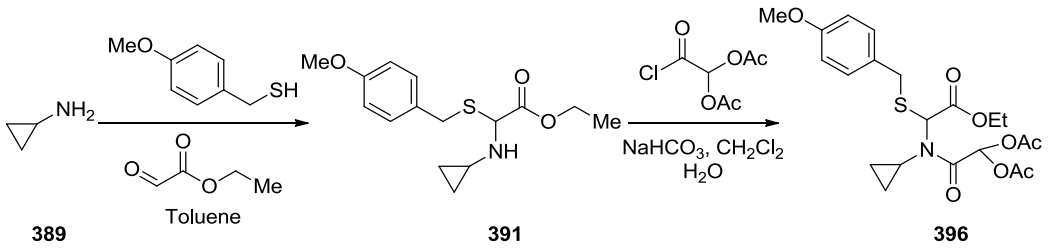
Scheme 132 – (3S,3'S,6S,6'S)-4,4'-(hexane-1,6-diyl)bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione) synthesis

Following purification by automatic flash column chromatography and recrystallisation, the dimeric ETP precursor (**497**) was obtained in a good yield of 33%. The following scheme outlines the proposed mechanistic pathway for the formation of the dimeric product (Scheme **133**).



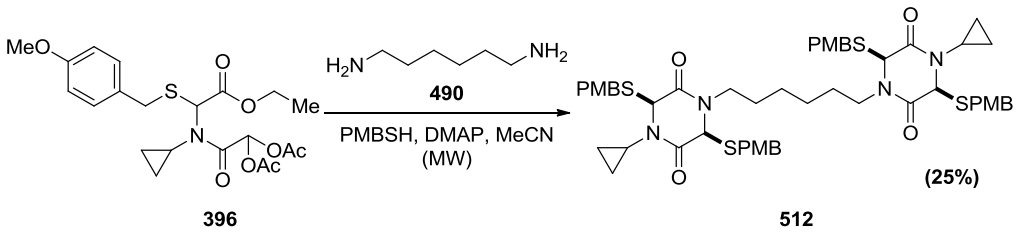
Scheme 133 – Molecular mechanism for the synthesis of (3S,3'S,6S,6'S)-4,4'-(hexane-1,6-diyl)bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione)

After achieving our first protected dimeric ETP, we decided to synthesise a second dimeric ETP (**512**) (Scheme **135**), which came from reaction of the cyclopropane substituted diacetate analogue (**396**) and a diamine linker. In the first instance, we synthesised cyclisation precursor (**396**) as described previously in 54% overall yield (Scheme **134**).



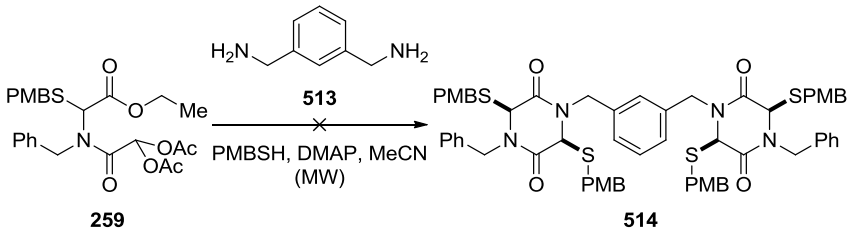
Scheme 134 – 2-(cyclopropyl(2-ethoxy-1-(4-methoxybenzylthio)-2-oxoethyl) amino)-2-oxoethane-1,1-diyl diacetate synthesis

Reaction of this diacetate precursor (**396**) with 1,6-diaminohexane (**490**), *para*-methoxybenzyl mercaptan and DMAP as catalyst followed. Conditions for this synthesis were similar to those used before for compound (**497**), stirring at room temperature for 10 min followed by heating at 130 °C for 30 minutes under microwave conditions. Following purification/recrystallisation, we observed efficient formation of the desired protected dimeric ETP (**512**) in a yield of 25% as the *cis* isomer, highlighting the relative ease with which dimeric ETP analogues may be synthesised using this synthetic approach (Scheme **135**).



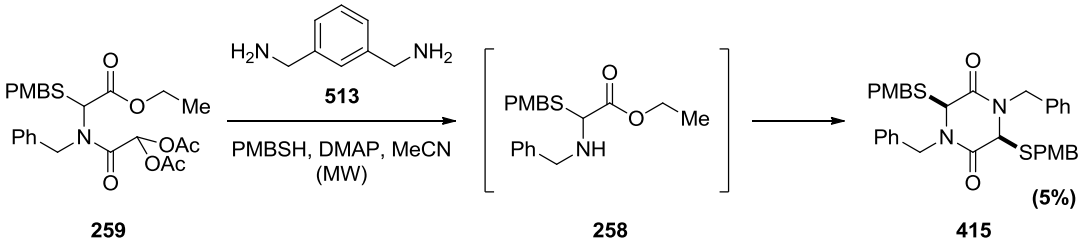
Scheme 135 – (3S,3'S,6S,6'S)-4,4'-(hexane-1,6-diyl)bis(1-cyclopropyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione) synthesis

The next attempt led to the synthesis of another protected dimeric ETP analogue (**514**) by reaction of diacetate (**529**) and a different diamine linker, *meta*-xylylenediamine (**513**) (Scheme **136**).



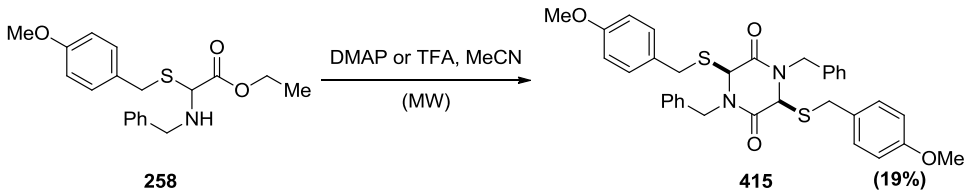
Scheme 136 – Model for (3S,6S)-1-benzyl-4-(3-(((2R,5R)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)methyl)benzyl)-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione synthesis

Following purification, analysis of the ^{1}H NMR spectrum surprisingly indicated the formation of a symmetrical monomeric ETP precursor (**415**) which was obtained from the dimerisation of two amines (**258**) (Scheme **137**).



Scheme 137 – 1,4-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione synthesis as a by-product of (3S,6S)-1-benzyl-4-(3-(((2R,5R)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)methyl)benzyl)-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione synthesis

This led to the investigation of a possible mechanism by which this dimerisation may have taken place. With this, a two component reaction using amine (**258**) in the presence of a catalyst and acetonitrile as a solvent was carried out to attempt the synthesis of (**415**) (Scheme **138**).

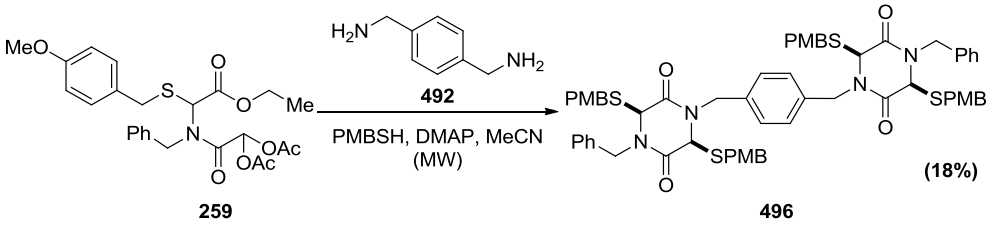


Scheme 138 – 1,4-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione synthesis using microwave conditions and different reaction catalysts from ethyl-2-(benzylamino)-2-((4-methoxybenzyl)thio)acetate as starting material

As anticipated, the ETP (**415**) was obtained using both types of catalyst although only in trace quantities (<20%).

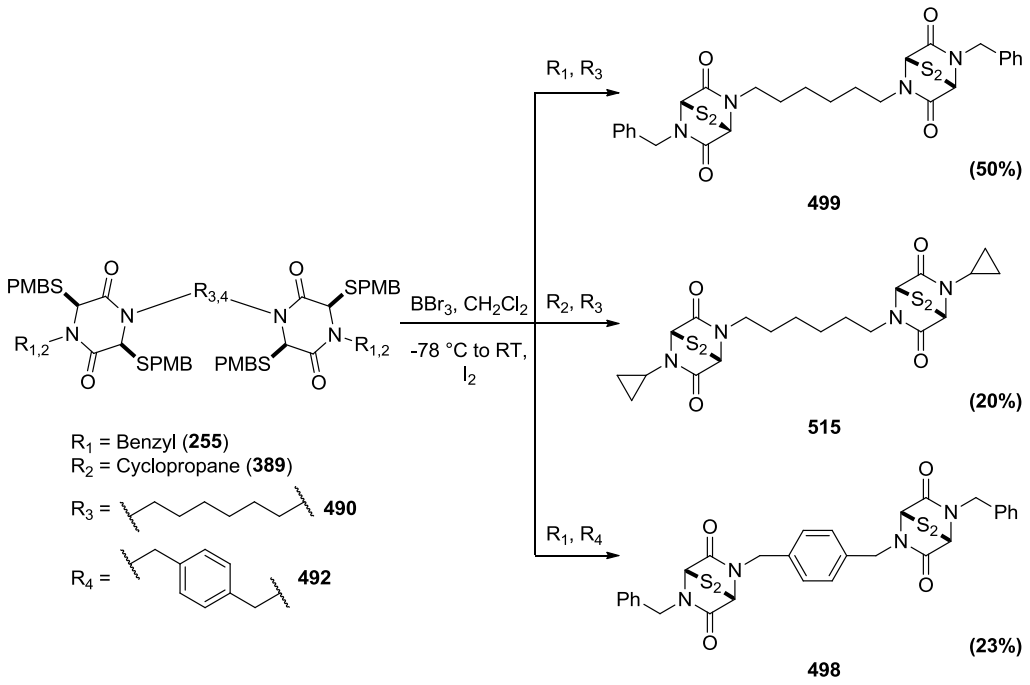
Following the previous reaction using *meta*-xylylenediamine (**513**), we then tried to synthesise the same type of ETP precursor however using *para*-xylylenediamine (**492**).

The reasons for choosing this linker were due to the amine positions that may avoid the previous outcome as for the putative synthesis of compound (**514**). The equidistant position of the amines could determine a better positioning for both nucleophilic attacks (Scheme **139**). The reaction gave an 18% yield of the *cis* isomeric form after recrystallisation.



Scheme 139 – (3S,3'S,6S,6'S)-4,4'-(1,4-phenylenebis(methylene))bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione) synthesis

After achieving three different protected ETPs, deprotection and oxidation was carried out for compounds (**497**), (**512**) and (**496**) to achieve the final ETP forms (**499**), (**515**) and (**498**), respectively. For this, boron tribromide solution was used to form tetra-thiol intermediates which were treated with methanol to increase the solubility of each reaction derivative. Sodium hydrogen carbonate or Rochelle's salt addition followed and oxidation using iodine enabled disulfide bridge formation for each final compound (Scheme **140**).



Scheme 140 – Thiol deprotection / oxidation of different dimeric ETPs

After reactions work up using sodium thiosulfate, purification using automated column chromatography and HLPC gave compounds (**499**), (**515**) and (**498**) in 50%, 20% and 23% yields, respectively. HPLC results shown below (Figure **69**) indicate the retention time for the three synthesised molecules.

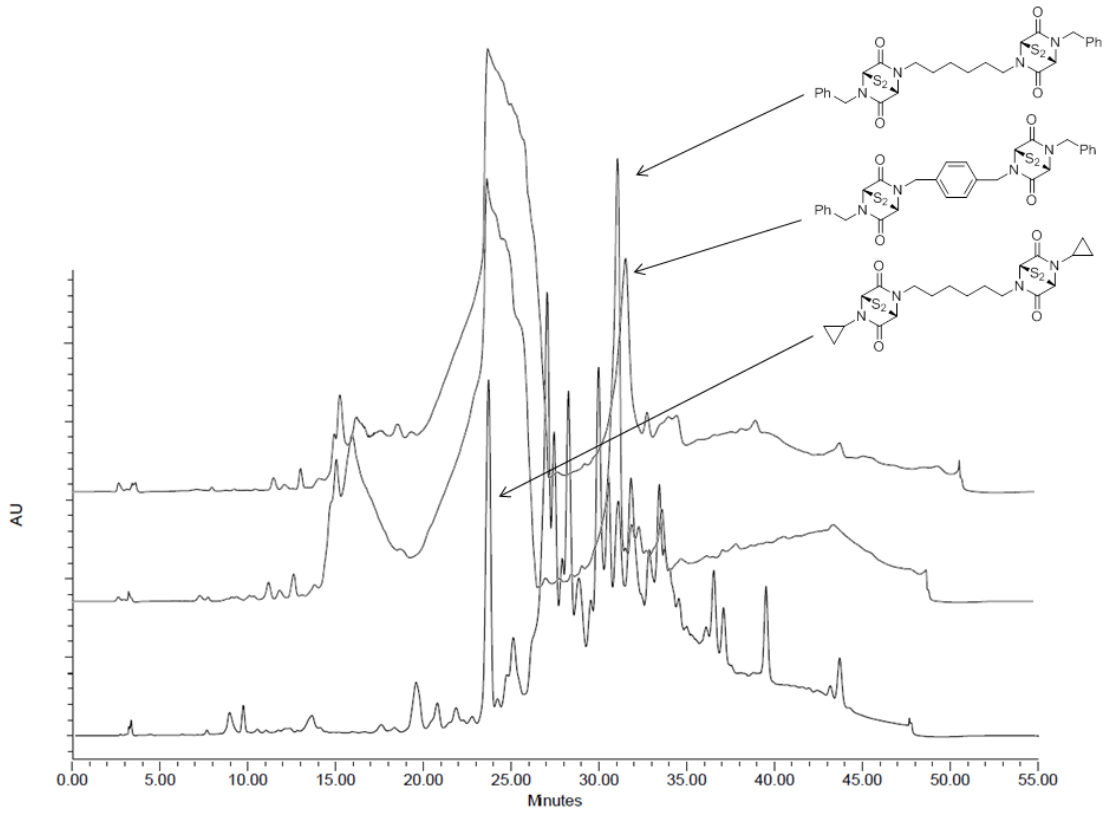


Figure 69 – HPLC separation of different examples of dimeric ETPs showing the correspondent retention times

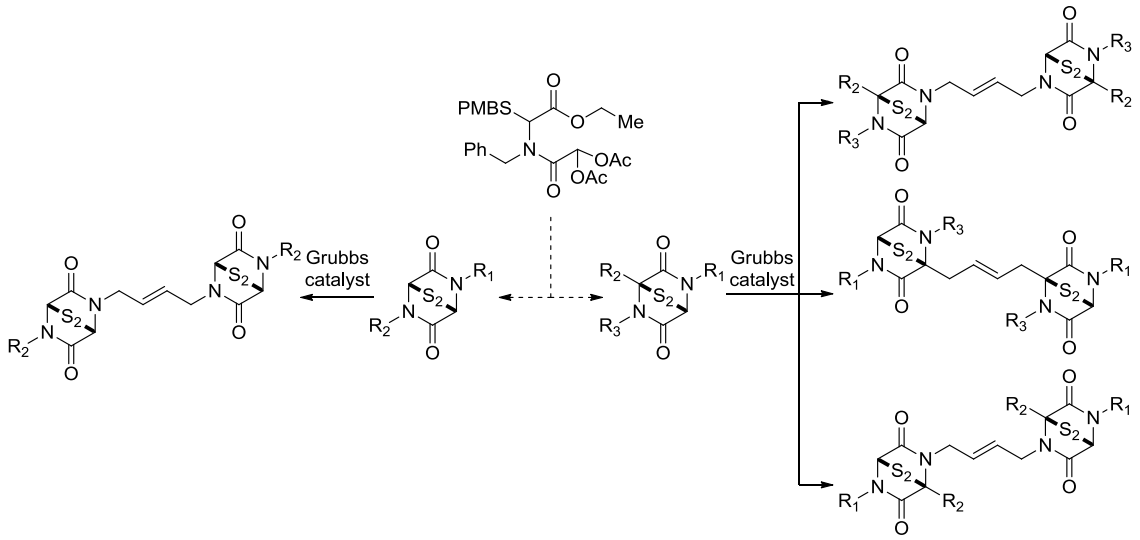
According to solvent rates throughout the separation, ETPs presented very similar solubility profiles with all molecules isolated from the reactions provingly to be extremely insoluble.

Another approach towards the synthesis of dimeric ETPs focused our attempts on olefin metathesis. For this, different starting materials, catalysts and conditions were used as described below.

3.4.3 Mono and disubstituted dimeric ETPs formation using cross metathesis

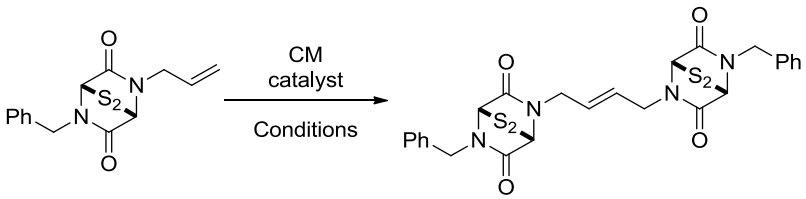
As described before, synthesis of both bi and tricyclic ETP systems was achieved using RCM reactions. We anticipated that the same principle could be applied to access dimeric ETP molecules by CM between an already built monomeric ETPs possessing one allyl substituent.[286] This idea was envisioned for two distinct classes of molecules:

di and tri-substituted ETPs. The first class would allow the synthesis of mono-substituted dimeric ETPs while the second class formed by tri-substituted ETPs would render disubstituted dimeric ETPs (Scheme **141**).



Scheme 141 – Model for the synthesis of dimeric ETPs using Grubbs catalysed reactions

We first started with the synthesis of the dimeric product formed by coupling two molecules of (±)-(1S,4S)-2-allyl-5-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (**416**). Using different conditions, we were able to achieve the mono-substituted dimeric ETP (**516**) that presented the same type of polarity when compared to previously synthesised dimeric molecules (Table **12**). Optimal conditions that enabled the synthesis of (**516**) involved the use of a microwave reaction at 150 °C for 2 hours and Grubbs second generation or Grubbs-Hoveyda second-generation catalysts.



431 **516**

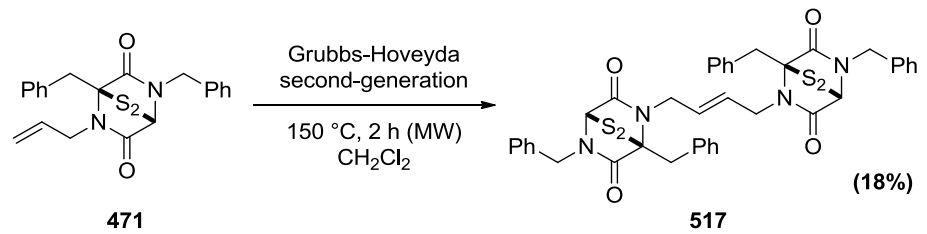
		Conditions		
CM catalyst	**Entry**	**Temperature (°C)**	**Time**	**Yield (%)**
Grubbs second generation	1	120 (Reflux)	24h	0
Grubbs second generation	2	70 (MW)	15min	0

Table 12 – Continues next page

Grubbs second generation	3	100 (MW)	20min	0
Grubbs second generation	4	120 (MW)	30min	0
Grubbs second generation	5	150 (MW)	2h	47
Grubbs-Hoveyda second-generation	6	120 (Reflux)	24h	0
Grubbs-Hoveyda second-generation	7	70 (MW)	15min	0
Grubbs-Hoveyda second-generation	8	100 (MW)	20min	0
Grubbs-Hoveyda second-generation	9	120 (MW)	30min	0
Grubbs-Hoveyda second-generation	10	150 (MW)	2h	22

Table 12 – Conditions for (±)-(1S,1'S,4S,4'S)-5,5'-((E)-but-2-ene-1,4-diyl)bis(2-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) synthesis

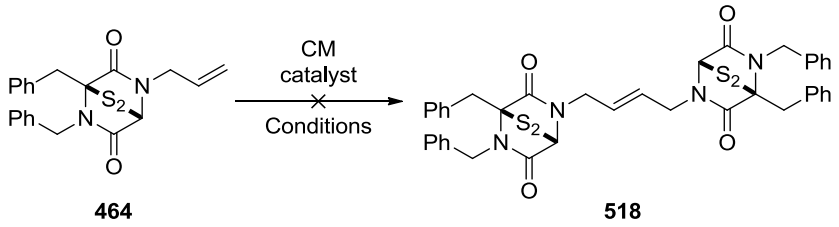
Synthesis of (**516**) showed the feasibility of the initial idea of using olefin metathesis to synthesise dimeric ETP molecules. In order to overcome the solubility problems encountered from previously produced dimeric compounds we decided to take the same coupling approach towards tri-substituted ETPs. The same conditions used to promote the cross coupling of two molecules of compound (**416**) to the desired prospect (**516**) were tried for monomeric ETP (**471**). By using Grubbs-Hoveyda second-generation catalyst under microwave conditions (150 °C for two hours), we were able to synthesise dimeric ETP (**517**) in 18% yield (Scheme **142**). This product (**517**) already showed different solubility since purification was achievable under less polar conditions with the use of a petroleum spirit / ethyl acetate gradient; when compare to dichloromethane / methanol system used for other dimeric ETPs.



471 **517**

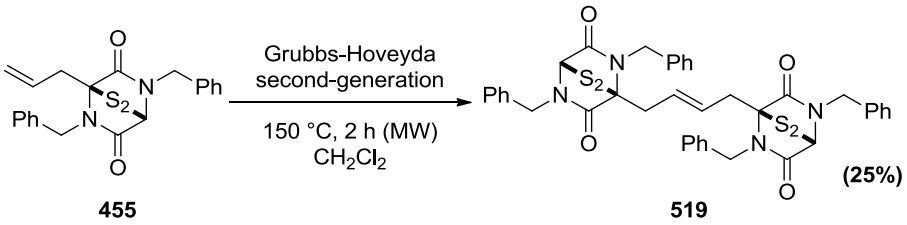
Scheme 142 – (±)-(1S,1'S,4S,4'S)-2,2'-((E)-but-2-ene-1,4-diyl)bis(1,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) synthesis

The same methodology was used to obtain dimeric ETP (**518**) by reacting two molecules of tri-substituted ETP (**464**). However, after several trials the product was not achieved. TLC, NMR and MS analysis only showed the presence of starting material after the same type of reaction conditions listed for the synthesis of compound (**517**) (Scheme **143**).



Scheme 143 – Model for (±)-(1S,1'S,4S,4'S)-5,5'-((E)-but-2-ene-1,4-diyl)bis(1,2-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) synthesis

Our unique approach for the synthesis of dimeric ETPs using olefin metathesis again proved successful for carbon allyl tri-substituted ETP (**455**), to give dimeric compound (**519**) in 25% yield (Scheme **144**). By using Grubbs-Hoveyda second-generation catalyst and microwave assisted reaction conditions (150 °C for two hours), dimeric ETP (**519**) was synthesised showing the potential of this approach. The polarity was similar to that presented for compound (**517**) demonstrating the correlation in between both molecular entities.



Scheme 144 – (±)-(1S,1'S,4S,4'S)-1,1'-((E)-but-2-ene-1,4-diyl)bis(2,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) synthesis

3.5 Biological assays

Following on from the development of novel synthetic routes to both monomeric and dimeric ETPs cores where we could vary four substituents, we next turned our attention towards the testing of the biological effects of these newly synthesised molecules. To achieve this, we worked closely with a number of different research groups to explore the scope of our ETP project in a number of areas that included: microbiology, biochemistry and virology.

In each specific area I would like first to express my deepest regards for the resources and time expended to obtain the data shown below in this chapter.

*For this I would like to thank Professor Peter Taylor from the Pharmaceutics Department at School of Pharmacy UCL for providing the facilities and expertise to develop all the microbiological work. I would also like to express my gratitude to Doctor João Moreira from Professor Taylor's group who took the task of investigating the ETPs microbiological activity against different strains of resistant bacteria. All the work shown below in section **3.5.1** was carried out in Professor Peter Taylor's laboratory in collaboration with Doctor João Moreira.*

3.5.1 ETPs Antibacterial assays

The antibacterial activity of ETPs was first reported in the early 1950' as demonstrated by Kidd and co-workers.[290] Gliotoxin (**1**) is perhaps the most well studied example of the ETP family of natural products and was tested against a wide range of resistant bacteria cell cultures which included: *Staphylococcus aureus*, *Streptococcus viridians* and different strains of *Mycobacterium tuberculosis*. All results gave minimal inhibitory concentrations (MIC) below 32 µg/mL which made gliotoxin (**1**) a suitable candidate for further exploration of its antibacterial activity.[290] The MIC is defined as the lowest concentration of drug that inhibits any visual bacterial growth after 16 to 20 hours.[291]

In order to test the antibacterial activity of our newly synthesised ETPs, four different strains of bacteria were chosen: one strain of methicillin-resistant *Staphylococcus aureus* (MRSA) – EMRSA-16, two clinical isolates from the Royal Free Hospital UCL of vancomycin-resistant *Enterococcus* (VRE) strains – VRE-1 and VRE-10 – and a surrogate for tuberculosis infection, *Mycobacterium aurum* – *M. aurum* A+. Selection of these strains was closely related to the importance that MRSA and VRE have in nosocomial infections and the emergence of resistance to new antibiotic treatments.[292]

The use of a *Mycobacterium* sp. strain in the study is linked to a disease responsible for a high human mortality rate worldwide, tuberculosis. *Mycobacterium aurum* provides a surrogate for the infectious pathogen *Mycobacterium tuberculosis* in a less hazardous and more economic approach for the primary screening of new tuberculostatic drugs.[293]

Using the Clinical and Laboratory Standards Institute (CLSI) guidelines, ETPs were analysed by one of two protocols for susceptibility testing, involving broth dilution: the microdilution procedure. This method was performed in a 96-well plate and required a small volume of 0.1 mL of each test compound. This technique guarantees a fast, reliable and high-throughput screening for different compounds. The CLSI also suggest the Mueller Hinton Broth (MHB) as the standard medium for susceptibility testing, since it shows good reproducibility between different batches and contains no sulfonamide, trimethoprim and tetracycline inhibitors.[291]

With the above requirements, MICs for different ETPs were determined using U-shaped 96-well microtitre plates as shown below (Figure **70**).

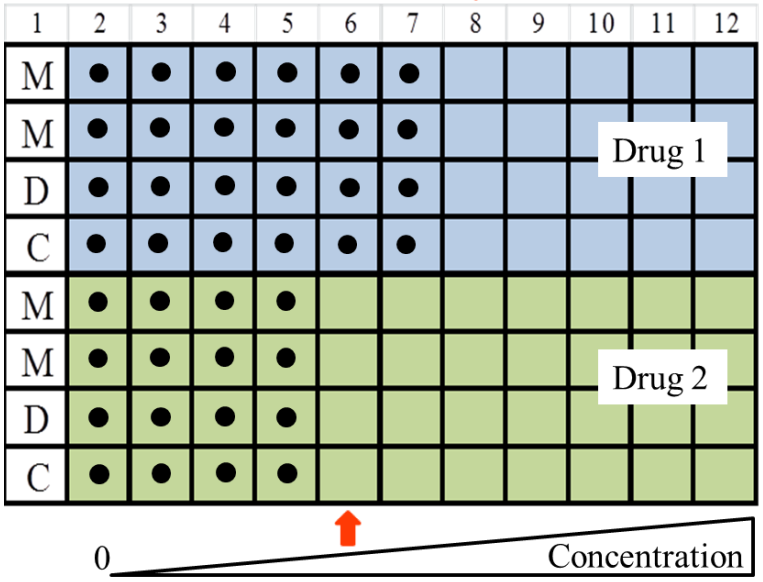


Figure 70 – Schematic representation of the U-shaped 96-well plate for MIC determinations. Increasing concentrations of the test drug are added from column 2 (0 mg/L) to column 12 (32 mg/L). M represents the sterility control for the medium used; D represents the test drug mixed with the medium used and C represents a standard antibiotic known to inhibit growth of the bacteria in study. Red arrows represent the MIC for the examples depicted.

In way of example, increasing concentrations of drug one, shown in blue, and two, represented in green, were tested from columns two to twelve against one bacterial isolate in quadruplicate. Controls for this procedure were included in column one. M referred to 200 µL of MHB and D for 200 µL of the tested drug in the absence of

bacteria. Wells C contained 100 µL of a conventional antibiotic in a concentration known to kill the bacteria in study while red arrows indicate the MIC in mg/L. Bacterial growth can be visualised in the form of bacterial clumps at the bottom of the well or by observation of turbidity. A clear well indicated inhibition of growth.

Stock solutions of ETPs (**425**), (**426**), (**427**), (**430**), (**434**), (**437**) and (**459**) (Table **13**) were prepared at a concentration of 3.2 mg/mL in 100% DMSO and stored at -20 °C until required. For each experiment, stock solutions were diluted to 64 mg/L in an appropriate volume of MHB and 200 µL dispensed into each well on column twelve. 100 µL of MHB were dispensed in all remaining wells. Two-fold serial dilutions of test compounds were prepared from columns twelve to three, for final concentrations between 32 and 0.0625 mg/L, and the remaining 100 µL were discarded. The initial range of drug concentrations tested was adjusted for the following technical replicates.

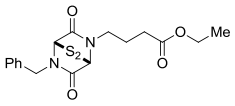
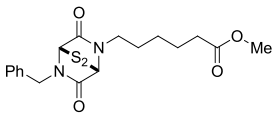
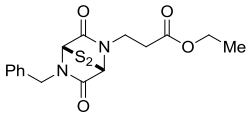
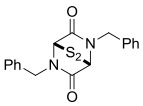
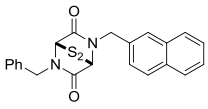
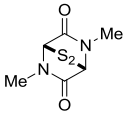
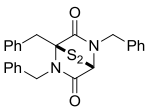
Compound	Structure
425	
426	
427	
430	
434	
437	
459	

Table 13 – ETP structures for microbiological assays

Bacterial suspensions were prepared using CLSI guidelines; bacteria were grown to a density equivalent to 0.5 McFarland (BioMérieux SA, Marcy l'Etoile, France) at 37 °C and then diluted in MHB to a final concentration of 3-7x10^5 colony-forming units *per* mL (CFU/mL). This bacterial suspension was added to all wells to a final volume of 200 µL. The plates were sealed with a plastic cover, shaken gently on an orbital shaker (Bibby Scientific, Stone, UK) to ensure proper re-suspension of the bacteria and incubated at 37 °C.

The MIC procedure for ETPs against *Mycobacterium aurum* was performed in a similar fashion to that described above, with the exception of the preparation of the bacterial suspension. The method used was based on that described by Seidel and Taylor.[294] MHB supplemented with 20 - 25 mg of Ca^{2+} and 10 - 12.5 mg of Mg^{2+} *per* litre (cation adjusted-MHB), was used as growth medium. Mycobacterial strains were plated on to MHA plates and incubated at 37 °C until colonies were visible. Bacterial suspensions were prepared by suspending colonies in a 0.9% saline solution. Glass beads (4 mm diameter, Sigma-Aldrich) were added and the suspensions shaken vigorously to disrupt clumps. Supernatants were adjusted to a 0.5 McFarland standard corresponding to OD_{600} of 0.08 - 0.1 and a bacterial concentration of 5x10^5 CFU/mL. Mycobacterial suspension was then added to all wells to a final volume of 200 µL. Column number one was maintained for control as described before. The incubation period for *M. aurum* A+ was five days. After incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye was added to facilitate MIC visualisation. Metabolically active cells reduce this tetrazolium dye to water-insoluble MTT formazan and converts the initial yellow colour of the dye to dark blue/purple. Bacterial growth was detected by addition of 20 µL of MTT methanol solution (5 g/L) to each well. Plates were incubated at 37 °C for a further 20 – 50 minutes and the MIC value was determined by visual observation. Eight examples of synthesised ETPs were used for these biological assays which included seven disubstituted ETPs (**434**, **430**, **437**, **426**, **427** and **425**) and one tri-substituted ETP (**459**). Test results are shown below (Table **14**).

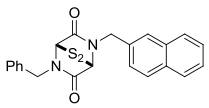
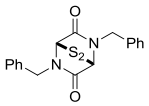
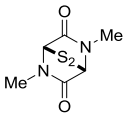
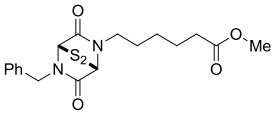
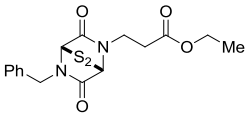
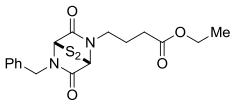
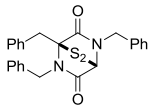
Compound	Structure	MIC (μg/mL)				MIC (μM)			
		EMRSA-16	VRE-1	VRE-10	*M. aurum* A+	EMRSA-16	VRE-1	VRE-10	*M. aurum* A+
434		32	>32	>32	8	79	>79	>79	20
430		32	>32	>32	8	90	>90	>90	22
437		16	>32	8	16	78	>157	39	78
426		32	>32	>32	32	81	>81	>81	81
427		32	>32	>32	16	87	>87	>87	44
425		16	>32	>32	16	42	>84	>84	42
459		16	>32	>32	8	36	>72	>72	18

Table 14 – MIC's for the ETPs microbiological assays using four different strains of resistant bacteria: EMRSA-16, VRE-1, VRE-10 and *M. aurum* A+

Our results showed that compounds (**437**), (**425**) and (**459**) demonstrate relevant activity against EMRSA-16 strain with values ranging to half the concentration needed to prevent bacterial growth when compared to the other tested examples. However, all compounds excluding (**437**) do not exhibit any significant antibacterial activity against both strains of VRE. This can be taken as a positive outcome, since specificity towards a single bacterial strain is crucial in antibacterial drug discovery. Excellent results were

obtained when ETPs were tested against the tuberculosis surrogate (*M. aurum* A+), with compound (**426**) showing the only result above 16 µg/mL for the MIC. All other tested molecules achieved values in the range of 16 to 8 µg/mL (MIC). In terms of structure relationship activity (SRA) for the tuberculosis assay, compounds that have longer chain substituents as (**426**), (**427**) and (**425**) show less activity (16 µg/mL) when compared to molecules with short length substituents (i.e. **430**). However, ETPs (**434**), (**430**) and (**459**) that both have bulkier built-in substituents (i. e. benzyl and naphthyl) showed better antimycobacterial outcomes (8 µg/mL) when compared to other molecules with only one benzylic substituent (eg. **427**). ETPs that contain more aromatic groups proved to have better antibacterial activity against *M. aurum* A+ than others with only aliphatic substituents (eg. **437**). Lipophilicity of the tested molecules was shown to be a relevant characteristic for SRA since a decrease in hydrophilicity was translated in an increase of antibacterial activity. Examples of this occurrence are demonstrated for compound (**437**) with a partition coefficient (CLogP) of 0.27 and a 16 µg/mL MIC when compared to compounds (**430**), (**434**) and (**459**) with 8 µg/mL MICs with CLogP values ranging between 3 – 5, as shown below (Figure **71**).

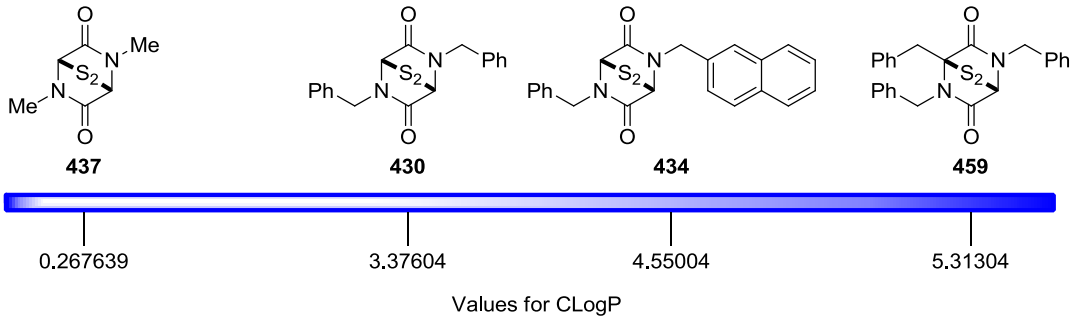


Figure 71 – CLogP of different ETPs tested against resistant bacteria

Molarity values showed by these molecules regarding their MICs are quite encouraging for antibacterial activity since they range from 22 µM (**430**) to 18 µM (**459**).[295, 296, 297]

3.5.2 ETPs Anticancer assays

For this I would like to thank Doctor Andy Wilderspin from the Pharmaceutical & Biological Chemistry Department at School of Pharmacy UCL for allowing me the use

*of his laboratory and developed assays to explore the anticancer testing of ETPs. I would also like to express my gratefulness to Andreia Guimarães from Doctor Wilderspin's group who took the effort and skills to investigate the anticancer activity of our ETPs using an in-house assay. All the work shown below in section **3.5.2** was developed in Doctor Andy Wilderspin's laboratory in collaboration with Andreia Guimarães.*

The widespread reported biological activity of the ETP family of natural products was first demonstrated by the anticancer associated activity of gliotoxin in 1936.[4] Since then, ETPs have proven to be potent anticancer agents with both the natural and synthetic examples reported.[258, 112]

In order to interpret the biological activity of the synthesised ETPs a homogeneous assay based on fluorescence resonance energy transfer (FRET) was used.[298] This assay developed by Doctor Andy Wilderspin's group detected protein-protein interactions associated with the *locus* where ETPs express their biological activity – the HIF-1α C-terminal activation (C-TAD) and the cysteine-histidine rich 1 (CH1) domains of the p300 co-activator. These two proteins were tagged with fluorescent markers. HIF-1α C-TAD was marked with a cyan fluorescent protein (CFPTEV HIF-1α C-TAD) and the p300 CH1 tagged with a yellow fluorescent protein (YFPTEV p300 CH1). The interaction between these two provided a FRET signal indicative of the proximity of both proteins.[299]

The assay consisted in a 1:1 ratio of CFPTEV HIF-1α C-TAD and YFPTEV p300 CH1 using a 96-well flat-bottom non-binding plate (Corning™) to form the complex. The apparatus used for measurements was a PerkinElmer EnVision 2101 Multilable reader (excitation 405 nm, emission 535 nm). Stock solutions of a small library of ETPs: (**18**), (**425**), (**426**), (**427**), (**429**), (**431**), (**432**), (**433**), (**437**), (**459**), (**483**) and (**488**) (Table **15**); were prepared in 10% methanol / 90% water and stored at -20 °C until required. DMSO was not used as a solvent since it interfered with the test results due to an intense colour change by the solution that was formed. For each experiment, stock solutions were diluted to 1000, 500, 300, 100, 50, 30, 10, 5, 3 and 1 μM in an appropriate volume of water.

Compound	Structure	Compound	Structure

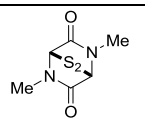
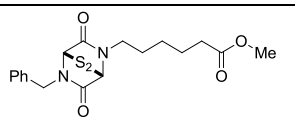
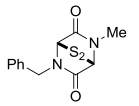
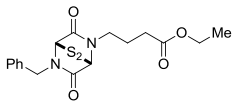
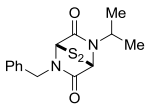
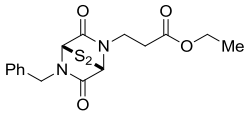
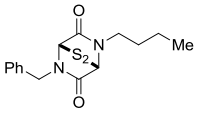
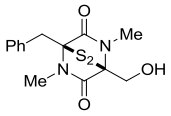
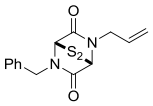
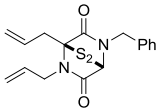
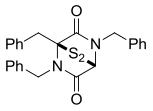
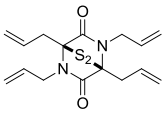
437		**426**	
433		**425**	
432		**427**	
429		**18**	
431		**483**	
459		**488**	

Table 15 – ETP structures for anticancer assay

The monitoring of this complex formation was carried out by FRET analysis enabling the fast screening of potential molecular inhibitors. Any hits from the screen were expected to disrupt the FRET signal by binding to the YFPTEV p300 CH1 domain, disabling the protein complex in this manner. Initial measurements from the screening corresponded to those from each of the isolated proteins: YFPTEV p300 CH1 and CFPTEV HIF-1α C-TAD; and the resulting protein-protein interaction complex, CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 (Chart **2**).

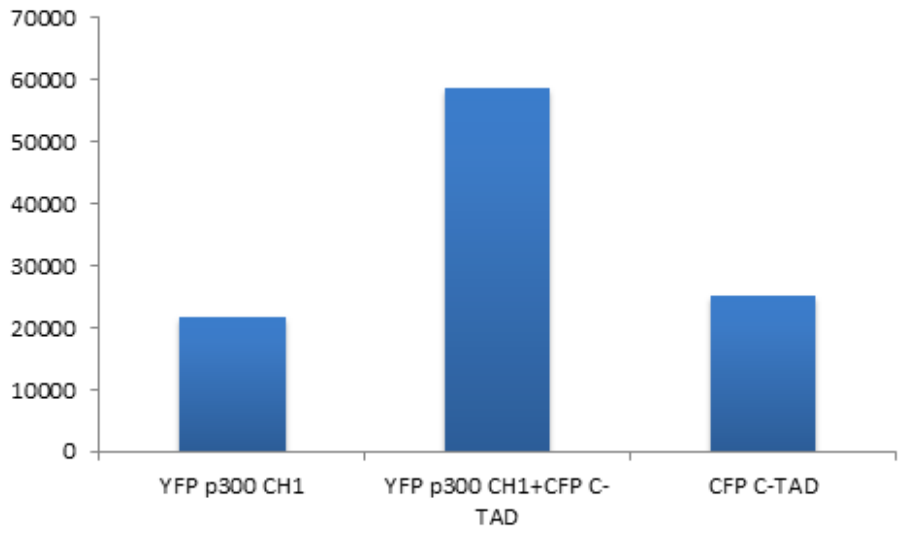


Chart 2 – FRET measurements for YFPTEV p300 CH1, CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex and CFPTEV HIF-1α C-TAD in RFU

As shown above (Chart **2**), YFPTEV p300 CH1 presented 21,776 relative fluorescence units (RFU) compare to CFPTEV HIF-1α C-TAD with 25,263 RFU, setting the value for the experimental threshold. The complex CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 resulted in 58,803 RFU that provided an end limit for the assay in a 1:1 ratio experiment with both labelled proteins.

Using these average values as the start and end points for our research, we next tested a small library of ETPs by applying the same methodology. All twelve ETPs used during this experiment were first mixed separately with each labelled protein (YFPTEV p300 CH1 and CFPTEV HIF-1α C-TAD) to demonstrate that no variation from the initial setup values was seen, providing us with a range of standards during the course of the tests. The outcomes were expressed by percentage of inhibition of the CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 interaction as shown below.

3.5.2.1 Compound **437**

Results for ETP (**437**) clearly show an inhibition of 48% of the CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex at 500 µM concentration. Results decrease to 7% in the lowest concentration tested of 1 µM (Chart **3**).

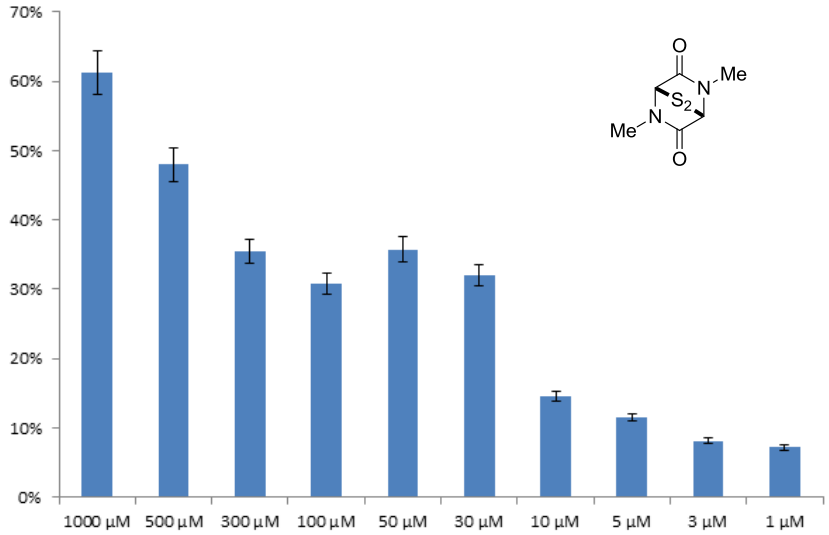


Chart 3 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (compound **437**)

3.5.2.2 Compound **433**

Significant changes were seen in compound (**433**) since 1000 µM of the ETP inhibited 77% of protein-protein interaction whereas using half this concentration (500 µM) only 41% inhibition occurred. Values were kept in a small range of variation between 500 and 30 µM (14%) with a decline in activity from the 10 µM tested concentration with 10% inhibition of the complex (Chart **4**).

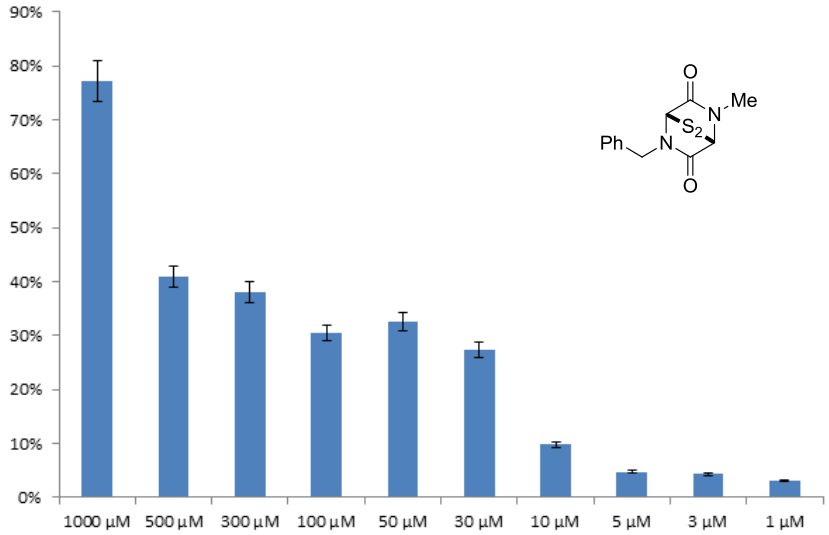


Chart 4 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (compound **433**)

3.5.2.3 Compound **432**

Compound (**432**) showed a key decrease in activity from 500 to 300 µM concentration with a 26% drop. However, the most significant loss of inhibition of the CFPTEV HIF-

1α C-TAD / YFPTEV p300 CH1 complex came with the 10 µM assay (11%) where the previous tested concentration (30 µM) showed results of 28% (Chart **5**).

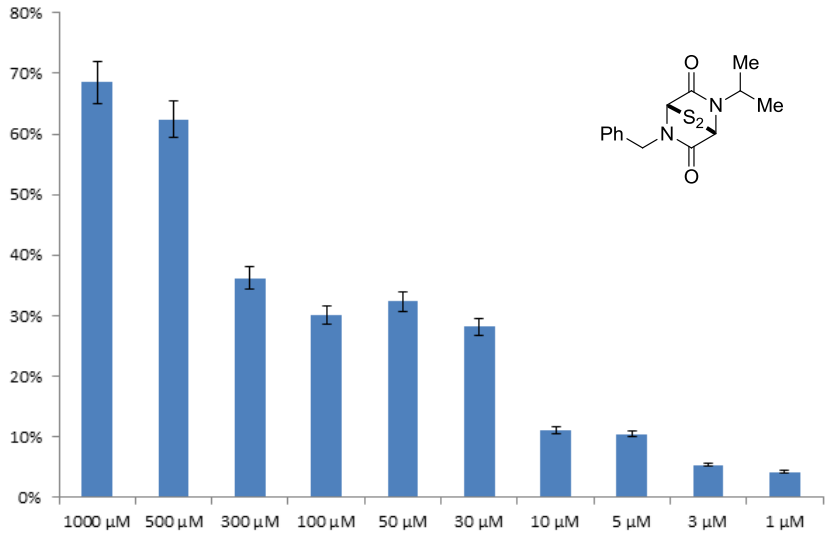


Chart 5 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (compound **432**)

3.5.2.4 Compound **429**

ETP (**429**) showed a linear increase of activity from 1 µM (12%) to 1000 µM (77%) concentration. The highest decrease in inhibition of protein-protein interaction was seen between 300 µM (62%) and 100 µM (44%) (Chart **6**).

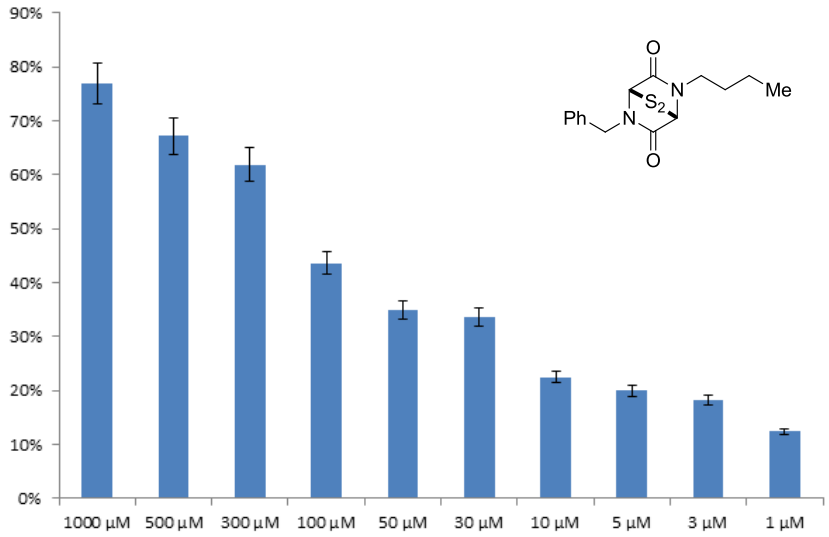


Chart 6 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (compound **429**)

3.5.2.5 Compound **431**

This asymmetric disubstituted ETP (**431**) showed promising results when compared to the previously tested molecules, since inhibition of the CFPTEV HIF-1α C-TAD /

YFPTEV p300 CH1 complex at 1 µM concentration was 38%. Results were maintained from an 80% inactivation of protein binding at 1000 µM to 77% at 500 µM. In the range of 300 to 50 µM a short reduction in activity was seen with a difference of 3% between the tests performed (Chart **7**).

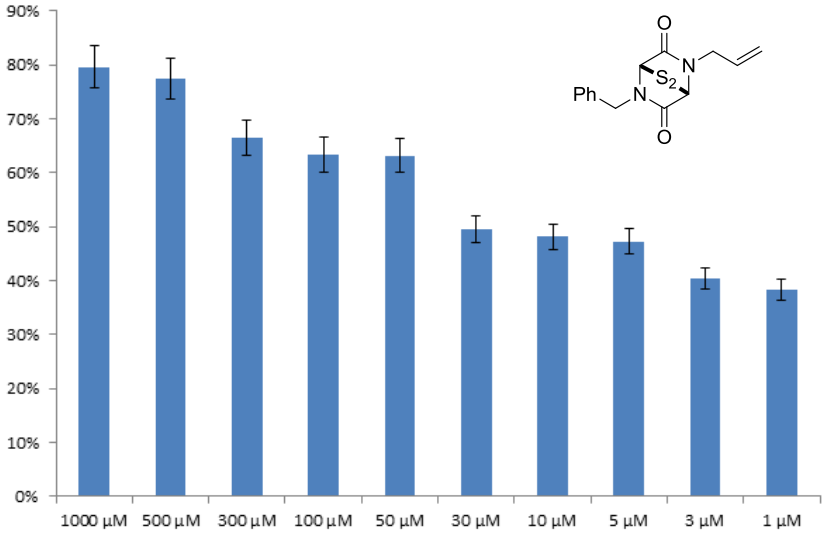


Chart 7 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (compound **431**)

3.5.2.6 Compound **459**

Tri-benzyl substituted ETP (**459**) gave the best results from this small library of compounds. The values for protein-protein inhibition ranged from 99 to 78%. Data shown below (Chart **8**) illustrates the potency of this analogue since dilution from 1000 to 1 µM in concentration only decreased the outcome of the assay by 19%, highlighting the potential of this compound to inhibit the binding of HIF-1α with p300.

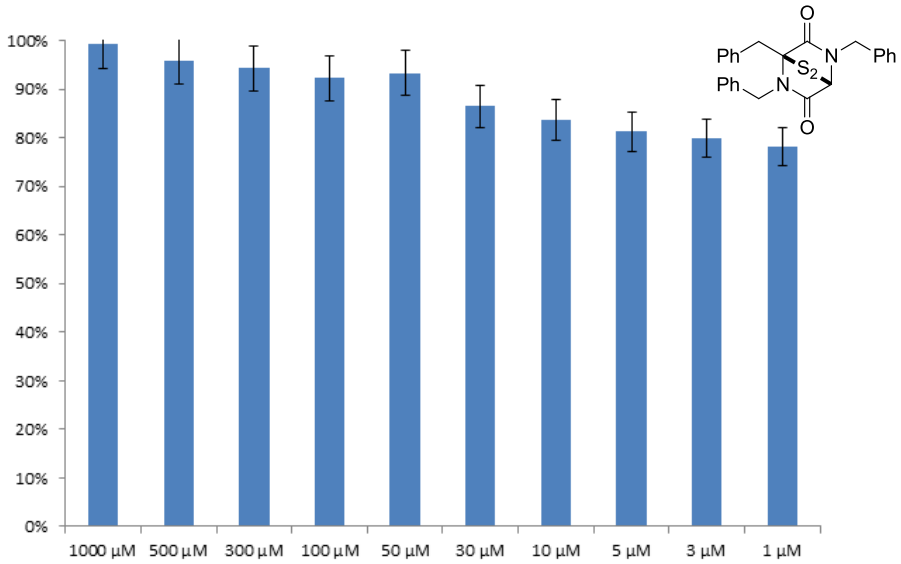


Chart 8 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (compound **459**)

3.5.2.7 Compound **426**

ETPs with different length carbon chain esters were also synthesised using the newly developed base catalysed approach. One of the examples – compound (**426**) – was tested and the inhibition of protein binding ranged between similar values to that seen before: 53% (1000 µM) and 16% (1 µM), respectively (Chart **9**).

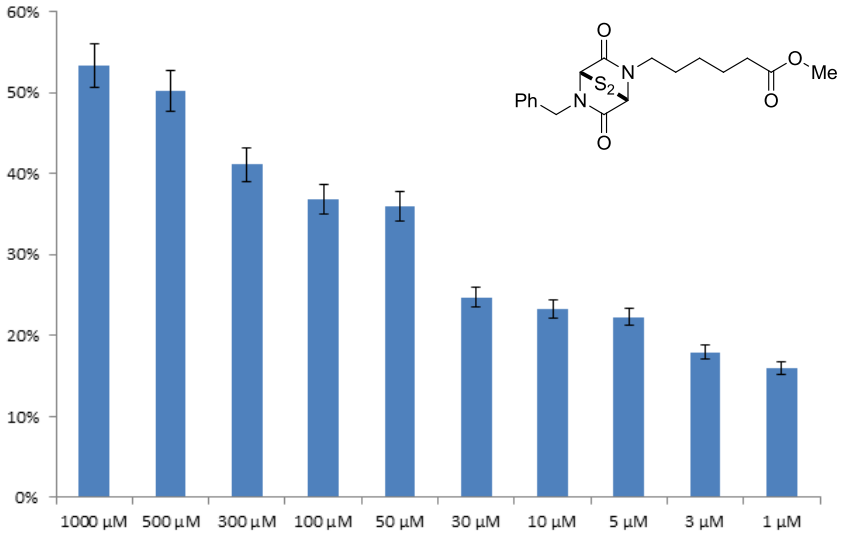


Chart 9 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (compound **426**)

3.5.2.8 Compound **425**

One of the other tested ETP substituted esters (**426**) showed similar results to compound (**425**). CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 inhibition was achieved at a maximum value of 52% at 1000 µM concentration. A significant decrease in activity was observed between 50 µM (30%) and 30 µM (20%) (Chart **10**).

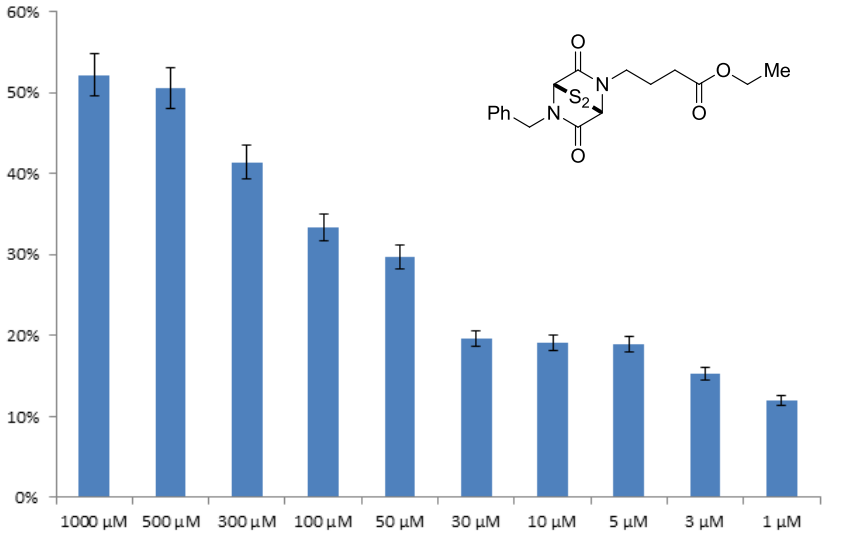


Chart 10 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (compound **425**)

3.5.2.9 Compound **427**

Compound (**427**) gave a similar profile in its protein-protein inhibition rate at 1000 µM (55%) as its ester substituted ETP congeners. This compound showed low activity from 30 µM (10%) to 1 µM (4%) concentration (Chart **11**).

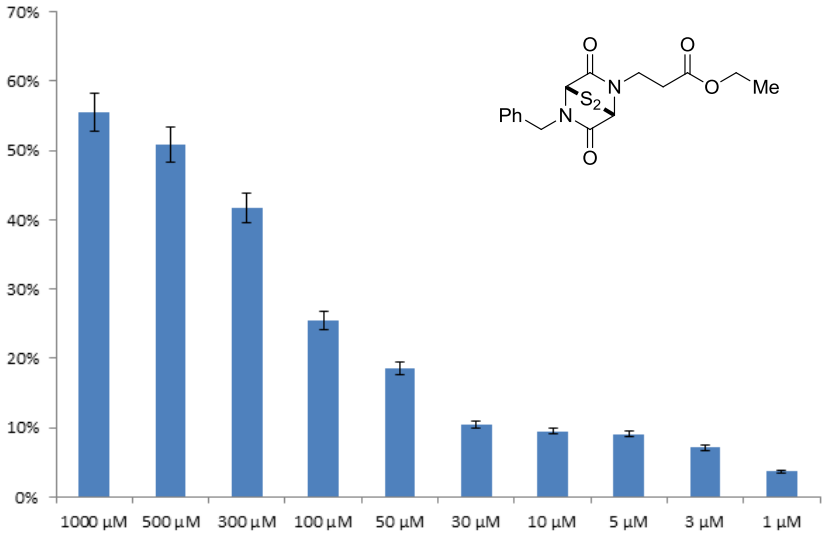


Chart 11 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (compound **427**)

3.5.2.10 Compound **18** (Hyalodendrin)

Hyalodendrin (**18**) was the only natural product used in the assay and gave results ranging from 53% (1000 µM) to 1% (1 µM) inhibition of the CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex. This compound showed a significant loss of activity between 300 µM (41%) and 100 µM (20%) (Chart **12**).

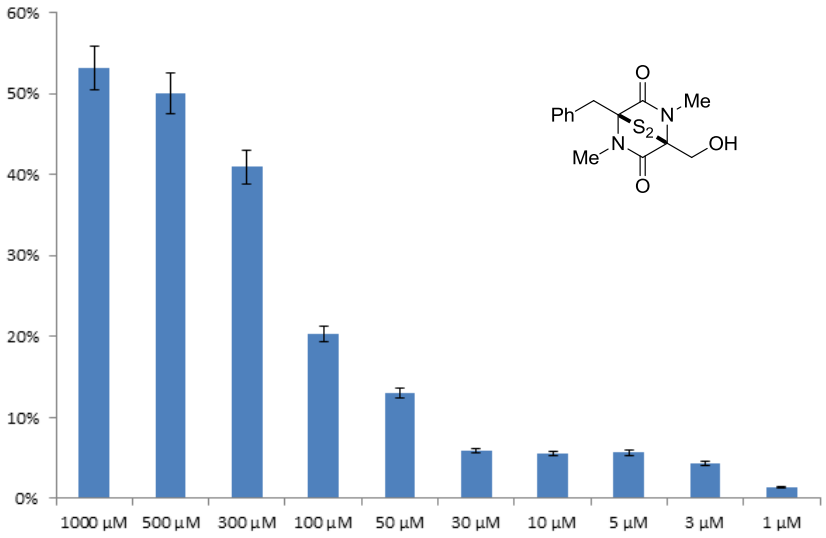


Chart 12 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (compound **18**)

3.5.2.11 Compound **483**

Tri-substituted ETP (**483**) showed percentages of protein inhibition in a range of 52% (1000 µM) to 4% (1 µM). Analogous to hyalodendrin (**18**), this compound also gave a decrease in activity between the 300 µM (42%) and 100 µM (25%) concentration values used for the assay (Chart **13**).

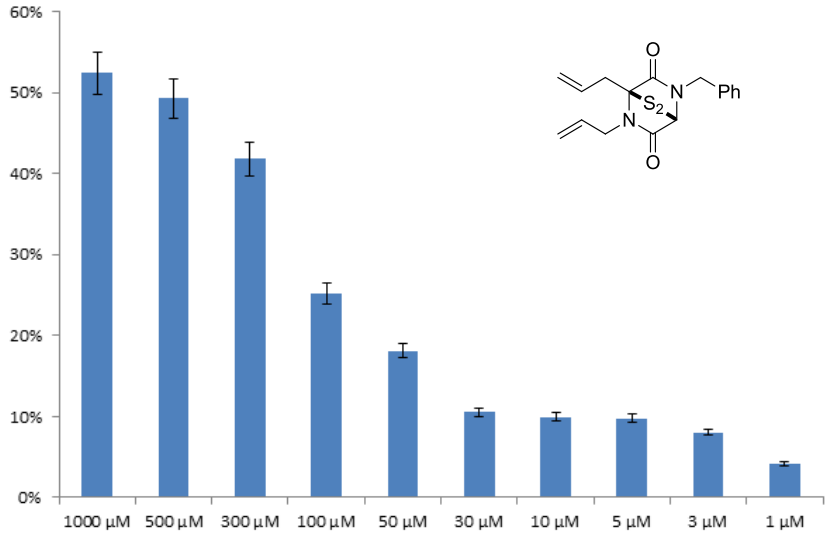


Chart 13 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (compound **483**)

3.5.2.12 Compound **488**

Tetra-substituted ETP (**488**) gave initial constant CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex inhibition results for concentrations 1000 µM (52%), 500 µM (49%) and 300 µM (49%). However, this last value declined to 25% for 100 µM concentration and a steady state was reached at 4% total activity at 1 µM (Chart **14**).

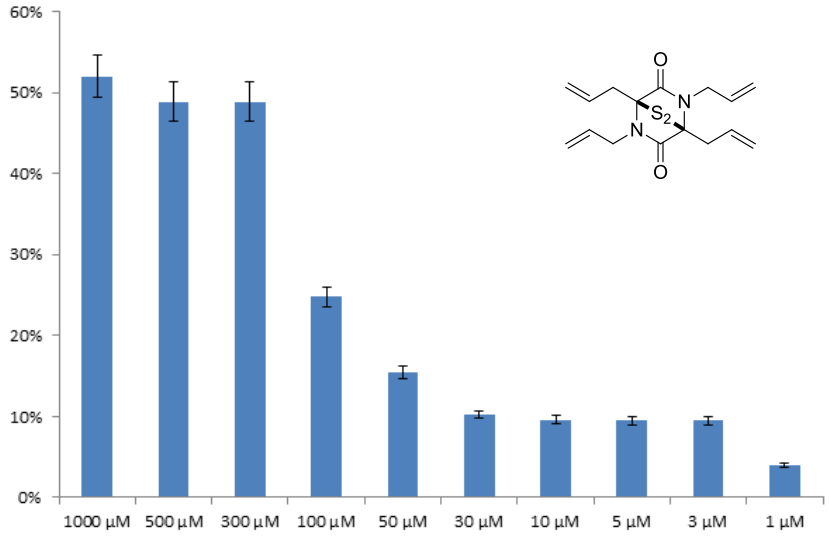


Chart 14 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (compound **488**)

Results from the FRET CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex inhibition assay clearly showed the dominance of compound (**459**) when compared to other tested compounds with an average of 78% inhibition at a 1 µM range (Chart **15**).

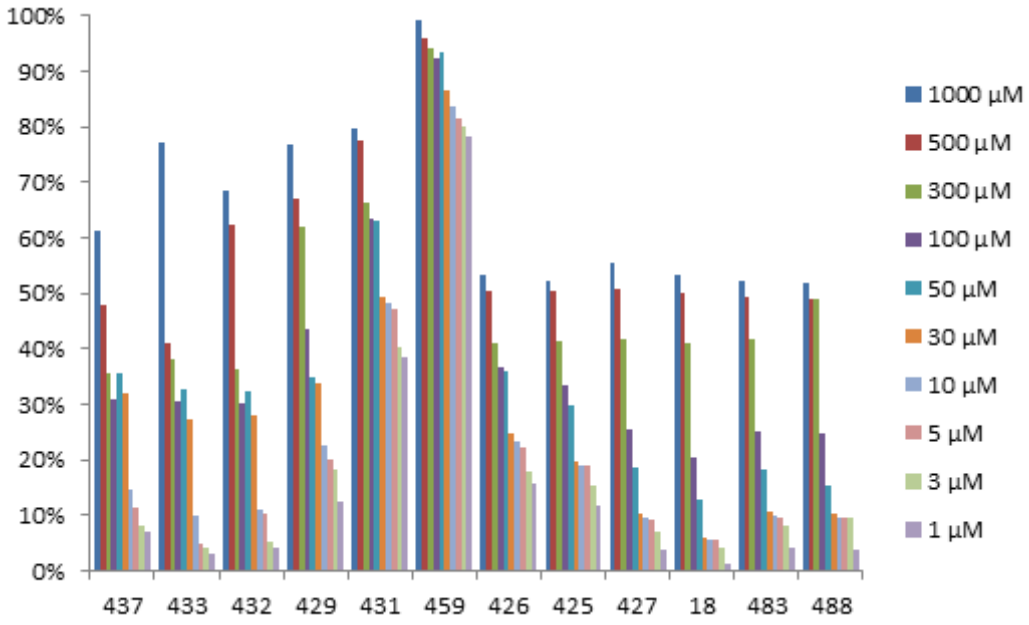


Chart 15 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (ETPs anticancer assay summary)

When compared to only aliphatic disubstituted ETP (**437**), compound (**459**) clearly highlighted the importance of aromatic substituents around the ETP core since inhibition of protein-protein interaction for (**437**) gave an average value of 7% at 1 µM concentration. The same low range of inhibition was also shown by hyalodendrin (**18**), the only ETP natural product tested, having the same core structure as (**437**) with variations on the remaining *3*- and *6*-positions of the ETP ring (Figure **72**).

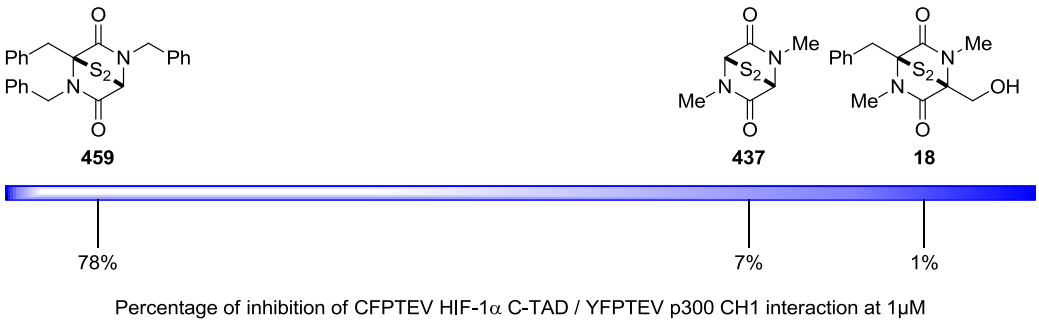


Figure 72 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (correlation between compounds **459**, **437** and **18**)

Compounds (**433**), (**429**) and (**431**) with percentage inhibitions above 75% at 1000 µM proved to be very good candidates at an initial stage. However, the values dropped to

significant lower percentages below 15% when the concentrations were lowered to 1 µM for both aliphatic saturated substituted ETPs (i. e. **433**, **429**). The same occurred for compound (**432**) which possessed similar molecular saturated features. A 38% inhibition of the CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex was achieved at 1 µM concentration for compound (**431**) proving the importance of the unsaturated carbon ETP substituent when compare to different saturated carbon chains of disubstituted ETPs (**433**), (**429**) and (**432**) (Figure **73**).

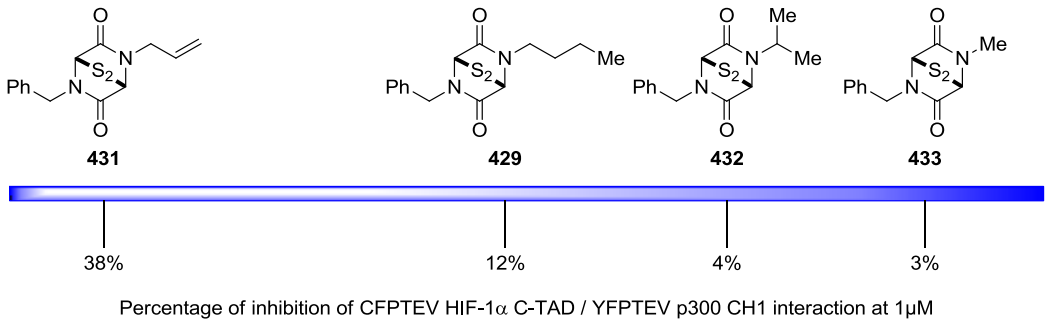


Figure 73 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (correlation between compounds **431**, **429**, **432** and **433**)

Based on these findings we decided to test different synthesised compounds that contained more unsaturated carbons in the core structure. ETPs (**483**) and (**488**) were chosen for this testing since both contained more than one allyl substituent. However, results indicated the absence of a correlation between ETPs with greater number of unsaturated carbons and an increase in protein-protein interaction inhibition as shown below (Figure **74**).

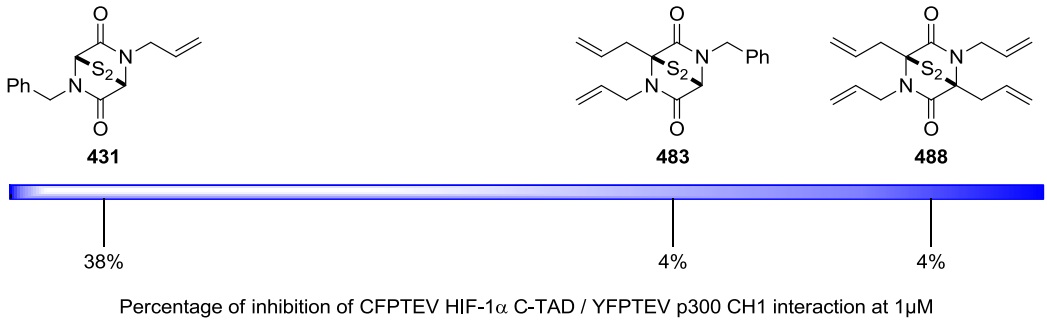


Figure 74 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (correlation between compounds **431**, **483** and **488**)

ETPs containing different ester substituents (**426**, **425** and **427**) showed that the length of the carbon chain had some influence on the outcome of the assay. The values for inhibition of protein-protein interaction were similar for the three tested compounds

until dilution reached 300 µM concentration where considerable changes in inhibition were seen. Shorter carbon chain ester compound (**427**) showed a reduction in activity from 42 to 25% when diluted from 300 to 100 µM concentration. Compounds (**426**) and (**425**) also showed significant decreases in activity from 50 to 30 µM concentration. However, the percentages of complex inhibition for these molecules clearly show the correlation between the lengths of the carbon chains associated with the ester substituents at these concentrations. Longer carbon chain ester ETP (**427**) gave a higher percentage of activity (16%) at 1 µM concentration that disubstituted ETP (**425**) (12%), which showed a higher percentage of inhibition when compared to a shorter carbon chain ester ETP (**426**) (4%) at the same tested concentration (Figure **75**).

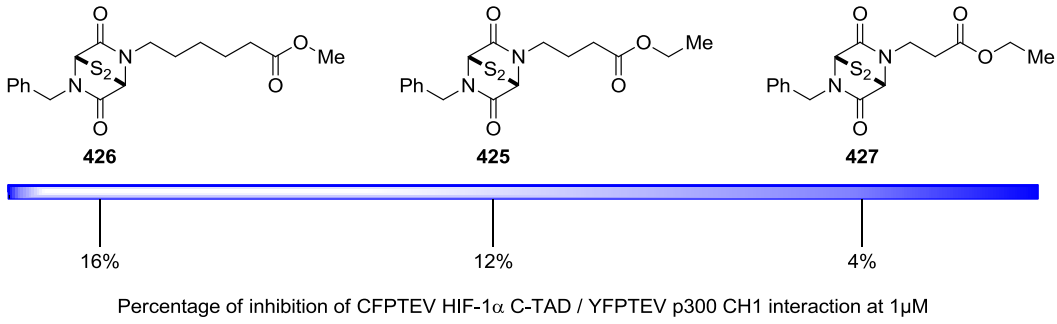


Figure 75 – Inhibition of CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex (correlation between compounds **426**, **425** and **427**)

3.5.3 ETPs Antiviral assay

*For this I would like to thank Professor Doctor Hofmann-Lehmann, Doctor M. L. Meli and T. Meili from the Clinical Laboratory, Vetsuisse Faculty, University of Zürich (Switzerland); Professor Antti Poso and Doctor J. T. Laitinen from the School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland (Finland); and, Doctor K. Allenspach from Veterinary Clinical Sciences, Royal Veterinary College (United Kingdom) for providing the services and knowledge to develop all the virology work. I would also like to express my appreciation to Christopher Asquith from Doctor Stephen Hilton's group that took the task of investigating the ETPs antiviral activity. All the work shown below in section **3.5.3** was developed in Professor Doctor Hofmann-Lehmann laboratory in collaboration with Christopher Asquith.*

As described before, retroviral nucleocapsid proteins contain two zinc finger motifs that mediate genomic RNA packaging into virions. As demonstrated for the p300 CH1 rich domain, ETPs have the ability to bind to zinc disabling its protein interactions.[253] Using

the same principle to target the RNA transcription stage could provide an alternative in the fight against HIV.[300, 301, 302]

Therapeutic resistance to anti-viral drugs via mutation is a major challenge affecting both human and veterinary medicine. Since its discovery in 1984, just one single species disease - human immunodeficiency virus (HIV) has caused 25 million deaths and 34 million infected worldwide with 2.6 million new infections in 2009. This clearly highlights the unique challenge in this research area. Amongst non-human vertebrates, feline immunodeficiency virus (FIV) infection is perhaps the closest biological model of HIV infection with an analogous late-stage AIDS-type progression.[303]

FIV infection primarily occurs through biting and during mating with about 44 million cats currently infected worldwide. Targeting the mutation resistant nucleocapsid protein in FIV that performs the same role as the NCp7 protein in HIV can determine a new therapeutic approach towards HIV treatment.[302]

3.5.3.1 Cytotoxicity for different cell lines

To test the cytotoxicity of ETPs, treatment of Crandell Rees feline kidney (CRFK) cell line was carried out using three higher level concentrations (100 µM, 10 µM and 1 µM) of compounds: (Gliotoxin (**1**)), (Hyalodendrin (**18**)), (Chaetocin A (**42**)), (**425**), (**426**), (**427**), (**429**), (**430**), (**431**), (**432**), (**433**), (**434**), (**435**), (**455**), (**459**), (**464**), (**471**), (**483**), (**487**), (**488**), (**499**), (**516**) and (**517**) (Table **16**).

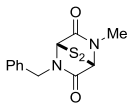
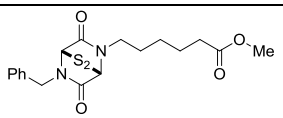
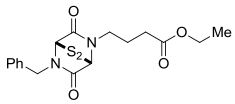
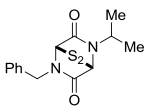
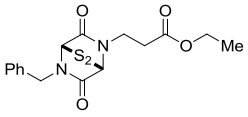
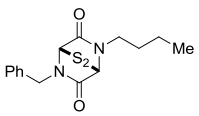
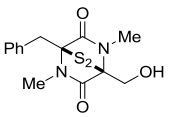
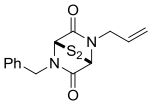
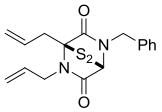
Compound	Structure	Compound	Structure
433		**426**	
		425	
432		**427**	
429		**18**	
431		**483**	

Table 16 – Continues next page

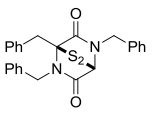
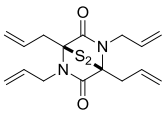
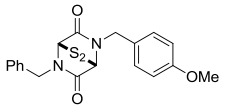
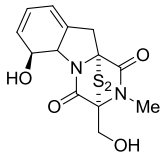
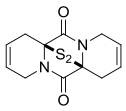
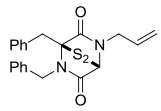
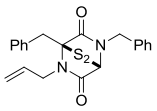
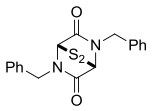
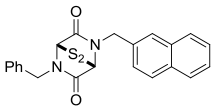
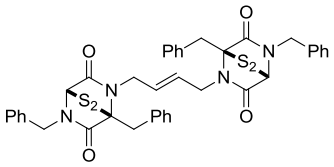
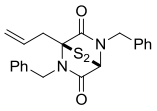
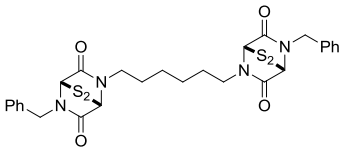
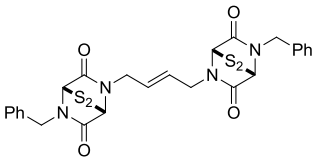
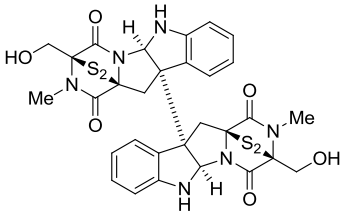
459		**488**	
435		**1**	
487		**464**	
471		**430**	
434		**517**	
455		**499**	
516		**42**	

Table 16 – ETP structures for antiviral assays

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the compounds' toxicity and viability of cells by MTT dye reduction.

First 10,000 CRFK cells were eluted with Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum 100 M/mL Glutamine and 1% v/v antibiotic antimycotic (Ab/Am) (total volume 200 µL) onto 96-well plates and incubated for 24 hours (37 ºC, 5% CO_2). The medium was then removed by vacuum and replaced with the specific dilution of the compounds to test (200 µL). Compounds were suspended in 2% DMSO and RPMI 1640 medium supplemented with 10% fetal bovine serum 100 M/mL Glutamine and 1% v/v antibiotic / antimycotic (Ab/Am) and diluted to the desired concentrations. After 24 hours, the wells were observed and the medium removed.

The cells were then suspended in RPMI 1640 phenol red-free medium (180 µL) and MTT (3 mg/mL) (20 µL) and incubated 4 hours at (37 ºC, 5% CO_2). The medium was then removed by vacuum and the cells were lyse with methanol (200 µL) to reveal a bright purple formazan product. The methanol-formazan absorbance was then determined at 570 nm using a BioTek Synergy HT plate reader with KC4 software.

Data was expressed as the percentage of viability (normalised to cells with no exposure) of compound-treated wells compared to that of untreated control wells. The same procedure was also carried out for the feline lymphoblastoid cell line 4 (FL-4) using six higher level concentrations (100 µM, 10 µM, 1 µM, 100 nM, 10 nM and 1 nM) of the previously reported compounds. In the FL-4 cell line, 50% of cytotoxic concentration (CC_{50}) is defined as the concentration of drug at which the tissue viability was reduced to 50% of the drug-free control value. Compounds that passed the initial 24 hours exposure test with a toxicity of less than 25% were then screened against FIV. The MTT assay is a colorimetric assay and that can be used for measuring the viability living cells. The mitochondrial reductase enzymes reduce the tetrazolium salt by cleavage of the central core to produce an insoluble formazan which gives a purple colour. This assay measures the cellular metabolic activity via nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) dependent cellular oxidoreductase enzymes. The amount of formazan dye is directly proportional to the amount of viable cells or cytotoxicity.[304]

ETP synthetic analogues showed that even after longer term exposure, it was possible to have compounds containing the ETP core that were non-toxic (Table **17**). This was significant since it highlighted the potential for further development and clearly showed the limitation of the harmful effects previously associated with ETPs. In contrast, natural products containing the ETP core showed high toxicity when tested. Gliotoxin (**1**) caused complete cell lysis at a concentration as low as 1 µM in CRFK cells and produced no detectable results on the MTT assay. Hyalodendrin (**18**) only showed significant results at 1 µM concentration.

All other monomeric synthetic ETPs were followed up for additional analysis apart from compounds (**430**) and (**431**). Remarkably, compound (**434**) showed no toxic effects even when tested at the limiting concentration of 100 µM in CRFK cell line. Chaetocin A (**42**) also showed heavy toxicity along with some of the dimeric ETPs tested: (**499**) and (**516**). However, dimeric ETP (**517**) showed good results at 10 µM

concentration leading to further testing. CC_{50} results for compounds (**433**), (**427**), (**426**), (**483**), (**464**) and (**517**) clearly showed the importance of synthetic analogues when compared to the homologous natural products.[253] The different toxicity profiles meant that the compounds were exposed to the cells and had significant pharmokinetic properties.

	CRFK cell line				FL-4 cell line						
Compound	100uM	10uM	1uM	Test FIV	100uM	10uM	1uM	100nM	10nM	1nM	CC50
435	32.3%	98.9%	>100	Yes							
432	49.0%	>100	>100	Yes							
429	35.0%	>100	>100	Yes							
Gliotoxin (1)	0%	0%	0%	No							
433	16.9%	>100	>100	Yes		>100	>100	>100	>100	>100	>10uM
431	48.8%	63.8%	50.2%	No							
459	49.3%	96.8%	>100	Yes		37.91	41.45	61.26	>100	>100	611.45nM
427	81.6%	>100	>100	Yes		63.60	>100	>100	>100	>100	>10uM
426	85.6%	>100	>100	Yes		93.65	>100	>100	>100	>100	>10uM
425	68.5%	>100	>100	Yes		31.07	99.28	>100	>100	>100	7.51uM
Hyalodendrin (18)	34.3%	86.5%	91.2%	No							
483	88.7%	>100	>100	Yes	62.18	98.70	>100	>100	>100	>100	>100uM
488	50.1%	99.4%	>100	Yes		43.37	95.09	>100	>100	>100	8.84uM
487	62.5%	>100	>100	Yes		34.13	89.96	94.61	>100	>100	7.44uM
464	87.6%	>100	>100	Yes	34.39	>100	>100	>100	>100	>100	99.98uM
471	71.0%	85.1%	>100	Yes		34.37	27.59	98.47	>100	>100	715.86nM
430	39.1%	52.5%	>100	No							
434	>100	>100	>100	Yes	36.79	30.05	94.05	>100	>100	>100	7.19uM
517	28.8%	>100	>100	Yes		61.38	>100	>100	92.51	91.39	>10uM
455	25.8%	>100	>100	Yes		25.54	81.10	>100	89.75	>100	6.04uM
499	29.7%	60.2%	77.7%	No							
516	29.7%	29.1%	77.7%	No							
Chaetocin A (42)	-	-	-	Yes		10.75	36.50	30.71	20.93	31.70	<1nM

Table 17 – Toxicity results from Initial CRFK Kidney cell line and from the subsequent FL-4 (FIV) Lymphocyte cell line. Toxic concentrations are shown in red, while mild/non-toxic are shown in green. The CC_{50} values correspond to the FL-4 (FIV) cell line.

The second stage of testing involved the testing of compounds at six concentrations (100 µM, 10 µM, 1 µM, 100 nM, 10 nM and 1 nM) unless otherwise determined by the 24 hours assay. The cells were exposed to the compounds for 7 days and sampled every day for each concentration. This cell supernatant sample was then extracted in a total nucleic acid (TNA) [304] extraction and subsequently used to determine the viral load by a polymerase chain reaction (PCR) for FIV RNA. The DNA and albumin were also tested as controls (results not shown) to detect any interferences. If the compound was active it should show a decrease in the detection value Ct (threshold cycle). After the 7 day screen, the cells were also checked for their viability to rule out any toxicity effects and validate the PCR result.[304]

3.5.3.2 FIV Ct values

To calculate Ct values, 10,000 FL-4 (FIV) cells were eluted with RPMI 1640 medium supplemented with 10% fetal bovine serum 100 M/mL Glutamine and 1% v/v antibiotic / antimycotic (Ab/Am) (total volume: 200 µL) onto 96-well plates and incubated for 24 hours (37 ºC, 5% CO_2). Compounds were suspended in 2% DMSO and RPMI 1640 medium supplemented with 10% fetal bovine serum 100 M/mL Glutamine and 1% v/v antibiotic antimycotic (Ab/Am) and diluted to the desired concentrations. On day zero the plate was first centrifuged (150 g, 5 minutes) and 100 µL of supernatant was removed from each well and replaced with twice the concentrated compound dilution in horizontal triplicate. Day 1 – 6 follow the same procedure but at the normal concentration; the 100 µL supernatant was pooled to create a 300 µL batch that was frozen immediately at -20 ºC.

The total nucleic acids were extracted as previously reported from 200 µL of supernatant and 300 µL lysis buffer using MagNA Pure LC Total Nucleic Acid (TNA) isolation kit and a MagNA Pure LC instrument (Roche Diagnostics, Rotkreuz, Switzerland), according to the manufacturer's recommendations. The elution volume was 90 µL. Extracted samples were stored at -80 ºC until analysis. Negative controls were used in all extractions and consisted of 200 µL phosphate-buffer saline solution prepared concurrently with each batch of samples to monitor for cross-contamination.

All TNA samples were tested by TaqMan® real-time PCR for the presence and load FIV RNA and FIV DNA on an ABI PRISM 7700/7500 Sequence Detection System (Applied Biosystems, Rotkreuz, Switzerland). PCR runs were performed with concurrent negative controls of RNA free water.

The PCR was performed in a reaction volume of 25 µL, using of master mix (12.5 µL) (Eurogentec), Primer FIV 552f (800 nM, 1 µL) (Microsynth, Switzerland), Primer FIV672r (800 nM, 1 µL) (Microsynth, Switzerland), Probe FIV 582p (160 nM, 0.4 µL) (Microsynth, Switzerland), Euroscript Reverse Transcriptase enzyme (0.125 µL) (Eurogentec) and RNA free water (4.975 µL) or RNA free water (5.1 µL) for RNA or DNA, respectively, followed by TNA (5 µL). The preparation was pipetted on ice at 0 ºC. The PCR cycle was ran with the following cycle parameters: 2 minutes at 50 ºC or 30 minutes 48 ºC for RNA and DNA, respectively, and 10 minutes at 95 ºC; followed by 45 cycles of 95 ºC for 15 seconds, 60 ºC for 1 min.

On Day 7 the final 100 µL was collected and pooled so medium RPMI 1640 (Phenol-Red free) (100 µL) could be added to each well, followed by MTT (3 mg/mL) (20 µL) and incubated 4 hours at (37 ºC, 5% CO_2). The medium was then removed by vacuum and the cells were lysied with methanol (200 µL) to reveal a dark purple formazan product. The methanol-formazan absorbance was then determined at 570 nm (Bio-Tek Synergy HT plate reader with KC4 software). Data was expressed as the percentage of viability (normalised to cells with no exposure) of compound-treated wells compared to that of untreated control wells. The 50% cytotoxic concentration (CC_{50}) is defined as before.[304]

We observed a full spectrum of results from inactive/toxic compounds at high concentration to highly active compounds at low concentration. The FIV screening assay measured the viral loading with a constant level of compound to see if there was a reduction or stabilization. Since Ct value is the detection limit of the virus by PCR, the longer it took for the virus to be detected the less virus was shed from the cells and hence the lower the viral load in the supernatant. Day one and seven (Table **18**) showed a marked progression towards higher Ct values and hence more virus was shed as the cells multiplied.

	Day 1						Day 7					
Number	100uM	10uM	1uM	100nM	10nM	1nM	100uM	10uM	1uM	100nM	10nM	1nM
435												
432	25.1	25.2	25.0				28.4	27.8	25.0			
429	25.1	25.2	24.9				27.6	28.1	18.3			
Gliotoxin (1)												
433		24.0	24.3	24.5	24.2	24.6		18.4	18.3	18.7	30.6	19.4
431												
459		24.0	24.2	24.5	24.2	24.3		27.3	30.4	25.9	20.4	19.2
427		23.5	24.3	24.4	24.3	24.4		29.0	18.7	18.7	18.1	17.6
426		24.1	22.9	24.0	23.2	23.0		19.1	18.6	18.6	18.3	17.6
425		25.0	23.9	23.4	23.6	23.7		28.3	18.4	17.8	17.8	18.0
Hyalodendrin (18)												
483	23.8	23.2	23.1	24.1	23.9	24.5	20.5	18.1	17.5	16.2	16.4	16.3
488		24.5	24.3	24.6	24.4	24.1		18.9	18.4	17.8	17.6	16.8
487		25.2	24.6	25.0	24.4	24.6		21.9	18.7	16.9	16.6	16.8
464	24.8	24.5	24.6	24.4	24.4	24.6	28.6	18.9	18.5	17.6	17.8	17.6
471		23.2	24.3	24.8	24.4	23.9		27.0	26.9	18.4	18.2	17.0
430												
434	25.0	23.9	23.7	24.4	25.1	25.3	27.8	24.8	17.7	17.3	17.0	16.7
517		25.2	25.1	25.5	26.7	25.4		19.7	18.3	17.8	16.1	16.9
455		24.6	24.6	23.8	24.7	24.3		24.6	18.9	18.5	17.6	17.5
499												
516												
Chaetocin A (42)		25.1	23.8	24.8	24.5	24.6		29.0	28.4	29.2	28.9	28.7
	Control			Negative Control			Control			Negative Control		
	C20	23.9		CD12	25.0		C20	17.6		CD12	26.8	
	C21	24.8		Extraction Control			C21	17.4		Extraction Control		
	C22	24.6		PBS	Undetermined		C22	17.1		PBS	Undetermined	
	Average	24.4		PBS	Undetermined		Average	17.4		PBS	Undetermined	

Table 18 – Primary Ct Values on day one and day seven after exposure to the compounds

There were two disruptions to this pathway, one was that the cells were destroyed by the compound and hence Ct values remained static or increased as the virus was incubated and became denatured with no fresh supply (these results are shown in red). The other option was that the compound could actively reduce the amount of virus shedded from the cells without toxicity and increasing significantly the replication of the virus. Relative to the non-treated cells (these are shown in blue), compounds of this series have shown very good efficacy (Table **19**).

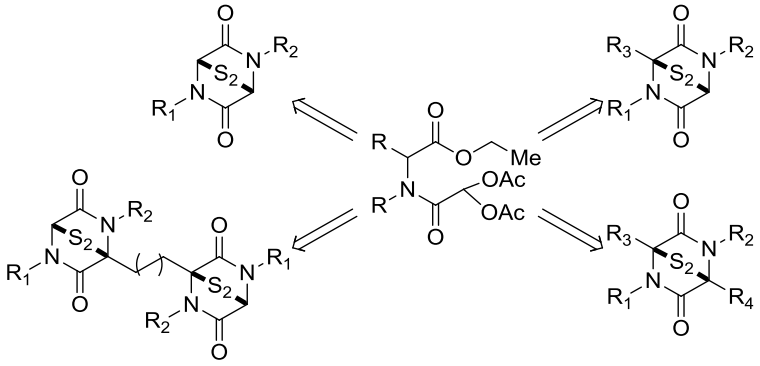
	Day 1						Day 7					
Number	100uM	10uM	1uM	100nM	10nM	1nM	100uM	10uM	1uM	100nM	10nM	1nM
435												
432	-0.7	-0.8	-0.6				11.0	10.5	7.6			
429	-0.7	-0.8	-0.5				10.2	10.8	1.0			
Gliotoxin (1)												
433		0.4	0.1	0.0	0.2	-0.2		1.0	1.0	1.3	13.2	2.0
431												
459		0.4	0.2	0.0	0.2	0.1		9.9	13.0	8.5	3.1	1.8
427		0.9	0.1	0.0	0.1	0.0		11.6	1.4	1.3	0.7	0.2
426		0.3	1.5	0.4	1.2	1.4		1.8	1.2	1.3	0.9	0.3
425		-0.6	0.5	1.0	0.8	0.7		10.9	9.5	0.4	0.4	0.6
Hyalodendrin (18)												
483	0.6	1.2	1.3	0.3	0.5	-0.1	3.1	0.7	0.1	-1.2	-1.0	-1.0
488		-0.1	0.1	-0.2	0.0	0.3		1.5	1.1	0.4	0.2	-0.5
487		-0.8	-0.2	-0.6	0.0	-0.2		4.5	1.3	-0.5	-0.7	-0.5
464	-0.4	-0.1	-0.2	0.1	0.0	-0.2	11.2	1.5	1.1	0.2	0.4	0.2
471		1.2	0.1	-0.4	0.0	0.5		9.7	9.5	1.0	0.9	-0.3
430												
434	-0.6	0.5	0.7	0.0	-0.7	-0.9	10.4	7.5	0.3	-0.1	-0.4	-0.6
517		-0.8	-0.6	-1.1	-2.3	-1.0		2.3	0.9	0.4	-1.3	-0.5
455		-0.2	-0.2	0.6	-0.3	0.1		7.2	1.5	1.1	0.2	0.1
499												
516												
Chaetocin A (42)		-0.7	0.6	-0.4	-0.1	-0.2		11.7	11.0	11.8	11.5	11.3

Table 19 - Difference in Ct values from the control untreated cells. Day seven shows the most active compounds highlighted in blue

ETP (**459**) showed a reduction of almost 10-fold reduction in viral loading at 10 nM and 5-fold at 1 nM. Compounds (**426**) and (**464**) also showed reductions of around 5-fold at 10 µM and ETP (**427**) showed a 4-fold reduction at 100 nM. This was shown to be very encouraging data for further clinical development of the ETPs as antiviral agents, but due to time constrains, we were unable to test any other members of this class of compounds. Nevertheless, the data clearly shows that the toxicity previously associated with the ETP can be abrogated and activity maintained.

4. Summary & Future Work

Despite widespread interest in the ETP family of natural products as a result of their potent biological activities, a comprehensive medicinal chemistry analysis of their potential has yet to be realised. However, in our approach to these compounds, we have clearly developed a number of synthetic strategies to enable ready access to both the natural products and synthetic analogues as shown below (Scheme **145**).

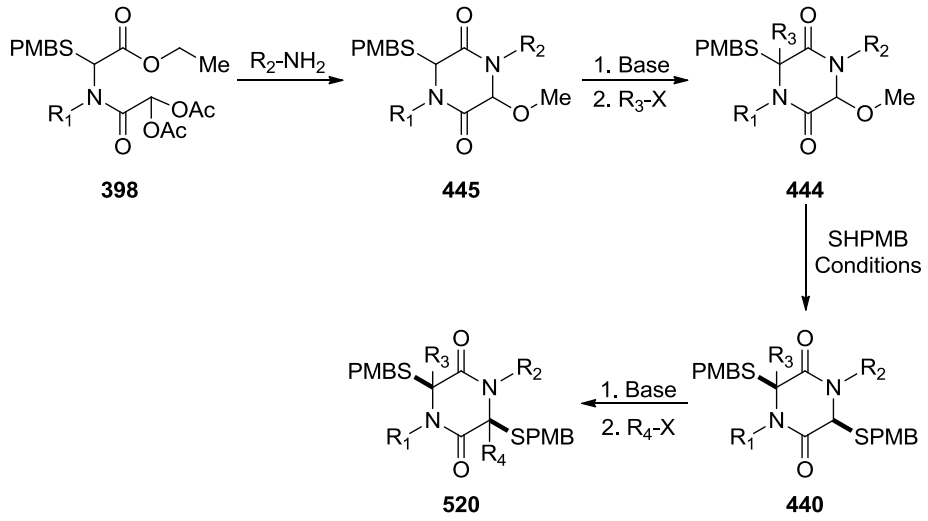


Scheme 145 – Different synthetic strategies developed to achieve ETPs

Our approach differs from others since it uses a short number of steps to achieve the fully functionalised ETP core. In section **3.3.1** a four step approach towards monomeric disubstituted ETPs was shown to be effective for all given examples. The introduction of sulfur at an initial stage combined with possible differentiation between symmetric and asymmetrical ETPs highlighted the well-designed method developed by ourselves. Our new base catalysed cyclisation reaction to form monomeric protected ETPs described in section **3.2.1** proved to be of use by increasing final reaction yields for previously synthesised compounds or by enabling us to access new examples of the ETP family of synthetic analogues such as the mono-substituted esters. This new methodology also led to the development of the first four-step synthetic reaction to achieve dimeric ETPs **3.4.2**. Using these new insights we were able to develop new ways of attaining the ETP core, as seen in section **4.4.1**, and this led to different examples of dimeric ETPs.

However, the increased polarity of these dimeric molecules and poor solubility rendered them ineffective for use in biological assays. This fortunately led us to the development of tri and tetra-substituted ETPs (Sections **3.3.2** and **3.3.3**). Our initial idea was to create a synthetic pathway in which every possible functionalisation of the ETP core could be achieved. The introduction of a methoxy diacetate cyclisation derivative proved to be successful, as it enabled the synthesis of various tri-substituted ETP analogues.

The same approach used to achieve tri-substituted ETPs permitted the synthesis of a bicyclic ETP system (Section **3.3.2.1**). We expected to use the knowledge acquired from this synthetic route to develop new examples of synthetic analogues since members of the ETP family of natural products show numerous examples of bicyclic systems fused at the central molecular core. We were also able to synthesise tetra-substituted ETPs (Section **3.3.3**) in an analogous manner to the tricyclic ETP system (Section **3.3.3.1**). Nevertheless, in the future we plan to revise our approach to tetra-substituted ETPs by using a similar pathway as for tri-substituted ETPs (Scheme **146**). By using the same methodology to achieve alkylation of the methoxy intermediate (**444**), a tetra-substituted protected ETP intermediate (**520**) with different substituents at all four substitution points can readily be obtained.

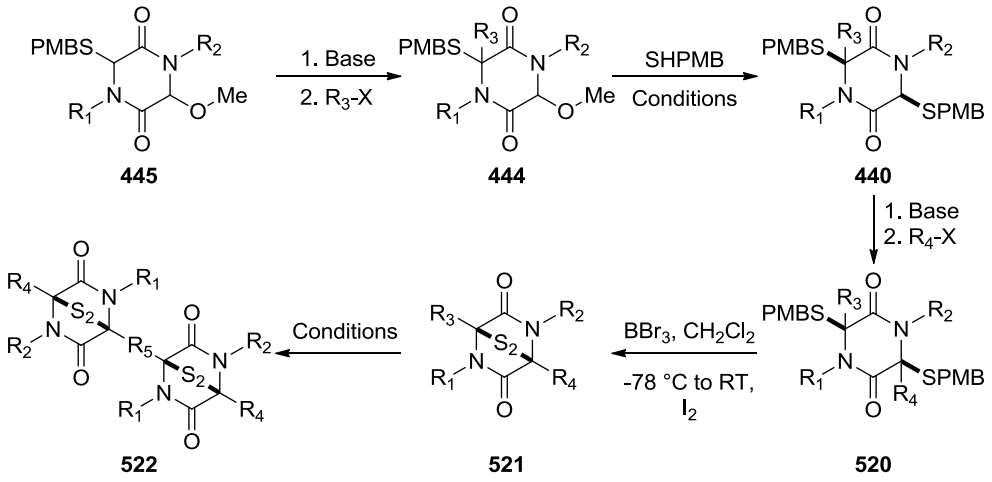


Scheme 146 – Model for the synthesis of tetrasubstituted ETPs

As described previously, mono-substituted dimeric ETPs showed weak solubility and therefore were not able to be used in most of the biological assays. The synthesis of disubstituted dimeric ETPs (Section **3.4.3**) had a very important impact on the solubility of the dimeric series of molecules. Using the same principles as for the synthesis of tri-substituted ETPs, here we were able to combine two monomeric tri-substituted ETP molecules into one disubstituted dimeric ETP. The addition of more aromatic substituents to the ETP core changed the solubility of the final molecules which will therefore be able to explore their biological potential.

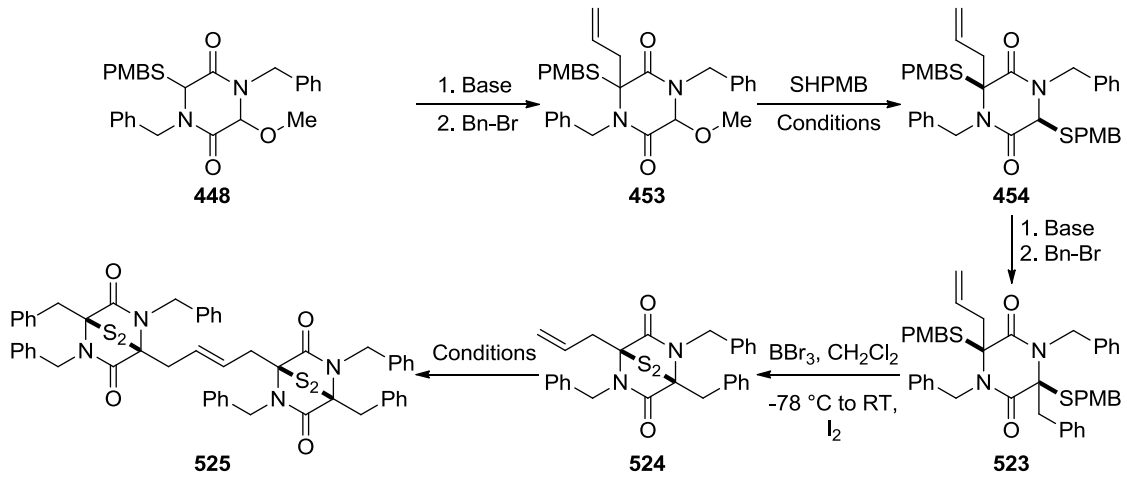
Using the same approach as for the tetra-substituted ETPs, intermediate (**520**) could be formed using two different stages of alkylation with different substituents. By coupling

two of these tetra-substituted ETPs (**521**), a new tri-substituted dimeric ETP can be obtained (**522**) (Scheme **147**).



Scheme 147 – Model for the synthesis of trisubstituted dimeric ETPs (1)

Following the potent activity displayed by compound (**459**), one of the first examples that should be synthesised for this class of molecules should comprise two ETPs with tribenzyl substituents into one single dimeric molecule using the chemistry outlined in sections **3.3.2** and **3.4.3** (Scheme **148**).



Scheme 148 – Model for the synthesis of trisubstituted dimeric ETPs (2)

The biological results presented in section **3.5** clearly demonstrate the potential that these newly synthesised molecules have for the treatment of hypoxic cancer, specific strains of bacteria and antiviral activity. Exceptional results were obtained with ETP (**459**) in every assay carried out. It showed specificity in the microbiological testing for *M. aurum* A+ (MIC: 18 μM) as well as inhibition of the CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex by 78% at 1 μM concentration. A preliminary structure

relationship activity (SRA) was achieved for results shown in section **3.5.1** since ETPs which contain more aromatic and cyclic substituents gave the best outcomes. Testing of a large number of compounds that would include dimeric ETPs should be performed to demonstrate the validity of this SRA.

It was clearly shown that ETPs containing longer aliphatic chain substituents had better biological activities when compared to small chain aliphatic substituted ETPs. Mono ester substituted ETPs also demonstrated this, since longer chain ester mono-substituted ETPs gave a higher inhibition of protein-protein interaction. However, an increase of unsaturated carbon substituents also diminished activity. With results as high as 38% for inhibition of the CFPTEV HIF-1α C-TAD / YFPTEV p300 CH1 complex at 1 μM for compound (**431**), the introduction of more allylic groups decreased inhibition of protein / protein interaction to an average of 4%. This clearly demonstrates the importance of a balance between both unsaturated and aromatic substituents surrounding the ETP core.

Preliminary antiviral studies (Section **3.5.3**) clearly showed significant differences in toxicity between the three tested natural products and all other synthetic ETPs for both cell lines. Ct values also highlighted the promise of synthetic ETP analogues when compared to their parent natural products.

5. Experimental

5.1 General details

All reactions requiring the use of dry conditions were carried out under an atmosphere of nitrogen and all glassware was pre-dried in an oven (110 °C) and cooled under nitrogen prior to use. Stirring was by internal magnetic follower unless otherwise stated. Microwave heated reactions were carried out in a Biotage™ Initiator 2.5. All reactions were followed by TLC and organic phases extracted were dried with anhydrous magnesium sulfate or sodium sulfate as required.

TLC analysis was carried out using Merck aluminium-backed plates coated with silica gel 60 F_{254}. Components were visualised using combinations of ultraviolet light, iodine and ceric ammonium molybdate stain.

All anhydrous solvents and reagents were purchased from Sigma-Aldrich and Fisher Scientific chemical companies and used with Oxford sure/seal valves attached to a standard Schlenk line for solvent removal.

Purification of compounds was carried out using two different methodologies: flash column chromatography (FCC) as reported by Still,[305] or by automatic column chromatography (ACC). Silica gel used for FCC was Merck 60 (230-400 mesh). ACC was carried out using a Biotage™ Horizon High Performance Flash Chromatography (HPFC). The use of this automated system required different pre-packed silica columns supported by the system and supplied by Biotage™. Columns used were: Flash 12+S, Flash 12+M, Flash 25+S, Flash 25+M, SNAP 10 g, SNAP 25 g, SNAP 50 g, SNAP 100 g, ZIP 5 g, ZIP 10 g, ZIP 30 g and ZIP 45 g cartridges. In order to simplify the use of ACC a series of tests were made using the different types of columns described above. These assessments led to the creation of an internal protocol that enabled the standardization of product purification. The outcome of our in-house procedure was a spreadsheet that aided in the selection of different solvent gradients which allowed the purification of the chosen product. Figure **76** shows the designed spreadsheet where Entry 1 represents the TLC analysis used for product separation. Rf values were collected with the solvent system used for elution of the reaction from the TLC plate, along with the mass of product to separate and entered into this section which provides automatic information to the remaining segments (Entries 2, 3 and 4, figure **76**). Entry 2 indicates the evaluation of the separation according to the values entered in Entry 1 in three different grades: difficult, typical and easy. Part 3 of the spreadsheet shows the columns highlighted in green that should be used for separation from the given product

information reported in entry 1. More information can be found in this section regarding the steps that should be input into the Biotage™ Horizon purification system along with the flow rates to be used so that the product could be readily separated. Entry 4 shows a visual breakdown of the solvent gradient for purification in a visible chart.

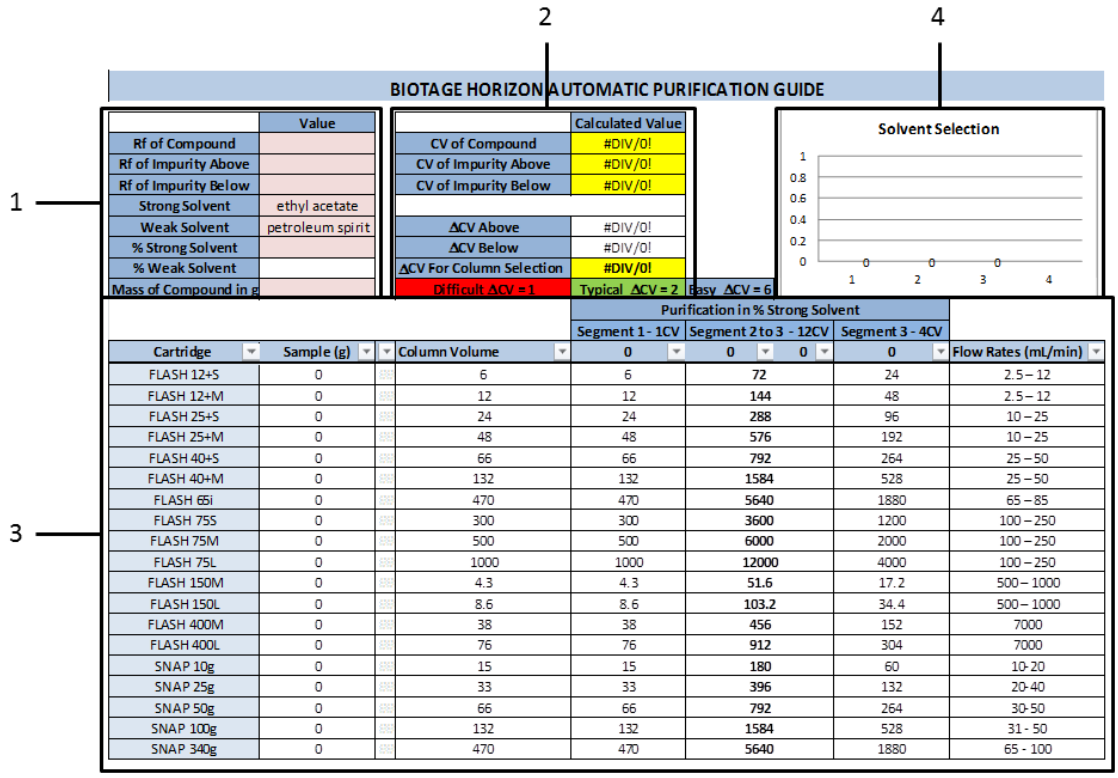


Figure 76 – Biotage™ Horizon HPFC purification guide

As an typical example of separation, 0.54 g of compound (**425**) gave an Rf value of 0.37 from TLC analysis for the product with impurities of 0.89 and 0.01 Rf above and below, respectively, using a 3 : 1 (petroleum ether / ethyl acetate) solvent system. Following entry of these values, the first section of our designed spreadsheet was populated accordingly (Figure **77**).

	Value
Rf of Compound	0.37
Rf of Impurity Above	0.89
Rf of Impurity Below	0.01
Strong Solvent	ethyl acetate
Weak Solvent	petroleum spirit
% Strong Solvent	25
% Weak Solvent	75
Mass of Compound in g	0.535

Figure 77 – Biotage™ Horizon HPFC purification guide (Entry 1)

Following the data loaded into these fields, Entry 2 of the above spreadsheet (Figure **76**) enables the user to evaluate the separation using the defined 3 : 1 (petroleum ether / ethyl acetate) solvent system and as can be seen, it suggested that this is an easy purification (Figure **78**).

	Calculated Value	
CV of Compound	2.70	
CV of Impurity Above	1.12	
CV of Impurity Below	100.00	
ΔCV Above	1.579	
ΔCV Below	97.297	
ΔCV For Column Selection	**1.58**	
Difficult ΔCV = 1	Typical ΔCV = 2	Easy ΔCV = 6

Figure 78 – Biotage™ Horizon HPFC purification guide (Entry 2)

Column choice for compound (**452**) is linked to the TLC analysis with the most suitable suggestion being the Flash 25+S, Flash 25+M and SNAP 50 g cartridges (Figure **79**). Each of these columns could be used to separate the molecule required with information for the setup of Biotage™ Horizon shown in every row of the chosen purification column (i. e. column volume (CV), segments in CV *per* strong solvent percentage (6.25 and 50% ethyl acetate) and flow rates (mL/min)).

				Purification in % Strong Solvent				
				Segment 1 - 1CV	Segment 2 to 3 - 12CV		Segment 3 - 4CV	
Cartridge	Sample (g)		Column Volume	6.25	6.25	50	50	Flow Rates (mL/min)
FLASH 12+S	0.075		6	6	72		24	2.5 – 12
FLASH 12+M	0.15		12	12	144		48	2.5 – 12
FLASH 25+S	0.375		24	24	288		96	10 – 25
FLASH 25+M	0.75		48	48	576		192	10 – 25
FLASH 40+S	0.825		66	66	792		264	25 – 50
FLASH 40+M	1.65		132	132	1584		528	25 – 50
FLASH 65i	6		470	470	5640		1880	65 – 85
FLASH 75S	3.5		300	300	3600		1200	100 – 250
FLASH 75M	6		500	500	6000		2000	100 – 250
FLASH 75L	11.7		1000	1000	12000		4000	100 – 250
FLASH 150M	46.9		4.3	4.3	51.6		17.2	500 – 1000
FLASH 150L	93.8		8.6	8.6	103.2		34.4	500 – 1000
FLASH 400M	330		38	38	456		152	7000
FLASH 400L	670		76	76	912		304	7000
SNAP 10g	0.15		15	15	180		60	10-20
SNAP 25g	0.15		33	33	396		132	20-40
SNAP 50g	0.825		66	66	792		264	30-50
SNAP 100g	1.5		132	132	1584		528	31 - 50
SNAP 340g	6		470	470	5640		1880	65 - 100

Figure 79 – Biotage™ Horizon HPFC purification guide (Entry 3)

Graphical analysis (Figure **80**) allowed confirmation of the methodology calculated for the purification of compound (**452**), facilitating the visualization of the steps required for the separation of impurities.

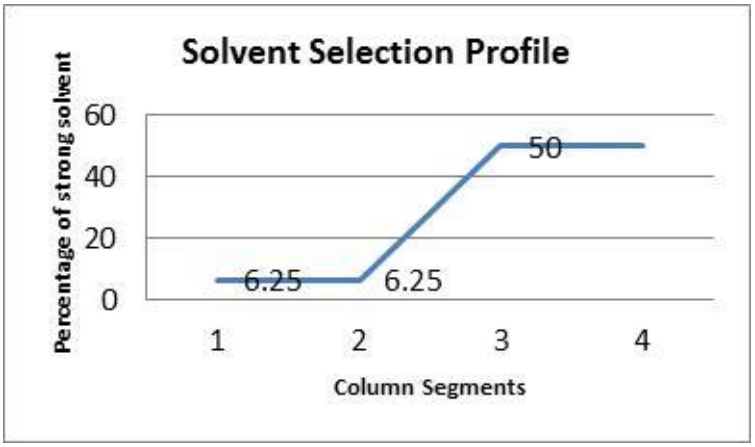


Figure 80 – Biotage™ Horizon HPFC purification guide (Entry 4)

Analytical Reversed-Phase HPLC was performed on a Waters Atlantis C_{18} column (250 mm x 4.6 mm). Separation was achieved using a linear gradient at a flow rate of 1.2 mL min^{-1} effected by a Waters 600S controller and 616 pump running solvent A [0.1% (v/v) TFA in H_2O], solvent B [0.16% (v/v) TFA in 90% MeCN]; 0% B to 100% B over 20 min then 100% B for 5 min then 100% B to 0% B over 7 min (System A). All data was managed using Millennium version 2.15 software.

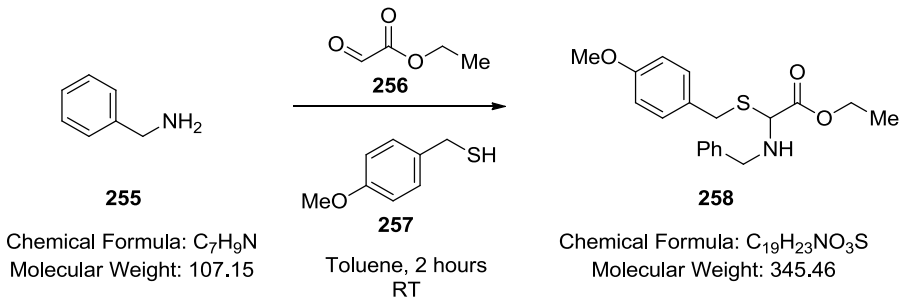
Semi-preparative reverse phase – HPLC (RP-HPLC) (<20 mg material) was achieved using a Waters Atlantis Prep LC C_{18} column (250.0 mm x 6.0 mm) at a flow rate of 5 mL min^{-1}. Preparative RP-HPLC (>20 mg material) was achieved using a Waters Atlantis Prep LC C_{18} column (250 mm x 19 mm) at a flow rate of 20.4 mL min^{-1}. Two Applied Biosystems 200 solvent Delivery System pumps were used, running 0% B to 100% B over 40 min then 100% B for 10 min then 100% B to 0% B over 7 min (System B). Semi-preparative and preparative RP-HPLC was also achieved using a Waters 600E Multi Solvent Delivery System: Waters Applied Biosystems 783A Programmable Absorbance Detector and Waters 2847 dual λ Absorbance Detector also using System B. Separation was monitored at 214 nm, 254 nm and 340 nm by Waters Millennium Chromatography Manager Software and Empower2 Pro Chromatography software. Fractions were collected using a Waters Fraction Collector III, and data managed using Empower2 Pro Software.

Infrared spectra were recorded on a Bruker Alpha FT-IR spectrophotometer using NaCl plates. ^{1}H NMR and ^{13}C NMR were recorded using either a Bruker AM400 spectrometer operating at 400 MHz for proton and 101 MHz for carbon or a Bruker AM500 spectrometer operating at 500 MHz for proton and 126 MHz for carbon.

Chemical shifts (δ_H and δ_C) are quoted as parts per million downfield from 0. The multiplicity of a ^{1}H NMR signal is designated by one of the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, br = broad and m = multiplet. Coupling constants (*J*) are expressed in Hertz.

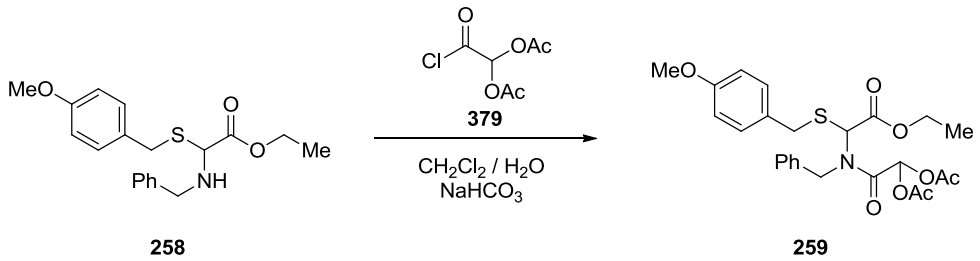
High-resolution mass spectra were carried out using either a Kratos MS89MS with Kratos DS90 software or a Jeol AX505W with Jeol complement data system. Samples were ionised electronically (EI), with an accelerating voltage of ≈6kV or by low resolution fast atom bombardment (FAB) in a thioglycerol matrix. High-resolution fast atom bombardment was carried out at the ULIRS mass spectrometry facility at UCL School of Pharmacy.

5.2 Ethyl-2-(benzylamino)-2-((4-methoxybenzyl)thio)acetate (258)[217]



Benzylamine (2.76 mL, 25.2 mmol) (**255**) was added to a solution of ethyl glyoxalate (5.00 mL of a 50% solution in toluene, 25.2 mmol) (**256**) in toluene (20.0 mL) at room temperature (RT) and stirred for 2 minutes, whereupon *para*-methoxybenzyl mercaptan (3.54 mL, 25.2 mmol) (**257**) was added and the mixture stirred for 2 hours. Solvent was removed under reduced pressure and the residue purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 50 g) to give ethyl-2-(benzylamino)-2-((4-methoxybenzyl)thio)acetate (7.56 g, 87%) (**258**) as a colourless oil; ν_{max}/cm^{-1} 3347 (N-H), 1732 (C=O); δ H (400 MHz; $CDCl_3$) 1.31 (3H, t, *J* 7.2, $CO_2CH_2CH_3$), 1.88 (1H, br s, N*H*), 3.72 (1H, d, *J* 13.1, SCH_2), 3.75 – 3.77 (2H, m, $NHCH_2$), 3.79 (3H, s, OCH_3), 3.87 (1H, d, *J* 13.1, SCH_2), 4.22 (2H, q, *J* 7.2, $CO_2CH_2CH_3$), 4.35 (1H, s, C*H*), 6.84 (2H, d, *J* 8.7, Ar-*H*), 7.23 – 7.29 (7H, m, Ar-*H*); δ C (101 MHz; $CDCl_3$) 14.15 ($CO_2CH_2\mathbf{C}H_3$), 33.23 ($S\mathbf{C}H_2$), 49.22 ($NH\mathbf{C}H_2$), 55.28 ($O\mathbf{C}H_3$), 61.41 ($CO_2\mathbf{C}H_2CH_3$), 63.71 (**C**H), 114.00 (Ar-**C**-H), 127.19 (Ar-**C**-H), 128.41 (Ar-**C**-H), 128.48 (Ar-**C**-H), 130.07 (Ar-**C**-H), 138.79 (quaternary **C**), 158.76 (quaternary **C**), 169.97 ($\mathbf{C}O_2CH_2CH_3$); *m/z* 346 (100%, $[M+H]^+$): Found $[M+H]^+$ 346.1477, $C_{19}H_{24}NO_3S$ requires 346.1482.

5.3 2-(Benzyl(2-ethoxy-1-(4-methoxybenzylthio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (259)[1]



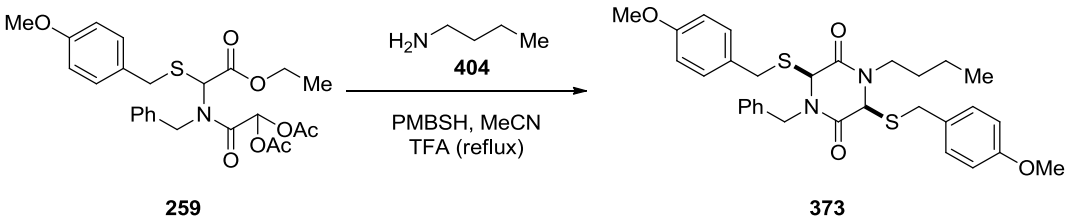
258 **259**

Chemical Formula: $C_{19}H_{23}NO_3S$
Molecular Weight: 345.46

Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

A solution of diacetoxyacetyl chloride (5.11 g, 26.3 mmol) (**379**) in dichloromethane (20.0 mL) was added dropwise to a rapidly stirred biphasic mixture of ethyl-2-(benzylamino)-2-((4-methoxybenzyl)thio)acetate (7.56 g, 21.9 mmol) (**258**) and sodium hydrogen carbonate (12.9 g, 153 mmol) in dichloromethane (150 mL) and water (100 mL) at RT and the resulting mixture stirred rapidly for 12 hours. The layers were separated and the aqueous phase extracted with dichloromethane (2 x 100 mL). The combined extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure to give 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (10.5 g, 95%) (**259**) as a colourless oil that did not require further purification; ν_{max}/cm^{-1} 1770 (C=O), 1739 (C=O), 1685 (C=O); δ H (400 MHz; $CDCl_3$) 1.15 (3H, t, *J* 7.1, CH_2CH_3), 1.95 (3H, s, OC(O)CH_3), 1.98 (3H, s, OC(O)CH_3), 3.72 (2H, s, SCH_2), 3.75 (3H, s, OCH_3), 3.98 – 4.02 (2H, m, CH_2CH$_3$), 4.59 (1H, d, *J* 17.4, NCH_2), 4.86 (1H, d, *J* 17.4, NCH_2), 5.85 (1H, s, C*H*S), 6.79 (2H, d, *J* 8.4, Ar-*H*), 6.92 (1H, s, C*H*$(OAc)_2$), 7.16 – 7.27 (7H, m, Ar-*H*); δ C (101 MHz; $CDCl_3$) 13.76 (CH_2**C**H_3), 20.20 (C(O)**C**H_3), 20.25 (C(O)**C**H_3), 35.49 (**C**H_2), 48.68 (**C**H_2), 55.18 (O**C**H_3), 61.65 (**C**HS), 62.21 (**C**H_2CH_3), 83.99 (**C**H$(OAc)_2$), 113.97 (Ar-**C**-H), 126.79 (Ar-**C**-H), 127.66 (Ar-**C**-H), 128.48 (Ar-**C**-H), 128.65 (quaternary **C**), 130.21 (Ar-**C**-H), 135.72 (quaternary **C**), 158.87 (quaternary **C**), 165.76 (**C**O), 167.16 (**C**O), 168.34 (**C**O), 168.62 (**C**O); *m/z* 504 (100%, $[M+H]^+$): Found $[M+H]^+$ 504.1688, $C_{25}H_{30}NO_8S$ requires 504.1692.

5.4 (3S,6S)-1-Benzyl-4-butyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (373)[1]



259

Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

373

Chemical Formula: $C_{31}H_{36}N_2O_4S_2$
Molecular Weight: 564.76

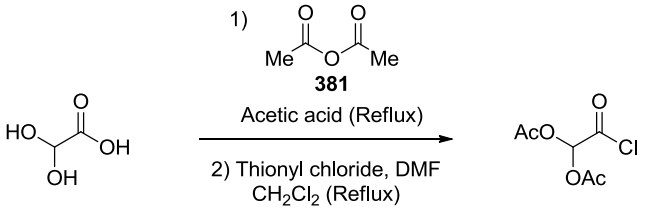
5.4.1 Method A:

n-Butylamine (0.57 mL, 5.72 mmol) (**404**) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.96 g, 1.91 mmol) (**259**) in acetonitrile (30.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.40 mL, 2.86 mmol) (**257**) and the resulting mixture stirred for 2 minutes. TFA (0.15 mL, 1.91 mmol) was added and the resulting mixture heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-benzyl-4-butyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.70 g, 65%) (**373**) as a colourless solid; m.p. 134-135 °C; ν_{max}/cm^{-1} 1670 (C=O); δ H (400 MHz; $CDCl_3$) 0.75 – 0.79 (3H, m, $NCH_2(CH_2)_2CH_3$), 0.97 – 1.13 (4H, m, $NCH_2(CH_2)_2CH_3$), 2.54 – 2.57 (1H, m, $NCH_2(CH_2)_2CH_3$), 3.54 – 3.57 (2H, m, $NCH_2(CH_2)_2CH_3$ & NCH_2-Ph), 3.77 (1H, s, SCH_2), 3.80 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.87 (1H, s, SCH_2), 3.94 (1H, d, *J* 13.9, SCH_2), 4.00 (1H, d, *J* 13.9, SCH_2), 4.28 (1H, s, CHS), 4.42 (1H, s, CHS), 5.14 (1H, d, *J* 14.5, NCH_2-Ph), 6.67 (2H, d, *J* 7.4, Ar-*H*), 6.84 – 6.91 (4H, m, Ar-*H*), 7.10 – 7.19 (3H, m, Ar-*H*), 7.33 – 7.41 (4H, m, Ar-*H*); δ C (101 MHz; $CDCl_3$) 13.51 ($CH_2\mathbf{C}H_3$), 19.86 ($\mathbf{C}H_2$), 28.75 ($\mathbf{C}H_2$), 36.17 ($\mathbf{C}H_2$), 36.71 ($\mathbf{C}H_2$), 43.40 ($\mathbf{C}H_2$), 46.01 ($\mathbf{C}H_2$), 55.22 ($O\mathbf{C}H_3$), 57.25 (**C**HS), 58.79 (**C**HS), 113.97 (Ar-**C**-H), 114.00 (Ar-**C**-H), 127.79 (Ar-**C**-H), 128.54 (Ar-**C**-H), 128.69 (quaternary **C**), 128.85 (quaternary **C**), 130.69 (Ar-**C**-H), 130.77 (Ar-**C**-H), 134.94 (quaternary **C**), 159.06 (quaternary **C**), 164.94 (**C**O), 165.32 (**C**O); *m/z* 565 (100%, $[M+H]^+$): Found $[M+H]^+$ 565.2175, $C_{31}H_{37}N_2O_4S_2$ requires 565.2195.

5.4.2 Method B:

n-Butylamine (0.18 mL, 1.82 mmol) (**404**) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (**259**) (0.31 g, 0.61 mmol) in acetonitrile (30.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.13 mL, 0.91 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.04 g, 0.30 mmol) was added in a single portion and the resulting mixture heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-benzyl-4-butyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.28 g, 81%) (**373**) as a colourless solid; identical spectroscopic data to that obtained previously for (**373**).

5.5 Diacetoxyacetyl chloride (379)[285]

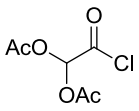


380 **379**

Chemical Formula: $C_2H_4O_4$
Molecular Weight: 92.05

Chemical Formula: $C_6H_7ClO_5$
Molecular Weight: 194.57

A solution of glyoxylic acid monohydrate (16.0 g, 174 mmol) in acetic anhydride (49.2 mL, 521 mmol) and acetic acid (29.9 mL, 521 mmol) was heated at 150 °C for 3 hours and then allowed to cool to RT over 1 hour. Excess reactants were removed under azeotropic distillation (toluene 2 x 100 mL) under reduced pressure. The residue was taken up in dichloromethane (200 mL) followed by sequential addition of thionyl chloride (25.4 mL, 348 mmol) and dimethylformamide (0.03 mL, 0.35 mmol). The mixture was heated under reflux conditions (50 °C) for varying time periods (Table **1**) until diacetoxyacetyl chloride was formed. Solvent and excess thionyl chloride were removed under reduced pressure and the crude product purified by distillation under reduced pressure (64-65 °C/ 1.0 mmHg) to give diacetoxyacetyl chloride (25.0 g, 75%) (**379**) as a colourless oil; ν_{max}/cm^{-1} 1763 (C=O), 1376 (CH_3), 1200 (C-C(O)-C), 718 (C-Cl); δ H (500 MHz; $CDCl_3$) 2.09 (6H, s, $CH(OC(O)C\mathit{H}_3)_2$), 6.79 (1H, s, C*H*); δ C (126 MHz; $CDCl_3$) 20.48 (**C**H_3), 88.03 (**C**$H(OAc)_2$), 167.67 (**C**O), 168.21 (**C**O); *m/z* 195 (100%, $[M+H]^+$): Found $[M+H]^+$ 195.5805, $C_6H_8ClO_5$ requires 195.5801.

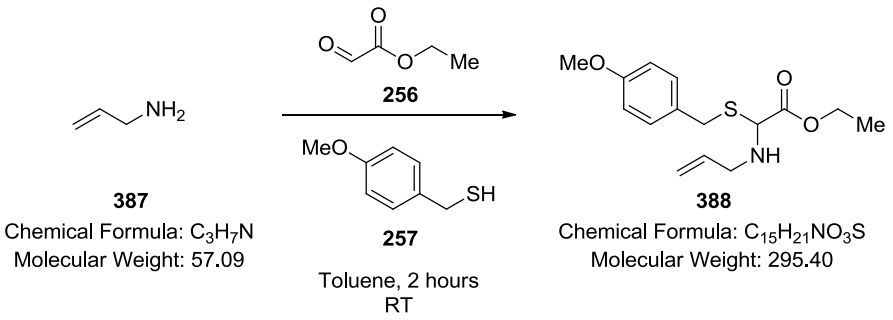


δ H (400 MHz; $CDCl_3$)

Time period (hours)	COO*H*	C*H*(OC(O)CH_3)$_2$	$CH(OC(O)C\mathit{H}_3)_2$
1.5	10.81 (1 H, s)	6.74 (1 H, s)	2.03 – 2.04 (6 H, s)
3.0	10.62 (0.5 H, s)	6.78 (1 H, s)	2.08 – 2.10 (6 H, s)
5.0	-	6.79 (1 H, s)	2.09 (6 H, s)

Table 20 – Diacetoxyacetyl chloride synthesis

5.6 Ethyl-2-(allylamino)-2-(4-methoxybenzylthio)acetate (388)

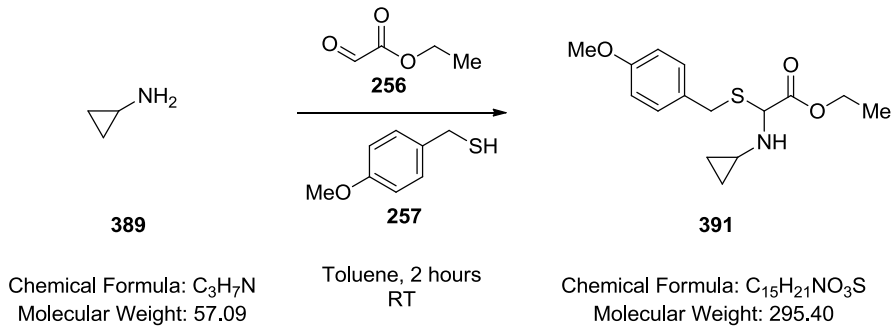


387
Chemical Formula: C_3H_7N
Molecular Weight: 57.09

388
Chemical Formula: $C_{15}H_{21}NO_3S$
Molecular Weight: 295.40

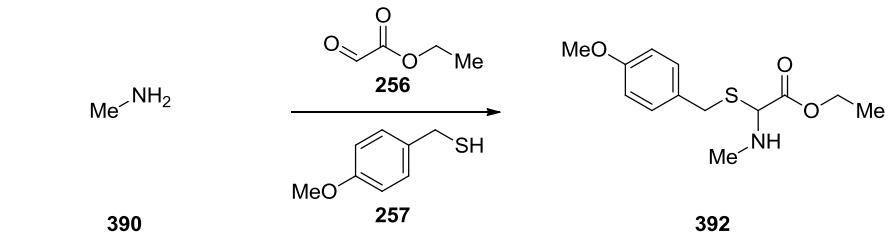
Allylamine (1.90 mL, 25.2 mmol) (**387**) was added to a solution of ethyl glyoxalate (5.00 mL of a 50% solution in toluene, 25.2 mmol) (**256**) in toluene (30.0 mL) at room temperature (RT) and the solution stirred for 2 minutes, whereupon *para*-methoxybenzyl mercaptan (3.51 mL, 25.22 mmol) (**257**) was added and the mixture stirred for 2 hours. Solvent was removed under reduced pressure and the residue purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 50 g) to give ethyl-2-(allylamino)-2-(4-methoxybenzylthio)acetate (6.00 g, 81%) (**388**) as a colourless oil; ν_{max}/cm^{-1} 3344 (N-H), 1732 (C=O); δ H (400 MHz; $CDCl_3$) 1.27 (3H, t, *J* 7.1, CH_2CH_3), 1.89 (1H, br s, N*H*), 3.11 (1H, dd, *J* 13.9 & 6.4, NCH_2), 3.28 (1H, dd, *J* 13.9 & 6.6, NCH_2), 3.69 (2H, s, SCH_2), 3.74 (3H, s, OCH_3), 4.18 (2H, q, *J* 7.1, CH_2CH_3), 4.32 (1H, s, C*H*S), 5.06 – 5.10 (2H, m, $CH=CH_2$), 5.72 – 5.77 (1H, m, $CH=CH_2$), 6.80 (2H, d, *J* 8.6, Ar-*H*), 7.23 (2H, d, *J* 8.6, Ar-*H*); δ C (101 MHz; $CDCl_3$) 13.89 (CH_2CH_3), 32.85 (CH_2), 47.59 (CH_2), 54.99 (OCH_3), 61.09 (CH_2), 63.36 (CHS), 113.66 (Ar-C-H), 116.64 ($CH=CH_2$), 129.72 (quaternary **C**), 129.78 (Ar-C-H), 135.07 ($CH=CH_2$), 158.46 (quaternary **C**), 169.68 (CO); *m/z* 296 (100%, $[M+H]^+$): Found $[M+H]^+$ 296.1322, $C_{15}H_{22}NO_3S$ requires 296.1320.

5.7 Ethyl-2-(cyclopropylamino)-2-(4-methoxybenzylthio)acetate (391)



Cyclopropylamine (1.76 mL, 25.2 mmol) (**389**) was added to a solution of ethyl glyoxalate (5.00 mL of a 50% solution in toluene, 25.2 mmol) (**256**) in toluene (25.0 mL) at RT and stirred for 2 minutes, whereupon *para*-methoxybenzyl mercaptan (3.51 mL, 25.2 mmol) (**257**) was added and the mixture stirred for 2 hours. Solvent was removed under reduced pressure and the residue purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 50 g) to give ethyl-2-(cyclopropylamino)-2-(4-methoxybenzylthio)acetate (5.07 g, 68%) (**391**) as a colourless oil; ν_{max}/cm^{-1} 3342 (N-H), 1732 (C=O); δ H (400 MHz; $CDCl_3$) 0.29 – 0.32 (1H, m, cyclopropyl-C*H*$_2$), 0.38 – 0.42 (1H, m, cyclopropyl-C*H*$_2$), 0.48 – 0.50 (2H, m, cyclopropyl-C*H*$_2$), 1.32 (3H, t, *J* 6.5, $CO_2CH_2CH_3$), 2.34 – 2.37 (1H, m, cyclopropyl-C*H*), 2.50 (1H, br s, N*H*), 3.82 – 3.93 (5H, m, SC*H*$_2$ & OC*H*$_3$), 4.23 (2H, q, *J* 6.5, $CO_2CH_2CH_3$), 4.42 (1H, s, C*H*), 6.88 (2H, d, *J* 8.4, Ar-*H*), 7.31 (2H, d, *J* 8.4, Ar-*H*); δ C (101 MHz; $CDCl_3$) 5.99 (**C**H$_2$), 6.52 (**C**H$_2$), 14.09 ($CO_2CH_2\mathbf{C}H_3$), 26.74 (NH**C**H-CH_2CH_2), 33.56 (S**C**H$_2$), 55.26 (O**C**H$_3$), 61.26 ($CO_2\mathbf{C}H_2CH_3$), 64.35 (**C**H), 113.94 (Ar-**C**-H), 129.86 (quaternary **C**), 130.05 (Ar-**C**-H), 158.77 (quaternary **C**), 170.08 (**C**$O_2CH_2CH_3$); *m/z* 296 (100%, $[M+H]^+$): Found $[M+H]^+$ 296.1236, $C_{15}H_{22}NO_3S$ requires 296.1237.

5.8 Ethyl-2-((4-methoxybenzyl)thio)-2-(methylamino)acetate (392)[217]

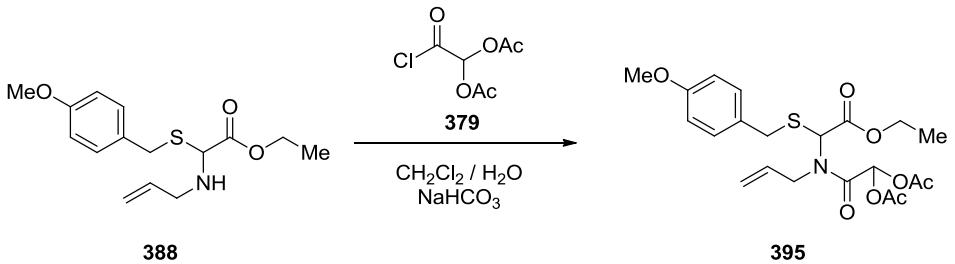


Chemical Formula: CH_5N
Molecular Weight: 31.06

Chemical Formula: $C_{13}H_{19}NO_3S$
Molecular Weight: 269.36

Methylamine (10.2 mL of a 2M solution in tetrahydrofuran, 20.4 mmol) (**390**) was added to a solution of ethyl glyoxalate (4.20 mL of a 50% solution in toluene, 22.3 mmol) (**256**) in toluene (50.0 mL) at RT and stirred for 2 minutes, whereupon *para*-methoxybenzyl mercaptan (3.10 mL, 22.3 mmol) (**257**) was added and the mixture heated to reflux under Dean and Stark water removal conditions for 5 hours. Solvent was removed under reduced pressure and the residue purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 50 g) to give ethyl-2-((4-methoxybenzyl)thio)-2-(methylamino)acetate (2.04 g, 37%) (**392**) as a colourless oil; ν_{max}/cm^{-1} 3358 (N-H), 1735 (C=O); δ H (400 MHz; $CDCl_3$) 1.29 (3H, t, *J* 7.2, $CO_2CH_2CH_3$), 1.76 (1H, br s, N*H*), 2.33 (3H, s, $NHCH_3$), 3.71 (2H, s, SCH_2), 3.78 (3H, s, OCH_3), 4.20 (2H, q, *J* 7.2, $CO_2CH_2CH_3$), 4.23 (1H, s, C*H*), 6.83 (2H, d, *J* 8.6, Ar-*H*), 7.25 (2H, d, *J* 8.6, Ar-*H*); δ C (101 MHz; $CDCl_3$) 14.13 ($CO_2CH_2\mathbf{C}H_3$), 32.19 ($N\mathbf{C}H_3$), 33.14 ($S\mathbf{C}H_2$), 55.27 ($O\mathbf{C}H_3$), 61.33 ($CO_2\mathbf{C}H_2CH_3$), 66.22 (**C**H), 113.91 (Ar-**C**-H), 129.92 (quaternary **C**), 130.03 (Ar-**C**-H), 158.72 (quaternary **C**), 169.99 (**C**O); *m/z* 270 (100%, $[M+H]^+$): Found $[M+H]^+$ 270.1162, $C_{13}H_{20}NO_3S$ requires 270.1164.

5.9 2-(Allyl(2-ethoxy-1-(4-methoxybenzylthio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (395)



388

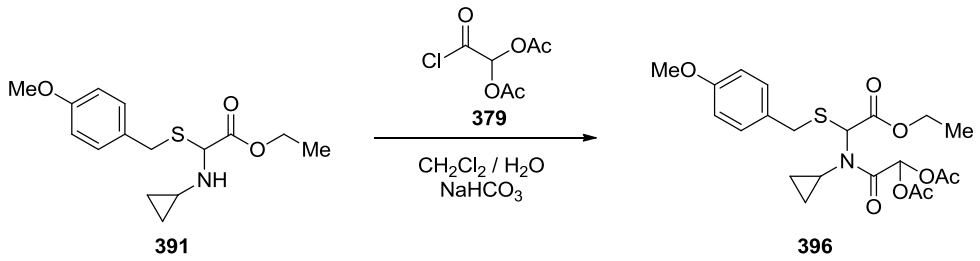
Chemical Formula: $C_{15}H_{21}NO_3S$
Molecular Weight: 295.40

395

Chemical Formula: $C_{21}H_{27}NO_8S$
Molecular Weight: 453.51

A solution of diacetoxyacetyl chloride (4.74 g, 24.4 mmol) (**379**) in dichloromethane (15.0 mL) was added dropwise to a rapidly stirred biphasic mixture of ethyl-2-(allylamino)-2-(4-methoxybenzylthio)acetate (6.00 g, 20.3 mmol) (**388**) and sodium hydrogen carbonate (11.9 g, 142 mmol) in dichloromethane (200 mL) and water (100 mL) at RT and the resulting mixture stirred rapidly for 12 hours. The layers were separated and the aqueous phase extracted with dichloromethane (2 x 100 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure to give 2-(allyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (8.93 g, 97%) (**395**) as a colourless oil that did not require further purification; ν_{max}/cm^{-1} 1770 (C=O), 1743 (C=O), 1681 (C=O); δ H (400 MHz; $CDCl_3$) 1.20 (3H, t, *J* 7.1, CH_2CH_3), 1.95 (3H, s, $OC(O)CH_3$), 2.11 (3H, s, $OC(O)CH_3$), 3.67 (2H, s, SCH_2), 3.73 (3H, s, OCH_3), 3.99 (1H, dd, *J* 17.7 & 5.4, NCH_2), 4.09 (2H, d, *J* 7.1, CH_2CH_3), 4.25 (1H, dd, *J* 17.7 & 5.5, NCH_2), 5.19 – 5.23 (2H, m, $CH=CH_2$), 5.84 – 5.89 (1H, m, $CH=CH_2$), 6.08 (1H, s, CHS), 6.80 (2H, d, *J* 8.6, Ar-*H*), 7.03 (1H, s, $CH(OAc)_2$), 7.19 (2H, d, *J* 8.6, Ar-*H*); δ C (101 MHz; $CDCl_3$) 13.70 ($CH_2\mathbf{C}H_3$), 20.29 ($C(O)\mathbf{C}H_3$), 34.90 ($\mathbf{C}H_2$), 47.12 ($\mathbf{C}H_2$), 54.99 ($O\mathbf{C}H_3$), 60.77 (**C**HS), 62.08 ($\mathbf{C}H_2$), 83.86 ($\mathbf{C}H(OAc)_2$), 113.80 (Ar-**C**-H), 117.56 ($CH=\mathbf{C}H_2$), 128.48 (quaternary **C**), 130.02 (Ar-**C**-H), 133.22 ($\mathbf{C}H=CH_2$), 158.73 (quaternary **C**), 165.41 (**C**O), 167.14 (**C**O), 168.37 (**C**O), 168.69 (**C**O); *m/z* 454 (100%, $[M+H]^+$): Found $[M+H]^+$ 454.1539, $C_{21}H_{28}NO_8S$ requires 454.1536.

5.10 2-(Cyclopropyl(2-ethoxy-1-(4-methoxybenzylthio)-2-oxoethyl) amino)-2-oxoethane-1,1-diyl diacetate (396)

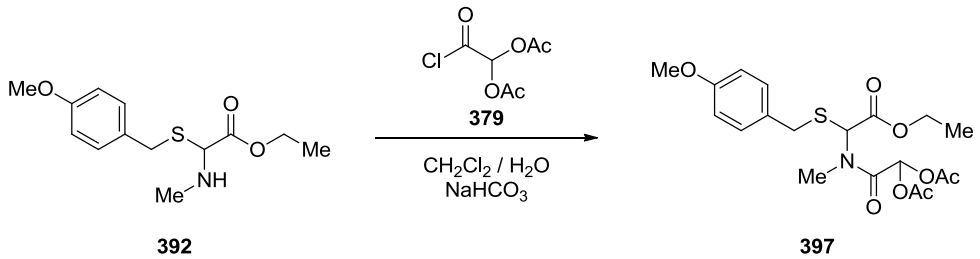


Chemical Formula: $C_{15}H_{21}NO_3S$
Molecular Weight: 295.40

Chemical Formula: $C_{21}H_{27}NO_8S$
Molecular Weight: 453.51

A solution of diacetoxyacetyl chloride (3.95 g, 20.3 mmol) (**379**) in dichloromethane (20.0 mL) was added dropwise to a rapidly stirred biphasic mixture of ethyl 2-(cyclopropylamino)-2-(4-methoxybenzylthio)acetate (5.00 g, 16.9 mmol) (**391**) and sodium hydrogen carbonate (9.95 g, 118 mmol) in dichloromethane (200 mL) and water (100 mL) at room temperature and the resulting mixture stirred rapidly for 12 hours. The layers were separated and the aqueous phase extracted with dichloromethane (2 x 100 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure to give 2-(cyclopropyl(2-ethoxy-1-(4-methoxybenzylthio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (6.02 g, 78%) (**396**) as a colourless oil that did not require further purification; ν_{max}/cm^{-1} 1768 (C=O), 1743 (C=O), 1693 (C=O); δ H (400 MHz; $CDCl_3$) 0.82 – 0.89 (3H, m, cyclopropyl-C*H*$_2$), 1.06 – 1.11 (1H, m, cyclopropyl-C*H*$_2$), 1.26 (3H, t, *J* 7.1, $CO_2CH_2CH_3$), 2.17 (3H, s, OC(O)C*H*$_3$), 2.18 (3H, s, OC(O)C*H*$_3$), 2.70 – 2.77 (1H, m, cyclopropyl-C*H*), 3.79 (3H, s, OC*H*$_3$), 3.83 (2H, s, SC*H*$_2$), 4.13 – 4.23 (2H, m, C*H*$_2$CH$_3$), 5.61 (1H, s, C*H*S), 6.85 (2H, d, *J* 8.7, Ar-*H*), 7.26 (2H, d, *J* 8.7, Ar-*H*), 7.55 (1H, s, C*H*(OAc)$_2$); δ C (101 MHz; $CDCl_3$) 8.11 (**C**H$_2$), 8.49 (**C**H$_2$), 13.83 (COCH$_2$**C**H$_3$), 20.37 (C(O)**C**H$_3$), 20.41 (C(O)**C**H$_3$), 29.01 (**C**H), 35.91 (S**C**H$_2$), 55.12 (O**C**H$_3$), 62.21 (CO**C**H$_2$CH$_3$), 62.46 (**C**HS), 83.82 (**C**H(OAc)$_2$), 113.86 (Ar-**C**-H), 128.79 (quaternary **C**), 130.12 (Ar-**C**-H), 158.78 (quaternary **C**), 166.60 (**C**O), 168.10 (**C**O), 168.69 (**C**O), 168.74 (**C**O); *m/z* 454 (100%, $[M+H]^+$): Found $[M+H]^+$ 454.1564, $C_{21}H_{28}NO_8S$ requires 454.1536.

5.11 2-((2-Ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)(methyl)amino)-2-oxoethane-1,1-diyl diacetate (397)



392

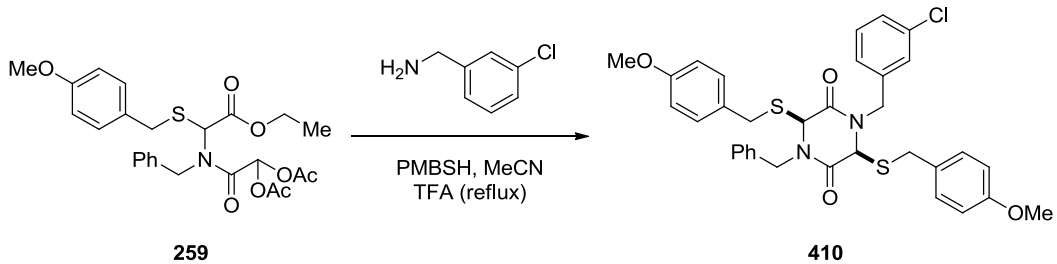
Chemical Formula: $C_{13}H_{19}NO_3S$
Molecular Weight: 269.36

397

Chemical Formula: $C_{19}H_{25}NO_8S$
Molecular Weight: 427.47

A solution of diacetoxyacetyl chloride (2.53 g, 13.0 mmol) (**379**) in dichloromethane (20.0 mL) was added dropwise to a rapidly stirred biphasic mixture of ethyl-2-((4-methoxybenzyl)thio)-2-(methylamino)acetate (3.50 g, 13.0 mmol) (**392**) and sodium hydrogen carbonate (1.09 g, 13.0 mmol) in dichloromethane (150 mL) and water (80.0 mL) at room temperature and the resulting mixture stirred rapidly for 12 hours. The layers were separated and the aqueous phase extracted with dichloromethane (2 x 100 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure to give 2-((2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)(methyl)amino)-2-oxoethane-1,1-diyl diacetate (5.41 g, 97%) (**397**) as a colourless oil that did not require further purification; ν_{max}/cm^{-1} 1774 (C=O), 1739 (C=O), 1685 (C=O); δ H (400 MHz; $CDCl_3$) 1.21 (3H, t, *J* 7.2, OCH_2CH_3), 2.15 (6H, s, $CH(OC(O)CH_3)_2$), 3.03 (3H, s, NCH_3), 3.59 (1H, d, *J* 13.4, SCH_2), 3.66 (1H, d, *J* 13.4, SCH_2), 3.74 (3H, s, OCH_3), 4.11 – 4.15 (2H, m, OCH_2CH_3), 6.35 (1H, s, CHS), 6.80 (2H, d, *J* 8.6, Ar-*H*), 7.11 (1H, s, $CH(OAc)_2$), 7.18 (2H, d, *J* 8.6, Ar-*H*); δ C (101 MHz; $CDCl_3$) 13.85 ($CH_2\mathbf{C}H_3$), 20.34 ($CH(OC(O)\mathbf{C}H_3)_2$), 30.28 ($N\mathbf{C}H_3$), 34.90 ($S\mathbf{C}H_2$), 55.06 ($O\mathbf{C}H_3$), 60.53 (**C**HS), 62.18 ($\mathbf{C}H_2CH_3$), 83.81 ($\mathbf{C}H(OAc)_2$), 113.85 (Ar-**C**-H), 128.58 (quaternary **C**), 130.01 (Ar-**C**-H), 158.76 (quaternary **C**), 164.81 (C**O**), 167.05 (C**O**), 168.39 (C**O**), 168.70 (C**O**); *m/z* 428 (100%, $[M+H]^+$): Found $[M+H]^+$ 428.1312, $C_{19}H_{26}NO_8S$ requires 428.1301.

5.12 (3S,6S)-1-Benzyl-4-(3-chlorobenzyl)-3,6-bis((4-methoxybenzyl) thio)piperazine-2,5-dione (410)[1]



259

Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

410

Chemical Formula: $C_{34}H_{33}ClN_2O_4S_2$
Molecular Weight: 633.22

5.12.1 Method A:

3-Chlorobenzylamine (0.04 mL, 0.45 mmol) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.11 g, 0.21 mmol) (**410**) in acetonitrile (30.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.04 mL, 0.32 mmol) (**257**) and the resulting mixture stirred for 2 minutes. TFA (0.01 mL, 0.15 mmol) was added and the resulting mixture heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-benzyl-4-(3-chlorobenzyl)-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.06 g, 60%) (**410**) as a colourless solid; m.p. 159-161 °C; ν_{max}/cm^{-1} 1670 (C=O), 736 (C-Cl); δ H (400 MHz; $CDCl_3$) 3.53 (1H, d, *J* 14.7, NC*H*$_2$), 3.58 (1H, d, *J* 14.6, NC*H*$_2$), 3.80 – 3.83 (2H, m, SC*H*$_2$), 3.84 (3H, s, OC*H*$_3$), 3.85 (3H, s, OC*H*$_3$), 3.95 – 4.02 (2H, m, SC*H*$_2$), 4.35 (1H, s, C*H*S), 4.38 (1H, s, C*H*S), 5.02 (1H, d, *J* 14.7, NC*H*$_2$), 5.14 (1H, d, *J* 14.6, NC*H*$_2$), 6.53 (1H, d, *J* 7.6, Ar-*H*), 6.65 – 6.71 (3H, m, Ar-*H*), 6.87 – 6.91 (4H, m, Ar-*H*), 7.06 (1H, m, Ar-*H*), 7.14 – 7.25 (4H, m, Ar-*H*), 7.34 – 7.38 (4H, m, Ar-*H*); δ C (101 MHz; $CDCl_3$) 36.30 (S**C**H$_2$), 36.36 (S**C**H$_2$), 45.73 (N**C**H$_2$), 46.10 (N**C**H$_2$), 55.22 (O**C**H$_3$), 55.25 (O**C**H$_3$), 57.32 (**C**HS), 57.87 (**C**HS), 114.14 (Ar-**C**-H), 126.53 (Ar-**C**-H), 127.85 (Ar-**C**-H), 128.11 (Ar-**C**-H), 128.15 (Ar-**C**-H), 128.41 (Ar-**C**-H), 128.61 (Ar-**C**-H), 128.69 (quaternary **C**), 129.79 (Ar-**C**-H), 130.70 (Ar-**C**-H), 130.77 (Ar-**C**-H), 134.44 (quaternary **C**), 134.80 (quaternary **C**), 137.07 (quaternary **C**), 159.13 (quaternary **C**), 159.14 (quaternary **C**), 164.93 (**C**O), 165.01 (**C**O); *m/z* 655.1494 (100%, $[M+Na]^+$), 656.1428 (53.3%, $[M+Na]^+$), 657.1417 (58.0%, $[M+Na]^+$), 658.1183 (123.6%, $[M+Na]^+$), 659.1173 (82.5%, $[M+Na]^+$), $C_{34}H_{33}ClN_2O_4S_2Na$ requires 655.1468.

5.12.2 Method B:

3-Chlorobenzylamine (0.18 mL, 1.48 mmol) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.25 g, 0.49 mmol) (**259**) in acetonitrile (15.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.10 mL, 0.74 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.03 g, 0.25 mmol) was added and the resulting mixture heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-benzyl-4-(3-chlorobenzyl)-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.23 g, 75%) (**410**) as a colourless solid; identical spectroscopic data to that obtained previously for (**410**).

5.12.3 Method C:

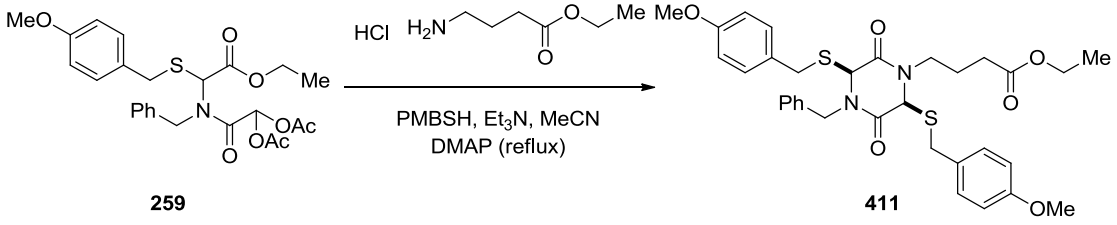
3-Chlorobenzylamine (0.30 mL, 2.44 mmol) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.41 g, 0.81 mmol) (**259**) in acetonitrile (5.00 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.17 mL, 1.22 mmol) (**257**) and the resulting mixture stirred for 2 minutes. TFA (0.03 mL, 0.41 mmol) was added and the mixture heated in the microwave at 150 °C for 5 minutes, allowed to cool to room temperature and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-benzyl-4-(3-chlorobenzyl)-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.27 g, 51%) (**410**) as a colourless solid; identical spectroscopic data to that obtained previously for (**410**).

5.12.4 Method D:

3-Chlorobenzylamine (0.13 mL, 1.07 mmol) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.18 g, 0.36 mmol) (**259**) in acetonitrile (5.00 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.07 mL, 0.53 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.02 g, 0.18 mmol) was added and the mixture heated in the microwave at 150 °C for 5 minutes, allowed to cool to room temperature and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1,

petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-benzyl-4-(3-chlorobenzyl)-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.15 g, 68%) (**410**) as a colourless solid; identical spectroscopic data to that obtained previously for (**410**).

5.13 Ethyl-4-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)butanoate (411)



259

Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

411

Chemical Formula: $C_{33}H_{38}N_2O_6S_2$
Molecular Weight: 622.79

5.13.1 Method A:

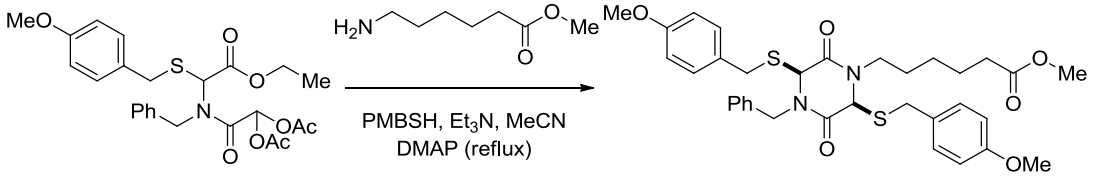
Ethyl-4-aminobutanoate hydrochloride (0.24 g, 1.40 mmol) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.24 g, 0.47 mmol) (**259**) in acetonitrile (15.0 mL) followed by addition of triethylamine (0.26 mL, 1.87 mmol) and *para*-methoxybenzyl mercaptan (0.10 mL, 0.70 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.03 g, 0.23 mmol) was added and the resulting mixture heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give ethyl 4-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)butanoate (0.20 g, 70%) (**411**) as a colourless solid; m.p. 105-107 °C; ν_{max}/cm^{-1} 2931 (C-H), 1730 (C=O), 1674 (C=O), 833 (C-H), 733 (C-H); δ H (400 MHz, $CDCl_3$) 1.13 (3H, t, *J* 7.1, OCH_2CH_3), 1.31 – 1.51 (2H, m, $CH_2CO_2CH_2CH_3$), 1.93 – 2.02 (2H, m, NCH_2CH_2), 2.54 – 2.66 (1H, m, NCH_2CH_2), 3.41– 3.54 (2H, m, NCH_2-Ph), 3.70 (1H, d, *J* 12.2, SCH_2), 3.73 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.78 – 3.83 (1H, m, SCH_2), 3.85 (1H, d, *J* 13.9, SCH_2), 3.90 – 4.01 (3H, m, $CO_2CH_2CH_3$ & SCH_2), 4.39 (1H, s, CHS), 4.21 (1H, s, CHS), 5.08 (1H, d, *J* 14.5, NCH_2), 6.61 – 6.65 (2H, m, Ar-*H*), 6.77 – 6.81 (2H, m, Ar-*H*), 6.80 – 6.84 (2H, m, Ar-*H*), 7.03 – 7.09 (2H, m, Ar-*H*), 7.11 – 7.13 (1H, m, Ar-*H*), 7.23 – 7.27 (2H, m, Ar-*H*), 7.30 – 7.34 (2H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 14.04 ($CO_2CH_2\mathbf{C}H_3$), 21.95 ($\mathbf{C}H_2$), 31.29 ($\mathbf{C}H_2$), 36.10 ($S\mathbf{C}H_2$), 36.80 ($S\mathbf{C}H_2$), 43.13 ($\mathbf{C}H_2$), 46.00 ($\mathbf{C}H_2$), 55.14 ($O\mathbf{C}H_3$), 55.15 ($O\mathbf{C}H_3$), 57.23 (**C**HS), 59.26 (**C**HS), 60.24 ($CO_2\mathbf{C}H_2CH_3$), 113.96 (Ar-**C**-H), 114.01 (Ar-**C**-H), 127.72 (Ar-**C**-H), 128.46 (Ar-**C**-H), 128.52 (Ar-**C**-H), 128.56 (quaternary **C**), 128.73 (quaternary **C**), 130.60 (Ar-**C**-H), 130.67 (Ar-**C**-H), 134.84 (quaternary **C**), 159.01 (quaternary **C**), 159.03 (quaternary **C**),

164.92 (**C**O), 165.07 (**C**O), 172.26 (**C**O_2CH_2); *m/z* 645 (100%, $[M+Na]^+$): Found $[M+Na]^+$ 645.2069, $C_{33}H_{38}N_2O_6S_2Na$ requires 645.2069.

5.13.2 Method B:

Ethyl 4-aminobutanoate hydrochloride (0.10 g, 0.61 mmol) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.24 g, 0.47 mmol) (**259**) in acetonitrile (3.00 mL) followed by addition of triethylamine (0.34 mL, 2.45 mmol) and *para*-methoxybenzyl mercaptan (0.10 mL, 0.73 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.04 g, 0.31 mmol) was added and the mixture heated in the microwave at 130 °C for 35 minutes, allowed to cool to room temperature and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give ethyl 4-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)butanoate (0.11 g, 28%) (**411**) as a colourless solid; identical spectroscopic data to that obtained previously for (**411**).

5.14 Methyl 6-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)hexanoate (412)



259

Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

412

Chemical Formula: $C_{34}H_{40}N_2O_6S_2$
Molecular Weight: 636.82

5.14.1 Method A:

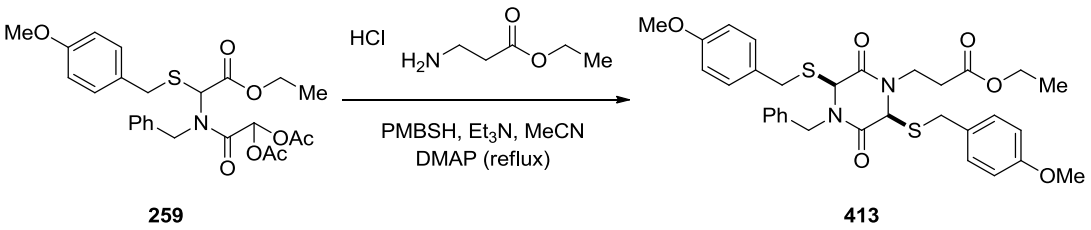
Aminocaproic methyl ester (0.23 g, 1.58 mmol) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.27 g, 0.53 mmol) (**259**) in acetonitrile (15.0 mL) followed by addition of triethylamine (0.29 mL, 2.11 mmol) and *para*-methoxybenzyl mercaptan (0.11 mL, 0.79 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.03 g, 0.26 mmol) was added and the resulting mixture heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give methyl 6-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)hexanoate (0.26 g, 76%) (**412**) as a colourless solid; m.p. 109-112 °C; ν_{max}/cm^{-1} 1641 (C=O), 1452 (C-H), 1174 (C-O); δ H (400 MHz, $CDCl_3$) 0.96 – 1.13 (3H, m, $NCH_2(CH_2)_3CH_2CO$), 1.14 – 1.26 (1H, m, $NCH_2(CH_2)_3CH_2CO$), 1.42 – 1.55 (2H, m, $NCH_2(CH_2)_3CH_2CO$), 2.24 (2H, t, *J* 2.3, $NCH_2(CH_2)_3CH_2CO$), 2.58 – 2.65 (1H, m, $NCH_2(CH_2)_3CH_2CO$), 3.47 – 3.55 (1H, m, $NCH_2(CH_2)_3CH_2CO$), 3.59 (1H, d, *J* 14.5, NCH_2-Ph), 3.68 (3H, s, CO_2CH_3), 3.83 (3H, s, OCH_3), 3.80 (1H, d, *J* 13.9, SCH_2), 3.85 – 3.92 (4H, m, SCH_2 & OCH_3), 3.96 (1H, d, *J* 13.9, SCH_2), 4.03 (1H, d, *J* 13.9, SCH_2), 4.31 (1H, s, CHS), 4.43 (1H, s, CHS), 5.17 (1H, d, *J* 14.5, NCH_2-Ph), 6.71 (2H, d, *J* 7.0, Ar-*H*), 6.85 – 6.96 (4H, m, Ar-*H*), 7.12 – 7.19 (2H, m, Ar-*H*), 7.19 – 7.25 (1H, m, Ar-*H*), 7.32 – 7.39 (2H, m, Ar-*H*), 7.39 – 7.45 (2H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 24.34 (**C**H_2), 26.12 (**C**H_2), 26.39 (**C**H_2), 33.68 (**C**H_2), 36.18 (**C**H_2), 36.70 (**C**H_2), 43.52 (**C**H_2), 46.04 (**C**H_2), 51.36 (CO_2**C**H_3),55.18 (O**C**H_3), 55.21 (O**C**H_3), 57.32 (**C**HS), 58.90 (**C**HS), 113.96 (Ar-**C**-H), 114.06 (Ar-**C**-H), 127.78 (Ar-**C**-H), 128.52 (Ar-**C**-H), 128.66 (quaternary **C**), 128.81 (quaternary **C**), 130.68 (Ar-**C**-H), 130.72 (Ar-**C**-H), 134.91 (quaternary **C**), 159.06

(quaternary **C**), 159.07 (quaternary **C**), 164.91 (**C**O), 165.22 (**C**O), 173.69 (**C**O_2CH_3); *m/z* 637 (100%, $[M+H]^+$): Found $[M+H]^+$ 637.2441, $C_{34}H_{41}N_2O_6S_2$ requires 637.2406.

5.14.2 Method B:

Aminocaproic methyl ester (0.26 g, 1.79 mmol) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.30 g, 0.60 mmol) (**259**) in acetonitrile (3.00 mL) followed by addition of triethylamine (0.34 mL, 2.38 mmol) and *para*-methoxybenzyl mercaptan (0.09 mL, 0.66 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.04 g, 0.30 mmol) was added and the mixture heated in the microwave at 130 °C for 25 minutes, allowed to cool to room temperature and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give methyl 6-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)hexanoate (0.17 g, 46%) (**412**) as a colourless solid; identical spectroscopic data to that obtained previously for (**412**).

5.15 Ethyl-3-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)propanoate (413)



259

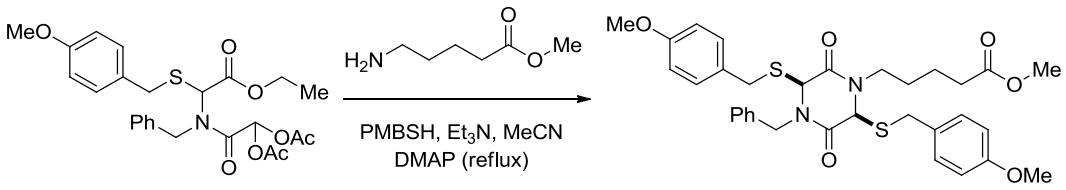
Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

413

Chemical Formula: $C_{32}H_{36}N_2O_6S_2$
Molecular Weight: 608.77

Ethyl-3-aminopropanoate hydrochloride (0.08 g, 0.52 mmol) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.26 g, 0.52 mmol) (**259**) in acetonitrile (15.0 mL) followed by addition of triethylamine (0.29 mL, 2.07 mmol) and *para*-methoxybenzyl mercaptan (0.11 mL, 0.77 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.03 g, 0.26 mmol) was added and the resulting mixture stirred for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give ethyl-3-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)propanoate (0.19 g, 61%) (**413**) as a light yellow solid; m.p. 127-131 °C; ν_{max}/cm^{-1} 1732 (C=O), 1672 (C=O), 1451 (C-H); δ H (400 MHz, $CDCl_3$) 1.11 (3H, t, *J* 7.1, $CO_2CH_2CH_3$), 2.24 – 2.38 (2H, m, $CH_2CO_2CH_2CH_3$), 2.90 – 3.06 (1H, m, NCH_2CH_2), 3.48 (1H, d, *J* 14.5, NCH_2Ph), 3.55 (1H, m, NCH_2CH_2), 3.67 – 3.76 (4H, m, SCH_2 & OCH_3), 3.77 (3H, s, OCH_3), 3.83 – 3.86 (2H, m, SCH_2), 3.95 – 3.98 (3H, m, $CO_2CH_2CH_3$ & SCH_2), 4.22 (1H, s, CHS), 4.55 (1H, s, CHS), 5.08 – 5.10 (1H, m, Ar-*H*), 6.56 – 6.69 (2H, m, Ar-*H*), 6.75 – 6.88 (4H, m, Ar-*H*), 7.03 – 7.09 (2H, m, Ar-*H*), 7.12 – 7.14 (1H, m, Ar-*H*), 7.26 (2H, d, *J* 8.6, Ar-*H*), 7.32 (2H, d, *J* 8.6, Ar-*H*); δ C (101 MHz, $CDCl_3$) 13.98 (CH_2CH_3) 31.77 (CH_2), 36.14 (CH_2), 36.91 (CH_2), 40.80 (CH_2), 46.02 (CH_2), 55.16 (OCH_3), 55.18 (OCH_3), 57.21 (CHS), 60.44 (CHS), 60.51 (CO_2CH_2), 114.03 (Ar-C-H), 127.75 (Ar-C-H), 128.47 (Ar-C-H), 128.50 (Ar-C-H), 128.71 (quaternary C), 130.51 (Ar-C-H), 130.69 (Ar-C-H), 134.88 (quaternary C), 159.05 (quaternary C), 164.96 (CO), 165.00 (CO), 170.64 (CO_2CH_2); *m/z* 609 (100%, $[M+H]^+$): Found $[M+H]^+$ 609.2103, $C_{32}H_{37}N_2O_6S_2$ requires 609.2093.

5.16 Methyl 5-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)pentanoate (414)



259

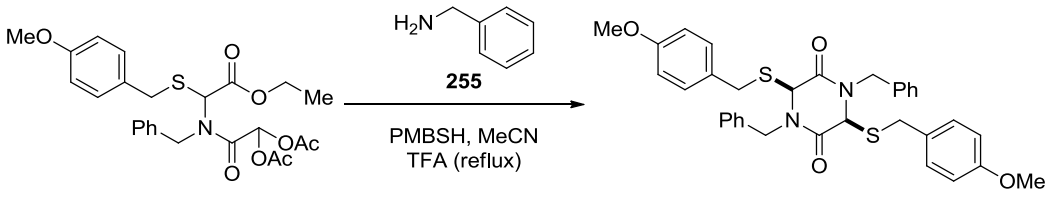
Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

414

Chemical Formula: $C_{33}H_{38}N_2O_6S_2$
Molecular Weight: 622.79

Aminovaleric methyl ester (0.16 g, 1.22 mmol) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.20 g, 0.41 mmol) (**259**) in acetonitrile (15.0 mL) followed by addition of triethylamine (0.23 mL, 1.62 mmol) and *para*-methoxybenzyl mercaptan (0.09 mL, 0.61 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.03 g, 0.20 mmol) was added and the resulting mixture heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give methyl 5-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)pentanoate (0.20 g, 78%) (**414**) as a colourless solid; m.p. 94-96 °C; ν_{max}/cm^{-1} 1732 (C=O), 1663 (C=O), 731 (C-H); δ H (400 MHz; $CDCl_3$) 1.08 – 1.36 (4H, m, $NCH_2(CH_2)_2CH_2CO$), 2.00 – 2.15 (2H, m, $NCH_2(CH_2)_2CH_2CO$), 2.46 – 2.60 (1H, m, $NCH_2(CH_2)_2CH_2CO$), 3.38 – 3.45 (1H, m, $NCH_2(CH_2)_2CH_2CO$), 3.49 (1H, d, *J* 14.5, NCH_2-Ph), 3.58 (3H, d, *J* 5.0, CO_2CH_3), 3.67 – 3.72 (1H, m, SCH_2), 3.73 (3H, s, OCH_3), 3.75 – 3.82 (4H, m, SCH_2 & OCH_3), 3.83 – 3.88 (1H, m, SCH_2), 3.93 (1H, d, *J* 13.1, SCH_2), 4.22 (1H, s, CHS), 4.35 (1H, s, CHS), 5.07 (1H, d, *J* 14.5, NCH_2-Ph), 6.59 – 6.64 (2H, m, Ar-*H*), 6.76 – 6.85 (5H, m, Ar-*H*), 7.03 – 7.14 (3H, m, Ar-*H*), 7.25 – 7.27 (1H, m, Ar-*H*), 7.30 – 7.34 (2H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 21.87 ($\mathbf{C}H_2$), 26.11 ($\mathbf{C}H_2$), 33.25 ($\mathbf{C}H_2$), 36.13 ($S\mathbf{C}H_2$), 36.69 ($S\mathbf{C}H_2$), 43.26 ($\mathbf{C}H_2$), 46.01 ($\mathbf{C}H_2$), 51.36 ($O\mathbf{C}H_3$), 55.13 ($O\mathbf{C}H_3$), 55.16 (**C**HS), 57.28 (**C**HS), 58.93 ($\mathbf{C}O_2CH_3$), 113.94 (Ar-**C**-H), 114.02 (Ar-**C**-H), 127.73(Ar-**C**-H), 128.48 (Ar-**C**-H), 128.74 (Ar-**C**-H), 130.63 (quaternary **C**), 130.68 (Ar-**C**-H), 134.84 (Ar-**C**-H), 159.02 (quaternary **C**), 159.05 (quaternary **C**), 164.89 (**C**O), 165.16 (**C**O), 173.26 ($\mathbf{C}O_2CH_3$); *m/z* 645 (100%, $[M+Na]^+$): Found $[M+Na]^+$ 645.2101, $C_{33}H_{38}N_2O_6S_2Na$ requires 645.2069.

5.17 1,4-Dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (415)[253]



259

Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

415

Chemical Formula: $C_{34}H_{34}N_2O_4S_2$
Molecular Weight: 598.77

5.17.1 Method A:

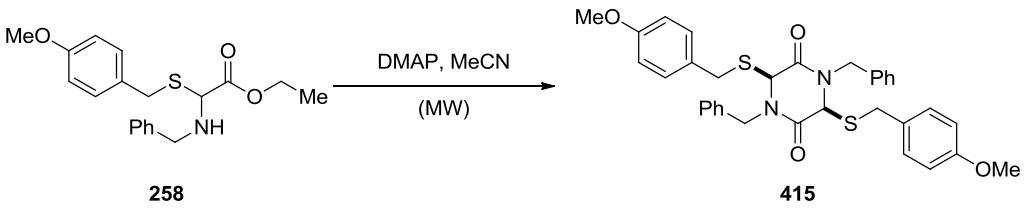
Benzylamine (0.66 mL, 5.99 mmol) (**255**) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (1.01 g, 1.99 mmol) (**259**) in acetonitrile (30.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.42 mL, 2.99 mmol) (**257**) and the resulting mixture stirred for 2 minutes. TFA (0.05 mL, 0.07 mmol) was added and the resulting mixture heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give 1,4-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.76 g, 64%) (**415**) as a colourless solid; m.p. 205-206 °C; ν_{max}/cm^{-1} 3231 (N-H), 1664 (C=O), 1504 (C=O); δ H (400 MHz; $CDCl_3$) 3.58 (2H, d, *J* 14.6, NC*H*$_2$), 3.83 (2H, d, *J* 14.0, SC*H*$_2$), 3.86 (6H, s, OC*H*$_3$), 3.99 (2H, d, *J* 14.0, SC*H*$_2$), 4.38 (2H, s, C*H*S), 5.13 (2H, d, *J* 14.6, NC*H*$_2$), 6.65 (4H, d, *J* 6.8, Ar-*H*), 6.89 (4H, d, *J* 8.6, Ar-*H*), 7.11 – 7.21 (6H, m, Ar-*H*), 7.36 (4H, d, *J* 8.6, Ar-*H*); δ C (101 MHz; $CDCl_3$) 36.30 (**C**H$_2$), 46.01 (**C**H$_2$), 55.25 (O**C**H$_3$), 57.37 (**C**HS), 114.11 (Ar-**C**-H), 127.80 (Ar-**C**-H), 128.47 (Ar-**C**-H), 128.56 (Ar-**C**-H), 128.81 (quaternary **C**), 130.80 (Ar-**C**-H), 134.86 (quaternary **C**), 159.09 (quaternary **C**), 165.00 (**C**O); *m/z* 599 (100%, $[M+H]^+$): Found $[M+H]^+$ 599.2065, $C_{34}H_{35}N_2O_4S_2$ requires 599.2038.

5.17.2 Method B:

Benzylamine (0.08 mL, 0.72 mmol) (**255**) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.12 g, 0.24 mmol) (**259**) in acetonitrile (5.00 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.05 mL, 0.72 mmol) (**257**) and the resulting mixture stirred for 2 minutes. TFA (0.03 mL, 0.41 mmol) was added and the mixture heated in the

microwave at 150 °C for 5 minutes, allowed to cool to room temperature and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give 1,4-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.09 g, 60%) (**415**) as a colourless solid; identical spectroscopic data to that obtained previously for (**415**).

5.18 1,4-Dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (415)



258 **415**

Chemical Formula: $C_{19}H_{23}NO_3S$
Molecular Weight: 345.46

Chemical Formula: $C_{34}H_{34}N_2O_4S_2$
Molecular Weight: 598.77

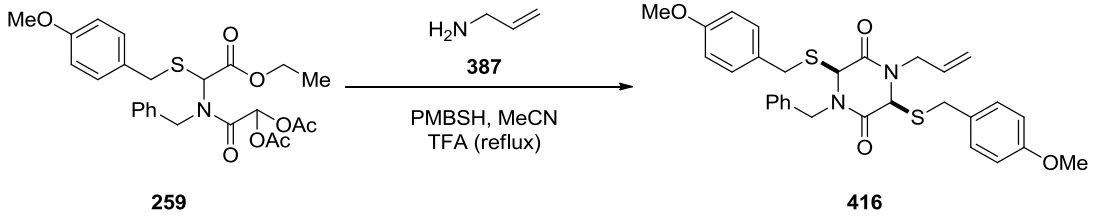
5.18.1 Method A:

DMAP (0.08 g, 0.61 mmol) was added to a solution of ethyl-2-(benzylamino)-2-((4-methoxybenzyl)thio)acetate (0.30 g, 0.87 mmol) (**258**) in acetonitrile (1.50 mL) and the mixture stirred for 10 minutes at RT and then heated in the microwave at 160 °C for 30 minutes. Solvent was removed under reduced pressure and the residue purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give 1,4-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.02 g, 8%) (**415**) as a colourless solid; identical spectroscopic data to that obtained previously for (**415**).

5.18.2 Method B:

TFA (0.05 mL, 0.66 mmol) was added to a solution of ethyl-2-(benzylamino)-2-((4-methoxybenzyl)thio)acetate (0.33 g, 0.94 mmol) (**258**) in acetonitrile (1.50 mL) and the mixture stirred for 10 minutes at RT and then heated in the microwave at 160 °C for 30 minutes. Solvent was removed under reduced pressure and the residue purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give 1,4-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.05 g, 17%) (**415**) as a colourless solid; identical spectroscopic data to that obtained previously for (**415**).

5.19 (3S,6S)-1-Allyl-4-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (416)[253]



259

Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

416

Chemical Formula: $C_{30}H_{32}N_2O_4S_2$
Molecular Weight: 548.72

5.19.1 Method A:

Allylamine (0.44 mL, 5.84 mmol) (**387**) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.98 g, 1.95 mmol) (**259**) in acetonitrile (30.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.41 mL, 2.92 mmol) (**257**) and the resulting mixture stirred for 2 minutes. TFA (0.06 mL, 0.78 mmol) was added and the solution stirred for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-allyl-4-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.58 g, 54%) (**416**) as a light yellow solid; m.p. 123-128 °C; ν_{max}/cm^{-1} 3030 (N-H), 1664 (C=C), 1608 (C=O); δ H (400 MHz, $CDCl_3$) 3.05 (1H, d, *J* 15.0 SCH_2), 3.46 – 3.52 (1H, m, SCH_2), 3.68 – 3.73 (4H, m, SCH_2 & OCH_3), 3.77 – 3.83 (4H, m, SCH_2 & OCH_3), 3.85 – 3.87 (1H, m, NCH_2), 3.91 – 3.97 (2H, m, NCH_2), 4.26 (1H, s, C*H*S), 4.32 – 4.34 (1H, m, NCH_2), 4.41 (1H, s, C*H*S), 4.88 (1H, d, *J* 10.1, $NCH_2CH=CH_2$), 5.03 – 5.13 (1H, m, $NCH_2CH=CH_2$), 5.35 – 5.37 (1H, m, $NCH_2CH=CH_2$), 6.61 – 6.65 (2H, m, Ar-*H*), 6.74 – 6.84 (4H, m, Ar-*H*), 7.08 – 7.12 (3H, m, Ar-*H*), 7.23 – 7.32 (4H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 36.46 (**C**H_2), 36.76 (**C**H_2), 45.78 (**C**H_2), 46.28 (**C**H_2), 55.45 (O**C**H_3), 55.52 (O**C**H_3), 57.56 (**C**HS), 58.27 (**C**HS), 114.26 (Ar-**C**-H), 114.32 (Ar-**C**-H), 119.63 (**C**H_2), 128.03(Ar-**C**-H), 128.77 (Ar-**C**-H), 129.02 (quaternary **C**), 130.87 (Ar-**C**-H), 131.00 (Ar-**C**-H), 135.17 (quaternary **C**), 159.32 (quaternary **C**), 164.96 (**C**O), 165.24 (**C**O); *m/z* 549 (100%, $[M+H]^+$); Found $[M+H]^+$ 549.1874, $C_{30}H_{33}N_2O_4S_2$ requires 549.1882.

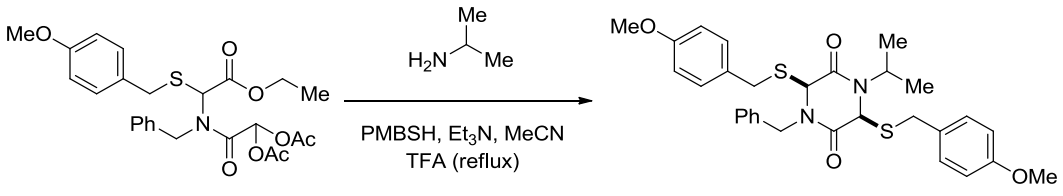
5.19.2 Method B:

Allylamine (0.09 mL, 1.15 mmol) (**387**) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.19 g, 0.52 mmol) (**259**) in acetonitrile (15.0 mL) followed by addition *para*-methoxybenzyl mercaptan (0.08 mL, 0.58 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.02 g, 0.19 mmol) was added and the resulting mixture stirred for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-allyl-4-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.11 g, 53%) (**416**) as a light yellow solid; identical spectroscopic data to that obtained previously for (**416**).

5.19.3 Method C:

Allylamine (0.18 mL, 2.33 mmol) (**387**) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.59 g, 1.17 mmol) (**259**) in acetonitrile (4.00 mL) followed by addition *para*-methoxybenzyl mercaptan (0.41 mL, 2.91 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.07 g, 0.58 mmol) was added and the mixture heated in the microwave at 140 °C for 30 minutes, allowed to cool to room temperature and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-allyl-4-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.49 g, 76%) (**416**) as a light yellow solid; identical spectroscopic data to that obtained previously for **(416)**.

5.20 (3S,6S)-1-Benzyl-4-isopropyl-3,6-bis((4-methoxybenzyl)thio) piperazine-2,5-dione (417)[217]



259

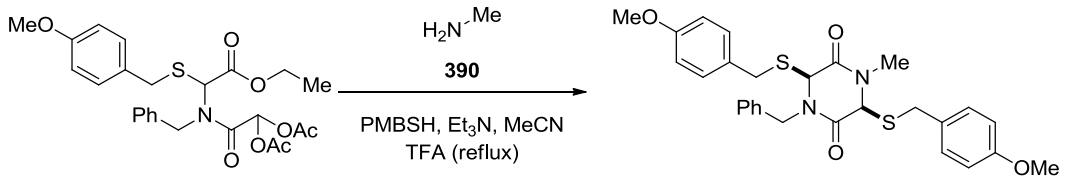
Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

417

Chemical Formula: $C_{30}H_{34}N_2O_4S_2$
Molecular Weight: 550.73

Isopropylamine (0.51 mL, 6.02 mmol) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (1.01 g, 1.99 mmol) (**259**) in acetonitrile (30.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.42 mL, 2.99 mmol) (**257**) and the resulting mixture stirred for 2 minutes. TFA (0.05 mL, 0.07 mmol) was added and the resulting solution heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-benzyl-4-isopropyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.76 g, 64%) (**417**) as a colourless solid; m.p. 119-121 °C; ν_{max}/cm^{-1} 1662 (C=O); δ H (400 MHz; $CDCl_3$) 0.89 (3H, d, *J* 6.8, $CH(CH_3)_2$), 1.12 (3H, d, *J* 6.8, $CH(CH_3)_2$), 3.53 (1H, d, *J* 14.5, NCH_2), 3.77 – 3.84 (8H, m, 2 x OCH_3 & SCH_2), 3.93 – 4.09 (3H, m, SCH_2 & $CH(CH_3)_2$), 4.30 (1H, s, CHS), 4.58 (1H, s, CHS), 5.10 (1H, d, *J* 14.5, NCH_2), 6.67 (2H, d, *J* 7.2, Ar-*H*), 6.84 – 6.90 (4H, m, Ar-*H*), 7.10 – 7.19 (3H, m, Ar-*H*), 7.33 (2H, d, *J* 8.6, Ar-*H*), 7.40 (2H, d, *J* 8.6, Ar-*H*); δ C (101 MHz; $CDCl_3$) 18.86 ($CH(\mathbf{C}H_3)_2$), 20.05 ($CH(\mathbf{C}H_3)_2$), 36.33 ($S\mathbf{C}H_2$), 36.58 ($S\mathbf{C}H_2$), 45.89 ($N\mathbf{C}H_2$), 47.92 ($\mathbf{C}H(CH_3)_2$), 55.21 ($O\mathbf{C}H_3$), 55.25 ($O\mathbf{C}H_3$), 56.02 (**C**HS), 58.10 (**C**HS), 113.88 (Ar-**C**-H), 114.06 (Ar-**C**-H), 127.72 (Ar-**C**-H), 128.49 (Ar-**C**-H), 128.62 (quaternary **C**), 128.95 (quaternary **C**), 130.76 (Ar-**C**-H), 130.78 (Ar-**C**-H), 135.03 (quaternary **C**), 158.93 (quaternary **C**), 159.04 (quaternary **C**), 164.75 (**C**O), 165.10 (**C**O); *m/z* 551 (100%, $[M+H]^+$): Found $[M+H]^+$ 551.2029, $C_{30}H_{35}N_2O_4S_2$ requires 551.2038.

5.21 (3S,6S)-1-Benzyl-3,6-bis((4-methoxybenzyl)thio)-4-methylpiperazine -2,5-dione (418)[217]



259

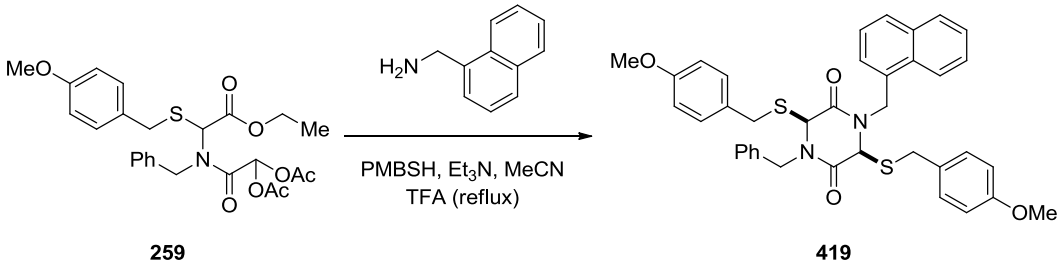
Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

418

Chemical Formula: $C_{28}H_{30}N_2O_4S_2$
Molecular Weight: 522.68

Methylamine (4.60 mL of a 2M solution in tetrahydrofuran, 9.17 mmol) (**390**) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (1.54 g, 3.06 mmol) (**259**) in acetonitrile (30.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (6.64 mL, 4.60 mmol) (**257**) and the resulting mixture stirred for 2 minutes. TFA (0.05 mL, 0.07 mmol) was added and the resulting mixture heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (1:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-benzyl-3,6-bis((4-methoxybenzyl)thio)-4-methylpiperazine-2,5-dione (0.53 g, 33%) (**418**) as a colourless solid; m.p. 127-129 °C; ν_{max}/cm^{-1} 1674 (C=O); δ H (400 MHz; $CDCl_3$) 2.63 (3H, s, NC*H*$_3$), 3.54 (1H, d, *J* 14.5, NC*H*$_2$-Ph), 3.79 – 3.81 (4H, m, OC*H*$_3$ & SC*H*$_2$), 3.83 (3H, s, OC*H*$_3$), 3.88 (1H, d, *J* 13.9, SC*H*$_2$), 3.95 (1H, d, *J* 13.9, SC*H*$_2$), 4.01 (1H, d, *J* 13.8, SC*H*$_2$), 4.29 (1H, s, C*H*S), 4.39 (1H, s, C*H*S), 5.16 (1H, d, *J* 14.5, NC*H*$_2$-Ph), 6.67 (1H, d, *J* 6.8, Ar-*H*), 6.86 (2H, d, *J* 8.6, Ar-*H*), 6.88 (2H, d, *J* 8.6, Ar-*H*), 7.09 – 7.18 (4H, m, Ar-*H*), 7.34 (2H, d, *J* 8.6, Ar-*H*), 7.38 (2H, d, *J* 8.6, Ar-*H*); δ C (101 MHz; $CDCl_3$) 31.50 (N**C**H$_3$), 36.22 (**C**H$_2$), 36.77 (**C**H$_2$), 46.01 (**C**H$_2$), 55.27 (2 x O**C**H$_3$), 57.06 (**C**HS), 61.53 (**C**HS), 113.98 (Ar-**C**-H), 114.10 (Ar-**C**-H), 127.86 (Ar-**C**-H), 128.56 (Ar-**C**-H), 128.63 (Ar-**C**-H), 128.82 (quaternary **C**), 130.64 (Ar-**C**-H), 130.81 (Ar-**C**-H), 134.92 (quaternary **C**), 159.04 (quaternary **C**), 159.09 (quaternary **C**), 165.00 (**C**O), 165.17 (**C**O); *m/z* 523 (100%, $[M+H]^+$): Found $[M+H]^+$ 523.1734, $C_{28}H_{31}N_2O_4S_2$ requires 523.1725.

5.22 (3S,6S)-1-Benzyl-3,6-bis((4-methoxybenzyl)thio)-4-(naphthalen-1-ylmethyl)piperazine-2,5-dione (419)[217]



259

Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

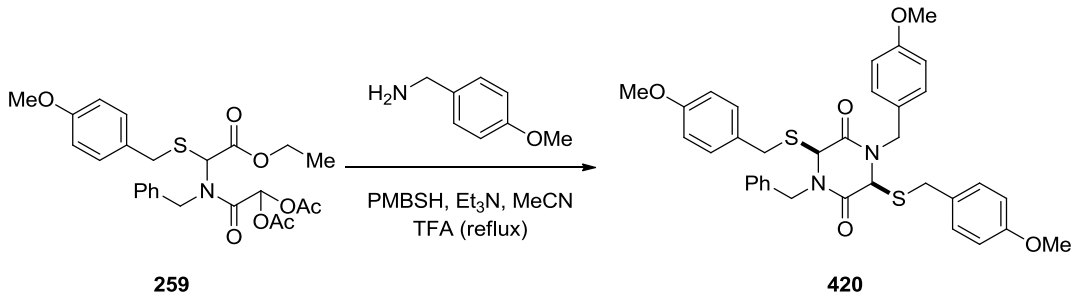
419

Chemical Formula: $C_{38}H_{36}N_2O_4S_2$
Molecular Weight: 648.83

Naphthylmethylamine (0.67 mL, 4.59 mmol) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.77 g, 1.53 mmol) (**259**) in acetonitrile (30.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.32 mL, 2.29 mmol) (**257**) and the resulting mixture stirred for 2 minutes. TFA (0.05 mL, 0.07 mmol) was added and the resulting solution heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (1:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-benzyl-3,6-bis((4-methoxybenzyl)thio)-4-(naphthalen-1-ylmethyl)piperazine-2,5-dione (0.57 g, 58%) (**419**) as a colourless solid; m.p. 138-140 °C; ν_{max}/cm^{-1} 1670 (C=O); δ H (400 MHz; $CDCl_3$) 3.55 (1H, d, *J* 14.9, NC*H*$_2$), 3.80 – 3.81 (1H, m, SC*H*$_2$), 3.82 (3H, s, OC*H*$_3$), 3.86 (3H, s, OC*H*$_3$), 3.89 (1H, d, *J* 13.9, SC*H*$_2$), 3.95 (1H, d, *J* 13.9, SC*H*$_2$), 4.04 (1H, d, *J* 13.9, SC*H*$_2$), 4.12 (1H, d, *J* 14.7, NC*H*$_2$), 4.42 (1H, s, C*H*S), 4.45 (1H, s, C*H*S), 5.11 (1H, d, *J* 14.9, NC*H*$_2$), 5.79 (1H, d, *J* 14.7, NC*H*$_2$), 6.51 (1H, d, *J* 6.9, Ar-*H*), 6.56 (2H, d, *J* 7.5, Ar-*H*), 6.81 (2H, d, *J* 8.5, Ar-*H*), 6.89 (2H, d, *J* 8.5, Ar-*H*), 7.08 – 7.23 (3H, m, Ar-*H*), 7.32 (2H, d, *J* 8.5, Ar-*H*), 7.37 (2H, d, *J* 8.5, Ar-*H*), 7.42 – 7.62 (3H, m, Ar-*H*), 7.74 (1H, d, *J* 8.2, Ar-*H*), 7.83 – 7.86 (2H, m, Ar-*H*); δ C (101 MHz; $CDCl_3$) 36.15 (**C**H$_2$), 36.67 (**C**H$_2$), 44.15 (**C**H$_2$), 45.75 (**C**H$_2$), 55.16 (O**C**H$_3$), 55.23 (O**C**H$_3$), 56.61 (**C**HS), 57.97 (**C**HS), 114.06 (Ar-**C**-H), 114.11 (Ar-**C**-H), 123.29 (Ar-**C**-H), 100.09 (Ar-**C**-H), 100.93 (Ar-**C**-H), 126.61 (Ar-**C**-H), 127.64 (Ar-**C**-H), 127.94 (Ar-**C**-H), 128.05 (Ar-**C**-H), 128.49 (Ar-**C**-H), 128.65 (Ar-**C**-H), 128.88 (Ar-**C**-H), 128.90 (quaternary **C**), 129.59 (quaternary **C**), 130.66 (Ar-**C**-H), 130.71 (Ar-**C**-H), 131.37 (quaternary **C**), 133.76 (quaternary **C**), 134.79 (quaternary **C**), 158.90 (quaternary **C**), 159.08 (quaternary **C**), 164.76 (**C**O),

165.26 (**C**O); *m/z* 649 (100%, $[M+H]^+$): Found $[M+H]^+$ 649.2203, $C_{38}H_{37}N_2O_4S_2$ requires 649.2195.

5.23 (3S,6S)-1-Benzyl-4-(4-methoxybenzyl)-3,6-bis((4-methoxybenzyl) thio)piperazine-2,5-dione (420)[217]



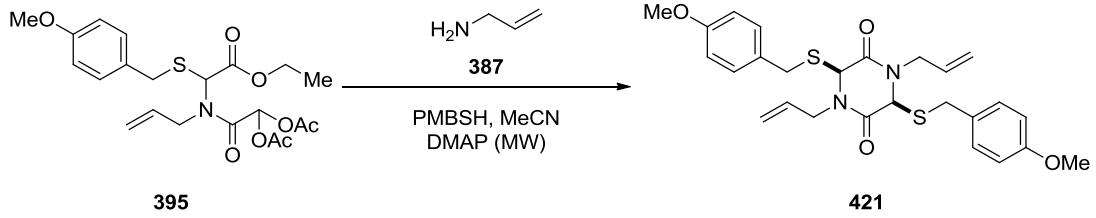
259 **420**

Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

Chemical Formula: $C_{35}H_{36}N_2O_5S_2$
Molecular Weight: 628.80

para-Methoxybenzylamine (0.89 mL, 6.80 mmol) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (1.14 g, 2.27 mmol) (**259**) in acetonitrile (30.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.47 mL, 3.40 mmol) (**257**) and the resulting mixture stirred for 2 minutes. TFA (0.05 mL, 0.07 mmol) was added and the resulting solution heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (1:3, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-benzyl-4-(4-methoxybenzyl)-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (1.20 g, 84%) (**420**) as a colourless solid; m.p. 141-143 °C; ν_{max}/cm^{-1} 1666 (C=O), 1510 (C-H), 829 (C-H); δ H (400 MHz; $CDCl_3$) 3.50 – 3.59 (2H, m, NC*H*$_2$Ar), 3.76 (3H, s, OC*H*$_3$), 3.79 (1H, s, SC*H*$_2$), 3.84 (1H, s, SC*H*$_2$), 3.85 (3H, s, OC*H*$_3$), 3.86 (3H, s, OC*H*$_3$), 3.99 (2H, s, SC*H*$_2$), 4.37 (1H, s, C*H*S), 4.38 (1H, s, C*H*S), 5.09 (1H, d, *J* 11.4, NC*H*$_2$), 5.13 (1H, d, *J* 11.5, NC*H*$_2$), 6.56 (2H, d, *J* 8.7, Ar-*H*), 6.65 – 6.67 (4H, m, Ar-*H*), 6.90 – 6.94 (4H, m, Ar-*H*), 7.11 – 7.21 (3H, m, Ar-*H*), 7.37 – 7.40 (4H, m, Ar-*H*); δ C (101 MHz; $CDCl_3$) 36.15 (S**C**H$_2$), 36.24 (S**C**H$_2$), 45.33 (N**C**H$_2$), 45.94 (N**C**H$_2$), 55.07 (O**C**H$_3$), 55.19 (2 x O**C**H$_3$), 56.96 (**C**HS), 57.42 (**C**HS), 113.88 (Ar-**C**-H), 114.06 (Ar-**C**-H), 126.82 (quaternary **C**), 127.72 (Ar-**C**-H), 128.42 (Ar-**C**-H), 128.49 (Ar-**C**-H), 128.78 (quaternary **C**), 128.85 (quaternary **C**), 129.86 (Ar-**C**-H), 130.73 (Ar-**C**-H), 130.79 (Ar-**C**-H), 134.83 (quaternary **C**), 159.04 (quaternary **C**), 159.10 (quaternary **C**), 164.85 (**C**O), 164.91 (**C**O); *m/z* 629 (100%, $[M+H]^+$): Found $[M+H]^+$ 629.2145, $C_{35}H_{37}N_2O_5S_2$ requires 629.2144.

5.24 (3S,6S)-1,4-Diallyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (421)



395

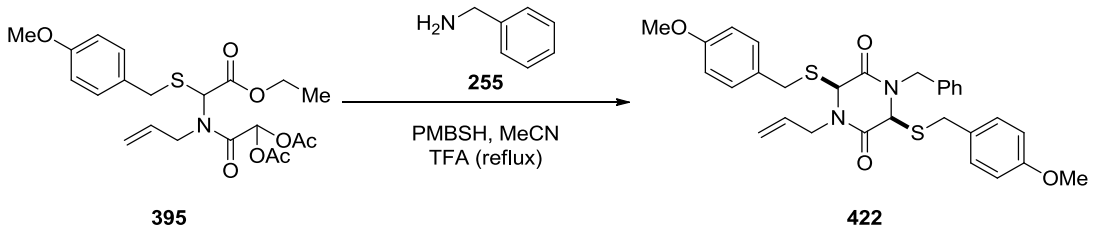
Chemical Formula: $C_{21}H_{27}NO_8S$
Molecular Weight: 453.51

421

Chemical Formula: $C_{26}H_{30}N_2O_4S_2$
Molecular Weight: 498.66

Allylamine (0.62 mL, 8.31 mmol) (**387**) was added to a solution of 2-(allyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (2.09 g, 4.62 mmol) (**395**) in acetonitrile (15.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (1.29 mL, 9.23 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.28 g, 2.31 mmol) was added and the mixture heated in the microwave at 140 °C for 30 minutes, allowed to cool to room temperature and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1,4-diallyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (1.67 g, 72%) (**421**) as a colourless solid; m.p. 70-71 °C; ν_{max}/cm^{-1} 1675 (C=O), 1609 (C=C), 1465 (CH_2), 832 (C-H); δ H (400 MHz, $CDCl_3$) 3.82 – 3.91 (12H, m, NC*H*$_2$ & OC*H*$_3$ & SC*H*$_2$), 3.98 – 4.02 (2H, m, NC*H*$_2$), 4.45 (2H, s, C*H*S), 4.62 – 4.68 (2H, m, CH=C*H*$_2$), 4.99 – 5.05 (2H, m, CH=C*H*$_2$), 5.48 – 5.54 (2H, m, C*H*=CH$_2$), 6.88 – 6.90 (4H, m, Ar-*H*), 7.36 – 7.38 (4H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 36.74 (**C**H$_2$), 45.85 (**C**H$_2$), 55.56 (O**C**H$_3$), 58.20 (**C**HS), 114.28 (**C**H=CH$_2$), 119.78 (**C**H$_2$), 129.04 (quaternary **C**), 130.91 (Ar-**C**-H), 130.95 (Ar-**C**-H), 159.36 (quaternary **C**), 165.09 (**C**O); *m/z* 499 (100%, $[M+H]^+$); Found $[M+H]^+$ 499.1723, $C_{26}H_{31}N_2O_4S_2$ requires 499.1725.

5.25 (3S,6S)-1-Allyl-4-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (416)[253]



395

Chemical Formula: $C_{21}H_{27}NO_8S$
Molecular Weight: 453.51

422

Chemical Formula: $C_{30}H_{32}N_2O_4S_2$
Molecular Weight: 548.72

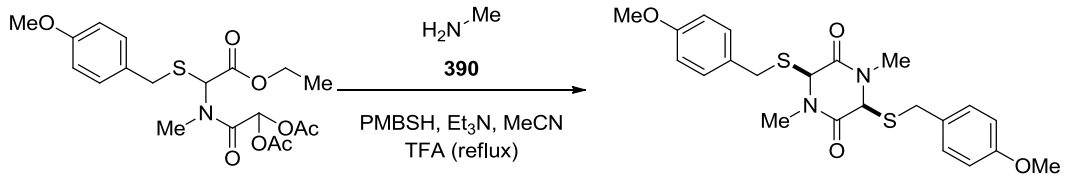
5.25.1 Method A:

Benzylamine (0.40 mL, 3.68 mmol) (**255**) was added to a solution of 2-(allyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.56 g, 1.23 mmol) (**395**) in acetonitrile (30.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.25 mL, 1.79 mmol) (**257**) and the resulting mixture stirred for 2 minutes. TFA (0.05 mL, 0.66 mmol) was added and the resulting mixture stirred for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-allyl-4-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.55 g, 80%) (**422**) as a light yellow solid; identical spectroscopic data to that obtained previously for (**416**).

5.25.2 Method B:

Benzylamine (0.23 mL, 2.07 mmol) (**255**) was added to a solution of 2-(allyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.63 g, 1.38 mmol) (**395**) in acetonitrile (30.0 mL) followed by addition *para*-methoxybenzyl mercaptan (0.39 mL, 2.77 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.08 g, 0.69 mmol) was added and the resulting mixture stirred for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-1-allyl-4-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.55 g, 72%) (**422**) as a light yellow solid; identical spectroscopic data to that obtained previously for (**416**).

5.26 (3S,6S)-3,6-Bis((4-methoxybenzyl)thio)-1,4-dimethylpiperazine-2,5-dione (423)



259

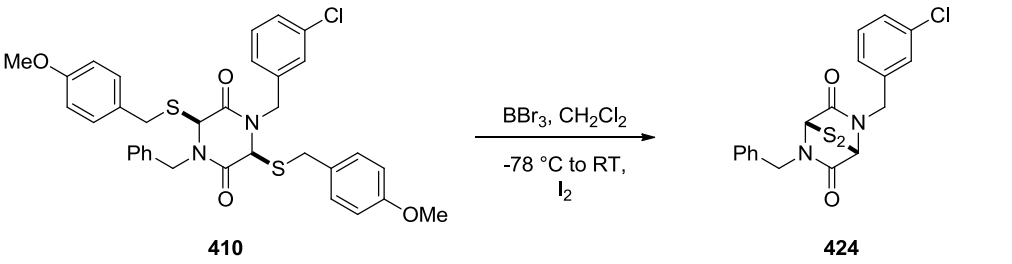
Chemical Formula: $C_{19}H_{25}NO_8S$
Molecular Weight: 427.47

423

Chemical Formula: $C_{22}H_{26}N_2O_4S_2$
Molecular Weight: 446.58

Methylamine (17.5 mL of a 2M solution in tetrahydrofuran, 35.0 mmol) (**390**) was added to a solution of 2-((2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)(methyl)amino)-2-oxoethane-1,1-diyl diacetate (4.98 g, 12.0 mmol) (**259**) in toluene (100 mL) followed by addition of *para*-methoxybenzyl mercaptan (2.44 mL, 17.5 mmol) (**257**) and the resulting mixture stirred for 2 minutes. TFA (0.05 mL, 0.07 mmol) was added and the resulting solution stirred for one hour and then heated to reflux under Dean and Stark water removal conditions for 24 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (1:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,6S)-3,6-bis((4-methoxybenzyl)thio)-1,4-dimethylpiperazine-2,5-dione (3.21 g, 62%) (**423**) as a colourless crystalline solid; m.p. 118-119 °C; ν_{max}/cm^{-1} 1651 (C=O); δ H (400 MHz; $CDCl_3$) 2.65 (6H, s, NC*H*$_3$), 3.78 (6H, s, OC*H*$_3$), 3.83 (2H, d, *J* 13.6, SC*H*$_2$), 3.96 (2H, d, *J* 13.6, SC*H*$_2$), 4.31 (2H, s, C*H*S), 6.85 (4H, d, *J* 8.6, Ar-*H*), 7.34 (4H, d, *J* 8.6, Ar-*H*); δ C (101 MHz; $CDCl_3$) 31.51 (N**C**H$_3$), 36.60 (**C**H$_2$), 55.21 (O**C**H$_3$), 61.36 (**C**HS), 113.90 (Ar-**C**-H), 128.55 (quaternary **C**), 130.57 (Ar-**C**-H), 158.97 (quaternary **C**), 165.14 (**C**O); *m/z* 447 (100%, $[M+H]^+$): Found $[M+H]^+$ 447.1353, $C_{22}H_{27}N_2O_4S_2$ requires 447.1334.

5.27 (±)-(1S,4S)-2-Benzyl-5-(3-chlorobenzyl)-7-thia-2,5-diazabicyclo [2.2.1]heptane-3,6-dione 7-sulfide (424)[217]



410

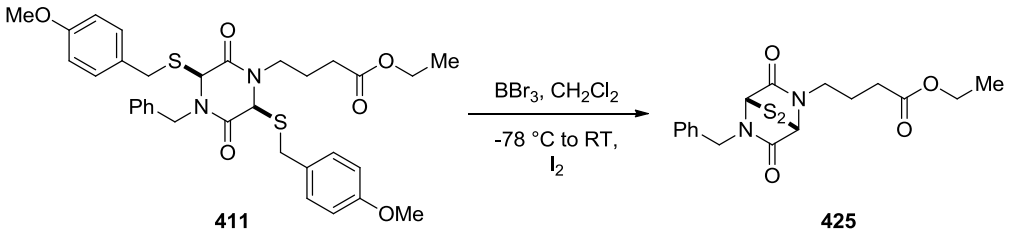
Chemical Formula: $C_{34}H_{33}ClN_2O_4S_2$
Molecular Weight: 633.22

424

Chemical Formula: $C_{18}H_{15}ClN_2O_2S_2$
Molecular Weight: 390.91

Boron tribromide (0.21 mL, 2.25 mmol) was added dropwise to a solution of (3S,6S)-1-benzyl-4-(3-chlorobenzyl)-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.72 g, 1.12 mmol) (**410**) in dichloromethane (40.0 mL) at -78 °C. The resulting mixture was stirred for 45 minutes whereupon aqueous ammonium chloride (15.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. A solution of saturated aqueous sodium thiosulfate was added, the mixture stirred for 30 minutes and diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (±)-(1S,4S)-2-benzyl-5-(3-chlorobenzyl)-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.14 g, 32%) (**424**) as a pale yellow solid; m.p. 147-149 °C; ν_{max}/cm^{-1} 1674 (C=O); δ H (400 MHz; $CDCl_3$) 4.50 – 4.52 (2H, m, NC*H*$_2$Ar), 4.72 (1H, d, *J* 11.1, NC*H*$_2$), 4.84 (1H, d, *J* 11.9, NC*H*$_2$), 5.26 (1H, s, C*H*S), 5.27 (1H, s, C*H*S), 7.18 – 7.19 (1H, m, Ar-*H*), 7.26 – 7.40 (8H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 47.24 (N**C**H$_2$), 47.71 (N**C**H$_2$), 64.49 (**C**HS), 64.85 (**C**HS), 126.30 (Ar-**C**-H), 128.36 (Ar-**C**-H), 128.43 (Ar-**C**-H), 128.58 (Ar-**C**-H), 128.74 (Ar-**C**-H), 129.14 (Ar-**C**-H), 130.36 (Ar-**C**-H), 134.01 (quaternary **C**), 134.91 (quaternary **C**), 136.27 (quaternary **C**), 163.58 (**C**O), 163.65 (**C**O); *m/z* 391.0853 (100%, $[M+H]^+$), 392.0931 (27.6%, $[M+H]^+$), 393.0873 (42.3%, $[M+H]^+$), 394.0934 (14.1%, $[M+H]^+$), 395.0991 (13.9%, $[M+H]^+$), $C_{18}H_{16}ClN_2O_2S_2$ requires 391.0842.

5.28 Ethyl 4-((±)-(1S,4S)-5-benzyl-3,6-dioxo-7-sulfido-7-thia-2,5-diazabicyclo [2.2.1]heptan-2-yl)butanoate (425)

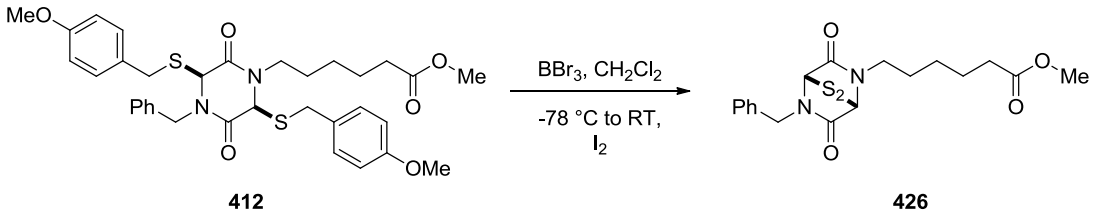


Chemical Formula: $C_{33}H_{38}N_2O_6S_2$
Molecular Weight: 622.79

Chemical Formula: $C_{17}H_{20}N_2O_4S_2$
Molecular Weight: 380.48

Boron tribromide (0.04 mL, 0.39 mmol) was added dropwise to a solution of ethyl 4-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)butanoate (0.12 g, 0.19 mmol) (**411**) in dichloromethane (10.0 mL) at -78 °C. The resulting mixture was stirred for 30 minutes whereupon sodium hydrogen carbonate (10.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 5 minutes. A solution of saturated aqueous sodium thiosulfate was added and the mixture stirred for 30 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further recrystallisation to give ethyl 4-((±)-(1S,4S)-5-benzyl-3,6-dioxo-7-sulfido-7-thia-2,5-diazabicyclo[2.2.1]heptan-2-yl)butanoate (0.05 g, 64%) (**425**) as a pale yellow solid; m.p. 82-84 °C; ν_{max}/cm^{-1} 1498 (CH_2), 1160 (C-C(O)-C); δ H (400 MHz; $CDCl_3$) 1.28 (3H, t, *J* 7.1, OCH_2CH_3), 1.99 – 2.09 (2H, m, $NCH_2CH_2CH_2CO$), 2.40 – 2.43 (2H, m, $NCH_2CH_2CH_2CO$), 3.52 – 3.67 (2H, m, $NCH_2CH_2CH_2CO$), 4.17 – 4.20 (2H, m, OCH_2CH_3), 4.53 (1H, d, *J* 12.0, NCH_2-Ph), 4.86 (1H, d, *J* 12.2, NCH_2-Ph), 5.20 (1H, s, CHS), 5.42 (1H, s, CHS), 7.29 – 7.32 (2H, m, Ar-*H*), 7.36 – 7.41 (3 H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 14.43 ($\mathbf{C}H_3$), 23.27 ($\mathbf{C}H_2$), 31.23 ($\mathbf{C}H_2$), 44.91 ($\mathbf{C}H_2$), 47.96 ($\mathbf{C}H_2$), 60.88 ($\mathbf{C}H_2$), 64.90 (**C**HS), 66.07 (**C**HS), 128.65 (Ar-**C**-H), 128.82 (Ar-**C**-H), 129.38 (Ar-**C**-H), 134.30 (quaternary **C**), 163.85 (**C**O), 164.10 (**C**O), 172.70 (**C**O); *m/z* 403 (100%, $[M+Na]^+$): Found $[M+Na]^+$ 403.1341, $C_{17}H_{20}N_2O_4S_2Na$ requires 403.0762.

5.29 Methyl 6-((±)-(1S,4S)-5-benzyl-3,6-dioxo-7-sulfido-7-thia-2,5-diazabicyclo[2.2.1]heptan-2-yl)hexanoate (426)



412

Chemical Formula: $C_{34}H_{40}N_2O_6S_2$
Molecular Weight: 636.82

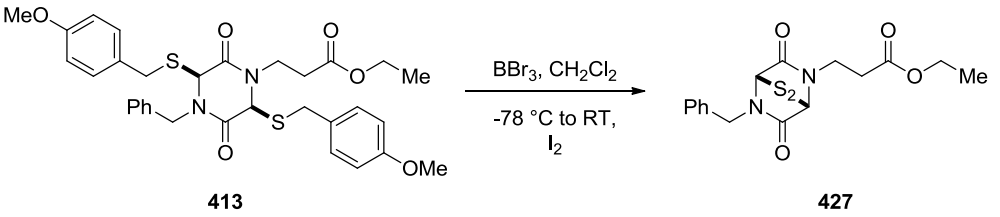
426

Chemical Formula: $C_{18}H_{22}N_2O_4S_2$
Molecular Weight: 394.51

Boron tribromide (0.03 mL, 0.35 mmol) was added dropwise to a solution of methyl 6-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)hexanoate (0.15 g, 0.24 mmol) (**412**) in dichloromethane (10.0 mL) at -78 °C. The resulting mixture was stirred for 30 minutes whereupon sodium hydrogen carbonate (10.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 5 minutes. A solution of saturated aqueous sodium thiosulfate was added and the mixture stirred for 30 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give methyl 6-((±)-(1S,4S)-5-benzyl-3,6-dioxo-7-sulfido-7-thia-2,5-diazabicyclo[2.2.1]heptan-2-yl)hexanoate (0.07 g, 75%) (**426**) as a pale yellow solid; m.p. 85-88 °C; ν_{max}/cm^{-1} 2933 (C-H), 1734 (C=O), 1674 (C=O), 1451 (CH_2), 733 (C-H); δ H (400 MHz; $CDCl_3$) 1.36 – 1.42 (2H, m, $NCH_2(CH_2)_3CH_2CO$), 2.34 (2H, t, *J* 7.4, $NCH_2(CH_2)_3CH_2CO$), 3.53 (2H, t, *J* 7.3, $NCH_2(CH_2)_3CH_2CO$), 3.68 – 3.69 (5H, m, $CH_2CO_2CH_3$), 3.73 – 3.80 (2H, m, $NCH_2(CH_2)_3CH_2CO$), 4.52 (1H, d, *J* 12.0, NCH_2-Ph), 4.85 (1H, d, *J* 12.0, NCH_2-Ph), 5.20 (1H, s, CHS), 5.37 (1H, s, CHS), 7.30 (2H, d, *J* 7.7, Ar-*H*), 7.37 – 7.40 (3H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 24.55 (**C**H_2), 26.31 (**C**H_2), 27.64 (**C**H_2), 33.95 (**C**H_2), 45.26 (**C**H_2), 47.94 (**C**H_2), 51.74 (**C**H_3), 64.93 (**C**HS), 65.84 (**C**HS), 128.59 (Ar-**C**-H), 128.78 (Ar-**C**-H), 129.34 (Ar-**C**-H),

134.32 (quaternary **C**), 163.77 (**C**O), 164.16 (**C**O), 174.01 (**C**O); *m/z* 417 (100%, $[M+Na]^+$): Found $[M+Na]^+$ 417.0939, $C_{18}H_{22}N_2O_4S_2Na$ requires 417.0919.

5.30 Ethyl 3-((±)-(1S,4S)-5-benzyl-3,6-dioxo-7-sulfido-7-thia-2,5-diazabicyclo [2.2.1]heptan-2-yl)propanoate (427)



413

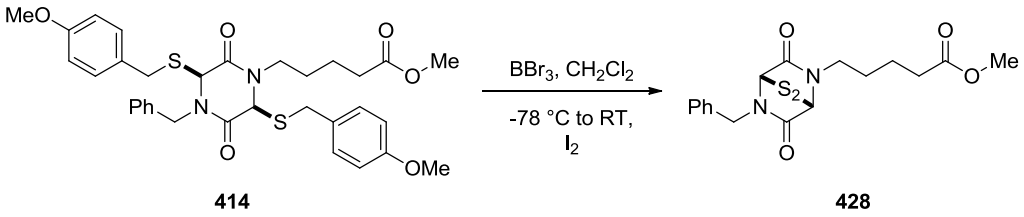
Chemical Formula: $C_{32}H_{36}N_2O_6S_2$
Molecular Weight: 608.77

427

Chemical Formula: $C_{16}H_{18}N_2O_4S_2$
Molecular Weight: 366.46

Boron tribromide (0.05 mL, 0.56 mmol) was added dropwise to a solution of ethyl 3-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)propanoate (0.17 g, 0.28 mmol) (**413**) in dichloromethane (10.0 mL) at -78 °C. The resulting mixture was stirred for 30 minutes whereupon sodium hydrogen carbonate (10.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 5 minutes. A solution of saturated aqueous sodium thiosulfate was added and the mixture stirred for 30 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give ethyl 3-((±)-(1S,4S)-5-benzyl-3,6-dioxo-7-sulfido-7-thia-2,5-diazabicyclo[2.2.1]heptan-2-yl)propanoate (0.05 g, 51%) (**427**) as a pale yellow solid; m.p. 100-104 °C; ν_{max}/cm^{-1} 1670 (C=O), 1496 (C-H); δ H (400 MHz; $CDCl_3$) 1.30 (3H, t, *J* 7.1, OCH_2CH_3), 2.71 (2H, t, *J* 6.2, NCH_2), 3.79 – 3.84 (2H, m, NCH_2CH_2), 4.22 (2H, q, *J* 7.1, OCH_2CH_3), 4.50 (1H, d, *J* 15.0, NCH_2), 4.86 (1H, d, *J* 15.0, NCH_2), 5.19 (1H, s, C*H*S), 5.67 (1H, s, C*H*S), 7.28 – 7.29 (1H, m, Ar-*H*), 7.30 (1H, d, *J* 2.0, Ar-*H*), 7.38 (2H, t, *J* 2.1, Ar-*H*), 7.39 – 7.40 (1H, m, Ar-*H*); δ C (101 MHz; $CDCl_3$) 14.39 (**C**H_3), 33.04 (**C**H_2), 41.88 (**C**H_2), 47.85 (**C**H_2), 61.45 (**C**H_2), 64.80 (**C**HS), 66.98 (**C**HS), 128.64 (Ar-**C**-H), 128.78 (Ar-**C**-H), 129.35 (Ar-**C**-H), 134.35 (quaternary **C**), 163.64 (**C**O), 164.12 (**C**O), 171.66 (**C**O); *m/z* 389 (100%, $[M+Na]^+$): Found $[M+Na]^+$ 389.0622, $C_{16}H_{18}N_2O_4S_2Na$ requires 389.0606.

5.31 Ethyl 3-((±)-(1S,4S)-5-benzyl-3,6-dioxo-7-sulfido-7-thia-2,5-diazabicyclo [2.2.1]heptan-2-yl)propanoate (428)



414 **428**

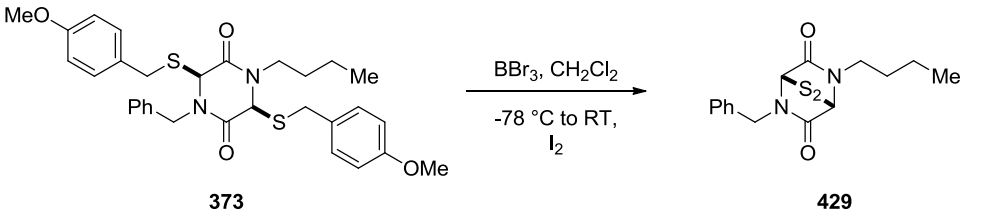
Chemical Formula: $C_{33}H_{38}N_2O_6S_2$
Molecular Weight: 622.79

Chemical Formula: $C_{17}H_{20}N_2O_4S_2$
Molecular Weight: 380.48

Boron tribromide (0.02 mL, 0.21 mmol) was added dropwise to a solution of methyl 5-((2S,5S)-4-benzyl-2,5-bis((4-methoxybenzyl)thio)-3,6-dioxopiperazin-1-yl)pentanoate (0.07 g, 0.11 mmol) (**414**) in dichloromethane (10.0 mL) at -78 °C. The resulting mixture was stirred for 45 minutes whereupon sodium hydrogen carbonate (10.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 3 minutes. A solution of saturated aqueous sodium thiosulfate was added and the mixture stirred for 30 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C : ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give methyl 5-((±)-(1S,4S)-5-benzyl-3,6-dioxo-7-sulfido-7-thia-2,5-diazabicyclo[2.2.1]heptan-2-yl)pentanoate (0.01 g, 29%) (**428**) as a pale yellow solid; m.p. 93-96 °C; ν_{max}/cm^{-1} 1732 (C=O), 1672 (C=O), 1453 (CH_2), 734 (C-H), 700 (C-H); δ H (400 MHz; $CDCl_3$) 1.28 – 1.31 (2H, m, $NCH_2(CH_2)_2CH_2CO$), 2.39 (2H, t, *J* 6.9, $NCH_2(CH_2)_2CH_2CO$), 3.55 (2H, t, *J* 6.6, $NCH_2(CH_2)_3CH_2CO$), 3.70 (4H, s, $CH_2CO_2CH_3$), 3.82 – 3.84 (1H, m, $CH_2CO_2CH_3$), 4.53 (1H, d, *J* 11.0, NCH_2-Ph), 4.87 (1H, d, *J* 10.0, NCH_2-Ph), 5.20 (1H, s, CHS), 5.36 (1H, s, CHS), 7.29 (2H, d, *J* 4.2, Ar-*H*), 7.38 – 7.43 (3H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 22.14 (**C**H_2), 27.40 (**C**H_2), 33.52 (**C**H_2), 45.04 (**C**H_2), 47.98 (**C**H_2), 51.86 (**C**H_3), 64.91 (**C**HS), 65.85 (**C**HS), 128.64 (Ar-**C**-H), 128.84 (Ar-**C**-H), 129.40 (Ar-**C**-H), 134.29 (quaternary **C**),

163.82 (**C**O), 164.14 (**C**O), 173.67 (**C**O); *m/z* 403 (100%, $[M+Na]^+$): Found $[M+Na]^+$ 403.1341, $C_{17}H_{20}N_2O_4S_2Na$ requires 403.0762.

5.32 (±)-(1S,4S)-2-Benzyl-5-butyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (429)[217]



373

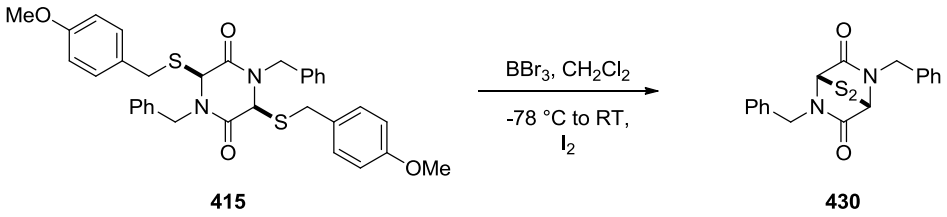
Chemical Formula: $C_{31}H_{36}N_2O_4S_2$
Molecular Weight: 564.76

429

Chemical Formula: $C_{15}H_{18}N_2O_2S_2$
Molecular Weight: 322.45

Boron tribromide (0.11 mL, 1.12 mmol) was added dropwise to a solution of (3S,6S)-1-benzyl-4-butyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.25 g, 0.45 mmol) (**373**) in dichloromethane (15.0 mL) at -78 °C. The resulting mixture was stirred for 30 minutes whereupon aqueous hydrochloric acid (15.0 mL of a 0.5M solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 15 minutes. A solution of saturated aqueous sodium thiosulfate was added and the mixture stirred for 30 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (2:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (±)-(1S,4S)-2-benzyl-5-butyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.08 g, 54%) (**429**) as a pale yellow solid; m.p. 119-121 °C; ν_{max}/cm^{-1} 1681 (C=O); δ H (400 MHz; $CDCl_3$) 0.95 (3H, t, *J* 7.3, $NCH_2(CH_2)_2CH_3$), 1.29 – 1.37 (2H, m, $NCH_2(CH_2)_2CH_3$), 1.61 – 1.72 (2H, m, $NCH_2(CH_2)_2CH_3$), 3.42 – 3.59 (2H, m, $NCH_2(CH_2)_2CH_3$), 4.51 (1H, d, *J* 14.9, NCH_2-Ph), 4.84 (1H, d, *J* 14.9, NCH_2-Ph), 5.18 (1H, s, C*H*S), 5.34 (1H, s, C*H*S), 7.26 – 7.29 (2H, m, Ar-*H*), 7.31 – 7.38 (3H, m, Ar-*H*); δ C (101 MHz; $CDCl_3$) 13.65 (**C**H_3), 19.90 (**C**H_2), 29.75 (**C**H_2), 44.90 (**C**H_2), 47.65 (**C**H_2), 64.69 (**C**HS), 65.55 (**C**HS), 128.36 (Ar-**C**-H), 128.56 (Ar-**C**-H), 129.12 (Ar-**C**-H), 134.08 (quaternary **C**), 163.53 (**C**O), 163.99 (**C**O); *m/z* 323 (100%, $[M+H]^+$): Found $[M+H]^+$ 323.0900, $C_{15}H_{19}N_2O_2S_2$ requires 323.0888.

5.33 (±)-(1S,4S)-2,5-Dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (430)[217]



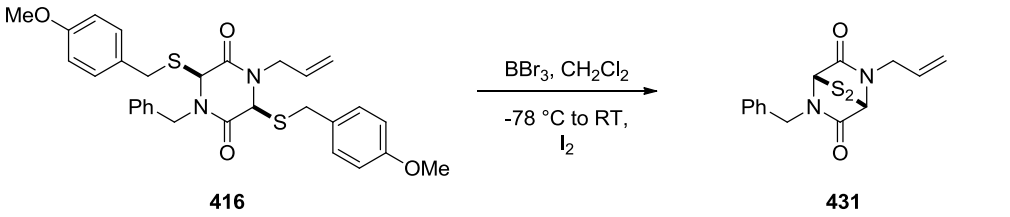
415 **430**

Chemical Formula: $C_{34}H_{34}N_2O_4S_2$
Molecular Weight: 598.77

Chemical Formula: $C_{18}H_{16}N_2O_2S_2$
Molecular Weight: 356.46

Boron tribromide (0.07 mL, 0.69 mmol) was added dropwise to a solution of (3S,6S)-1,4-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.21 g, 0.34 mmol) (**415**) in dichloromethane (5.00 mL) at -78 °C. The resulting mixture was stirred for 45 minutes whereupon aqueous ammonium chloride (15.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. A solution of saturated aqueous sodium thiosulfate was added and the mixture stirred for 30 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (±)-(1S,4S)-2,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.10 g, 85%) (**430**) as a pale yellow solid; m.p. 166-188 °C; ν_{max}/cm^{-1} 1685 (C=O); δ H (400 MHz; $CDCl_3$) 4.63 (2H, d, *J* 12.0, C*H*$_2$Ar), 4.99 (2H, d, *J* 12.0 Hz, C*H*$_2$Ar), 5.39 (2H, s, C*H*S), 7.40 – 7.54 (10H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 48.14 (**C**H_2), 65.14 (**C**H), 128.85 (Ar-**C**-H), 129.03 (Ar-**C**-H), 129.59 (Ar-**C**-H), 134.54 (quaternary **C**), 164.19 (**C**O); *m/z* 357 (100%, $[M+H]^+$): Found $[M+H]^+$ 357.0651, $C_{18}H_{17}N_2O_2S_2$ requires 357.0653.

5.34 (±)-(1S,4S)-2-Allyl-5-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (431)[253]



416

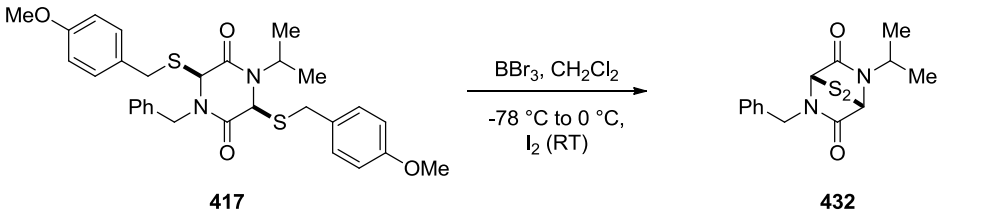
Chemical Formula: $C_{30}H_{32}N_2O_4S_2$
Molecular Weight: 548.72

431

Chemical Formula: $C_{14}H_{14}N_2O_2S_2$
Molecular Weight: 306.40

Boron tribromide (0.03 mL, 0.36 mmol) was added dropwise to a solution of (3S,6S)-1-allyl-4-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.10 g, 0.18 mmol) (**416**) in dichloromethane (10.0 mL) at -78 °C. The resulting mixture was stirred for 45 minutes whereupon aqueous ammonium chloride (15 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. A solution of saturated aqueous sodium thiosulfate was added and the mixture stirred for 30 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further recrystallisation to give (±)-(1S,4S)-2-allyl-5-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.03 g, 60%) (**431**) as a pale yellow solid; m.p. 116-118 °C; ν_{max}/cm^{-1} 1685 (C=O); δ H (400 MHz; $CDCl_3$) 3.96 (1H, dd, *J* 15.4 & 8.6, NC*H*$_2$CH=CH$_2$), 4.25 (1H, dd, *J* 15.4 & 7.6, NC*H*$_2$CH=CH$_2$), 4.49 (1H, d, *J* 11.7, NC*H*$_2$), 4.85 (1H, d, *J* 11.9, NC*H*$_2$), 5.22 (1H, s, C*H*S), 5.33 – 5.34 (2H, m, CH=C*H*$_2$), 5.36 (1H, s, C*H*S), 5.38 – 5.42 (1H, m, C*H*=CH$_2$), 7.26 – 7.29 (2H, m, Ar-*H*), 7.32 – 7.39 (3H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 46.38 (**C**H$_2$), 47.52 (**C**H$_2$), 64.36 (**C**HS), 64.55 (**C**HS), 120.65 (CH**C**H$_2$), 128.33 (Ar-**C**-H), 128.49 (Ar-**C**-H), 129.07 (Ar-**C**-H), 130.74 (**C**H=CH$_2$), 134.05 (quaternary **C**), 163.38 (**C**O), 163.82 (**C**O); *m/z* 307 (100%, $[M+H]^+$): Found $[M+H]^+$ 307.0484, $C_{14}H_{15}N_2O_2S_2$ requires 307.0497.

5.35 (±)-(1S,4S)-2-Benzyl-5-isopropyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (432)[217]



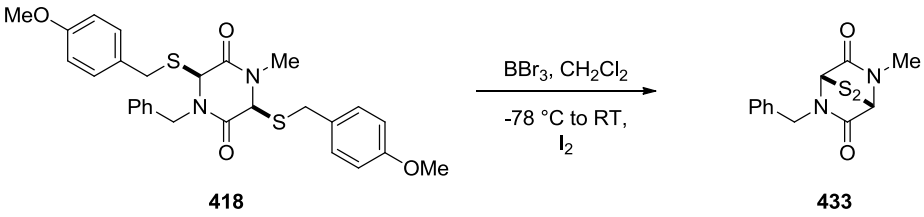
417 **432**

Chemical Formula: $C_{30}H_{34}N_2O_4S_2$
Molecular Weight: 550.73

Chemical Formula: $C_{14}H_{16}N_2O_2S_2$
Molecular Weight: 308.42

Boron tribromide (0.50 mL, 0.50 mmol, 1M solution in dichloromethane) was added dropwise to a solution of (3S,6S)-1-benzyl-4-isopropyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.11 g, 0.20 mmol) (**417**) in dichloromethane (2.00 mL) at -78 °C and stirred for 15 minutes. The resulting mixture was stirred for 30 minutes whereupon aqueous hydrochloric acid (15.0 mL of a 0.5M solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 5 minutes. A solution of saturated aqueous sodium thiosulfate was added and the mixture stirred for 30 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (±)-(1S,4S)-2-benzyl-5-isopropyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.04 g, 65%) (**432**) as a pale yellow solid; m.p. 177-180 °C; ν_{max}/cm^{-1} 1662 (C=O), 1609 (C=C), 1434 (C-H), 731 (C-H); δ H (400 MHz; $CDCl_3$) 1.35 (6H, dd, *J* 12.9 & 6.8, CH(CH_3)$_2$), 1.61 (1H, s, C*H*(CH_3)$_2$), 4.53 (1H, d, *J* 12.6, NCH_2-Ph), 4.83 (1H, d, *J* 12.9, NCH_2-Ph), 5.19 (1H, s, C*H*S), 5.48 (1H, s, C*H*S), 7.30 (3H, d, *J* 10.0, Ar-*H*), 7.39 (2H, d, *J* 7.4, Ar-*H*); δ C (101 MHz, $CDCl_3$) 19.78 (**C**H_3), 20.74 (**C**H_3), 46.63 (**C**H), 47.95 (**C**H_2), 61.03 (**C**HS), 65.29 (**C**HS), 128.62 (Ar-**C**-H), 128.76 (Ar-**C**-H), 129.33 (Ar-**C**-H), 134.35 (quaternary **C**), 163.02 (**C**O), 164.26 (**C**O); *m/z* 331 (100%, $[M+Na]^+$): Found $[M+Na]^+$ 331.0569, $C_{14}H_{16}N_2O_2S_2Na$ requires 331.0551.

5.36 (±)-(1S,4S)-2-Benzyl-5-methyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (433)[217]



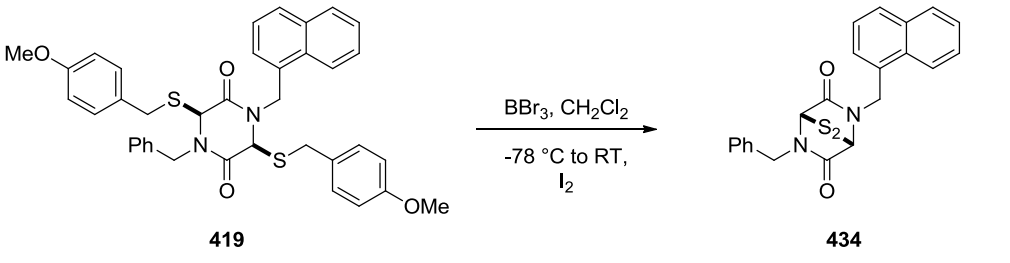
418 **433**

Chemical Formula: $C_{28}H_{30}N_2O_4S_2$
Molecular Weight: 522.68

Chemical Formula: $C_{12}H_{12}N_2O_2S_2$
Molecular Weight: 280.37

Boron tribromide (0.05 mL, 0.57 mmol) was added dropwise to a solution of (3S,6S)-1-benzyl-3,6-bis((4-methoxybenzyl)thio)-4-methylpiperazine-2,5-dione (0.15 g, 0.29 mmol) (**418**) in dichloromethane (8.00 mL) at -78 °C. The resulting mixture was stirred for 45 minutes whereupon aqueous ammonium chloride (15.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. A solution of saturated aqueous sodium thiosulfate was added and the mixture stirred for 30 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (±)-(1S,4S)-2-benzyl-5-methyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.07 g, 65%) (**433**) as a pale yellow solid; m.p. 109-111 °C; ν_{max}/cm^{-1} 1699 (C=O); δ H (400 MHz; $CDCl_3$) 3.12 (3H, s, NC*H*$_3$), 4.49 (1H, d, *J* 12.9, NC*H*$_2$), 4.90 (1H, d, *J* 12.9, NC*H*$_2$), 5.20 (1H, s, C*H*S), 5.32 (1H, s, C*H*S), 7.28 – 7.39 (5H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 31.40 (N**C**H$_3$), 47.48 (**C**H$_2$), 64.34 (**C**HS), 67.53 (**C**HS), 128.39 (Ar-**C**-H), 128.58 (Ar-**C**-H), 129.15 (Ar-**C**-H), 134.04 (quaternary **C**), 163.85 (**C**O), 163.99 (**C**O); *m/z* 281 (100%, $[M+H]^+$): Found $[M+H]^+$ 281.0415, $C_{12}H_{13}N_2O_2S_2$ requires 281.0418.

5.37 (±)-(1S,4S)-2-Benzyl-5-(naphthalen-1-ylmethyl)-7-thia-2,5-diazabicyclo [2.2.1]heptane-3,6-dione 7-sulfide (434)[217]



419

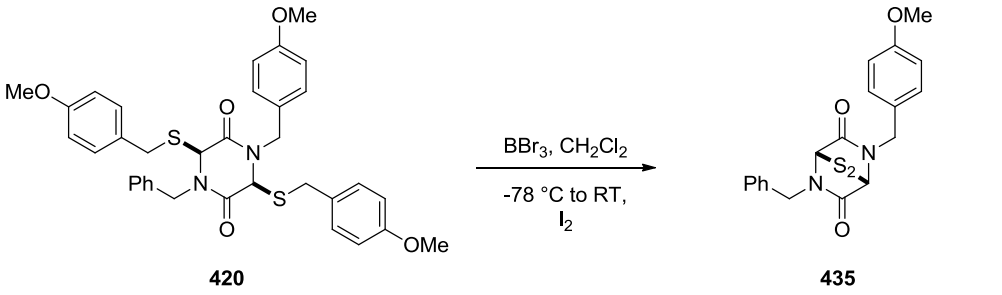
Chemical Formula: $C_{38}H_{36}N_2O_4S_2$
Molecular Weight: 648.83

434

Chemical Formula: $C_{22}H_{18}N_2O_2S_2$
Molecular Weight: 406.52

Boron tribromide (0.10 mL, 1.02 mmol) was added dropwise to a solution of (3S,6S)-1-benzyl-3,6-bis((4-methoxybenzyl)thio)-4-(naphthalen-1-ylmethyl)piperazine-2,5-dione (0.33 g, 0.51 mmol) (**419**) in dichloromethane (7.00 mL) at -78 °C. The resulting mixture was stirred for 45 minutes whereupon aqueous ammonium chloride (15.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. A solution of saturated aqueous sodium thiosulfate was added and the mixture stirred for 30 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) to give ((±)-(1S,4S)-2-benzyl-5-(naphthalen-1-ylmethyl)-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.18 g, 85%) (**434**) as a pale yellow solid; m.p. 161-163 °C; ν_{max}/cm^{-1} 1685 (C=O); δ H (400 MHz; $CDCl_3$) 4.40 (1H, d, *J* 12.6, NC*H*$_2$), 4.79 – 4.84 (3H, m, NC*H*$_2$), 5.24 (1H, s, C*H*S), 5.30 (1H, s, C*H*S), 7.20 – 7.25 (2H, m, Ar-*H*), 7.34 – 7.39 (3H, m, Ar-*H*), 7.44 – 7.47 (2H, m, Ar-*H*), 7.53 – 7.58 (2H, m, Ar-*H*), 7.88 – 7.92 (2H, m, Ar-*H*), 7.95 – 8.00 (1H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 45.09 (**C**H$_2$), 47.31 (**C**H$_2$), 63.36 (**C**HS), 64.64 (**C**HS), 123.05 (Ar-**C**-H), 100.10 (Ar-**C**-H), 126.32 (Ar-**C**-H), 126.94 (Ar-**C**-H), 128.13 (Ar-**C**-H), 128.35 (Ar-**C**-H), 128.39 (Ar-**C**-H), 128.82 (Ar-**C**-H), 128.98 (Ar-**C**-H), 129.48 (quaternary **C**), 129.88 (Ar-**C**-H), 131.48 (quaternary **C**), 133.84 (quaternary **C**), 134.11 (quaternary **C**), 163.43 (**C**O), 163.48 (**C**O); *m/z* 407 (100%, $[M+H]^+$): Found $[M+H]^+$ 407.0791, $C_{22}H_{19}N_2O_2S_2$ requires 407.0810.

5.38 (±)-(1S,4S)-2-Benzyl-5-(4-methoxybenzyl)-7-thia-2,5-diazabicyclo[2.2.1] heptane-3,6-dione 7-sulfide (435)[217]



420 **435**

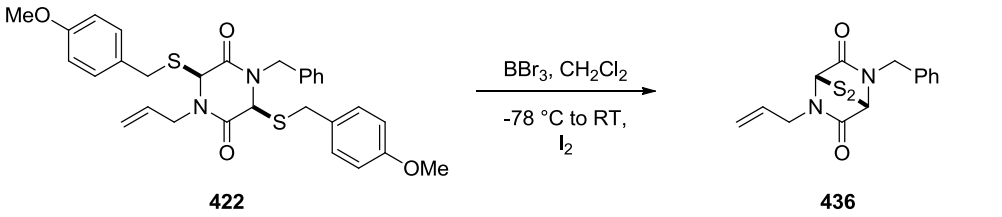
Chemical Formula: $C_{35}H_{36}N_2O_5S_2$
Molecular Weight: 628.80

Chemical Formula: $C_{19}H_{18}N_2O_3S_2$
Molecular Weight: 386.49

Boron tribromide (0.11 mL, 1.22 mmol) was added dropwise to a solution of (3S,6S)-1-benzyl-4-(4-methoxybenzyl)-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.38 g, 0.61 mmol) (**420**) in dichloromethane (8.00 mL) at -78 °C. The resulting mixture was stirred for 45 minutes whereupon aqueous ammonium chloride (15.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. A solution of saturated aqueous sodium thiosulfate was added and the mixture stirred for 30 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (±)-(1S,4S)-2-benzyl-5-(4-methoxybenzyl)-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.11 g, 48%) (**435**) as a pale yellow solid; m.p. 146-148 °C; ν_{max}/cm^{-1} 2991 (C-H), 1681 (C=O); δ H (400 MHz; $CDCl_3$) 3.80 (3H, s, OC*H*$_3$), 4.43 (1H, d, *J* 12.7, C*H*$_2$), 4.48 (1H, d, *J* 13.0, C*H*$_2$), 4.75 (1H, d, *J* 12.7, C*H*$_2$), 4.82 (1H, d, *J* 13.0, C*H*$_2$), 5.25 (1H, s, C*H*S), 5.26 (1H, s, C*H*S), 6.90 (2H, d, *J* 8.6, Ar-*H*), 7.21 (2H, d, *J* 8.6, Ar-*H*), 7.27 – 7.29 (2H, m, Ar-*H*), 7.35 – 7.36 (3H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 47.09 (**C**H$_2$), 47.55 (**C**H$_2$), 55.20 (O**C**H$_3$), 64.27 (**C**HS), 64.67 (**C**HS), 114.45 (Ar-**C**-H), 100.84 (quaternary **C**), 128.31 (Ar-**C**-H), 128.47 (Ar-**C**-H), 129.05 (Ar-**C**-H), 129.86 (Ar-**C**-H), 134.10 (quaternary **C**), 159.72

(quaternary **C**), 163.64 (**C**O), 163.77 (**C**O); *m/z* 387 (100%, $[M+H]^+$): Found $[M+H]^+$ 387.0833, $C_{19}H_{19}N_2O_3S_2$ requires 387.0837.

5.39 (±)-(1S,4S)-2-Allyl-5-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (436)[253]



422

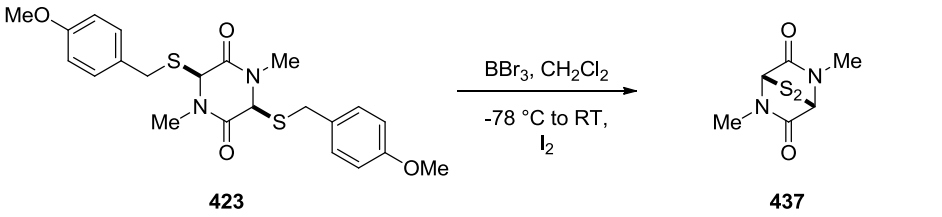
Chemical Formula: $C_{30}H_{32}N_2O_4S_2$
Molecular Weight: 548.72

436

Chemical Formula: $C_{14}H_{14}N_2O_2S_2$
Molecular Weight: 306.40

Boron tribromide (0.09 mL, 0.94 mmol) was added dropwise to a solution of (3S,6S)-1-allyl-4-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.26 g, 0.47 mmol) (**416**) in dichloromethane (7.00 mL) at -78 °C. The resulting mixture was stirred for 45 minutes whereupon aqueous ammonium chloride (15 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. A solution of saturated aqueous sodium thiosulfate was added and the mixture stirred for 30 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (±)-(1S,4S)-2-allyl-5-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.10 g, 67%) (**436**) as a pale yellow solid; identical spectroscopic data to that obtained previously for (**431**).

5.40 (±)-(1S,4S)-2,5-Dimethyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (437)



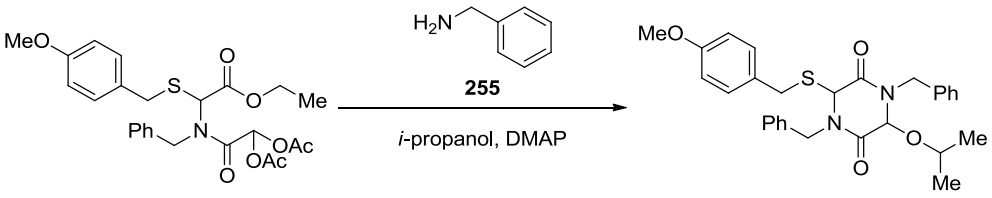
423 **437**

Chemical Formula: $C_{22}H_{26}N_2O_4S_2$
Molecular Weight: 446.58

Chemical Formula: $C_6H_8N_2O_2S_2$
Molecular Weight: 204.27

Boron tribromide (0.10 mL, 1.03 mmol) was added dropwise to a solution of (3S,6S)-3,6-bis((4-methoxybenzyl)thio)-1,4-dimethylpiperazine-2,5-dione (0.23 g, 0.52 mmol) (**423**) in dichloromethane (8.00 mL) at -78 °C. The resulting mixture was stirred for 45 minutes whereupon aqueous ammonium chloride (15.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 40 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. A solution of saturated aqueous sodium thiosulfate was added and the mixture stirred for 30 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (±)-(1S,4S)-2,5-dimethyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.07 g, 68%) (**437**) as a pale yellow solid; m.p. 202-205 °C; ν_{max}/cm^{-1} 1686 (C=O), 1390 (CH_3); δ H (400 MHz; $CDCl_3$) 3.05 (6H, s, NC*H*$_3$), 5.15 (2H, s, C*H*S); δ C (101 MHz, $CDCl_3$) 31.59 (N**C**H_3), 67.60 (**C**HS), 164.32 (**C**O); *m/z* 202 (100%, $[M-H]^-$): Found $[M-H]^-$ 202.9957, $C_6H_7N_2O_2S_2$ requires 202.9949.

5.41 1,4-Dibenzyl-3-isopropoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (446)



259

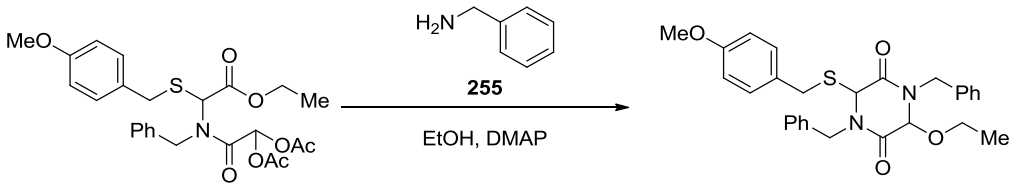
Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

446

Chemical Formula: $C_{29}H_{32}N_2O_4S$
Molecular Weight: 504.64

Benzylamine (0.18 mL, 1.67 mmol) (**255**) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.70 g, 1.39 mmol) (**259**) in *iso*-propanol (15.0 mL) and the resulting mixture stirred for 2 minutes. DMAP (0.09 g, 0.70 mmol) was added and the resulting mixture stirred for 24 hours at RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) to give 1,4-dibenzyl-3-isopropoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.42 g, 60%) (**446**) as a colourless oil; ν_{max}/cm^{-1} 3031 (C-H), 1677 (C=O), 1359 (CH_3), 1206 (C-C(O)-C), 1002 (C-C(O)-C), 733 (C-H), 700 (C-H); δ H (400 MHz, $CDCl_3$) 1.09 (3H, d, *J* 6.0, OCH(C*H*$_3$)$_2$), 1.28 (3H, d, *J* 6.2, OCH(C*H*$_3$)$_2$), 3.36 – 3.39 (1H, m, OC*H*(CH$_3$)$_2$), 3.75 (3H, s, OC*H*$_3$), 3.75 (2H, s, SC*H*$_2$), 4.33 (2H, d, *J* 4.2, NC*H*$_2$), 4.36 – 4.43 (2H, m, NC*H*$_2$), 4.69 (1H, s, C*H*S), 5.42 (1H, s, C*H*O), 6.78 (2H, d, *J* 1.2, Ar-*H*), 6.79 – 6.80 (2H, m, Ar-*H*), 7.11 – 7.13 (5H, m, Ar-*H*), 7.25 – 7.27 (5H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 21.50 (**C**H$_3$), 23.08 (**C**H$_3$), 36.44 (**C**H$_2$), 47.73 (**C**H$_2$), 49.81 (**C**H$_2$), 55.47 (O**C**H$_3$), 57.09 (**C**HS), 58.05 (**C**HO), 71.37 (O**C**H(CH$_3$)$_2$), 128.24 (Ar-**C**-H), 128.61 (Ar-**C**-H), 128.68 (Ar-**C**-H), 128.84 (Ar-**C**-H), 129.04 (Ar-**C**-H), 129.12 (Ar-**C**-H), 130.88 (Ar-**C**-H), 130.93 (Ar-**C**-H), 134.49 (quaternary **C**), 135.24 (quaternary **C**), 135.93 (quaternary **C**), 139.02 (quaternary **C**), 165.28 (**C**O), 166.92 (**C**O); *m/z* 505 (100%, $[M+H]^+$); Found $[M+H]^+$ 505.2163, $C_{29}H_{33}N_2O_4S$ requires 505.2161.

5.42 1,4-Dibenzyl-3-ethoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (447)[217]



259

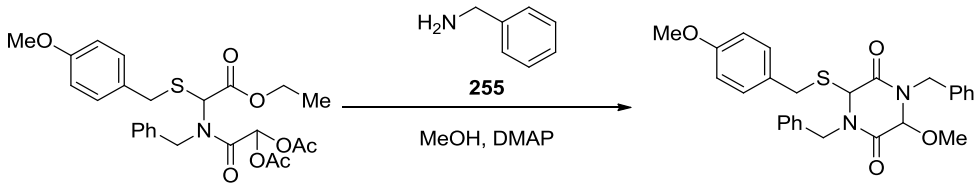
Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

447

Chemical Formula: $C_{28}H_{30}N_2O_4S$
Molecular Weight: 490.61

Benzylamine (0.14 mL, 1.26 mmol) (**255**) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.53 g, 1.05 mmol) (**259**) in ethanol (15.0 mL) and the resulting mixture stirred for 2 minutes. DMAP (0.06 g, 0.53 mmol) was added and the resulting mixture stirred for 24 hours at RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) to give 1,4-dibenzyl-3-ethoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.38 g, 74%) (**447**) as a colourless oil; ν_{max}/cm^{-1} 1683 (C=O), 729 (C-H); δ H (400 MHz, $CDCl_3$) 1.26 (3H, t, *J* 7.0, OCH_2CH_3), 3.64 (2H, d, *J* 6.7, SCH_2), 3.87 – 3.89 (7H, m, NCH_2 & OCH_3), 4.07 – 4.12 (2H, m, OCH_2CH_3), 4.47 (1H, s, C*H*S), 4.77 (1H, s, C*H*O), 6.76 – 6.82 (3H, m, Ar-*H*), 6.91 – 6.93 (3H, m, Ar-*H*), 7.18 – 7.22 (3H, m, Ar-*H*), 7.37 – 7.40 (5H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 15.17 ($OCH_2\mathbf{C}H_3$), 36.35 ($\mathbf{C}H_2$), 45.95 ($\mathbf{C}H_2$), 48.08 ($\mathbf{C}H_2$), 55.41 ($\mathbf{C}H_3$), 57.87 (**C**HS), 65.63 ($\mathbf{C}H_2$), 84.15 (**C**HO), 114.31 (Ar-**C**-H), 128.13 (Ar-**C**-H), 128.43 (Ar-**C**-H), 128.64 (Ar-**C**-H), 128.76 (Ar-**C**-H), 128.81 (Ar-**C**-H), 128.99 (Ar-**C**-H), 130.90 (Ar-**C**-H), 135.09 (quaternary **C**), 135.53 (quaternary **C**), 159.28 (quaternary **C**), 163.27 (quaternary **C**), 165.24 (**C**O), 166.51 (**C**O); *m/z* 491 (100%, $[M+H]^+$); Found $[M+H]^+$ 491.1992, $C_{28}H_{31}N_2O_4S$ requires 491.2004.

5.43 1,4-Dibenzyl-3-methoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (448)



259

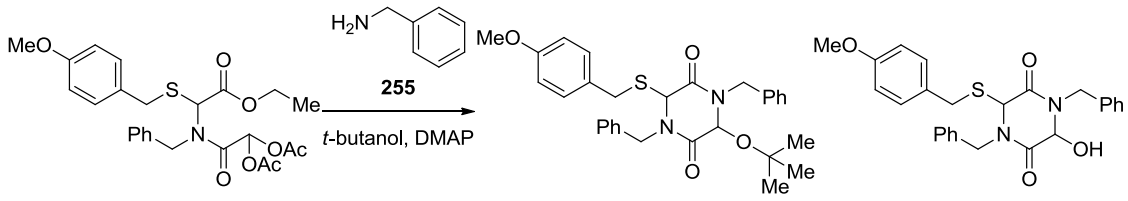
Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

448

Chemical Formula: $C_{27}H_{28}N_2O_4S$
Molecular Weight: 476.59

Benzylamine (0.21 mL, 1.93 mmol) (**255**) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.65 g, 1.29 mmol) (**259**) in methanol (15.0 mL) and the resulting mixture stirred for 2 minutes. DMAP (0.08 g, 0.64 mmol) was added and the resulting mixture stirred for 5 hours at RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give 1,4-dibenzyl-3-methoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.49 g, 79%) (**448**) as a colourless solid; m.p. 85-88 °C; ν_{max}/cm^{-1} 3030 (C-H), 3005 (C-H), 1671 (C=O), 1357 (CH_3), 1002 (C-O-C), 832 (C-H); δ H (400 MHz, $CDCl_3$) 3.53 (3H, s, OC*H*$_3$), 3.62 (1H, d, *J* 14.5, NC*H*$_2$), 3.87 – 3.90 (4H, m, SC*H*$_2$ & OC*H*$_3$), 4.09 (1H, d, *J* 13.8, SC*H*$_2$), 4.25 (1H, d, *J* 14.9, NC*H*$_2$), 4.66 (1H, s, C*H*O), 4.45 (1H, s, C*H*S), 5.18 (2H, d, *J* 14.7, NC*H*$_2$), 6.91 – 6.95 (2H, m, Ar-*H*), 7.16 – 7.29 (6H, m, Ar-*H*), 7.36 (6H, dd, *J* 16.4 & 9.2, Ar-*H*); δ C (101 MHz, $CDCl_3$) 36.29 (**C**H$_2$), 45.93 (**C**H$_2$), 48.07 (**C**H$_2$), 55.39 (O**C**H$_3$), 57.66 (O**C**H$_3$), 85.50 (**C**HS), 114.29 (**C**HO), 128.03 (Ar-**C**-H), 128.19 (Ar-**C**-H), 128.53 (Ar-**C**-H), 128.66 (Ar-**C**-H), 128.75 (Ar-**C**-H), 129.01 (Ar-**C**-H), 130.88 (Ar-**C**-H), 134.98 (quaternary **C**), 135.30 (quaternary **C**), 159.27 (quaternary **C**), 162.73 (**C**O), 166.29 (**C**O); *m/z* 477 (100%, $[M+H]^+$); Found $[M+H]^+$ 477.1841, $C_{27}H_{29}N_2O_4S$ requires 477.1848.

5.44 1,4-Dibenzyl-3-hydroxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (450)



259 **449** **450**

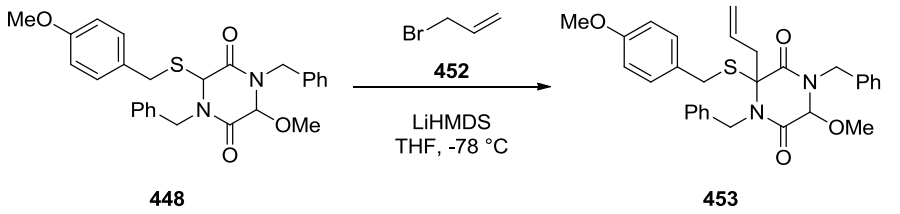
Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

Chemical Formula: $C_{30}H_{34}N_2O_4S$
Molecular Weight: 518.67

Chemical Formula: $C_{26}H_{26}N_2O_4S$
Molecular Weight: 462.56

Benzylamine (0.11 mL, 1.03 mmol) (**255**) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.43 g, 0.85 mmol) (**259**) in *tert*-butanol (15.0 mL) and the resulting mixture stirred for 2 minutes. DMAP (0.05 g, 0.43 mmol) was added and the resulting mixture stirred for 24 hours at RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) which further recrystallisation did not give 1,4-dibenzyl-3-(*tert*-butoxy)-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (**449**) but gave 1,4-dibenzyl-3-hydroxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.23 g, 59%) (**450**) as a colourless solid; m.p. 99-100 °C; ν_{max}/cm^{-1} 3322 (OH), 3030 (C-H), 1672 (C=O), 1001 (C-O), 832 (C-H), 731 (C-H), 699 (C-H); δ H (400 MHz, $CDCl_3$) 2.52 (1H, br s, O*H*), 3.88 – 3.95 (5H, m, OCH_3 & SCH_2), 4.12 (2H, d, *J* 7.5, NCH_2), 4.16 (2H, d, *J* 7.6, NCH_2), 4.46 (1H, s, C*H*S), 4.56 (1H, s, C*H*O), 6.77 – 6.82 (2H, m, Ar-*H*), 6.92 – 6.94 (2H, m, Ar-*H*), 7.32 – 7.42 (10H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 46.23 (**C**H_2), 46.46 (**C**H_2), 49.80 (**C**H_2), 55.46 (O**C**H_3), 57.08 (**C**HS), 69.13 (**C**HO), 127.57 (Ar-**C**-H), 127.89 (Ar-**C**-H), 128.16 (Ar-**C**-H), 128.32 (Ar-**C**-H), 128.67 (Ar-**C**-H), 128.83 (Ar-**C**-H), 129.11(Ar-**C**-H), 130.92 (Ar-**C**-H), 135.06 (quaternary **C**), 135.93 (quaternary **C**), 139.02 (quaternary **C**), 159.34 (quaternary **C**), 165.27 (**C**O), 165.16 (**C**O); *m/z* 485 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 485.1494, $C_{26}H_{26}N_2O_4SNa$ requires 485.1511.

5.45 3-Allyl-1,4-dibenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine -2,5-dione (453)



448 **453**

Chemical Formula: $C_{27}H_{28}N_2O_4S$
Molecular Weight: 476.59

Chemical Formula: $C_{30}H_{32}N_2O_4S$
Molecular Weight: 516.65

5.45.1 Method A:

LiHMDS (0.34 mL of a 1M solution in tetrahydrofuran, 0.34 mmol) was added dropwise to a solution of 1,4-dibenzyl-3-methoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.08 g, 0.17 mmol) (**448**) and allyl bromide (0.07 mL, 0.84 mmol) (**452**) in tetrahydrofuran (2.00 mL) at -78 °C and the resulting mixture stirred at this temperature for 1 hour and 1.5 hours at 0 °C. Saturated aqueous sodium hydrogen carbonate (10.0 mL) was added and solvent removed under reduced pressure. The residue was partitioned between water (10.0 mL) and dichloromethane (20.0 mL). The aqueous phase was extracted with dichloromethane (2 x 10.0 mL) and the combined extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) to give 3-allyl-1,4-dibenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.07 g, 79%) (**453**) as a pale yellow oil; ν_{max}/cm^{-1} 2934 (C-H), 1670 (C=O), 1610 (C=C), 1249 (C-O-C), 834 (C-H), 727 (C-H); δ H (400 MHz, $CDCl_3$) 2.62 (1H, dd, *J* 14.3 & 6.3, C*H*$_2$CH=CH), 3.03 (1H, dd, *J* 14.2 & 7.7, C*H*$_2$CH=CH), 3.50 (3H, s, OC*H*$_3$), 3.53 – 3.57 (1H, m, SC*H*$_2$), 3.69 (3H, s, OC*H*$_3$), 3.70 – 3.74 (1H, m, SC*H*$_2$), 4.06 (2H, d, *J* 14.5, NC*H*$_2$), 4.57 (1H, s, C*H*O), 4.74 (2H, d, *J* 14.5, NC*H*$_2$), 4.82 – 4.88 (2H, m, CH=C*H*$_2$), 4.98 – 5.09 (1H, m, C*H*=CH$_2$), 6.69 – 6.72 (2H, m, Ar-*H*), 6.95 – 6.98 (2H, m, Ar-*H*), 7.20 – 7.25 (8H, m, Ar-*H*), 7.34 – 7.37 (2H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 34.45 (**C**H$_2$), 42.10 (**C**H$_2$), 47.05 (**C**H$_2$), 47.51 (**C**H$_2$), 55.45 (O**C**H$_3$), 58.51 (O**C**H$_3$), 75.34 (quaternary **C**), 83.71 (**C**HO), 114.13 (**C**H=CH$_2$), 121.55 (**C**H$_2$), 127.65 (Ar-**C**-H), 127.77 (quaternary **C**), 128.32 (Ar-**C**-H), 128.61 (Ar-**C**-H), 128.99 (Ar-**C**-H), 129.01 (Ar-**C**-H), 129.21 (Ar-**C**-H), 129.86 (Ar-**C**-H), 130.58 (Ar-**C**-H), 135.26 (quaternary **C**), 137.65 (quaternary **C**), 159.06 (quaternary **C**), 164.25 (**C**O), 165.30 (**C**O); *m/z* 517 (100%, $[M+H]^+$); Found $[M+H]^+$ 517.2172, $C_{30}H_{33}N_2O_4S$ requires 517.2161.

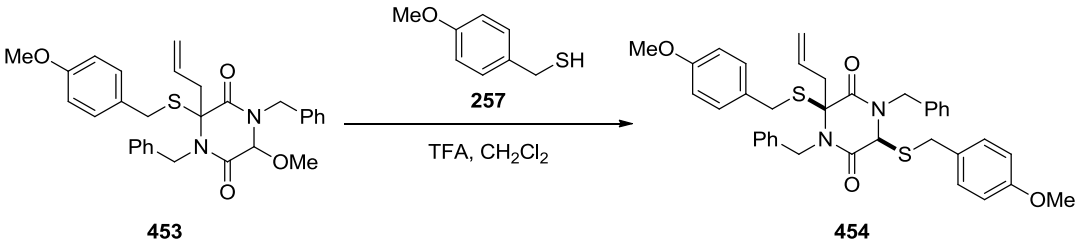
5.45.2 Method B:

LDA (0.13 mL of a 2M solution in tetrahydrofuran /heptane/ethylbenzene, 0.25 mmol) was added dropwise to a solution of 1,4-dibenzyl-3-methoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.10 g, 0.21 mmol) (**448**) and allyl bromide (0.04 mL, 0.42 mmol) (**452**) in tetrahydrofuran (2.00 mL) at -78 °C and the resulting mixture stirred at this temperature for 1 hour and 1.5 hours at 0 °C. Saturated aqueous sodium hydrogen carbonate (10.0 mL) was added and solvent removed under reduced pressure. The residue was partitioned between water (10.0 mL) and dichloromethane (20.0 mL). The aqueous phase was extracted with dichloromethane (2 x 10.0 mL) and the combined extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) to give 3-allyl-1,4-dibenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.04 g, 32%) (**453**) as a pale yellow oil; identical spectroscopic data to that obtained previously for (**453**).

5.45.3 Method C:

NaH (0.01 g, 0.24 mmol) was added dropwise to a solution of 1,4-dibenzyl-3-methoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.10 g, 0.20 mmol) (**448**) and allyl bromide (0.03 mL, 0.40 mmol) (**452**) in tetrahydrofuran (2.00 mL) at -78 °C and the resulting mixture stirred at this temperature for 1 hour and 1.5 hours at 0 °C. Saturated aqueous sodium hydrogen carbonate (10.0 mL) was added and solvent removed under reduced pressure. The residue was partitioned between water (10.0 mL) and dichloromethane (20.0 mL). The aqueous phase was extracted with dichloromethane (2 x 10.0 mL) and the combined extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) to give 3-allyl-1,4-dibenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.02 g, 22%) (**453**) as a pale yellow oil; identical spectroscopic data to that obtained previously for (**453**).

5.46 3-Allyl-1,4-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (454)



453

Chemical Formula: $C_{30}H_{32}N_2O_4S$
Molecular Weight: 516.65

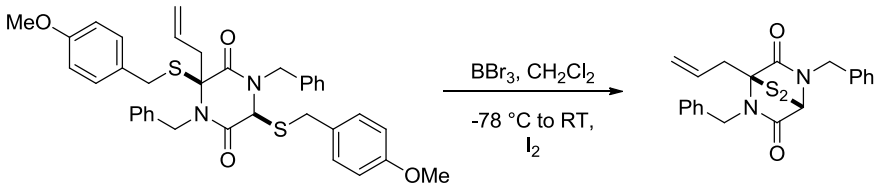
454

Chemical Formula: $C_{37}H_{38}N_2O_4S_2$
Molecular Weight: 638.84

TFA (0.71 mL, 9.17 mmol) was added to a solution of 3-allyl-1,4-dibenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.32 g, 0.61 mmol) (**453**) in dichloromethane (10.0 mL) at RT followed by the addition of *para*-methoxybenzyl mercaptan (0.13 mL, 0.92 mmol) (**257**) and the resulting mixture stirred at this temperature for 18 hours. After, the solution was diluted in dichloromethane (10.0 mL) and washed with saturated aqueous sodium hydrogen carbonate (10.0 mL). The aqueous phase was extracted with dichloromethane (2 x 10.0 mL) and the combined extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give 3-allyl-1,4-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.32 g, 83%) (**454**) as a pale yellow solid; m.p. 124-127 °C; ν_{max}/cm^{-1} 1667 (C=O), 1610 (C=C), 1001 (C-O-C), 843 (C-H), 725 (C-H), 701 (C-H); δ H (400 MHz, $CDCl_3$) 3.36 – 3.40 (1H, m, C*H*$_2$CH=CH$_2$), 3.49 – 3.53 (1H, m, C*H*$_2$CH=CH$_2$), 3.79 (3H, s, OC*H*$_3$), 3.81 (2H, s, SC*H*$_2$), 3.87 (3H, s, OC*H*$_3$), 3.89 (2H, d, *J* 5.9, SC*H*$_2$), 3.93 (1H, d, *J* 7.9, NC*H*$_2$), 4.03 – 4.07 (1H, m, NC*H*$_2$), 4.54 (1H, s, C*H*S), 5.20 – 5.25 (2H, m, CH=C*H*$_2$), 5.31 (1H, d, *J* 14.5, NC*H*$_2$), 5.43 (1H, d, *J* 14.2, NC*H*$_2$), 5.79 (1H, t, *J* 1.4, C*H*=CH$_2$), 6.75 – 6.78 (2H, m, Ar-*H*), 6.83 – 6.87 (2H, m, Ar-*H*), 6.91 – 6.94 (5H, m, Ar-*H*), 7.16 – 7.20 (5H, m, Ar-*H*), 7.35 – 7.37 (4H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 34.13 (**C**H$_2$), 34.83 (**C**H$_2$), 35.77 (**C**H$_2$), 41.00 (**C**H$_2$), 47.20 (**C**H$_2$), 55.43 (O**C**H$_3$), 55.52 (O**C**H$_3$), 58.50 (**C**HS), 68.84 (quaternary **C**), 114.36 (Ar-**C**-H), 121.60 (**C**H=CH), 123.27 (**C**H=CH), 128.43 (Ar-**C**-H), 128.72 (Ar-**C**-H), 128.79 (Ar-**C**-H), 128.98 (Ar-**C**-H), 129.33 (Ar-**C**-H), 130.19 (Ar-**C**-H), 130.54 (Ar-**C**-H), 130.85 (Ar-**C**-H), 131.00 (Ar-**C**-H), 135.42 (quaternary **C**), 158.99 (quaternary **C**), 159.07 (quaternary **C**), 159.33 (quaternary **C**), 163.47 (quaternary **C**), 165.49 (quaternary **C**), 165.61 (**C**O), 166.14

(**C**O); *m/z* 661 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 661.2162, $C_{37}H_{38}N_2O_4S_2Na$ requires 661.2170.

5.47 (±)-(1S,4S)-1-Allyl-2,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (455)



454 **455**

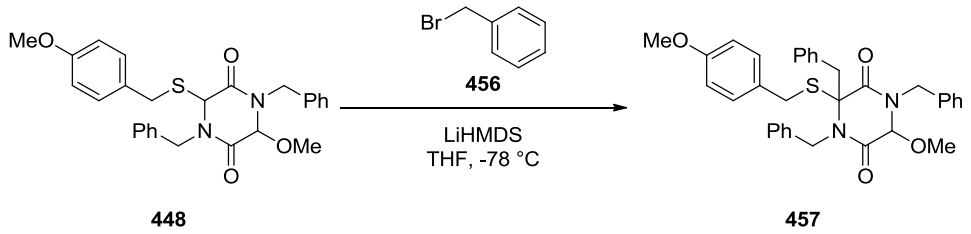
Chemical Formula: $C_{37}H_{38}N_2O_4S_2$
Molecular Weight: 638.84

Chemical Formula: $C_{21}H_{20}N_2O_2S_2$
Molecular Weight: 396.53

Boron tribromide (0.27 mL, 2.81 mmol) was added dropwise to a solution of 3-allyl-1,4-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.90 g, 1.40 mmol) (**455**) in dichloromethane (20.0 mL) at -78 °C. The resulting mixture was stirred for 15 minutes whereupon sodium hydrogen carbonate (10.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 10 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. Sodium thiosulfate was added and the mixture stirred for 10 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (±)-(1S,4S)-1-allyl-2,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.13 g, 24%) (**455**) as a pale yellow solid; m.p. 63-65 °C; ν_{max}/cm^{-1} 1687 (C=O, C=C), 1453 (CH_2), 730 (C-H), 697 (C-H); δ H (400 MHz, $CDCl_3$) 3.07 – 3.14 (1H, m, C*H*$_2$CH=CH_2), 3.25 – 3.31 (1H, m, C*H*$_2$CH=CH_2), 4.53 – 4.58 (1H, m, NC*H*$_2$-Ph), 4.63 – 4.68 (1H, m, NC*H*$_2$-Ph), 4.95 (1H, d, *J* 15.9, NC*H*$_2$-Ph), 5.13 (1H, d, *J* 16.1, NC*H*$_2$-Ph), 5.28 – 5.35 (2H, m, CH=C*H*$_2$), 5.41 (1H, s, C*H*S), 6.16 – 6.20 (1H, m, C*H*=CH_2), 7.27 – 7.30 (3H, m, Ar-*H*), 7.32 – 7.36 (4H, m, Ar-*H*), 7.39 – 7.43 (3H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 36.35 (**C**H_2), 45.35 (**C**H_2), 48.66 (**C**H_2), 64.24 (**C**HS), 75.21 (quaternary **C**), 120.89 (**C**H_2), 127.10 (Ar-**C**-H), 128.00 (Ar-**C**-H), 128.65 (Ar-**C**-H), 128.73 (Ar-**C**-H), 128.93 (Ar-**C**-H), 129.31 (Ar-**C**-H), 131.60 (**C**H=CH_2), 134.52

(quaternary **C**), 136.08 (quaternary **C**), 164.61 (**C**O), 165.14 (**C**O); *m/z* 419 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 419.0852, $C_{21}H_{20}N_2O_2S_2Na$ requires 419.0864.

5.48 1,3,4-Tribenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (457)



448

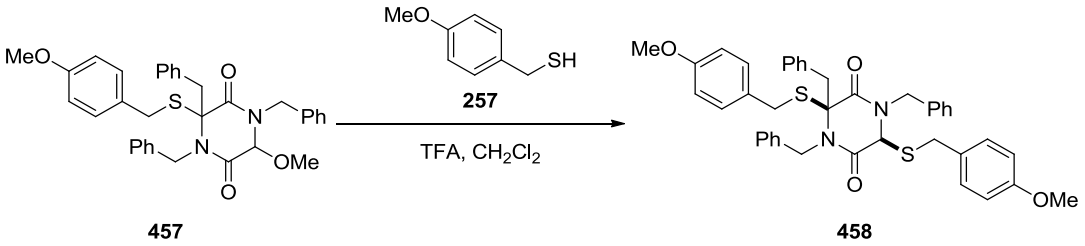
Chemical Formula: $C_{27}H_{28}N_2O_4S$
Molecular Weight: 476.59

457

Chemical Formula: $C_{34}H_{34}N_2O_4S$
Molecular Weight: 566.71

LiHMDS (0.50 mL of a 1M solution in tetrahydrofuran, 0.50 mmol) was added dropwise to a solution of 1,4-dibenzyl-3-methoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.20 g, 0.41 mmol) (**448**) and benzyl bromide (0.05 mL, 0.41 mmol) (**456**) in tetrahydrofuran (2.00 mL) at -78 °C and the resulting mixture stirred at this temperature for 1.5 hours and 1 hour at 0 °C. Saturated aqueous sodium hydrogen carbonate (10.0 mL) was added and solvent removed under reduced pressure. The residue was partitioned between water (10.0 mL) and dichloromethane (20.0 mL). The aqueous phase was extracted with dichloromethane (2 x 10.0 mL) and the combined extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) to give 1,3,4-tribenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.14 g, 59%) (**457**) as a pale yellow oil; ν_{max}/cm^{-1} 3004 (C-H), 1670 (C=O), 1453 (CH_2), 1357 (CH_3), 1000 (C-O-C); δ H (400 MHz, $CDCl_3$) 3.18 (1H, d, *J* 10.9, C*H*$_2$), 3.32 (1H, d, *J* 14.2, C*H*$_2$), 3.38 (1H, d, *J* 8.8, SC*H*$_2$), 3.44 (3H, s, OC*H*$_3$), 3.73 (1H, d, *J* 9.1, SC*H*$_2$), 3.78 (3H, s, OC*H*$_3$), 4.05 (1H, d, *J* 14.2, NC*H*$_2$), 4.13 (1H, s, C*H*O), 4.65 (1H, d, *J* 14.7, NC*H*$_2$), 4.75 (1H, d, *J* 14.7, NC*H*$_2$), 5.30 (1H, d, *J* 14.7, NC*H*$_2$), 7.01 (6H, dd, *J* 11.5 & 4.5, Ar-*H*), 7.14 – 7.17 (3H, m, Ar-*H*), 7.29 – 7.33 (8H, m, Ar-*H*), 7.54 – 7.57 (2H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 34.61 (**C**H$_2$), 43.51 (**C**H$_2$), 47.21 (**C**H$_2$), 47.91 (**C**H$_2$), 55.41 (O**C**H$_3$), 82.93 (O**C**H$_3$), 113.98 (**C**HO), 127.63 (Ar-**C**-H), 128.13 (Ar-**C**-H), 128.36 (Ar-**C**-H), 128.60 (Ar-**C**-H), 128.82 (Ar-**C**-H), 129.03 (Ar-**C**-H), 129.39 (Ar-**C**-H), 129.56 (Ar-**C**-H), 130.19 (Ar-**C**-H), 130.48 (Ar-**C**-H), 130.65 (Ar-**C**-H), 133.87 (quaternary **C**), 134.70 (quaternary **C**), 135.44 (quaternary **C**), 137.89 (quaternary **C**), 159.02 (quaternary **C**), 164.23 (quaternary **C**), 165.06 (**C**O), 166.07 (**C**O); *m/z* 589 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 589.2107, $C_{34}H_{34}N_2O_4SNa$ requires 589.2137.

5.49 (3S,6S)-1,3,4-Tribenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (458)

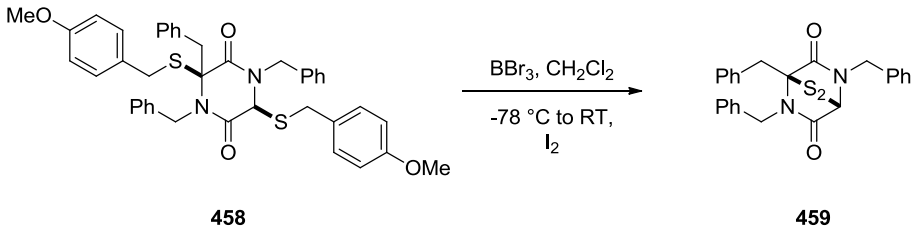


Chemical Formula: $C_{34}H_{34}N_2O_4S$
Molecular Weight: 566.71

Chemical Formula: $C_{41}H_{40}N_2O_4S_2$
Molecular Weight: 688.90

TFA (1.00 mL, 13.0 mmol) was added to a solution of 1,3,4-tribenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.18 g, 0.32 mmol) (**457**) in dichloromethane (5.00 mL) at RT followed by the addition of *para*-methoxybenzyl mercaptan (0.13 mL, 0.95 mmol) (**257**) and the resulting mixture stirred at this temperature for 18 hours. After, the solution was diluted in dichloromethane (10.0 mL) and washed with saturated aqueous sodium hydrogen carbonate (10.0 mL). The aqueous phase was extracted with dichloromethane (2 x 10.0 mL) and the combined extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) to give (3S,6S)-1,3,4-tribenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.20 g, 91%) (**458**) as a pale yellow oil; ν_{max}/cm^{-1} 3032 (C-H), 3005 (C-H), 1666 (C=O), 1356 (CH_3), 1001 (C-O-C), 833 (C-H), 730 (C-H); δ H (400 MHz, $CDCl_3$) 3.77 – 3.78 (2H, m, C*H*$_2$), 3.79 (3H, s, OC*H*$_3$), 3.84 (3H, s, OC*H*$_3$), 3.87 – 3.89 (4H, m, SC*H*$_2$ & NC*H*$_2$), 3.97 – 4.06 (2H, m, SC*H*$_2$), 4.27 (1H, s, C*H*S), 4.62 (1H, d, *J* 15.1, NC*H*$_2$), 5.28 (1H, d, *J* 15.1, NC*H*$_2$), 6.51 – 6.54 (3H, m, Ar-*H*), 6.75 – 6.81 (4H, m, Ar-*H*), 6.84 – 6.88 (4H, m, Ar-*H*), 7.00 – 7.05 (4H, m, Ar-*H*), 7.13 – 7.20 (4H, m, Ar-*H*), 7.27 – 7.31 (2H, m, Ar-*H*), 7.59 – 7.63 (2H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 35.31 (**C**H$_2$), 36.93 (**C**H$_2$), 43.44 (**C**H$_2$), 46.98 (**C**H$_2$), 47.70 (**C**H$_2$), 55.38 (O**C**H$_3$), 58.20 (O**C**H$_3$), 75.78 (quaternary **C**), 113.96 (**C**HS), 127.84 (Ar-**C**-H), 128.50 (Ar-**C**-H), 128.58 (Ar-**C**-H), 128.62 (Ar-**C**-H), 128.81 (Ar-**C**-H), 128.90 (Ar-**C**-H), 129.17 (Ar-**C**-H), 129.86 (Ar-**C**-H), 130.35 (Ar-**C**-H), 130.65 (Ar-**C**-H), 130.74 (Ar-**C**-H), 130.81 (Ar-**C**-H), 130.96 (Ar-**C**-H), 133.96 (quaternary **C**), 134.27 (quaternary **C**), 134.41 (quaternary **C**), 135.74 (quaternary **C**), 137.79 (quaternary **C**), 158.96 (quaternary **C**), 159.17 (quaternary **C**), 163.98 (**C**O), 165.99 (**C**O); *m/z* 711 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 711.2355, $C_{41}H_{40}N_2O_4S_2Na$ requires 711.2327.

5.50 (±)-(1S,4S)-1,2,5-Tribenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (459)



458

Chemical Formula: $C_{41}H_{40}N_2O_4S_2$
Molecular Weight: 688.90

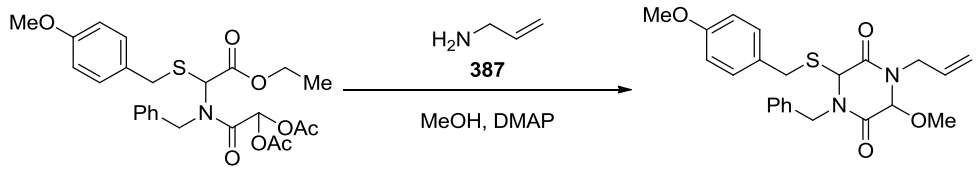
459

Chemical Formula: $C_{25}H_{22}N_2O_2S_2$
Molecular Weight: 446.58

Boron tribromide (0.70 mL of a 1M solution in dichloromethane, 0.70 mmol) was added dropwise to a solution of (3S,6S)-1,3,4-tribenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.12 g, 0.17 mmol) (**458**) in dichloromethane (2.00 mL) at -78 °C. The resulting mixture was stirred for 15 minutes whereupon aqueous sodium hydrogen carbonate (10.0 mL of a saturated aqueous solution) and methanol (1.00 mL) were added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 10 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. Sodium thiosulfate was added and the mixture stirred for 10 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g), followed by HPLC separation and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (±)-(1S,4S)-1,2,5-tribenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.04 g, 50%) (**459**) as a colourless solid; m.p. 122-124 °C; ν_{max}/cm^{-1} 1641 (C=O), 1438 (CH_2), 749 (C-H); δ H (400 MHz; $CDCl_3$) 3.62 – 3.68 (1H, m, C*H*$_2$), 3.73 – 3.79 (1H, m, C*H*$_2$), 4.23 (1H, d, *J* 15.2, NC*H*$_2$), 4.34 (1H, d, *J* 15.0, NC*H*$_2$), 4.86 (1H, d, *J* 14.9, NC*H*$_2$), 5.12 (1H, d, *J* 15.0, NC*H*$_2$), 5.26 (1H, s, C*H*S), 7.12 – 7.24 (15H, m, Ar-*H*); δ C (101 MHz; $CDCl_3$) 36.39 (**C**H_2), 46.11 (**C**H_2), 48.78 (**C**H_2), 64.13 (**C**HS), 76.53 (quaternary **C**), 127.38 (Ar-**C**-H), 127.98 (Ar-**C**-H), 128.17 (Ar-**C**-H), 128.68 (Ar-**C**-H), 128.74 (Ar-**C**-H), 128.80 (Ar-**C**-H), 128.98 (Ar-**C**-H), 129.40 (Ar-**C**-H), 130.42 (Ar-**C**-H), 133.96 (quaternary **C**), 134.54 (quaternary **C**), 136.00

(quaternary **C**), 164.83 (**C**O), 165.31 (**C**O); *m/z* 469 (100%, $[M+Na]^+$): Found $[M+Na]^+$ 469.1097, $C_{25}H_{22}N_2O_2S_2Na$ requires 469.1101.

5.51 1-Allyl-4-benzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (461)



259

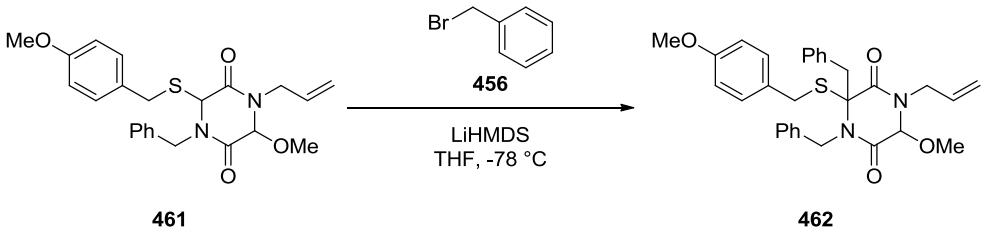
Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

461

Chemical Formula: $C_{23}H_{26}N_2O_4S$
Molecular Weight: 426.53

Allylamine (0.78 mL, 10.4 mmol) (**387**) was added to a solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (3.50 g, 6.94 mmol) (**259**) in methanol (25.0 mL) and the resulting mixture stirred for 2 minutes. DMAP (0.42 g, 3.47 mmol) was added and the resulting mixture stirred for 5 hours at RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 50 g) to give 1-allyl-4-benzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (2.26 g, 76%) (**461**) as a colourless oil; ν_{max}/cm^{-1} 1654 (C=O), 1458 (C=C), 1121 (C-O-C); δ H (400 MHz, $CDCl_3$) 3.48 (3H, s, OC*H*$_3$), 3.80 – 3.88 (5H, m, SC*H*$_2$ & OC*H*$_3$), 4.09 (1H, d, *J* 13.7, NC*H*$_2$), 4.22 (1H, d, *J* 14.8, NC*H*$_2$), 4.40 (2H, d, *J* 14.9, NC*H*$_2$), 4.54 (1H, s, C*H*S), 4.60 (1H, s, C*H*O), 4.68 (1H, d, *J* 15.0, CH=C*H*$_2$), 5.01 – 5.04 (1H, m, CH=C*H*$_2$), 5.23 – 5.26 (1H, m, *J* 14.8, C*H*=CH$_2$), 6.90 (2H, d, *J* 8.7, Ar-*H*), 7.30 – 7.32 (3H, m, Ar-*H*), 7.34 – 7.39 (4H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 36.49 (**C**H$_2$), 45.46 (**C**H$_2$), 48.03 (**C**H$_2$), 55.46 (O**C**H$_3$), 57.60 (O**C**H$_3$), 58.24 (**C**HS), 85.38 (**C**HO), 114.23 (Ar-**C**-H), 119.79 (CH=**C**H$_2$), 128.22 (Ar-**C**-H), 128.63 (Ar-**C**-H), 129.03 (Ar-**C**-H), 130.74 (**C**H=CH$_2$), 130.78 (Ar-**C**-H), 135.38 (quaternary **C**), 159.27 (quaternary **C**), 162.48 (**C**O), 166.33 (**C**O); *m/z* 449 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 449.1512, $C_{23}H_{26}N_2O_4SNa$ requires 449.1511.

5.52 1-Allyl-3,4-dibenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine -2,5-dione (462)



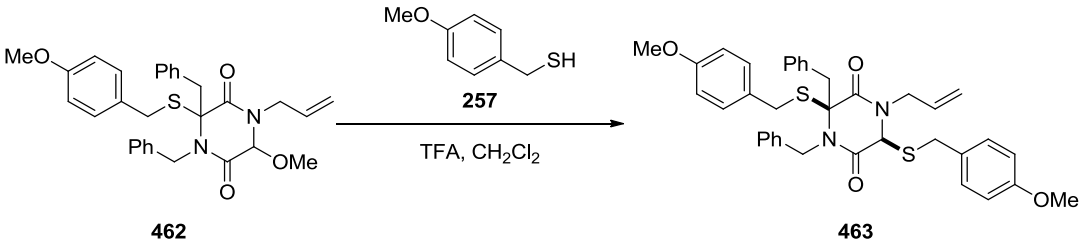
461 **462**

Chemical Formula: $C_{23}H_{26}N_2O_4S$
Molecular Weight: 426.53

Chemical Formula: $C_{30}H_{32}N_2O_4S$
Molecular Weight: 516.65

LiHMDS (6.35 mL of a 1M solution in tetrahydrofuran, 6.35 mmol) was added dropwise to a solution of 1-allyl-4-benzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (2.26 g, 5.29 mmol) (**461**) and benzyl bromide (0.94 mL, 7.94 mmol) (**456**) in tetrahydrofuran (2.00 mL) at -78 °C and the resulting mixture stirred at this temperature for 1.5 hours and 1 hour at 0 °C. Saturated aqueous sodium hydrogen carbonate (10.0 mL) was added and solvent removed under reduced pressure. The residue was partitioned between water (10.0 mL) and dichloromethane (20.0 mL). The aqueous phase was extracted with dichloromethane (2 x 10.0 mL) and the combined extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) to give 1-allyl-3,4-dibenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (1.33 g, 49%) (**462**) as a pale yellow oil; ν_{max}/cm^{-1} 3031 (C-H), 1671 (C=O), 1609 (C=C), 1000 (C-O-C), 834 (C-H), 748 (C-H), 700 (C-H); δ H (400 MHz, $CDCl_3$) 3.15 (1H, d, *J* 14.0, C*H*$_2$-Ph), 3.21 (1H, d, *J* 14.8, C*H*$_2$-Ph), 3.33 (3H, s, OC*H*$_3$), 3.44 – 3.47 (2H, m, SC*H*$_2$), 3.63 (3H, s, OCH_3), 4.01 (1H, s, C*H*O), 4.49 – 4.54 (1H, m, NC*H*$_2$), 4.60 – 4.64 (1H, m, NC*H*$_2$), 4.94 (1H, d, *J* 17.1, NC*H*$_2$), 5.02 (1H, d, *J* 17.1, NC*H*$_2$), 5.08 – 5.12 (1H, m, CH=C*H*$_2$), 5.18 – 5.22 (1H, m, CH=C*H*$_2$), 5.35 – 5.40 (1 H, m, C*H*=CH_2), 6.58 – 6.72 (6H, m, Ar-*H*), 7.12 – 7.15 (4H, m, Ar-*H*), 7.19 – 7.23 (4H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 34.64 (**C**H_2), 43.79 (**C**H_2), 46.71 (**C**H_2), 47.98 (**C**H_2), 55.44 (O**C**H_3), 57.18 (O**C**H_3), 83.22 (**C**HO), 113.98 (**C**H=CH_2), 119.92 (**C**H_2), 127.27 (quaternary **C**), 127.52 (Ar-**C**-H), 128.03 (Ar-**C**-H), 128.64 (Ar-**C**-H), 128.83 (Ar-**C**-H), 129.03 (Ar-**C**-H), 129.48 (Ar-**C**-H), 130.32 (Ar-**C**-H), 130.68 (Ar-**C**-H), 133.85 (quaternary **C**), 138.00 (quaternary **C**), 138.13 (quaternary **C**), 159.05 (quaternary **C**), 164.34 (**C**O), 164.65 (**C**O); *m/z* 517 (100%, $[M+H]^+$); Found $[M+H]^+$ 517.2155, $C_{30}H_{33}N_2O_4S$ requires 517.2161.

5.53 1-Allyl-3,4-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (463)

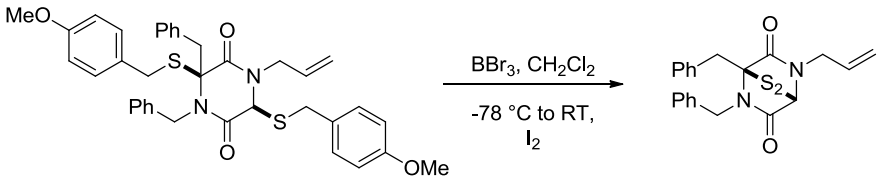


Chemical Formula: $C_{30}H_{32}N_2O_4S$
Molecular Weight: 516.65

Chemical Formula: $C_{37}H_{38}N_2O_4S_2$
Molecular Weight: 638.84

TFA (2.96 mL, 38.5 mmol) was added to a solution of 1-allyl-3,4-dibenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (1.33 g, 2.56 mmol) (**462**) in dichloromethane (20.0 mL) at RT followed by the addition of *para*-methoxybenzyl mercaptan (0.54 mL, 3.85 mmol) (**257**) and the resulting mixture stirred at this temperature for 18 hours. After, the solution was diluted in dichloromethane (10.0 mL) and washed with saturated aqueous sodium hydrogen carbonate (10.0 mL). The aqueous phase was extracted with dichloromethane (2 x 10.0 mL) and the combined extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give 1-allyl-3,4-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (1.33 g, 81%) (**463**) as a pale yellow solid; m.p. 124-126 °C; ν_{max}/cm^{-1} 1665 (C=O), 1609 (C=C), 1000 (C-O-C), 833 (C-H), 728 (C-H), 699 (C-H); δ H (400 MHz, $CDCl_3$) 3.33 – 3.39 (2H, m, C*H*$_2$-Ph), 3.47 (2H, s, SC*H*$_2$), 3.77 (6H, s, OC*H*$_3$), 3.81 (2H, s, SC*H*$_2$), 3.82 – 3.84 (2H, m, NC*H*$_2$CH=CH), 4.15 (1H, s, C*H*S), 4.57 – 4.65 (2H, m, NC*H*$_2$-Ph), 5.31 – 5.37 (2H, m, CH=C*H*$_2$), 5.43 – 5.54 (1H, m, C*H*=CH$_2$), 6.72 – 6.90 (11H, m, Ar-*H*), 7.07 – 7.15 (3H, m, Ar-*H*), 7.33 – 7.37 (4H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 34.64 (**C**H$_2$), 35.23 (**C**H$_2$), 37.10 (**C**H$_2$), 43.80 (**C**H$_2$), 46.71 (**C**H$_2$), 55.45 (O**C**H$_3$), 58.62 (O**C**H$_3$), 76.70 (quaternary **C**), 83.23 (**C**HS), 114.01 (**C**H=CH$_2$), 119.96 (CH=**C**H$_2$), 127.52 (Ar-**C**-H), 128.65 (Ar-**C**-H), 128.83 (Ar-**C**-H), 129.04 (Ar-**C**-H), 130.19 (Ar-**C**-H), 130.32 (Ar-**C**-H), 130.52 (Ar-**C**-H), 130.69 (Ar-**C**-H), 130.93 (Ar-**C**-H), 131.28 (Ar-**C**-H), 133.86 (quaternary **C**), 134.21 (quaternary **C**), 138.00 (quaternary **C**), 159.05 (quaternary **C**), 159.29 (quaternary **C**), 163.72 (quaternary **C**), 164.65 (**C**O), 166.18 (**C**O); *m/z* 661 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 661.2159, $C_{37}H_{38}N_2O_4S_2Na$ requires 661.2170.

5.54 (±)-(1S,4S)-5-Allyl-1,2-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (464)



463

Chemical Formula: $C_{37}H_{38}N_2O_4S_2$
Molecular Weight: 638.84

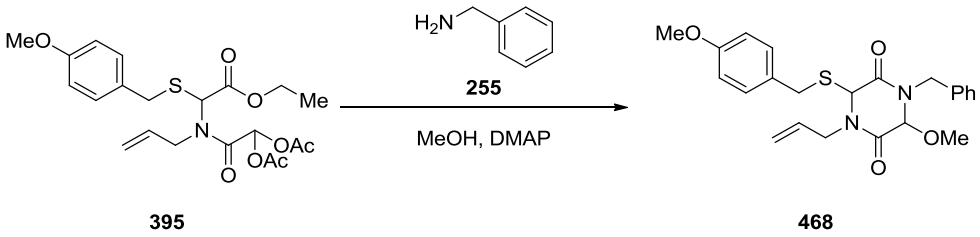
464

Chemical Formula: $C_{21}H_{20}N_2O_2S_2$
Molecular Weight: 396.53

Boron tribromide (0.34 mL, 4.170 mmol) was added dropwise to a solution of 1-allyl-3,4-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (1.33 g, 2.09 mmol) (**463**) in dichloromethane (30.0 mL) at -78 °C. The resulting mixture was stirred for 15 minutes whereupon sodium hydrogen carbonate (10.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 10 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. Sodium thiosulfate was added and the mixture stirred for 10 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (±)-(1S,4S)-5-allyl-1,2-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.19 g, 23%) (**464**) as a pale yellow solid; m.p. 62-64 °C; ν_{max}/cm^{-1} 1685 (C=O), 1628 (C=C), 734 (C-H), 697 (C-H); δ H (400 MHz, $CDCl_3$) 3.84 (2H, s, C*H*$_2$-Ph), 3.86 – 3.90 (1H, m, NC*H*$_2$CH=CH$_2$), 4.00 – 4.05 (1H, m, NC*H*$_2$CH=CH$_2$), 4.09 (1H, d, *J* 15.0, NC*H*$_2$-Ph), 4.64 (1H, s, C*H*S), 4.75 (1 H, d, *J* 17.1, CH=C*H*$_2$), 5.03 (1H, d, *J* 17.1, CH=C*H*$_2$), 5.22 (1H, d, *J* 15.0, NC*H*$_2$-Ph), 5.55 – 5.67 (1H, m, C*H*=CH$_2$), 6.89 – 6.92 (4H, m, Ar-*H*), 7.36 – 7.42 (6H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 35.94 (**C**H$_2$), 46.05 (**C**H$_2$), 48.00 (**C**H$_2$), 55.58 (**C**HS), 59.30 (**C**H=CH$_2$), 114.35 (Ar-**C**-H), 119.50 (CH=**C**H$_2$), 123.94 (Ar-**C**-H), 127.70 (Ar-**C**-H), 128.71 (Ar-**C**-H), 129.66 (Ar-**C**-H), 130.78 (Ar-**C**-H), 133.97 (quaternary **C**), 136.45 (quaternary **C**), 159.40 (quaternary **C**),

163.57 (**C**O), 165.65 (**C**O); *m/z* 397 (100%, $[M+H]^+$); Found $[M+H]^+$ 397.1078, $C_{21}H_{21}N_2O_2S_2$ requires 397.1045.

5.55 1-Allyl-4-benzyl-3-methoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (468)



395

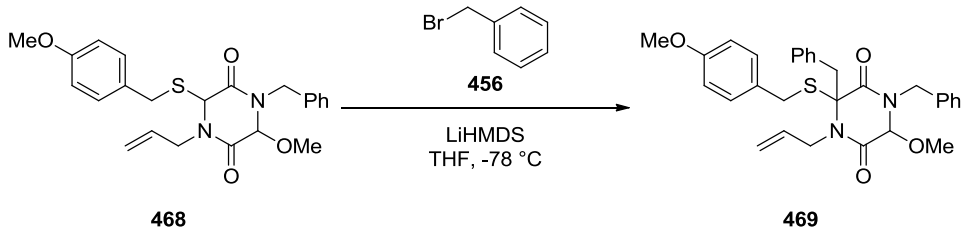
Chemical Formula: $C_{21}H_{27}NO_8S$
Molecular Weight: 453.51

468

Chemical Formula: $C_{23}H_{26}N_2O_4S$
Molecular Weight: 426.53

Benzylamine (1.42 mL, 13.0 mmol) (**255**) was added to a solution of 2-(allyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (3.92 g, 8.65 mmol) (**395**) in methanol (40.0 mL) and the resulting mixture stirred for 2 minutes. DMAP (0.53 g, 4.33 mmol) was added and the resulting mixture stirred for 12 hours at RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 50 g) to give 1-allyl-4-benzyl-3-methoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (3.14 g, 85%) (**468**) as a colourless oil; ν_{max}/cm^{-1} 1654 (C=O), 1458 (C=C), 1121 (C-O-C); δ H (400 MHz, $CDCl_3$) 3.48 (3H, s, OC*H*$_3$), 3.80 – 3.88 (5H, m, SC*H*$_2$ & OC*H*$_3$), 4.09 (1H, d, *J* 13.7, NC*H*$_2$), 4.22 (1H, d, *J* 14.8, NC*H*$_2$), 4.40 (2H, d, *J* 14.9, NC*H*$_2$), 4.54 (1H, s, C*H*S), 4.60 (1H, s, C*H*O), 4.68 (1H, d, *J* 15.0, CH=C*H*$_2$), 5.01 – 5.04 (1H, m, CH=C*H*$_2$), 5.23 – 5.26 (1H, m, *J* 14.8, C*H*=CH$_2$), 6.90 (2H, d, *J* 8.7, Ar-*H*), 7.30 – 7.32 (3H, m, Ar-*H*), 7.34 – 7.39 (4H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 36.49 (**C**H$_2$), 45.46 (**C**H$_2$), 48.03 (**C**H$_2$), 55.46 (O**C**H$_3$), 57.60 (O**C**H$_3$), 58.24 (**C**HS), 85.38 (**C**HO), 114.23 (Ar-**C**-H), 119.79 (CH=**C**H$_2$), 128.22 (Ar-**C**-H), 128.63 (Ar-**C**-H), 129.03 (Ar-**C**-H), 130.74 (**C**H=CH$_2$), 130.78 (Ar-**C**-H), 135.38 (quaternary **C**), 159.27 (quaternary **C**), 162.48 (**C**O), 166.33 (**C**O); *m/z* 449 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 449.1512, $C_{23}H_{26}N_2O_4SNa$ requires 449.1511.

5.56 4-Allyl-1,3-dibenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine -2,5-dione (469)



468

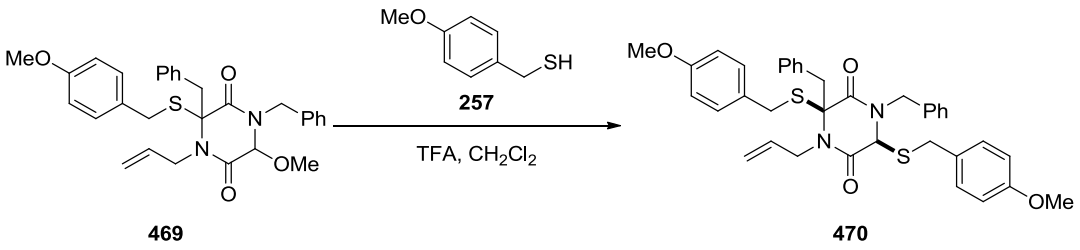
Chemical Formula: $C_{23}H_{26}N_2O_4S$
Molecular Weight: 426.53

469

Chemical Formula: $C_{30}H_{32}N_2O_4S$
Molecular Weight: 516.65

LiHMDS (8.62 mL of a 1M solution in tetrahydrofuran, 8.62 mmol) was added dropwise to a solution of 1-allyl-4-benzyl-3-methoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (3.06 g, 7.18 mmol) (**468**) and benzyl bromide (1.28 mL, 10.8 mmol) (**456**) in tetrahydrofuran (30.0 mL) at -78 °C and the resulting mixture stirred at this temperature for 1.5 hours and 1 hour at 0 °C. Saturated aqueous sodium hydrogen carbonate (10.0 mL) was added and solvent removed under reduced pressure. The residue was partitioned between water (10.0 mL) and dichloromethane (20.0 mL). The aqueous phase was extracted with dichloromethane (2 x 10.0 mL) and the combined extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) to give 4-allyl-1,3-dibenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (2.89 g, 78%) (**469**) as a pale yellow oil; ν_{max}/cm^{-1} 3032 (C-H), 1669 (C=O), 1609 (C=C), 1000 (C-O-C), 746 (C-H), 701 (C-H); δ H (400 MHz, $CDCl_3$) 3.03 – 3.07 (1H, d, *J* 14.3, C*H*$_2$-Ph), 3.26 (3H, s, OC*H*$_3$), 3.46 (1H, d, *J* 14.1, C*H*$_2$-Ph), 3.63 (2H, s, SC*H*$_2$), 3.64 (3H, s, OC*H*$_3$), 3.65 – 3.66 (1H, m, NC*H*$_2$CH=CH), 3.83 – 3.87 (1H, m, NC*H*$_2$CH=CH), 3.96 (1H, s, C*H*O), 4.93 – 5.06 (2H, m, NC*H*$_2$-Ph), 5.23 – 5.33 (2H, m, CH=C*H*$_2$), 5.89 – 5.97 (1H, m, C*H*=CH$_2$), 6.64 – 6.68 (2H, m, Ar-*H*), 6.86 – 6.95 (4H, m, Ar-*H*), 7.05 – 7.10 (8H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 34.55 (**C**H$_2$), 43.27 (**C**H$_2$), 46.96 (**C**H$_2$), 47.56 (**C**H$_2$), 55.50 (O**C**H$_3$), 57.57 (O**C**H$_3$), 76.43 (quaternary **C**), 82.68 (**C**HO), 114.23 (**C**H=CH$_2$), 119.28 (**C**H$_2$), 128.68 (Ar-**C**-H), 128.85 (Ar-**C**-H), 129.16 (Ar-**C**-H), 129.65 (Ar-**C**-H), 130.28 (Ar-**C**-H), 130.62 (Ar-**C**-H), 130.84 (Ar-**C**-H), 133.02 (Ar-**C**-H), 133.24 (quaternary **C**), 134.14 (quaternary **C**), 134.65 (quaternary **C**), 159.16 (quaternary **C**), 163.26 (**C**O), 165.00 (**C**O); *m/z* 517 (100%, $[M+H]^+$); Found $[M+H]^+$ 517.2171, $C_{30}H_{33}N_2O_4S$ requires 517.2161.

5.57 4-Allyl-1,3-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (470)



469

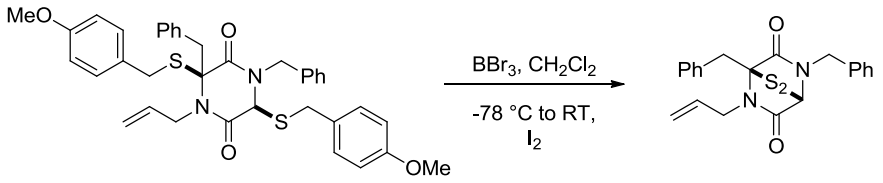
Chemical Formula: $C_{30}H_{32}N_2O_4S$
Molecular Weight: 516.65

470

Chemical Formula: $C_{37}H_{38}N_2O_4S_2$
Molecular Weight: 638.84

TFA (6.45 mL, 84.0 mmol) was added to a solution of 4-allyl-1,3-dibenzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (2.89 g, 5.58 mmol) (**469**) in dichloromethane (20.0 mL) at RT followed by the addition of *para*-methoxybenzyl mercaptan (1.17 mL, 8.38 mmol) (**257**) and the resulting mixture stirred at this temperature for 18 hours. After, the solution was diluted in dichloromethane (10.0 mL) and washed with saturated aqueous sodium hydrogen carbonate (10.0 mL). The aqueous phase was extracted with dichloromethane (2 x 10.0 mL) and the combined extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give 4-allyl-1,3-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (2.94 g, 82%) (**470**) as a pale yellow solid; m.p. 124-126 °C; ν_{max}/cm^{-1} 3004 (C-H), 1665 (C=O), 1609 (C=C), 1001 (C-O-C), 833 (C-H), 747 (C-H), 701 (C-H); δ H (400 MHz, $CDCl_3$) 3.42 (2H, d, *J* 16.5, C*H*$_2$-Ph), 3.79 (2H, s, SC*H*$_2$), 3.82 – 3.84 (6H, m, OC*H*$_3$), 3.84 (2H, s, SC*H*$_2$), 3.86 – 3.93 (2H, m, NC*H*$_2$), 4.00 – 4.07 (2H, m, NC*H*$_2$), 4.22 (1H, s, C*H*S), 4.39 – 4.51 (2H, m, CH=C*H*$_2$), 6.10 – 6.20 (1H, m, C*H*=CH$_2$), 6.76 – 6.84 (4H, m, Ar-*H*), 7.10 – 7.21 (6H, m, Ar-*H*), 7.26 – 7.33 (8H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 34.04 (**C**H$_2$), 35.43 (**C**H$_2$), 37.04 (**C**H$_2$), 43.23 (**C**H$_2$), 47.48 (**C**H$_2$), 55.44 (O**C**H$_3$), 55.48 (O**C**H$_3$), 57.94 (**C**HS), 74.92 (quaternary **C**), 114.25 (**C**H=CH$_2$), 118.65 (CH=**C**H$_2$), 128.62 (Ar-**C**-H), 128.74 (Ar-**C**-H), 128.82 (Ar-**C**-H), 129.22 (Ar-**C**-H), 129.99 (Ar-**C**-H), 130.33 (Ar-**C**-H), 130.67 (Ar-**C**-H), 130.97 (Ar-**C**-H), 133.14 (Ar-**C**-H), 133.90 (Ar-**C**-H), 134.13 (quaternary **C**), 134.57 (quaternary **C**), 135.80 (quaternary **C**), 159.16 (quaternary **C**), 159.25 (quaternary **C**), 163.95 (quaternary **C**), 165.52 (**C**O), 165.65 (**C**O); *m/z* 661 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 661.2168, $C_{37}H_{38}N_2O_4S_2Na$ requires 661.2170.

5.58 (±)-(1S,4S)-2-Allyl-1,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (471)



470

Chemical Formula: $C_{37}H_{38}N_2O_4S_2$
Molecular Weight: 638.84

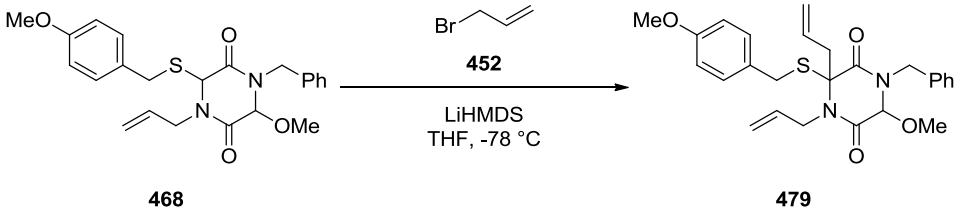
471

Chemical Formula: $C_{21}H_{20}N_2O_2S_2$
Molecular Weight: 396.53

Boron tribromide (0.87 mL, 9.20 mmol) was added dropwise to a solution of 4-allyl-1,3-dibenzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (2.94 g, 4.60 mmol) (**470**) in dichloromethane (50.0 mL) at -78 °C. The resulting mixture was stirred for 15 minutes whereupon sodium hydrogen carbonate (10.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 10 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. Sodium thiosulfate was added and the mixture stirred for 10 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (±)-(1S,4S)-2-allyl-1,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.38 g, 21%) (**471**) as a pale yellow solid; m.p. 63-64 °C; ν_{max}/cm^{-1} 1672 (C=O), 1627 (C=C), 832 (C-H), 698 (C-H); δ H (400 MHz, $CDCl_3$) 3.84 (2H, s, C*H*$_2$-Ph), 3.87 – 3.91 (2H, m, NC*H*$_2$CH=CH$_2$), 4.04 (1H, d, *J* 6.9, NC*H*$_2$-Ph), 4.65 (1H, s, C*H*S), 4.73 (1H, d, *J* 10.1, CH=C*H*$_2$), 5.04 (1H, d, *J* 10.0, CH=C*H*$_2$), 5.21 (1H, d, *J* 7.1, NC*H*$_2$-Ph), 5.56 – 5.70 (1H, m, C*H*=CH$_2$), 6.85 – 6.90 (2H, m, Ar-*H*), 6.91 – 6.97 (4H, m, Ar-*H*), 7.37 – 7.40 (4H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 44.00 (**C**H$_2$), 45.99 (**C**H$_2$), 47.92 (**C**H$_2$), 55.52 (**C**HS), 59.25 (**C**H=CH$_2$), 119.43 (CH=**C**H$_2$), 123.87 (Ar-**C**-H), 127.64 (Ar-**C**-H), 128.65 (Ar-**C**-H), 128.84 (Ar-**C**-H), 129.60 (Ar-**C**-H), 130.72 (Ar-**C**-H), 133.90 (quaternary **C**), 136.39 (quaternary **C**), 159.33 (quaternary **C**), 163.50 (**C**O), 165.58

(**C**O); *m/z* 419 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 419.0973, $C_{21}H_{20}N_2O_2S_2Na$ requires 419.0864.

5.59 3,4-Diallyl-1-benzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine -2,5-dione (479)



468

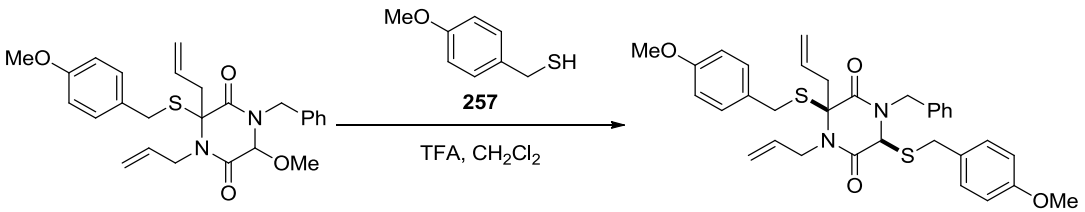
Chemical Formula: $C_{23}H_{26}N_2O_4S$
Molecular Weight: 426.53

479

Chemical Formula: $C_{26}H_{30}N_2O_4S$
Molecular Weight: 466.59

LiHMDS (7.73 mL of a 1M solution in tetrahydrofuran, 7.73 mmol) was added dropwise to a solution of 1-allyl-4-benzyl-3-methoxy-6-((4-methoxybenzyl)thio)piperazine-2,5-dione (2.75 g, 6.45 mmol) (**468**) and allyl bromide (0.84 mL, 9.67 mmol) (**452**) in tetrahydrofuran (30.0 mL) at -78 °C and the resulting mixture stirred at this temperature for 1.5 hours and 1 hour at 0 °C. Saturated aqueous sodium hydrogen carbonate (10.0 mL) was added and solvent removed under reduced pressure. The residue was partitioned between water (10.0 mL) and dichloromethane (20.0 mL). The aqueous phase was extracted with dichloromethane (2 x 10.0 mL) and the combined extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) to give 3,4-diallyl-1-benzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (2.05 g, 68%) (**479**) as a colourless oil; ν_{max}/cm^{-1} 2933 (C-H), 1671 (C=O, C=C), 1357 (CH_3), 1249 (C-O-C), 835 (C-H); δ H (400 MHz, $CDCl_3$) 2.60 (1H, dd, *J* 14.2 & 6.1, C*H*$_2$CH=CH$_2$), 3.05 (1H, dd, *J* 14.2 & 7.2, C*H*$_2$CH=CH$_2$), 3.49 (3H, s, OC*H*$_3$), 3.64 (1H, d, *J* 11.9, SC*H*$_2$), 3.70 (3H, s, OC*H*$_3$), 3.74 (1H, d, *J* 11.5, SC*H*$_2$), 3.99 (2H, d, *J* 10.7, NC*H*$_2$), 4.09 (1H, d, *J* 10.5, NC*H*$_2$), 4.48 (1H, s, C*H*O), 5.01 – 5.09 (1H, m, NC*H*$_2$), 5.11 – 5.18 (2H, m, CH=C*H*$_2$), 5.26 (1H, d, *J* 14.2, CH=C*H*$_2$), 5.32 (1H, d, *J* 14.5, CH=C*H*$_2$), 5.35 – 5.44 (1H, m, C*H*=CH$_2$), 5.92 – 5.94 (1H, m, C*H*=CH$_2$), 7.12 (2H, d, *J* 8.7, Ar-*H*), 7.18 – 7.27 (7H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 34.22 (**C**H$_2$), 41.74 (**C**H$_2$), 46.67 (**C**H$_2$), 47.28 (**C**H$_2$), 55.41 (O**C**H$_3$), 58.47 (O**C**H$_3$), 74.69 (quaternary **C**), 83.30 (**C**HO), 114.13 (**C**H=CH$_2$), 118.82 (CH=**C**H$_2$), 121.64 (CH=**C**H$_2$), 127.90 (**C**H=CH$_2$), 128.29 (quaternary **C**), 128.94 (Ar-**C**-H), 129.14 (Ar-**C**-H), 130.13 (Ar-**C**-H), 130.48 (Ar-**C**-H), 133.12 (quaternary **C**), 135.12 (Ar-**C**-H), 159.00 (quaternary **C**), 163.27 (**C**O), 165.22 (**C**O); *m/z* 467 (100%, $[M+H]^+$); Found $[M+H]^+$ 467.2023, $C_{26}H_{31}N_2O_4S$ requires 467.2004.

5.60 3,4-Diallyl-1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (480)



479

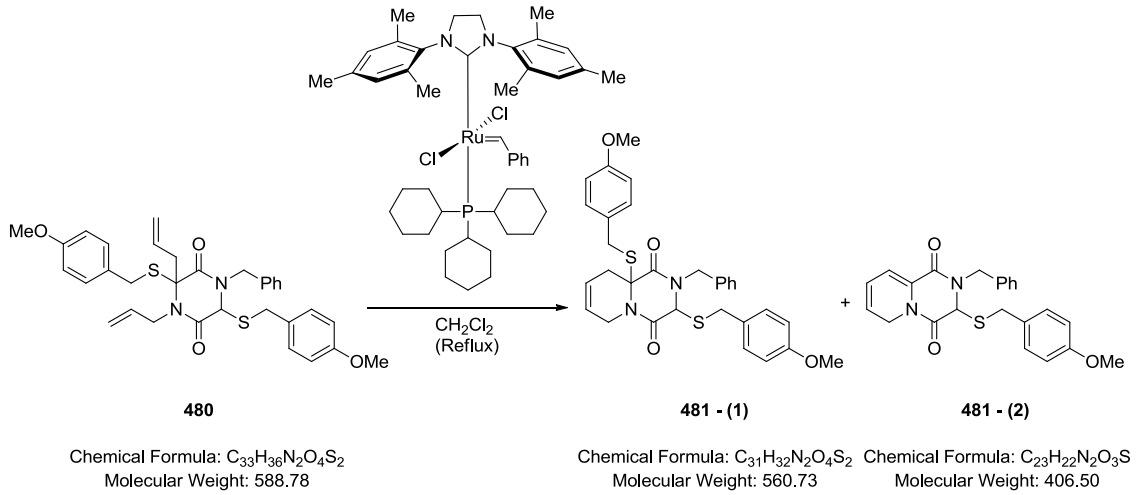
Chemical Formula: $C_{26}H_{30}N_2O_4S$
Molecular Weight: 466.59

480

Chemical Formula: $C_{33}H_{36}N_2O_4S_2$
Molecular Weight: 588.78

TFA (1.00 mL, 13.0 mmol) was added to a solution of 3,4-diallyl-1-benzyl-6-methoxy-3-((4-methoxybenzyl)thio)piperazine-2,5-dione (0.24 g, 0.51 mmol) (**479**) in dichloromethane (3.00 mL) at RT followed by the addition of *para*-methoxybenzyl mercaptan (0.22 mL, 1.54 mmol) (**257**) and the resulting mixture stirred at this temperature for 18 hours. After, the solution was diluted in dichloromethane (10.0 mL) and washed with saturated aqueous sodium hydrogen carbonate (10.0 mL). The aqueous phase was extracted with dichloromethane (2 x 10.0 mL) and the combined extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) to give 3,4-diallyl-1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.23 g, 76%) (**480**) as a pale yellow oil; ν_{max}/cm^{-1} 3004 (C-H), 1666 (C=O), 1609 (C=C), 1442 (CH_2), 834 (C-H), 750 (C-H); δ H (400 MHz, $CDCl_3$) 2.65 – 2.76 (2H, m, C*H*$_2$CH=CH$_2$), 2.96 – 3.03 (2H, m, SC*H*$_2$), 3.29 – 3.42 (2H, m, SC*H*$_2$), 3.73 – 3.76 (2H, m, NC*H*$_2$), 3.79 (3H, s, OC*H*$_3$), 3.86 – 3.87 (2H, m, NC*H*$_2$), 3.88 (3H, s, OC*H*$_3$), 4.00 – 4.34 (4H, m, CH=CC*H*$_2$), 4.47 (1H, s, C*H*S), 5.82 – 5.94 (1H, m, C*H*=CH$_2$), 5.97 – 6.11 (1H, m, C*H*=CH$_2$), 6.72 (2H, d, *J* 7.0, Ar-*H*), 6.75 – 6.78 (2H, m, Ar-*H*), 6.88 (2H, d, *J* 3.2, Ar-*H*), 6.93 – 6.97 (3H, m, Ar-*H*), 7.35 – 7.38 (2H, m, Ar-*H*), 7.39 – 7.43 (2H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 34.03 (**C**H$_2$), 35.29 (**C**H$_2$), 37.07 (**C**H$_2$), 41.36 (**C**H$_2$), 46.99 (**C**H$_2$), 55.49 (O**C**H$_3$), 57.65 (O**C**H$_3$), 58.08 (**C**HS), 73.50 (quaternary **C**), 114.46 (**C**H=CH$_2$), 118.22 (CH=**C**H$_2$), 121.57 (CH=**C**H$_2$), 128.35 (**C**H=CH$_2$), 129.33 (Ar-**C**-H), 130.15 (Ar-**C**-H), 130.61 (Ar-**C**-H), 130.89 (Ar-**C**-H), 131.07 (Ar-**C**-H), 133.11 (quaternary **C**), 133.51 (quaternary **C**), 135.22 (Ar-**C**-H), 135.83 (Ar-**C**-H), 159.14 (quaternary **C**), 159.44 (quaternary **C**), 164.18 (quaternary **C**), 165.11 (**C**O), 166.01 (**C**O); *m/z* 611 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 611.1988, $C_{33}H_{36}N_2O_4S_2Na$ requires 611.2014.

5.61 2-Benzyl-3,9a-bis((4-methoxybenzyl)thio)-2,3,9,9a-tetrahydro-1H-pyrido[1,2-a]pyrazine-1,4(6H)-dione (481)



480

Chemical Formula: $C_{33}H_{36}N_2O_4S_2$
Molecular Weight: 588.78

481 - (1)

Chemical Formula: $C_{31}H_{32}N_2O_4S_2$
Molecular Weight: 560.73

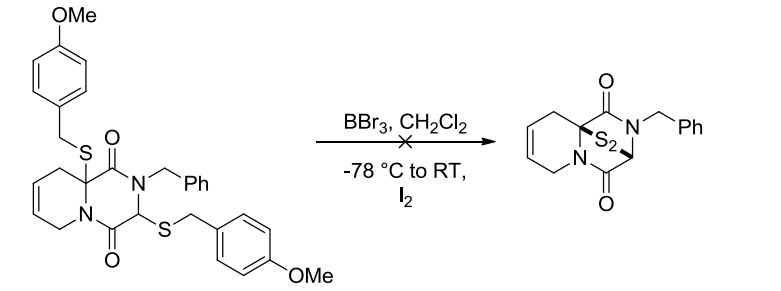
481 - (2)

Chemical Formula: $C_{23}H_{22}N_2O_3S$
Molecular Weight: 406.50

A solution of 3,4-diallyl-1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.09 g, 0.15 mmol) (**480**) in dichloromethane (5.00 mL) was degassed and taken under nitrogen atmosphere. To this (1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(phenylmethylene) (tricyclohexylphosphine) ruthenium (Grubbs second-generation catalyst) (0.01 g, 0.02 mmol) was added and the resulting mixture heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give 2-benzyl-3,9a-bis((4-methoxybenzyl)thio)-2,3,9,9a-tetrahydro-1H-pyrido[1,2-a]pyrazine-1,4(6H)-dione (0.03 g, 40%) (**481 – (1)**) and 2-benzyl-3-((4-methoxybenzyl)thio)-2,3-dihydro-1H-pyrido[1,2-a]pyrazine-1,4(6H)-dione (0.01 g, 3%) (**482 – (2)**) as pale yellow solids; Data for **481 – (1)**: m.p. 112-115 °C; ν_{max}/cm^{-1} 3033 (C-H), 2934 (C-H), 2836 (C-H), 1665 (C=O), 834 (C-H), 734 (C-H); δ H (400 MHz, $CDCl_3$) 3.36 – 3.40 (1H, m, C*H*$_2$CH=CH), 3.49 – 3.53 (1H, m, C*H*$_2$CH=CH), 3.79 (3H, s, OC*H*$_3$), 3.81 (2H, d, *J* 5.7, SC*H*$_2$), 3.87 (3H, s, OC*H*$_3$), 3.89 (2H, d, *J* 5.9, SC*H*$_2$), 3.93 (1H, d, *J* 7.9, NC*H*$_2$), 4.03 – 4.07 (1H, m, NC*H*$_2$), 4.54 (1H, s, C*H*S), 5.31 (1H, d, *J* 14.5, NC*H*$_2$CH=CH), 5.43 (1H, d, *J* 14.2, NC*H*$_2$CH=CH), 5.79 (2H, d, *J* 1.4, C*H*=C*H*), 6.75 – 6.78 (2H, m, Ar-*H*), 6.83 – 6.87 (2H, m, Ar-*H*), 6.91 – 6.94 (4H, m, Ar-*H*), 7.16 – 7.20 (3H, m, Ar-*H*), 7.35 – 7.37 (2H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 34.13 (**C**H$_2$), 34.83 (**C**H$_2$), 35.77 (**C**H$_2$), 41.00 (**C**H$_2$), 47.20 (**C**H$_2$), 55.43 (O**C**H$_3$), 55.52 (O**C**H$_3$), 58.50 (**C**HS), 68.84 (quaternary **C**), 114.36 (Ar-**C**-H), 121.60 (**C**H=CH), 123.27 (**C**H=CH), 128.43 (Ar-**C**-H), 128.79 (Ar-**C**-H), 128.98 (Ar-

C-H), 129.33 (Ar-**C**-H), 130.19 (Ar-**C**-H), 130.85 (Ar-**C**-H), 135.42 (quaternary **C**), 159.07 (quaternary **C**), 159.33 (quaternary **C**), 163.47 (quaternary **C**), 165.61 (quaternary **C**), 165.61 (**C**O), 166.14 (**C**O); *m/z* 583 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 583.1694, $C_{31}H_{32}N_2O_4S_2Na$ requires 583.1701; Data for **381 – (2)**: m.p. 92-95 °C; ν_{max}/cm^{-1} 2932 (C-H), 1680 (C=O), 1639 (C=C), 1609 (C=C), 834 (C-H), 734 (C-H), 701 (C-H); δ H (400 MHz, $CDCl_3$) 3.65 – 3.67 (1H, d, *J* 5.0, SCH_2), 3.70 (1H, d, *J* 5.0, SCH_2), 3.72 – 3.74 (1H, m, NCH_2), 3.76 (3H, s, OCH_3), 3.81 – 3.85 (1H, m, NCH_2), 4.00 (1H, d, *J* 12.6, NCH_2), 4.46 (1H, s, C*H*S), 4.76 (1H, d, *J* 12.5, NCH_2), 5.81 – 5.85 (1H, m, C*H*=CH), 6.01 – 6.04 (1H, m, C*H*=CH), 6.38 (1H, d, *J* 5.9, C*H*-CH), 6.77 – 6.81 (4H, m, Ar-*H*), 7.13 – 7.16 (2H, m, Ar-*H*), 7.22 – 7.26 (3H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 35.09 (**C**H_2), 43.30 (**C**H_2), 46.20 (**C**H_2), 55.50 (O**C**H_3), 58.71 (**C**HS), 113.44 (**C**H-CH), 114.30 (Ar-**C**-H), 121.99 (**C**H=CH), 100.69 (**C**H=CH), 127.20 (quaternary **C**), 128.23 (Ar-**C**-H), 128.58 (quaternary **C**), 128.93 (Ar-**C**-H), 129.06 (Ar-**C**-H), 130.64 (Ar-**C**-H), 135.00 (quaternary **C**), 158.97 (quaternary **C**), 159.27 (**C**O), 162.73 (**C**O); *m/z* 429 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 429.1237, $C_{23}H_{22}N_2O_3SNa$ requires 429.1249.

5.62 (±)-(3S,9aS)-2-Benzyl-2,3-dihydro-3,9a-epithiopyrido[1,2-a]pyrazine-1,4(6H,9H)-dione 10-sulfide (482)

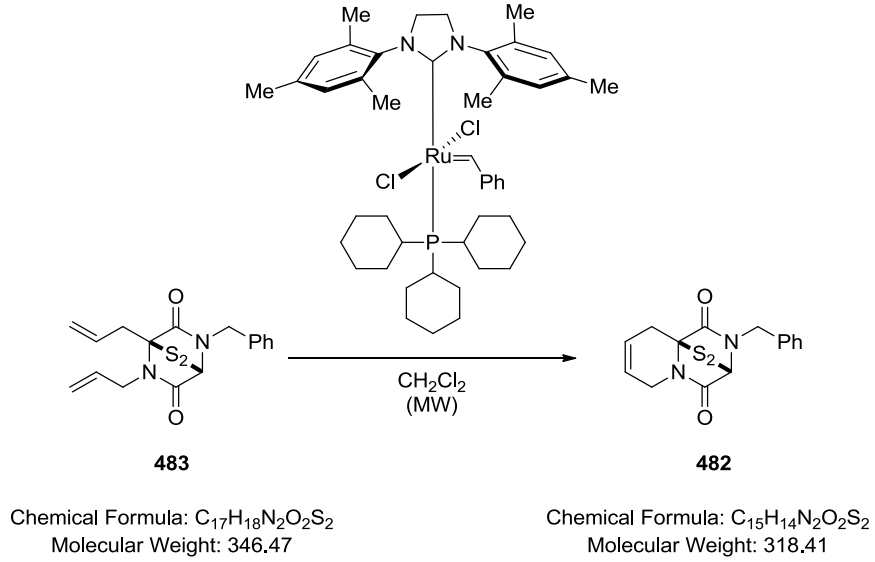


481 - (1)
Chemical Formula: $C_{31}H_{32}N_2O_4S_2$
Molecular Weight: 560.73

482
Chemical Formula: $C_{15}H_{14}N_2O_2S_2$
Molecular Weight: 318.41

Boron tribromide (0.24 mL of a 1M solution in dichloromethane, 0.24 mmol) was added dropwise to a solution of 2-benzyl-3,9a-bis((4-methoxybenzyl)thio)-2,3,9,9a-tetrahydro-1H-pyrido[1,2-a]pyrazine-1,4(6H)-dione (0.03 g, 0.06 mmol) (**481 – (1)**) in dichloromethane (2.00 mL) at -78 °C. The resulting mixture was stirred for 15 minutes whereupon aqueous sodium hydrogen carbonate (5.00 mL of a saturated aqueous solution) and methanol (2.00 mL) were added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 10 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. Sodium thiosulfate was added and the mixture stirred for 10 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) which did not give (3S,9aS)-2-benzyl-2,3-dihydro-3,9a-epithiopyrido[1,2-a]pyrazine-1,4(6H,9H)-dione 10-sulfide (**482**).

5.63 (±)-(3S,9aS)-2-Benzyl-2,3-dihydro-3,9a-epithiopyrido[1,2-a]pyrazine-1,4(6H,9H)-dione 10-sulfide (482)



483

Chemical Formula: $C_{17}H_{18}N_2O_2S_2$
Molecular Weight: 346.47

482

Chemical Formula: $C_{15}H_{14}N_2O_2S_2$
Molecular Weight: 318.41

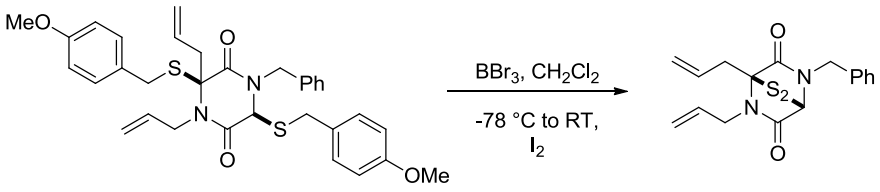
5.63.1 Method A:

A solution of 3,4-diallyl-1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.05 g, 0.15 mmol) (**483**) in dichloromethane (2.00 mL) was degassed and taken under nitrogen atmosphere. To this (1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(phenylmethylene) (tricyclohexylphosphine)ruthenium (Grubbs second-generation catalyst) (0.01 g, 0.02 mmol) was added and the resulting mixture heated at 80 °C for 30 minutes in the microwave, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,9aS)-2-benzyl-2,3-dihydro-3,9a-epithiopyrido[1,2-a]pyrazine-1,4(6H,9H)-dione 10-sulfide (0.01 g, 8%) (**482**) as a colourless solid; m.p. 119-121 °C; ν_{max}/cm^{-1} 1682 (C=O), 1639 (C=C), 746 (C-H); δ H (400 MHz, $CDCl_3$) 2.57 – 2.65 (2H, m, CH_2), 3.40 (2H, dd, *J* 13.4 & 5.8, NCH_2), 4.44 (1H, d, *J* 14.9, NCH_2), 4.88 (1H, d, *J* 14.9, NCH_2), 5.19 (1H, s, C*H*S), 5.93 (2H, ddd, *J* 6.9 & 4.3 & 2.2, C*H*=C*H*), 7.24 (2H, d, *J* 2.1, Ar-*H*), 7.29 – 7.32 (3H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 28.64 (**C**H_2), 41.64 (**C**H_2), 48.61 (**C**H_2), 64.59 (**C**HS), 70.72 (quaternary **C**), 114.24 (Ar-**C**-H), 121.41 (**C**H=CH), 122.59 (**C**H=CH), 128.74 (Ar-**C**-H), 129.38 (Ar-**C**-H), 130.58 (quaternary **C**), 190.40 (**C**O), 193.85 (**C**O); *m/z* 341 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 341.0520, $C_{15}H_{14}N_2O_2S_2Na$ requires 341.0515.

5.63.2 Method B:

A solution of 3,4-diallyl-1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.05 g, 0.15 mmol) (**483**) in dichloromethane (2.00 mL) was degassed and taken under nitrogen atmosphere. To this (1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(*o*-isopropoxyphenylmethylene) ruthenium (Grubbs-Hoveyda second-generation catalyst) (0.01 g, 0.02 mmol) was added and the resulting mixture heated at 80 °C for 30 minutes in the microwave, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,9aS)-2-benzyl-2,3-dihydro-3,9a-epithiopyrido[1,2-a]pyrazine-1,4(6H,9H)-dione 10-sulfide (0.03 g, 40%) (**482**) as a colourless solid; identical spectroscopic data to that obtained previously for (**482**).

5.64 (±)-(1S,4S)-1,2-Diallyl-5-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (483)



480

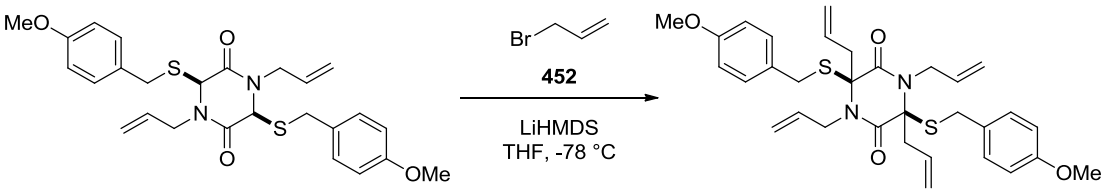
Chemical Formula: $C_{33}H_{36}N_2O_4S_2$
Molecular Weight: 588.78

483

Chemical Formula: $C_{17}H_{18}N_2O_2S_2$
Molecular Weight: 346.47

Boron tribromide (0.52 mL, 5.43 mmol) was added dropwise to a solution of 3,4-diallyl-1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (1.60 g, 2.72 mmol) (**480**) in dichloromethane (40.0 mL) at -78 °C. The resulting mixture was stirred for 15 minutes whereupon sodium hydrogen carbonate (10.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 10 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. Sodium thiosulfate was added and the mixture stirred for 10 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) to give ((±)-(1S,4S)-1,2-diallyl-5-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.266 g, 28%) (**483**) as a pale yellow oil; ν_{max}/cm^{-1} 1674 (C=O), 1609 (C=C), 833 (C-H), 735 (C-H), 701 (C-H); δ H (400 MHz, $CDCl_3$) 3.23 – 3.29 (1H, m, C*H*$_2$CH=CH$_2$), 3.90 – 3.99 (1H, m, C*H*$_2$CH=CH$_2$), 4.41 – 4.48 (2H, m, NC*H*$_2$), 4.82 (1H, d, *J* 14.9, NC*H*$_2$), 5.15 (1H, d, *J* 14.5, NC*H*$_2$), 5.21 (1H, s, C*H*S), 5.22 – 5.27 (2H, m, CH=C*H*$_2$), 5.30 – 5.33 (2H, m, CH=C*H*$_2$), 5.76 – 5.85 (1H, m, C*H*=CH$_2$), 6.07 – 6.12 (1H, m, C*H*=CH$_2$), 7.21 – 7.24 (2H, m, Ar-*H*), 7.27 – 7.31 (3H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 36.16 (**C**H$_2$), 44.96 (**C**H$_2$), 48.62 (**C**H$_2$), 64.28 (**C**HS), 75.25 (quaternary **C**), 118.20 (**C**H$_2$), 120.82 (**C**H$_2$), 128.68 (**C**H), 128.74 (Ar-**C**-H), 129.32 (**C**H), 131.71 (Ar-**C**-H), 131.91 (Ar-**C**-H), 134.48 (quaternary **C**), 164.52 (**C**O), 164.66 (**C**O); *m/z* 369 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 369.0726, $C_{17}H_{18}N_2O_2S_2Na$ requires 369.0707.

5.65 (3S,6S)-1,3,4,6-Tetraallyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (485)



421

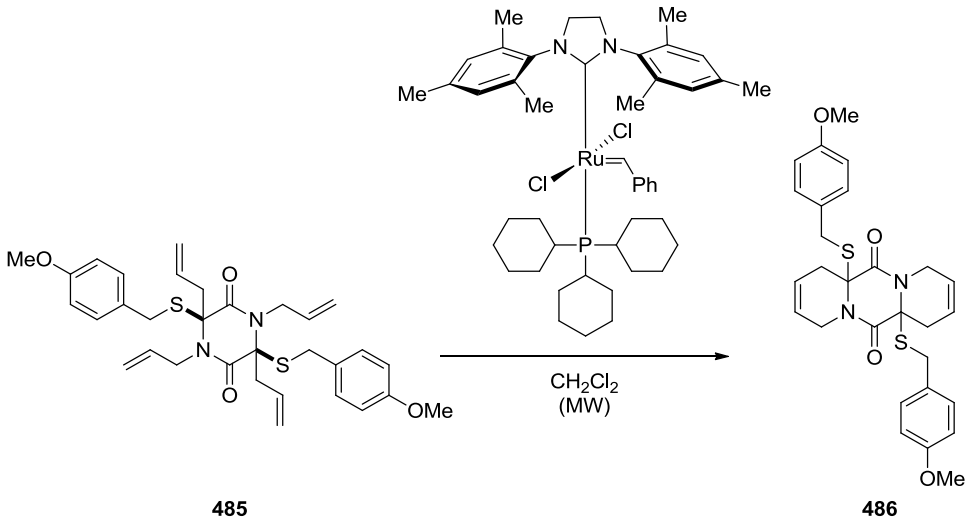
Chemical Formula: $C_{26}H_{30}N_2O_4S_2$
Molecular Weight: 498.66

485

Chemical Formula: $C_{32}H_{38}N_2O_4S_2$
Molecular Weight: 578.79

LiHMDS (4.14 mL of a 1M solution in tetrahydrofuran, 4.14 mmol) was added dropwise to a solution of (3S,6S)-1,4-diallyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.83 g, 1.65 mmol) (**421**) and allyl bromide (0.43 mL, 4.96 mmol) (**452**) in tetrahydrofuran (20.0 mL) at -78 °C and the resulting mixture stirred at this temperature for 1 hour and 1.5 hours at 0 °C. Saturated aqueous sodium hydrogen carbonate (10.0 mL) was added and solvent removed under reduced pressure. The residue was partitioned between water (10.0 mL) and dichloromethane (20.0 mL). The aqueous phase was extracted with dichloromethane (2 x 10.0 mL) and the combined extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) to give (3S,6S)-1,3,4,6-tetraallyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.53 g, 55%) (**485**) as a pale yellow oil; ν_{max}/cm^{-1} 1656 (C=O), 1610 (C=C), 1001 (C-O-C), 835 (C-H); δ H (400 MHz, $CDCl_3$) 2.69 (2H, d, *J* 14.2, C*H*$_2$CH=CH$_2$), 3.21 (2H, d, *J* 14.1, C*H*$_2$CH=CH$_2$), 3.79 (6H, s, OC*H*$_3$), 3.94 – 3.99 (2H, m, SC*H*$_2$), 4.05 – 4.09 (2H, m, SC*H*$_2$), 4.23 – 4.27 (4H, m, NC*H*$_2$), 5.13 – 5.22 (2H, m, CH=C*H*$_2$), 5.25 (4H, dd, *J* 10.2 & 1.2, CH=C*H*$_2$), 5.40 (2H, dd, *J* 10.3 & 1.4, CH=C*H*$_2$), 5.52 – 5.63 (2H, m, C*H*=CH$_2$), 6.02 – 6.07 (2H, m, C*H*=CH$_2$), 6.81 – 6.85 (4H, m, Ar-*H*), 7.20 – 7.24 (4H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 35.66 (**C**H$_2$), 41.66 (**C**H$_2$), 47.69 (**C**H$_2$), 55.42 (O**C**H$_3$), 73.15 (quaternary **C**), 114.20 (**C**H=CH$_2$), 118.63 (**C**H$_2$), 121.15 (**C**H$_2$), 128.06 (quaternary **C**), 130.53 (**C**H=CH$_2$), 131.28 (Ar-**C**-H), 133.62 (Ar-**C**-H), 159.05 (quaternary **C**), 164.94 (**C**O); *m/z* 601 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 601.2176, $C_{32}H_{38}N_2O_4S_2Na$ requires 601.2170.

5.66 6a,12a-Bis((4-methoxybenzyl)thio)-1,6a,7,12a-tetrahydrodipyrido [1,2-a:1',2'-d]pyrazine-6,12(4H,10H)-dione (486)



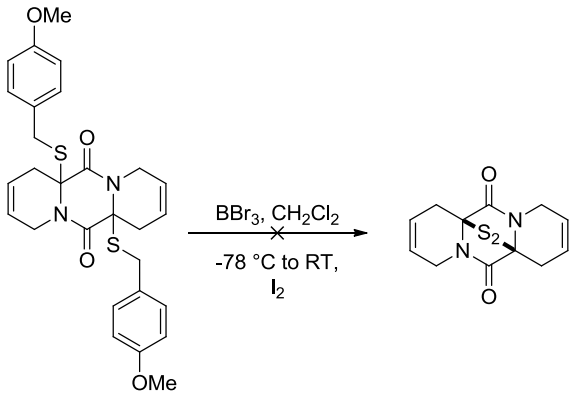
485 **486**

Chemical Formula: $C_{32}H_{38}N_2O_4S_2$
Molecular Weight: 578.79

Chemical Formula: $C_{28}H_{30}N_2O_4S_2$
Molecular Weight: 522.68

A solution of (3S,6S)-1,3,4,6-tetraallyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.20 g, 0.35 mmol) (**485**) in toluene (2.00 mL) was degassed and taken under nitrogen atmosphere. To this (1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(phenylmethylene) (tricyclohexylphosphine)ruthenium (Grubbs second-generation catalyst) (0.06 g, 0.07 mmol) was added and the resulting mixture heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) to give 6a,12a-bis((4-methoxybenzyl)thio)-1,6a,7,12a-tetrahydrodipyrido[1,2-a:1',2'-d]pyrazine-6,12(4H,10H)-dione (0.05 g, 28%) (**486**) as a pale yellow oil; ν_{max}/cm^{-1} 3001 (C-H), 2934 (C-H), 2836 (C-H), 1659 (C=O), 1609 (C=C), 835 (C-H); δ H (400 MHz, $CDCl_3$) 2.56 – 2.66 (2H, m, C*H*$_2$CH=CH), 2.88 – 2.97 (2H, m, C*H*$_2$CH=CH), 3.78 (6H, s, OC*H*$_3$), 3.85 – 3.92 (2H, m, SC*H*$_2$), 3.92 – 3.96 (2H, m, SC*H*$_2$), 4.02 – 4.06 (2H, m, NC*H*$_2$), 4.61 – 4.73 (2H, m, NC*H*$_2$), 5.72 – 5.78 (2H, m, C*H*=CH), 5.79 – 5.87 (2H, m, C*H*=CH), 6.79 – 6.82 (4H, m, Ar-*H*), 7.20 – 7.24 (4H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 34.71 (**C**H$_2$), 35.91 (**C**H$_2$), 41.26 (**C**H$_2$), 55.43 (O**C**H$_3$), 65.70 (quaternary **C**), 114.12 (Ar-**C**-H), 121.02 (**C**H=CH$_2$), 123.12 (**C**H=CH$_2$), 128.13 (quaternary **C**), 130.56 (Ar-**C**-H), 159.00 (quaternary **C**), 165.66 (**C**O); *m/z* 545 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 545.1529, $C_{28}H_{30}N_2O_4S_2Na$ requires 545.1545.

5.67 (±)-(6aS,12aS)-7,10-Dihydro-6a,12a-epithiodipyrido[1,2-a:1',2'-d] pyrazine-6,12(1H,4H)-dione 13-sulfide (487)



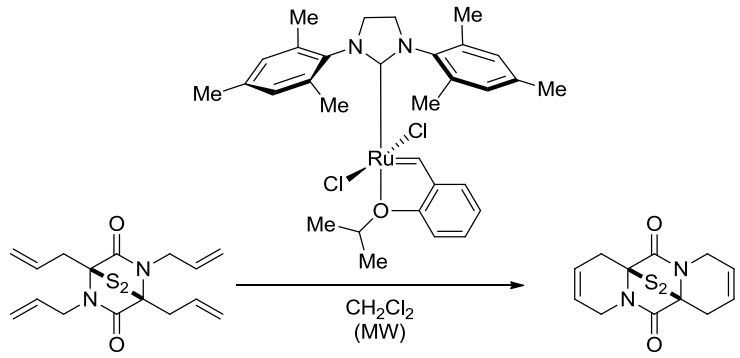
486 **487**

Chemical Formula: $C_{28}H_{30}N_2O_4S_2$
Molecular Weight: 522.68

Chemical Formula: $C_{12}H_{12}N_2O_2S_2$
Molecular Weight: 280.37

Boron tribromide (0.86 mL of a 1M solution in dichloromethane, 0.86 mmol) was added dropwise to a solution of 6a,12a-bis((4-methoxybenzyl)thio)-1,6a,7,12a-tetrahydrodipyrido[1,2-a:1',2'-d]pyrazine-6,12(4H,10H)-dione (0.15 g, 0.29 mmol) (**486**) in dichloromethane (2.00 mL) at -78 °C. The resulting mixture was stirred for 15 minutes whereupon aqueous sodium hydrogen carbonate (5.00 mL of a saturated aqueous solution) and methanol (2.00 mL) were added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 10 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. Sodium thiosulfate was added and the mixture stirred for 10 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) which did not give (6aS,12aS)-7,10-dihydro-6a,12a-epithiodipyrido[1,2-a:1',2'-d]pyrazine-6,12(1H,4H)-dione 13-sulfide (**487**).

5.68 (±)-(6aS,12aS)-7,10-Dihydro-6a,12a-epithiodipyrido[1,2-a:1',2'-d] pyrazine-6,12(1H,4H)-dione 13-sulfide (487)



488 **487**

Chemical Formula: $C_{16}H_{20}N_2O_2S_2$
Molecular Weight: 336.47

Chemical Formula: $C_{12}H_{12}N_2O_2S_2$
Molecular Weight: 280.37

5.68.1 Method A:

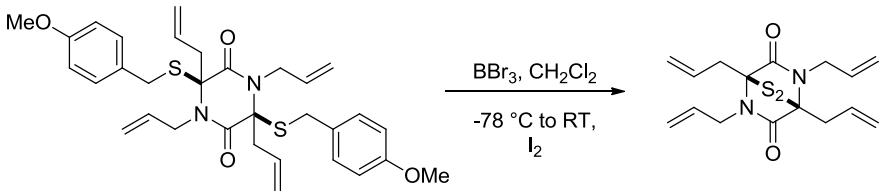
A solution of (±)-(1S,4S)-1,2,4,5-tetraallyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.04 g, 0.12 mmol) (**488**) in dichloromethane (2.00 mL) was degassed and taken under nitrogen atmosphere. To this (1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(*o*-isopropoxyphenylmethylene)ruthenium (Grubbs-Hoveyda second-generation catalyst) (0.01 g, 0.01 mmol) was added and the resulting mixture heated at 80 °C for 30 minutes in the microwave, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; pipette column) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (6aS,12aS)-7,10-dihydro-6a,12a-epithiodipyrido[1,2-a:1',2'-d]pyrazine-6,12(1H,4H)-dione 13-sulfide (0.01 g, 39%) (**487**) as a colourless solid; m.p. 135-137 °C; ν_{max}/cm^{-1} 1683 (C=O, C=C), 1351 (C-H), 1321 (C-H); δ H (400 MHz, $CDCl_3$) 2.60 – 2.72 (2H, m, C*H*$_2$CH=CH), 3.29 – 3.40 (2H, m, C*H*$_2$CH=CH), 3.87 – 4.08 (4H, m, NC*H*$_2$), 5.86 – 5.97 (4H, m, C*H*=C*H*); δ C (101 MHz, $CDCl_3$) 28.60 (**C**H$_2$), 42.30 (**C**H$_2$), 70.29 (quaternary **C**), 121.14 (**C**H=CH), 122.32 (**C**H=CH), 165.15 (**C**O); *m/z* 303 (100%, $[M+Na]^+$); Found $[M+Na]^+$ 303.0253, $C_{12}H_{12}N_2O_2S_2Na$ requires 303.0238.

5.68.2 Method B:

A solution of (±)-(1S,4S)-1,2,4,5-tetraallyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.04 g, 0.13 mmol) (**488**) in dichloromethane (2.00 mL) was degassed and taken under nitrogen atmosphere. To this (1,3-bis(2,4,6-trimethylphenyl)-2-

imidazolidinylidene)dichloro (phenylmethylene)(tricyclohexylphosphine)ruthenium (Grubbs second-generation catalyst) (0.01 g, 0.01 mmol) was added and the resulting mixture heated at 80 °C for 30 minutes in the microwave, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 5 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (6aS,12aS)-7,10-dihydro-6a,12a-epithiodipyrido[1,2-a:1',2'-d]pyrazine-6,12(1H,4H)-dione 13-sulfide (0.01 g, 7%) (**487**) as a colourless solid; identical spectroscopic data to that obtained previously for (**487**).

5.69 (±)-(1S,4S)-1,2,4,5-Tetraallyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (488)



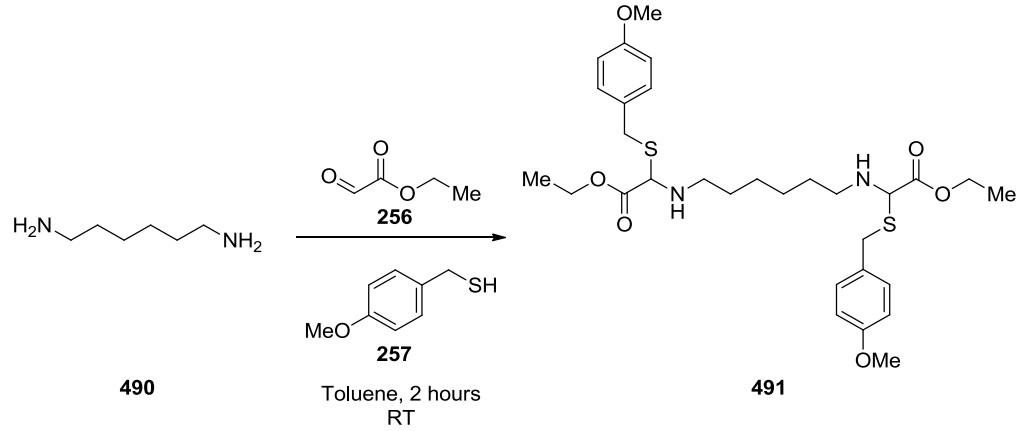
485 **488**

Chemical Formula: $C_{32}H_{38}N_2O_4S_2$
Molecular Weight: 578.79

Chemical Formula: $C_{16}H_{20}N_2O_2S_2$
Molecular Weight: 336.47

Boron tribromide (0.14 mL, 1.50 mmol) was added dropwise to a solution of 1,3,4,6-tetraallyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione (0.44 g, 0.75 mmol) (**485**) in dichloromethane (20.0 mL) at -78 °C. The resulting mixture was stirred for 15 minutes whereupon Rochelle's salt (20.0 mL of a saturated aqueous solution) was added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 10 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. Sodium thiosulfate was added and the mixture stirred for 10 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (±)-(1S,4S)-1,2,4,5-tetraallyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.10 g, 41%) (**488**) as a colourless solid; m.p. 180-182 °C; ν_{max}/cm^{-1} 1639 (C=O, C=C), 1462 (CH_2), 749 (CH_2); δ H (400 MHz, $CDCl_3$) 3.20 – 3.27 (2H, m, C*H*$_2$CH=CH), 3.28 – 3.36 (2H, m, C*H*$_2$CH=CH), 4.04 (2H, dd, *J* 16.4 & 6.8, NC*H*$_2$), 4.62 (2H, dd, *J* 16.2 & 4.8, NC*H*$_2$), 5.21 – 5.41 (8H, m, CH=C*H*$_2$), 5.89 – 5.94 (2H, m, C*H*=CH_2), 6.12 – 6.17 (2H, m, C*H*=CH_2); δ C (101 MHz, $CDCl_3$) 36.44 (**C**H_2), 45.46 (**C**H_2), 74.50 (quaternary **C**), 118.31 (CH=**C**H_2), 120.60 (CH=**C**H_2), 131.74 (**C**H=CH_2), 132.01 (**C**H=CH_2), 165.02 (**C**O); *m/z* 337 (100%, $[M+H]^+$); Found $[M+H]^+$ 337.1456, $C_{16}H_{21}N_2O_2S_2$ requires 337.1448.

5.70 Ethyl 2-((6-((2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino) hexyl)amino)-2-((4-methoxy benzyl)thio)acetate (491)

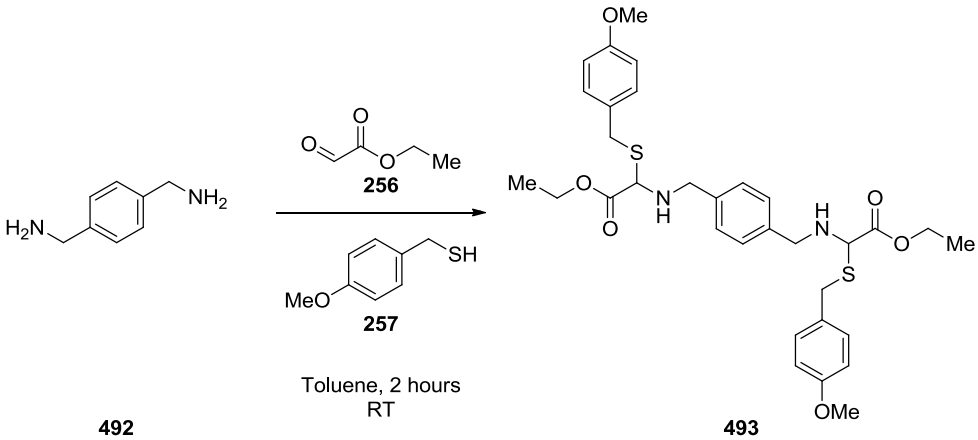


Chemical Formula: $C_6H_{16}N_2$
Molecular Weight: 116.20

Chemical Formula: $C_{30}H_{44}N_2O_6S_2$
Molecular Weight: 592.81

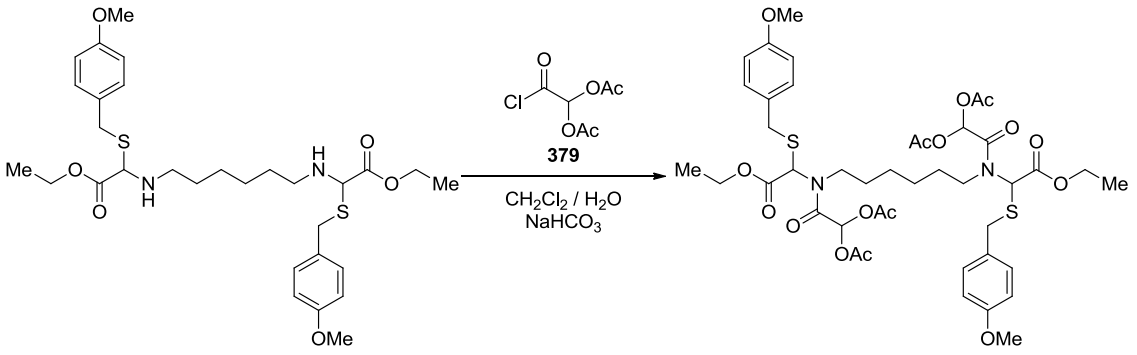
1,6-Diaminohexane (0.59 g, 5.07 mmol) (**490**) was added to a solution of ethyl glyoxalate (2.00 mL of a 50% solution in toluene, 10.1 mmol) (**256**) in toluene (20.0 mL) at RT and stirred for 2 minutes, whereupon *para*-methoxybenzyl mercaptan (1.41 mL, 10.1 mmol) (**257**) was added and the mixture stirred for 2 hours. Solvent was removed under reduced pressure and the residue purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 50 g) to give ethyl 2-((6-((2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)hexyl)amino)-2-((4-methoxybenzyl)thio)acetate (1.73 g, 58%) (**491**) as a colourless oil; ν_{max}/cm^{-1} 2953 (C-H), 2858 (C-H), 1736 (C=O), 1644 (C=O), 1463 (CH_2), 730 (CH_2); δ H (400 MHz; $CDCl_3$) 1.17 – 1.29 (8H, m, $NHCH_2(CH_2)_4CH_2NH$), 1.32 (6H, t, *J* 7.1, OCH_2CH_3), 1.67 – 1.77 (2H, m, N*H*), 2.37 – 2.45 (2H, m, $NHCH_2(CH_2)_4CH_2NH$), 2.68 – 2.74 (2H, m, $NHCH_2(CH_2)_4CH_2NH$), 3.74 (4H, d, *J* 1.9, SCH_2), 3.79 (6H, s, OCH_3), 4.23 (4H, q, *J* 7.2, OCH_2CH_3), 4.34 (2H, s, C*H*S), 6.82 – 6.87 (4H, m, Ar-*H*), 7.25 – 7.31 (4H, m, Ar-*H*); δ C (101 MHz; $CDCl_3$) 14.29 (**C**H_3), 27.24 (**C**H_2), 29.55 (**C**H_2), 33.36 (**C**H_2), 45.61 (**C**H_2), 55.43 (O**C**H_3), 61.47 (**C**H_2), 64.71 (**C**HS), 114.06 (Ar-**C**-H), 130.14 (Ar-**C**-H), 130.27 (quaternary **C**), 158.86 (quaternary **C**), 170.18 (**C**O); *m/z* 593 (100%, $[M+H]^+$): Found $[M+H]^+$ 593.5126, $C_{30}H_{45}N_2O_6S_2$ requires 593.5102.

5.71 Diethyl 2,2'-((1,4-phenylenebis(methylene))bis(azanediyl))bis(2-((4-methoxybenzyl)thio)acetate) (493)



p-Xylylenediamine (0.69 g, 5.04 mmol) (**492**) was added to a solution of ethyl glyoxalate (2.00 mL of a 50% solution in toluene, 10.1 mmol) (**256**) in toluene (20.0 mL) at RT and stirred for 2 minutes, whereupon *para*-methoxybenzyl mercaptan (1.41 mL, 10.1 mmol) (**257**) was added and the mixture stirred for 2 hours. Solvent was removed under reduced pressure and the residue purified via Biotage™ Horizon (3:1, petroleum spirit 40-60 °C: ethyl acetate; snap 50 g) to give diethyl 2,2'-((1,4-phenylenebis(methylene))bis(azanediyl))bis(2-((4-methoxybenzyl)thio)acetate) (2.43 g, 79%) (**493**) as a colourless oil; ν_{max}/cm^{-1} 2983 (C-H), 1729 (C=O), 1644 (N-H), 1176 (C-C(O)-C), 1108 (C-N), 832 (C-H); δ H (400 MHz; $CDCl_3$) 1.21 (6H, t, *J* 7.2, OCH_2CH_3), 2.04 (2H, br s, N*H*), 3.56 – 3.61 (2H, m, $NHCH_2$), 3.65 (4H, d, *J* 2.4, SCH_2), 3.69 (6H, d, *J* 0.8, OCH_3), 3.71 – 3.76 (2H, m, $NHCH_2$), 4.12 (4H, q, *J* 7.1, OCH_2CH_3), 4.24 (2H, s, C*H*S), 6.71 – 6.76 (4H, m, Ar-*H*), 7.06 – 7.09 (4H, m, Ar-*H*), 7.15 – 7.19 (4H, m, Ar-*H*); δ C (101 MHz; $CDCl_3$) 14.26 (**C**H_3), 33.33 (**C**H_2), 49.03 (**C**H_2), 55.42 (O**C**H_3), 61.52 (**C**H_2), 63.82 (**C**HS), 114.11 (Ar-**C**-H), 128.63 (Ar-**C**-H), 130.17 (Ar-**C**-H), 137.85 (quaternary **C**), 158.88 (quaternary **C**), 170.06 (**C**O); *m/z* 613 (100%, $[M+H]^+$): Found $[M+H]^+$ 613.2413, $C_{32}H_{41}N_2O_6S_2$ requires 613.2406.

5.72 (5,14-Bis((4-methoxybenzyl)thio)-4,15-dioxo-3,16-dioxa-6,13-diazaoctadecane-6,13-diyl)bis(2-oxoethane-2,1,1-triyl) tetraacetate (494)



491

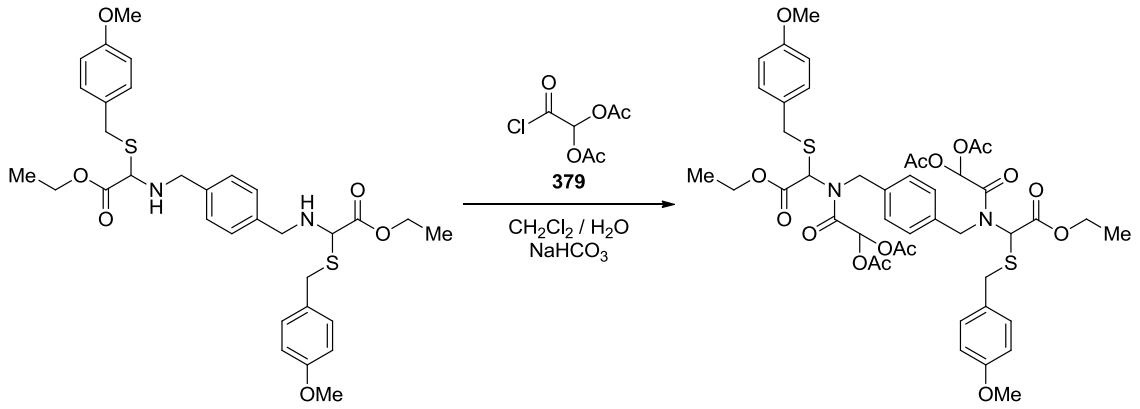
Chemical Formula: $C_{30}H_{44}N_2O_6S_2$
Molecular Weight: 592.81

494

Chemical Formula: $C_{42}H_{56}N_2O_{16}S_2$
Molecular Weight: 909.03

A solution of diacetoxyacetyl chloride (1.36 g, 7.00 mmol) (**379**) in dichloromethane (20.0 mL) was added dropwise to a rapidly stirred biphasic mixture of ethyl 2-((6-((2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)hexyl)amino)-2-((4-methoxybenzyl)thio) acetate (1.73 g, 2.92 mmol) (**491**) and sodium hydrogen carbonate (0.98 g, 11.7 mmol) in dichloromethane (200 mL) and water (100 mL) at RT and the resulting mixture stirred rapidly for 3 hours. The layers were separated and the aqueous phase extracted with dichloromethane (2 x 100 mL). The combined extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure to give (5,14-bis((4-methoxybenzyl)thio)-4,15-dioxo-3,16-dioxa-6,13-diazaoctadecane-6,13-diyl)bis(2-oxoethane-2,1,1-triyl) tetraacetate (2.40 g, 90%) (**494**) as a colourless oil that did not require further purification; ν_{max}/cm^{-1} 1736 (C=O), 1664 (C=O), 1463 (CH_2), 1374 (CH_3), 1248 (C-C(O)-C), 733 (C-H); δ H (400 MHz; $CDCl_3$) 1.28 (6H, t, *J* 7.1, OCH_2CH_3), 2.12 – 2.16 (8H, m, $NCH_2(CH_2)_4CH_2N$), 3.57 – 3.60 (4H, m, SCH_2), 3.72 – 3.83 (22H, m, 2 x $OC(O)CH_3$ & OCH_3 & NCH_2), 4.15 (4H, q, *J* 7.3, OCH_2CH_3), 5.06 (2H, s, CHS), 6.86 – 6.90 (4H, m, Ar-*H*), 6.92 (2H, s, $CH(OAc)_2$), 7.23 – 7.25 (4H, m, Ar-*H*); δ C (101 MHz; $CDCl_3$) 14.13 (**C**H_3), 14.19 (**C**H_3), 15.41 (**C**H_3), 21.26 (**C**H_2), 26.37 (**C**H_2), 34.94 (**C**H_2), 36.84 (**C**H_2), 42.94 (O**C**H_3), 55.45 (**C**H_2), 66.33 (**C**HS), 72.53 (**C**H$(OAc)_2$), 114.13 (quaternary **C**), 130.21 (Ar-**C**-H), 130.31 (Ar-**C**-H), 130.34 (quaternary **C**), 158.72 (**C**O), 159.82 (**C**O), 171.20 (**C**O), 175.77 (**C**O); *m/z* 909 (100%, $[M+H]^+$): Found $[M+H]^+$ 909.8103, $C_{42}H_{57}N_2O_{16}S_2$ requires 909.8123.

5.73 ((1,4-Phenylenebis(methylene))bis((2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)azanediyl))bis(2-oxoethane-2,1,1-triyl) tetraacetate (495)



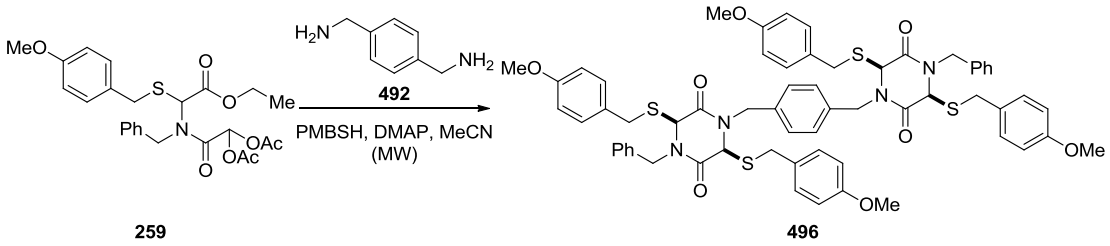
Chemical Formula: $C_{32}H_{40}N_2O_6S_2$
Molecular Weight: 612.80

Chemical Formula: $C_{44}H_{52}N_2O_{16}S_2$
Molecular Weight: 929.02

A solution of diacetoxyacetyl chloride (1.45 g, 7.44 mmol) (**379**) in dichloromethane (20.0 mL) was added dropwise to a rapidly stirred biphasic mixture of diethyl 2,2'-((1,4-phenylenebis(methylene))bis(azanediyl))bis(2-((4-methoxybenzyl)thio)acetate) (1.90 g, 3.10 mmol) (**493**) and sodium hydrogen carbonate (1.04 g, 12.4 mmol) in dichloromethane (200 mL) and water (100 mL) at RT and the resulting mixture stirred rapidly for 3 hours. The layers were separated and the aqueous phase extracted with dichloromethane (2 x 100 mL). The combined extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure to give ((1,4-phenylenebis(methylene))bis((2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)azanediyl))bis(2-oxoethane-2,1,1-triyl) tetraacetate (2.45 g, 85%) (**495**) as a colourless oil that did not require further purification; ν_{max}/cm^{-1} 1739 (C=O), 1639 (C=O), 1371 (CH_3), 1246 (C-C(O)-C); δ H (400 MHz; $CDCl_3$) 1.22 (6H, t, *J* 7.0, OCH_2CH_3), 2.01 – 2.16 (12H, m, $CH(C(O)CH_3)_2$), 3.82 – 3.85 (10H, m, OCH_3 & OCH_2CH_3), 3.99 – 4.10 (4H, m, SCH_2), 4.56 – 4.65 (2H, m, NCH_2), 4.86 – 4.95 (2H, m, NCH_2), 5.85 (2H, s, CHS), 6.84 – 6.88 (4H, m, Ar-*H*), 6.96 (2H, s, $CH(OAc)_2$), 7.21 – 7.29 (8H, m, Ar-*H*); δ C (101 MHz; $CDCl_3$) 14.08 (**C**H_3), 20.54 (**C**H_3), 27.51 (**C**H_3), 35.91 (**C**H_2), 48.51 (**C**H_2), 55.50 (O**C**H_3), 61.86 (**C**H_2), 62.50 (**C**HS), 84.24 (**C**H$(OAc)_2$), 85.52 (Ar-**C**-H), 114.34 (Ar-**C**-H), 127.29 (quaternary **C**), 128.91 (quaternary **C**), 130.49 (Ar-**C**-H), 159.24 (quaternary **C**), 160.76 (**C**O), 168.64 (**C**O),

167.45 (**C**O), 168.88 (**C**O); *m/z* 951 (100%, $[M+Na]^+$): Found $[M+Na]^+$ 951.2670, $C_{44}H_{52}N_2O_{16}S_2Na$ requires 951.2656.

5.74 (3S,3'S,6S,6'S)-4,4'-(1,4-Phenylenebis(methylene))bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione) (496)



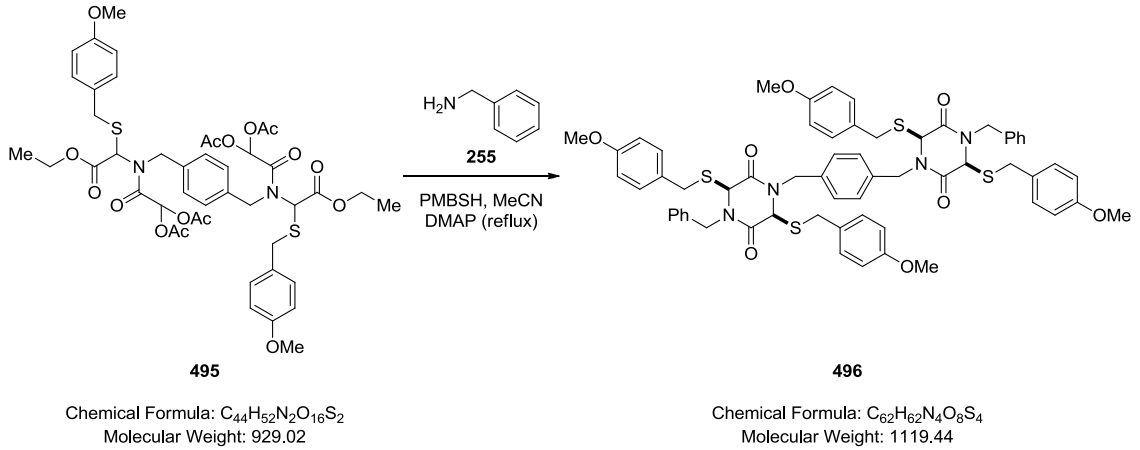
259 **496**

Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

Chemical Formula: $C_{62}H_{62}N_4O_8S_4$
Molecular Weight: 1119.44

A mixture of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.40 g, 0.79 mmol) (**259**), *p*-xylylenediamine (0.06 g, 0.47 mmol) (**492**), *para*-methoxybenzyl mercaptan (0.16 mL, 1.18 mmol) (**257**) and DMAP (0.02 g, 0.20 mmol) in acetonitrile (3.00 mL) was stirred at room temperature for 10 minutes and heated at 130 °C for 30 minutes in the microwave. The residue was purified via Biotage™ Horizon (2:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,3'S,6S,6'S)-4,4'-(1,4-phenylenebis(methylene))bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio) piperazine-2,5-dione) (0.04 g, 18%) (**496**) as a colourless solid; m.p. 63-65 °C; ν_{max}/cm^{-1} 1672 (C=O), 830 (C-H), 730 (C-H); δ H (400 MHz, $CDCl_3$) 3.49 (2H, d, *J* 12.3, SC*H*$_2$), 3.63 (2H, d, *J* 8.6, SC*H*$_2$), 3.80 (10H, d, *J* 6.2, SC*H*$_2$ & OC*H*$_3$), 3.87 (10H, d, *J* 4.5, NC*H*$_2$ & OC*H*$_3$), 4.01 (2H, d, *J* 13.9, NC*H*$_2$), 4.41 (2H, s, C*H*S), 4.53 (2H, s, C*H*S), 5.15 (2H, d, *J* 12.5, NC*H*$_2$), 6.88 – 6.93 (12H, m, Ar-*H*), 7.08 (4H, d, *J* 8.5, Ar-*H*), 7.13 – 7.19 (6H, m, Ar-*H*), 7.29 (8H, d, *J* 5.7, Ar-*H*); δ C (101 MHz, $CDCl_3$) 36.57 (**C**H$_2$), 36.68 (**C**H$_2$), 46.05 (**C**H$_2$), 46.36 (**C**H$_2$), 55.34 (O**C**H$_3$), 55.53 (O**C**H$_3$), 57.68 (**C**HS), 58.15 (**C**HS), 114.40 (Ar-**C**-H), 128.08 (Ar-**C**-H), 128.78 (Ar-**C**-H), 128.88 (Ar-**C**-H), 129.08 (quaternary **C**), 130.91 (Ar-**C**-H), 131.05 (Ar-**C**-H), 134.72 (quaternary **C**), 135.16 (quaternary **C**), 159.41 (quaternary **C**), 165.05 (**C**O), 165.27 (**C**O); *m/z* 1142 (100%, $[M+Na]^+$): Found $[M+Na]^+$ 1143.4436, $C_{62}H_{62}N_4O_8S_4Na$ requires 1143.4410.

5.75 (3S,3'S,6S,6'S)-4,4'-(1,4-Phenylenebis(methylene))bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione) (496)



495

Chemical Formula: $C_{44}H_{52}N_2O_{16}S_2$
Molecular Weight: 929.02

496

Chemical Formula: $C_{62}H_{62}N_4O_8S_4$
Molecular Weight: 1119.44

5.75.1 Method A:

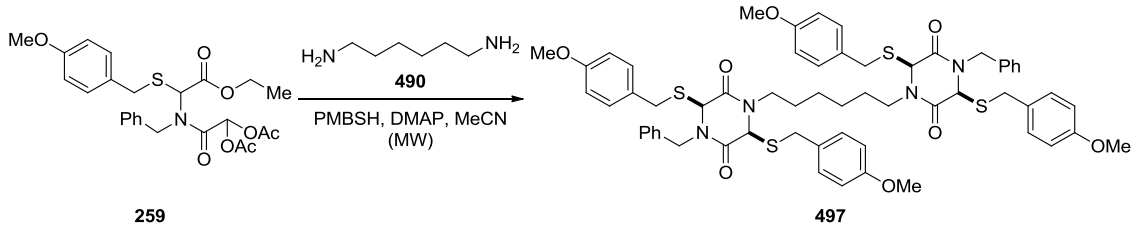
Benzylamine (0.15 mL, 1.41 mmol) (**255**) was added to a solution of ((1,4-phenylenebis(methylene))bis((2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)azanediyl))bis(2-oxoethane-2,1,1-triyl) tetraacetate (0.29 g, 0.31 mmol) (**495**) in acetonitrile (15.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.13 mL, 0.94 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.02 g, 0.15 mmol) was added and the resulting mixture heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (2:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,3'S,6S,6'S)-4,4'-(1,4-phenylenebis(methylene))bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio) piperazine-2,5-dione) (0.15 g, 43%) (**496**) as a colourless solid; identical spectroscopic data to that obtained previously for (**496**).

5.75.2 Method B:

Benzylamine (0.10 mL, 0.92 mmol) (**255**) was added to a solution of ((1,4-phenylenebis(methylene))bis((2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)azanediyl))bis(2-oxoethane-2,1,1-triyl) tetraacetate (0.19 g, 0.21 mmol) (**495**) in acetonitrile (3.00 mL) followed by addition *para*-methoxybenzyl mercaptan (0.09 mL, 0.61 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.01 g, 0.10 mmol) was added and the mixture heated in the microwave 130 °C for 30 minutes, allowed to cool to room temperature and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (2:1, petroleum spirit 40-60 °C: ethyl

acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,3'S,6S,6'S)-4,4'-(1,4-phenylenebis(methylene))bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio) piperazine-2,5-dione) (0.11 g, 48%) (**496**) as a colourless solid; identical spectroscopic data to that obtained previously for (**496**).

5.76 (3S,3'S,6S,6'S)-4,4'-(Hexane-1,6-diyl)bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione) (497)



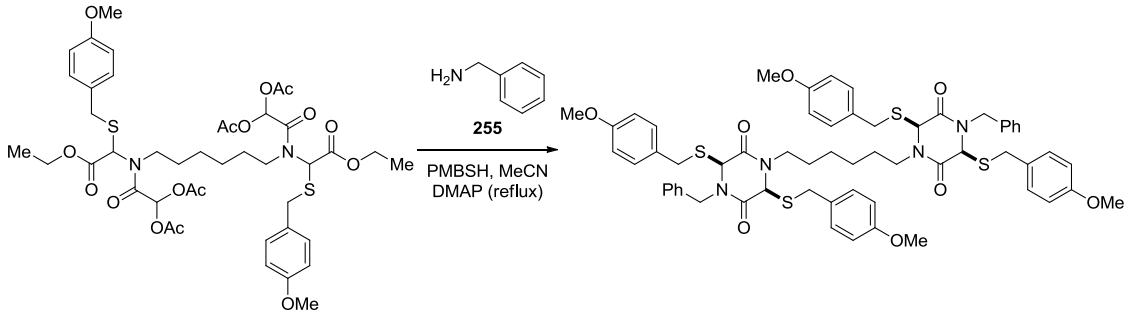
259 **497**

Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

Chemical Formula: $C_{60}H_{66}N_4O_8S_4$
Molecular Weight: 1099.45

A mixture of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.22 g, 0.43 mmol) (**259**), 1,6-diaminohexane (0.05 g, 0.43 mmol) (**490**), *para*-methoxybenzyl mercaptan (0.18 mL, 1.29 mmol) (**257**) and DMAP (0.03 g, 0.21 mmol) in acetonitrile (3.00 mL) was stirred at room temperature for 10 minutes and heated at 130 °C for 30 minutes in the microwave. The residue was purified via Biotage™ Horizon (2:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,3'S,6S,6'S)-4,4'-(hexane-1,6-diyl)bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione) (0.08 g, 33%) (**497**) as a colourless solid; m.p. 101-104 °C; ν_{max}/cm^{-1} 3003 (C-H), 1673 (C=O), 1451 (CH_2), 733 (CH_2); δ H (400 MHz, $CDCl_3$) 1.64 – 1.66 (2H, m, $NCH_2(CH_2)_4CH_2N$), 2.53 – 2.62 (2H, m, $NCH_2(CH_2)_4CH_2N$), 3.42 – 3.52 (2H, m, $NCH_2(CH_2)_4CH_2N$), 3.60 – 3.63 (2H, m, $NCH_2(CH_2)_4CH_2N$), 3.73 (4H, d, *J* 7.5, SCH_2), 3.80 (8H, d, *J* 12.9, OCH_3 & SCH_2), 3.88 – 3.94 (10H, m, SCH_2 & NCH_2 & OCH_3), 3.97 (2H, d, *J* 12.9, NCH_2), 4.04 (2H, d, *J* 13.8, NCH_2), 4.32 (2H, s, CHS), 4.44 (2H, s, CHS), 5.17 (2H, d, *J* 13.6, NCH_2), 6.72 (4H, d, *J* 7.6, Ar-*H*), 6.89 (10H, d, *J* 8.6, Ar-*H*), 7.37 – 7.42 (12H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 26.55 ($\mathbf{C}H_2$), 26.98 ($\mathbf{C}H_2$), 36.49 ($S\mathbf{C}H_2$), 37.01 ($S\mathbf{C}H_2$), 44.03 ($\mathbf{C}H_2$), 46.36 ($\mathbf{C}H_2$), 55.52 ($O\mathbf{C}H_3$), 57.67 (**C**HS), 59.37 (**C**HS), 114.26 (Ar-**C**-H), 114.37 (Ar-**C**-H), 128.83 (Ar-**C**-H), 129.14 (quaternary **C**), 130.94 (Ar-**C**-H), 131.04 (Ar-**C**-H), 135.24 (quaternary **C**), 159.36 (quaternary **C**), 165.12 (**C**O), 165.47 (**C**O); *m/z* 1121 (100%, $[M+Na]^+$): Found $[M+Na]^+$ 1121.3680, $C_{60}H_{66}N_4O_8S_4Na$ requires 1121.3661.

5.77 (3S,3'S,6S,6'S)-4,4'-(Hexane-1,6-diyl)bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione) (497)



494

Chemical Formula: $C_{42}H_{56}N_2O_{16}S_2$
Molecular Weight: 909.03

497

Chemical Formula: $C_{60}H_{66}N_4O_8S_4$
Molecular Weight: 1099.45

5.77.1 Method A:

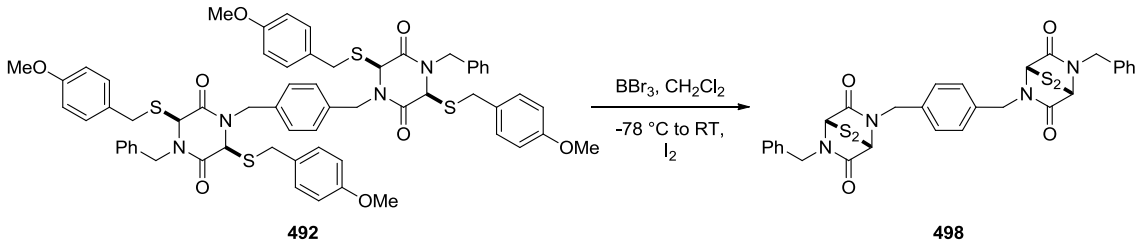
Benzylamine (0.11 mL, 1.04 mmol) (**255**) was added to a solution of (5,14-bis((4-methoxybenzyl)thio)-4,15-dioxo-3,16-dioxa-6,13-diazaoctadecane-6,13-diyl)bis(2-oxoethane-2,1,1-triyl) tetraacetate (0.21 g, 0.23 mmol) (**494**) in acetonitrile (15.0 mL) followed by addition of *para*-methoxybenzyl mercaptan (0.10 mL, 0.69 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.01 g, 0.12 mmol) was added and the resulting mixture heated at reflux for 16 hours, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (2:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,3'S,6S,6'S)-4,4'-(hexane-1,6-diyl)bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio) piperazine-2,5-dione) (0.13 g, 50%) (**497**) as a colourless solid; identical spectroscopic data to that obtained previously for (**497**).

5.77.2 Method B:

Benzylamine (0.10 mL, 0.94 mmol) (**255**) was added to a solution of (5,14-bis((4-methoxybenzyl)thio)-4,15-dioxo-3,16-dioxa-6,13-diazaoctadecane-6,13-diyl)bis(2-oxoethane-2,1,1-triyl) tetraacetate (0.19 g, 0.21 mmol) (**494**) in acetonitrile (3.00 mL) followed by addition *para*-methoxybenzyl mercaptan (0.09 mL, 0.63 mmol) (**257**) and the resulting mixture stirred for 2 minutes. DMAP (0.01 g, 0.11 mmol) was added and the mixture heated in the microwave 130 °C for 30 minutes, allowed to cool to room temperature and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (2:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give

(3S,3'S,6S,6'S)-4,4'-(hexane-1,6-diyl)bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione) (0.11 g, 47%) (**497**) as a colourless solid; identical spectroscopic data to that obtained previously for (**497**).

5.78 (±)-(1S,1'S,4S,4'S)-5,5'-(1,4-Phenylenebis(methylene))bis(2-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) (498)



492

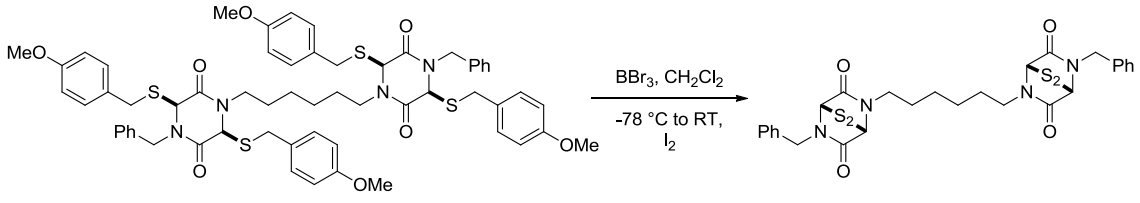
Chemical Formula: $C_{62}H_{62}N_4O_8S_4$
Molecular Weight: 1119.44

498

Chemical Formula: $C_{30}H_{26}N_4O_4S_4$
Molecular Weight: 634.81

Boron tribromide (0.03 mL, 0.36 mmol) was added dropwise to a solution of (3S,3'S,6S,6'S)-4,4'-(1,4-phenylenebis(methylene))bis(1-benzyl-3,6-bis((4-methoxybenzyl)thio) piperazine-2,5-dione) (0.10 g, 0.09 mmol) (**492**) in dichloromethane (3.00 mL) at -78 °C. The resulting mixture was stirred for 15 minutes whereupon Rochelle's salt (10.0 mL of a saturated aqueous solution) and methanol (2.00 mL) were added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 10 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. Sodium thiosulfate was added and the mixture stirred for 10 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (9:1, dichloromethane: methanol; snap 10 g), followed by HPLC separation and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (1S,1'S,4S,4'S)-5,5'-(1,4-phenylenebis(methylene))bis(2-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) (0.01 g, 23%) (**498**) as a colourless solid; m.p. 148-151 °C; ν_{max}/cm^{-1} 2919 (C-H), 1678 (C=O); δ H (500 MHz; $CDCl_3$) 4.44 – 4.54 (8H, m, NCH_2), 5.23 (4H, s, C*H*S), 6.45 – 6.46 (2H, m, Ar-*H*), 6.48 – 6.49 (2H, m, Ar-*H*), 7.02 – 7.03 (10H, m, Ar-*H*); *m/z* 657 (100%, $[M+Na]^+$): Found $[M+Na]^+$ 657.3334, $C_{30}H_{26}N_4O_4S_4Na$ requires 657.0735.

5.79 (±)-(1S,1'S,4S,4'S)-5,5'-(Hexane-1,6-diyl)bis(2-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) (499)



497

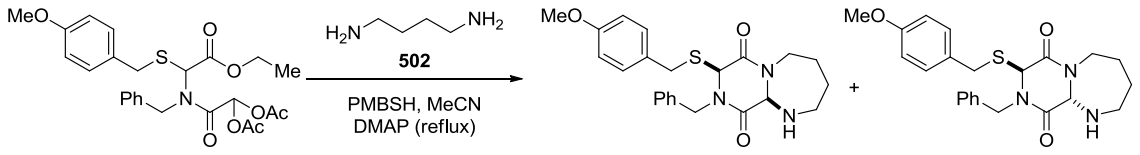
Chemical Formula: $C_{60}H_{66}N_4O_8S_4$
Molecular Weight: 1099.45

499

Chemical Formula: $C_{28}H_{30}N_4O_4S_4$
Molecular Weight: 614.82

Boron tribromide (0.68 mL of a 1M solution in dichloromethane, 0.68 mmol) was added dropwise to a solution of (3S,3'S,6S,6'S)-4,4'-(hexane-1,6-diyl)bis(1-benzyl-3,6-bis((4-methoxybenzyl) thio)piperazine-2,5-dione) (0.10 g, 0.11 mmol) (**497**) in dichloromethane (20.0 mL) at -78 °C. The resulting mixture was stirred for 15 minutes whereupon aqueous sodium hydrogen carbonate (10.0 mL of a saturated aqueous solution) and methanol (10.0 mL) were added and the biphasic mixture stirred for 15 minutes until the yellow colour had dissipated. The resulting mixture was stirred at room temperature for 10 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. Sodium thiosulfate was added and the mixture stirred for 10 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (9:1, dichloromethane: methanol; snap 10 g), followed by HPLC separation and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (1S,1'S,4S,4'S)-5,5'-(hexane-1,6-diyl)bis(2-benzyl-7-thia-2,5-diazabicyclo[2.2.1] heptane-3,6-dione 7-sulfide) (0.04 g, 50%) (**499**) as a colourless solid; m.p. 175-178 °C; ν_{max}/cm^{-1} 2092 (C-H), 1642 (C=O); δ H (500 MHz; $CDCl_3$) 2.01 – 2.17 (2H, m, $NCH_2(CH_2)_4CH_2N$), 2.40 – 2.42 (2H, m, $NCH_2(CH_2)_4CH_2N$), 3.52 – 3.55 (6H, m, NCH_2 & $NCH_2(CH_2)_4CH_2N$), 3.80 – 3.82 (2H, m, NCH_2), 4.54 (2H, d, *J* 14.8, NCH_2), 4.86 (2H, d, *J* 14.9, NCH_2), 5.20 (2H, s, C*H*S), 5.36 (2H, s, C*H*S), 7.38 – 7.45 (10H, m, Ar-*H*); *m/z* 613 (100%, $[M-H]^-$): Found $[M-H]^-$ 613.2100, $C_{28}H_{29}N_4O_4S_4$ requires 613.1072.

5.80 (8S,10a)-9-Benzyl-8-((4-methoxybenzyl)thio) octahydropyrazino[1,2-a][1,3] diazepine-7,10-dione (504) & (505)



259

Chemical Formula: $C_{25}H_{29}NO_8S$
Molecular Weight: 503.56

504

Chemical Formula: $C_{23}H_{27}N_3O_3S$
Molecular Weight: 425.54

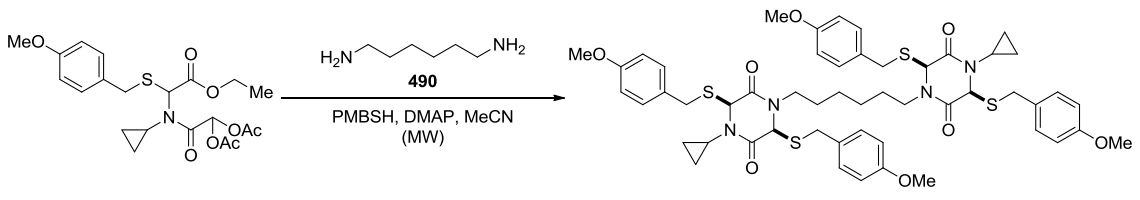
505

Chemical Formula: $C_{23}H_{27}N_3O_3S$
Molecular Weight: 425.54

A solution of 2-(benzyl(2-ethoxy-1-((4-methoxybenzyl)thio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.20 g, 0.40 mmol) (**259**), 1,4-diaminobutane (0.04 mL, 0.40 mmol) (**502**), *para*-methoxybenzyl mercaptan (0.08 mL, 0.60 mmol) (**257**) and DMAP (0.02 g, 0.20 mmol) in acetonitrile (3.00 mL) was stirred at room temperature for 10 minutes and heated at 130 °C for 25 minutes in the microwave. Solvent was removed under reduced pressure and the residue purified via Biotage™ Horizon (2:1, petroleum spirit 40-60 °C: ethyl acetate; snap 10 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (8S,10aR)-9-benzyl-8-((4-methoxybenzyl)thio)octahydropyrazino[1,2-a][1,3]diazepine-7,10-dione (0.06 g, 36%) (**504**) and (8S,10aS)-9-benzyl-8-((4-methoxybenzyl)thio) octahydropyrazino[1,2-a][1,3]diazepine-7,10-dione (0.02 g, 12%) (**505**) both as colourless solids; Data for (**504**): m.p. 122-123 °C; ν_{max}/cm^{-1} 3424 (NH), 1663 (C=O), 1511 (NH), 1249 (C-C(O)-C), 1080 (C-N), 732 (CH_2); δ H (400 MHz, $CDCl_3$) 1.59 – 1.77 (4H, m, $NCH_2(CH_2)_2CH_2NH$), 2.81 – 2.97 (3H, m, $NHCH_2$ & $NCH_2(CH_2)_2CH_2NH$), 3.70 – 3.80 (2H, m, SCH_2), 3.64 (1H, d, *J* 13.2, $NCH_2(CH_2)_2CH_2NH$), 3.86 (3H, s, OCH_3), 3.91 (1H, d, *J* 13.1, $NCH_2(CH_2)_2CH_2NH$), 3.99 (1H, d, *J* 13.8, NCH_2-Ph), 4.43 (1H, s, CHS), 4.84 (1H, s, CHNH), 5.19 (1H, d, *J* 13.5, NCH_2-Ph), 6.78 (1H, d, *J* 1.4, Ar-*H*), 6.79 – 6.80 (1H, m, Ar-*H*), 6.87 – 6.89 (1H, m, Ar-*H*), 6.89 – 6.91 (1H, m, Ar-*H*), 7.16 – 7.21 (2H, m, Ar-*H*), 7.24 – 7.25 (1H, m, Ar-*H*), 7.34 – 7.39 (2H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 27.43 ($\mathbf{C}H_2$), 30.58 ($\mathbf{C}H_2$), 34.81 ($S\mathbf{C}H_2$), 42.43 ($\mathbf{C}H_2$), 46.81 ($\mathbf{C}H_2$), 47.58 ($\mathbf{C}H_2$), 55.18 ($O\mathbf{C}H_3$), 58.08 (**C**HS), 72.06 (**C**HN), 113.98 (Ar-**C**-H), 127.82 (Ar-**C**-H), 128.43 (Ar-**C**-H), 128.57 (Ar-**C**-H), 128.60 (quaternary **C**), 130.60 (Ar-**C**-H), 134.73 (quaternary **C**), 158.99 (quaternary **C**), 163.50 (**C**O), 166.86 (**C**O); *m/z* 426 (100%, $[M+H]^+$): Found $[M+H]^+$ 426.1850, $C_{23}H_{28}N_3O_3S$ requires 426.1852; Data for (**505**): m.p. 121-123 °C; ν_{max}/cm^{-1} 3425 (NH), 1658 (C=O), 1511 (NH), 1249 (C-C(O)-C), 1032 (C-N); δ H (400 MHz, $CDCl_3$) 1.63 – 1.98 (4H, m, $NCH_2(CH_2)_2CH_2NH$), 2.02 –

2.16 (1H, m, $NCH_2(CH_2)_2CH_2NH$), 2.89 – 2.91 (1H, m, $NCH_2(CH_2)_2CH_2NH$), 2.99 – 3.09 (1H, m, $NCH_2(CH_2)_2CH_2NH$), 3.26 (1H, d, *J* 13.2, SCH_2), 3.59 (1H, d, *J* 13.1, SCH_2), 3.80 – 3.85 (1H, m, $NCH_2(CH_2)_2CH_2NH$), 3.86 (3H, s, OCH_3), 4.10 (1H, d, *J* 13.7, $NCH_2(CH_2)_2CH_2NH$), 4.24 (1H, d, *J* 12.9, NCH_2-Ph), 4.38 (1H, s, CHS), 4.67 (1H, s, CHNH), 5.19 (1H, d, *J* 13.0, NCH_2-Ph), 6.76 (2H, d, *J* 7.2, Ar-*H*), 6.89 (2H, d, *J* 8.6, Ar-*H*), 7.15 (1H, d, *J* 6.6, Ar-*H*), 7.18 (1H, d, *J* 6.0, Ar-*H*), 7.21 – 7.30 (1H, m, Ar-*H*), 7.37 (2H, d, *J* 8.6, Ar-*H*); δ C (101 MHz, $CDCl_3$) 25.77 (**C**H_2), 30.08 (**C**H_2), 35.81 (S**C**H_2), 45.01 (**C**H_2), 46.08 (**C**H_2), 48.25 (**C**H_2), 55.17 (O**C**H_3), 57.02 (**C**HS), 72.48 (**C**HN), 114.02 (Ar-**C**-H), 127.76 (Ar-**C**-H), 128.48 (Ar-**C**-H), 128.56 (Ar-**C**-H), 128.60 (quaternary **C**), 130.64 (Ar-**C**-H), 134.78 (quaternary **C**), 159.03(**C**O), 165.20 (**C**O); *m/z* 426 (100%, $[M+H]^+$): Found $[M+H]^+$ 426.1836, $C_{23}H_{28}N_3O_3S$ requires 426.1852.

5.81 (3S,3'S,6S,6'S)-4,4'-(Hexane-1,6-diyl)bis(1-cyclopropyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione) (512)



396

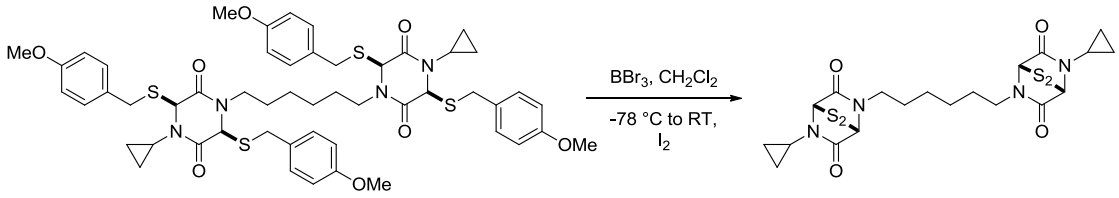
Chemical Formula: $C_{21}H_{27}NO_8S$
Molecular Weight: 453.51

512

Chemical Formula: $C_{52}H_{62}N_4O_8S_4$
Molecular Weight: 999.33

A mixture of 2-(cyclopropyl(2-ethoxy-1-(4-methoxybenzylthio)-2-oxoethyl)amino)-2-oxoethane-1,1-diyl diacetate (0.40 g, 0.88 mmol) (**396**), 1,6-diaminohexane (0.10 g, 0.88 mmol) (**490**), *para*-methoxybenzyl mercaptan (0.37 mL, 2.65 mmol) (**257**) and DMAP (0.05 g, 0.44 mmol) in acetonitrile (3.00 mL) was stirred at room temperature for 10 minutes and heated at 130 °C for 30 minutes in the microwave. The residue was purified via Biotage™ Horizon (2:1, petroleum spirit 40-60 °C: ethyl acetate; snap 25 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (3S,3'S,6S,6'S)-4,4'-(hexane-1,6-diyl)bis(1-cyclopropyl-3,6-bis((4-methoxybenzyl)thio)piperazine-2,5-dione) (0.11 g, 25%) (**512**) as a colourless solid; m.p. 98-102 °C; ν_{max}/cm^{-1} 2934 (C-H), 2856 (C-H), 2836 (C-H), 1666 (C=O), 1464(CH_2), 733 (C-H); δ H (400 MHz, $CDCl_3$) 0.13 (2H, dt, *J* 6.8 & 4.3, cyclopropyl-C*H*$_2$), 0.23 – 0.38 (2H, m, cyclopropyl-C*H*$_2$), 0.72 – 0.83 (2H, m, cyclopropyl-C*H*$_2$), 0.88 – 0.91 (4H, m, NCH$_2$(C*H*$_2$)$_4$CH$_2$N), 1.09 (2H, dt, *J* 6.2 & 4.4, cyclopropyl-C*H*$_2$), 1.28 – 1.30 (2H, m, NCH$_2$(C*H*$_2$)$_4$CH$_2$N), 1.60 – 1.61 (2H, m, cyclopropyl-C*H*), 2.49 – 2.58 (2H, m, NCH$_2$(C*H*$_2$)$_4$CH$_2$N), 2.62 – 2.64 (2H, m, SC*H*$_2$), 3.44 – 3.57 (2H, m, SC*H*$_2$), 3.78 (6H, s, OC*H*$_3$), 3.81 – 3.85 (8H, m, SC*H*$_2$ & OC*H*$_3$), 3.88 (2H, d, *J* 2.5, SC*H*$_2$), 4.01 (2H, d, *J* 8.7, NC*H*$_2$), 4.05 (2H, d, *J* 8.8, NC*H*$_2$), 4.30 (2H, s, C*H*S), 4.49 (2H, s, C*H*S), 6.85 – 6.92 (8H, m, Ar-*H*), 7.39 – 7.45 (8H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 4.76 (**C**H$_2$), 7.94 (**C**H$_2$), 26.47 (**C**H$_2$), 26.84 (**C**H$_2$), 28.39 (**C**H), 36.98 (S**C**H$_2$), 37.10 (S**C**H$_2$), 43.68 (N**C**H$_2$), 55.52 (O**C**H$_3$), 55.57 (O**C**H$_3$), 59.31 (**C**HS), 60.76 (**C**HS), 114.21 (Ar-**C**-H), 128.97 (quaternary **C**), 130.97 (Ar-**C**-H), 159.28 (quaternary **C**), 165.42 (**C**O), 167.47 (**C**O); *m/z* 999 (100%, $[M+H]^+$): Found $[M+H]^+$ 999.3549, $C_{52}H_{63}N_4O_8S_4$ requires 999.3529.

5.82 (±)-(1S,1'S,4S,4'S)-5,5'-(Hexane-1,6-diyl)bis(2-cyclopropyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) (515)



512

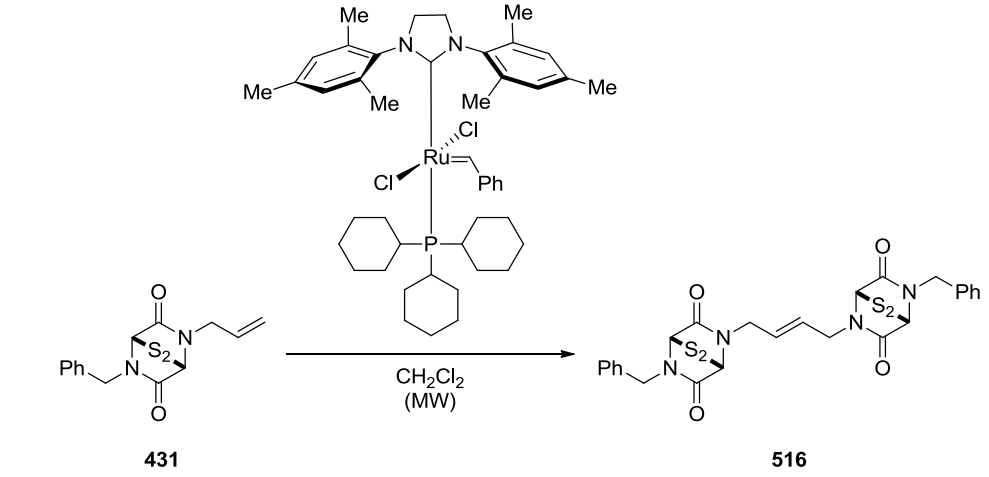
Chemical Formula: $C_{52}H_{62}N_4O_8S_4$
Molecular Weight: 999.33

515

Chemical Formula: $C_{20}H_{26}N_4O_4S_4$
Molecular Weight: 514.70

Boron tribromide (0.74 mL of a 1M solution in dichloromethane, 0.74 mmol) was added dropwise to a solution of (3S,3'S,6S,6'S)-4,4'-(hexane-1,6-diyl)bis(1-cyclopropyl-3,6-bis((4-methoxybenzyl) thio)piperazine-2,5-dione) (0.15 g, 0.15 mmol) (**512**) in dichloromethane (2.00 mL) at -78 °C. The resulting mixture was stirred for 15 minutes whereupon aqueous sodium hydrogen carbonate (10.0 mL of a saturated aqueous solution) and methanol (2.00 mL) were added and the biphasic mixture stirred for 15 minutes until the pale yellow colour had dissipated. The resulting mixture was stirred at room temperature for 10 minutes, whereupon iodine was added portionwise until the colour due to iodine just persisted and stirring was maintained for 2 minutes. Sodium thiosulfate was added and the mixture stirred for 10 minutes, diluted with dichloromethane (10.0 mL) and water (20.0 mL). The layers were separated and the aqueous phase extracted with dichloromethane (2 x 20.0 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (9:1, dichloromethane: methanol; snap 10 g), followed by HPLC separation and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (1S,1'S,4S,4'S)-5,5'-(hexane-1,6-diyl)bis(2-cyclopropyl-7-thia-2,5-diazabicyclo [2.2.1]heptane-3,6-dione 7-sulfide) (0.02 g, 20%) (**515**) as a colourless solid; m.p. 168-170 °C; ν_{max}/cm^{-1} 2924 (C-H), 2853 (C-H), 1684 (C=O), 1458 (CH_2); δ H (500 MHz; $CDCl_3$) 0.75 – 0.83 (2H, m, cyclopropyl-CH_2), 0.86 – 0.95 (2H, m, cyclopropyl-CH_2), 0.98 – 1.11 (4H, m, cyclopropyl-CH_2), 1.38 – 1.39 (2H, m, cyclopropyl-C*H*), 1.64 – 1.66 (8H, m, $NCH_2(CH_2)_4CH_2N$), 2.89 (2H, t, *J* 7.0, NCH_2), 3.50 (2H, t, *J* 7.1, NCH_2), 5.27 (2H, s, C*H*S), 5.38 (2H, s, C*H*S); δ C (126 MHz; $CDCl_3$) 6.14 (**C**H_2), 7.39 (**C**H_2), 26.25 (**C**H), 27.78 (**C**H_2), 28.58 (**C**H_2), 45.19 (**C**H_2), 66.34 (**C**HS), 67.01 (**C**HS), 164.05 (**C**O), 164.81 (**C**O); *m/z* 515 (100%, $[M+H]^+$): Found $[M+H]^+$ 515.1381, $C_{20}H_{27}N_4O_4S_4$ requires 515.0915.

5.83 (±)-(1S,1'S,4S,4'S)-5,5'-((*E*)-But-2-ene-1,4-diyl)bis(2-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) (516)



431

Chemical Formula: $C_{14}H_{14}N_2O_2S_2$
Molecular Weight: 306.40

516

Chemical Formula: $C_{26}H_{24}N_4O_4S_4$
Molecular Weight: 584.75

5.83.1 Method A:

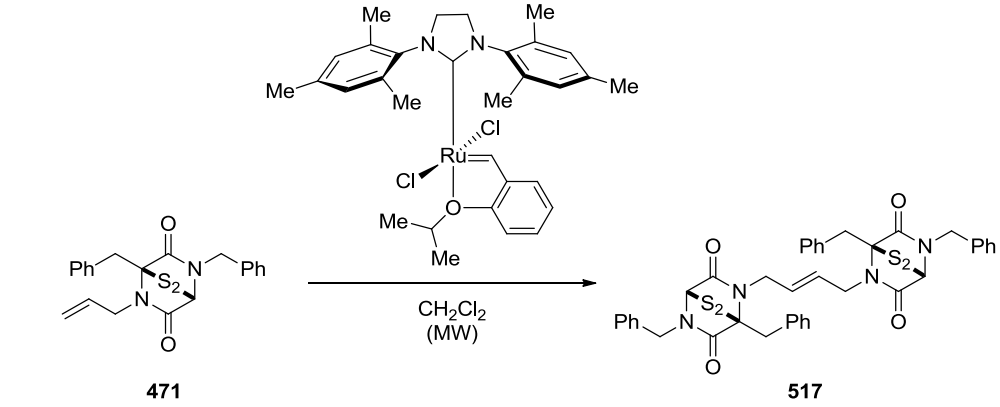
A solution of (±)-(1S,4S)-2-allyl-5-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.10 g, 1.20 mmol) (**431**) in dichloromethane (2.00 mL) was degassed and taken under nitrogen atmosphere. To this (1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro (phenylmethylene)(tricyclohexylphosphine)ruthenium (Grubbs second-generation catalyst) (0.01 g, 0.01 mmol) was added and the resulting mixture heated at 80 °C for 30 minutes in the microwave, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; pipette column) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (1S,1'S,4S,4'S)-5,5'-((*E*)-but-2-ene-1,4-diyl)bis(2-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) (0.04 g, 47%) (**516**) as a dark yellow solid; m.p. 198-200 °C; ν_{max}/cm^{-1} 3061 (CH=CH), 3029 (C-H), 1692 (C=O), 1606 (C=C), 1445 (CH_2), 724 (C-H), 699 (C-H); δ H (400 MHz, $CDCl_3$) 4.15 – 4.17 (4H, m, NC*H*$_2$), 4.54 (2H, d, *J* 7.4, NC*H*$_2$), 4.85 (2H, d, *J* 7.2, NC*H*$_2$), 5.23 (2H, s, C*H*S), 5.40 (2H, s, C*H*S), 5.87 – 5.91 (2H, m, C*H*=CH_2), 7.37 – 7.43 (10H, m, Ar-*H*); *m/z* 585 (100%, $[M+H]^+$): Found $[M+H]^+$ 585.0738, $C_{26}H_{25}N_4O_4S_4$ requires 585.0734.

5.83.2 Method B:

A solution of (±)-(1S,4S)-2-allyl-5-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.10 g, 0.119 mmol) (**431**) in dichloromethane (2.00 mL) was degassed

and taken under nitrogen atmosphere. To this (1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(*o*-isopropoxyphenylmethylene)ruthenium (Grubbs-Hoveyda second-generation catalyst) (0.01 g, 0.01 mmol) was added and the resulting mixture heated at 80 °C for 30 minutes in the microwave, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; pipette column) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (1S,1'S,4S,4'S)-5,5'-((*E*)-but-2-ene-1,4-diyl)bis(2-benzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) (0.02 g, 22%) (**516**) as a dark yellow solid; identical spectroscopic data to that obtained previously for (**516**).

5.84 (±)-(1S,1'S,4S,4'S)-2,2'-((*E*)-But-2-ene-1,4-diyl)bis(1,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) (517)



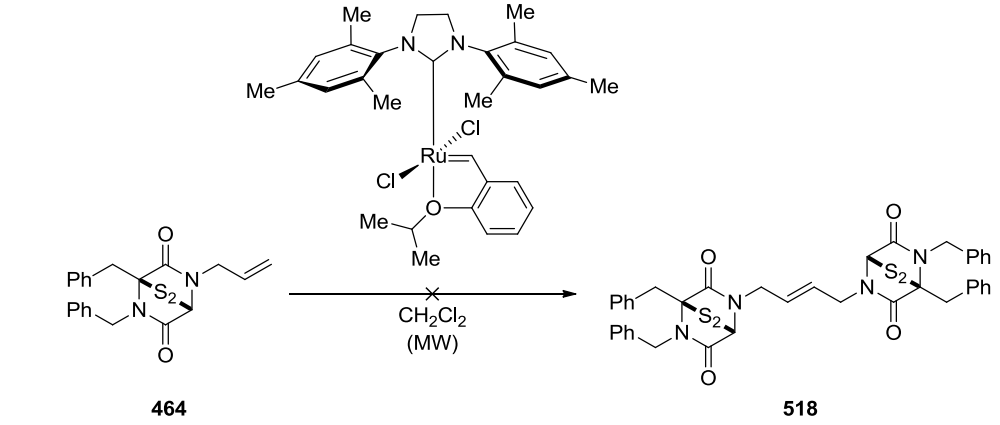
471 **517**

Chemical Formula: $C_{21}H_{20}N_2O_2S_2$
Molecular Weight: 396.53

Chemical Formula: $C_{40}H_{36}N_4O_4S_4$
Molecular Weight: 765.00

A solution of (±)-(1S,4S)-2-allyl-1,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.11 g, 0.28 mmol) (**471**) in dichloromethane (5.00 mL) was degassed and taken under nitrogen atmosphere. To this (1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(*o*-isopropoxyphenylmethylene)ruthenium (Grubbs-Hoveyda second-generation catalyst) (0.01 g, 0.02 mmol) was added and the resulting mixture heated at 150 °C for 2 hours in the microwave, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 5 g) and further purified by recrystallisation (petroleum spirit 40-60 °C: ethyl acetate) to give (1S,1'S,4S,4'S)-2,2'-((*E*)-but-2-ene-1,4-diyl)bis(1,5-dibenzyl-7-thia-2,5-diazabicyclo [2.2.1]heptane-3,6-dione 7-sulfide) (0.02 g, 18%) (**517**) as a colourless solid; m.p. 108-110 °C; ν_{max}/cm^{-1} 1670 (C=O), 1608 (C=C), 1453 (CH_2), 734 (C-H), 699 (C-H); δ H (400 MHz, $CDCl_3$) 3.47 (2H, d, *J* 14.1, NC*H*$_2$CH=CH), 3.65 (2H, d, *J* 13.8, NC*H*$_2$CH=CH), 3.69 – 3.73 (2H, m, NC*H*$_2$-Ph), 3.78 (4H, s, C*H*$_2$-Ph), 3.89 – 3.95 (2H, m, NC*H*$_2$-Ph), 4.31 (2H, s, C*H*S), 5.16 – 5.22 (2H, m, C*H*=C*H*), 6.61 – 6.64 (4H, m, Ar-*H*), 6.78 – 6.82 (4H, m, Ar-*H*), 7.05 – 7.10 (4H, m, Ar-*H*), 7.25 – 7.28 (8H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 36.41 (**C**H$_2$), 45.70 (**C**HS), 46.26 (**C**H$_2$), 50.48 (**C**H$_2$), 55.53 (quaternary **C**), 58.27 (**C**H=CH), 128.22 (Ar-**C**-H), 128.62 (Ar-**C**-H), 128.88 (Ar-**C**-H), 128.92 (Ar-**C**-H), 131.02 (Ar-**C**-H), 131.29 (Ar-**C**-H), 135.05 (quaternary **C**), 159.47 (quaternary **C**), 163.41 (**C**O), 167.21 (**C**O); *m/z* 765 (100%, $[M+H]^+$); Found $[M+H]^+$ 765.1645, $C_{40}H_{37}N_4O_4S_4$ requires 765.1629.

5.85 (±)-(1S,1'S,4S,4'S)-5,5'-((*E*)-But-2-ene-1,4-diyl)bis(1,2-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) (518)



464

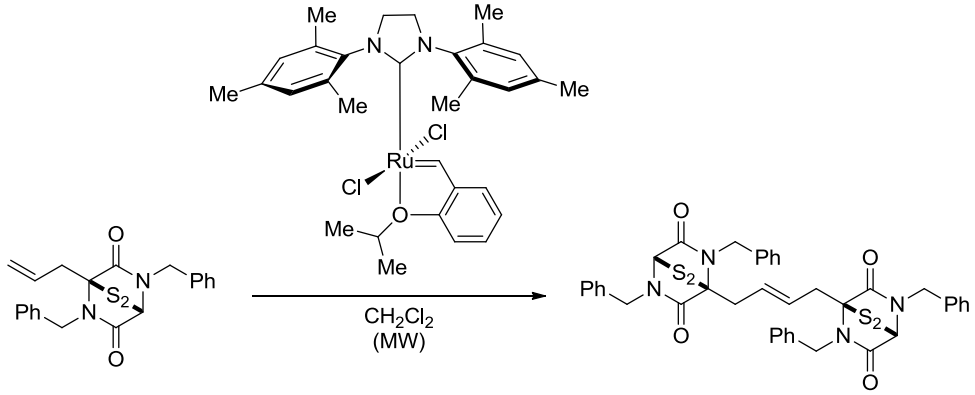
Chemical Formula: $C_{21}H_{20}N_2O_2S_2$
Molecular Weight: 396.53

518

Chemical Formula: $C_{40}H_{36}N_4O_4S_4$
Molecular Weight: 765.00

A solution of (±)-(1S,4S)-5-allyl-1,2-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.03 g, 0.08 mmol) (**464**) in dichloromethane (5.00 mL) was degassed and taken under nitrogen atmosphere. To this (1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(*o*-isopropoxyphenylmethylene) ruthenium (Grubbs-Hoveyda second-generation catalyst) (0.01 g, 0.01 mmol) was added and the resulting mixture heated at 150 °C for 2 hours in the microwave, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 5 g) which did not give (6aS,12aS)-7,10-dihydro-6a,12a-epithiodipyrido[1,2-a:1',2'-d]pyrazine-6,12(1H,4H)-dione 13-sulfide (**518**).

5.86 (±)-(1S,1'S,4S,4'S)-1,1'-((*E*)-But-2-ene-1,4-diyl)bis(2,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) (519)



455

Chemical Formula: $C_{21}H_{20}N_2O_2S_2$
Molecular Weight: 396.53

519

Chemical Formula: $C_{40}H_{36}N_4O_4S_4$
Molecular Weight: 765.00

A solution of (±)-(1S,4S)-1-allyl-2,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide (0.05 g, 0.08 mmol) (**455**) in dichloromethane (5.00 mL) was degassed and taken under nitrogen atmosphere. To this (1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(*o*-isopropoxyphenylmethylene)ruthenium (Grubbs-Hoveyda second-generation catalyst) (0.01 g, 0.01 mmol) was added and the resulting mixture heated at 150 °C for 2 hours in the microwave, cooled to RT and solvent removed under reduced pressure. The residue was purified via Biotage™ Horizon (4:1, petroleum spirit 40-60 °C: ethyl acetate; snap 5 g) to give (1S,1'S,4S,4'S)-1,1'-((*E*)-but-2-ene-1,4-diyl)bis(2,5-dibenzyl-7-thia-2,5-diazabicyclo[2.2.1]heptane-3,6-dione 7-sulfide) (0.01 g, 25%) (**519**) as a colourless oil; ν_{max}/cm^{-1} 3032 (C-H), 1684 (C=O), 1609 (C=C), 730 (CH_2), 700 (C-H); δ H (400 MHz, $CDCl_3$) 2.63 – 2.69 (2H, m, C*H*$_2$CH=CH), 2.75 – 2.81 (2H, m, C*H*$_2$CH=CH), 3.88 (2H, s, C*H*S), 4.79 – 4.85 (4H, m, NC*H*$_2$), 4.89 (2H, s, NC*H*$_2$), 5.03 (2H, s, NC*H*$_2$), 5.11 – 5.18 (2H, m, C*H*=CH$_2$), 6.89 – 6.93 (2H, m, Ar-*H*), 7.13 – 7.20 (2H, m, Ar-*H*), 7.30 – 7.47 (16H, m, Ar-*H*); δ C (101 MHz, $CDCl_3$) 44.41 (**C**H$_2$), 46.08 (**C**H$_2$), 46.75 (**C**H$_2$), 55.33 (**C**HS), 71.87 (quaternary **C**), 128.21 (Ar-**C**-H), 128.39 (Ar-**C**-H), 128.63 (Ar-**C**-H), 128.79 (Ar-**C**-H), 129.13 (Ar-**C**-H), 129.59 (Ar-**C**-H), 135.03 (**C**H=CH$_2$), 156.01 (quaternary **C**), 159.15 (quaternary **C**), 165.06 (**C**O), 169.88 (**C**O); *m/z* 765 (100%, $[M+H]^+$); Found $[M+H]^+$ 765.1634, $C_{40}H_{37}N_4O_4S_4$ requires 765.1629.

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