Design, Synthesis and Evaluation of Novel C2-Aryl Substituted Pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) Dimers

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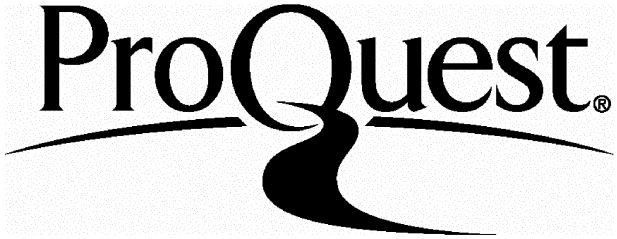

ABSTRACT

Synthetic introduction of an aryl substituent at the C2-position has dramatically enhanced the *in vivo* activity of pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) monomers. As dimerizing PBDs was known to enhance sequence selectivity and antitumour activity, the principal objective and work presented in this thesis focuses on developing new approaches to the synthesis of novel C2-aryl PBDs dimers via a new and improved methodology.

During the course of the research project extensive method development was performed which resulted in the development and optimisation of an efficient, concise and versatile synthetic route which greatly reduced the number of synthetic steps of previously reported literature methods. Consequently, six novel C2-aryl PBD imine dimers, a deuterated DSB-120 analogue and a novel example of a PBD tetralactam C2-aryl dimer were successfully synthesized via this new and improved tetralactam route. The synthetic approach involved the use and development of model systems to optimise the critical C11-lactam reduction step of SEM-protected dilactams to generate the N10-C11 imine moiety, critical for biological activity of PBDs. In addition, the Suzuki cross-coupling reaction was used to introduce aryl substituents at the C2 position of PBD dimers. The use of such a versatile coupling reaction at a late stage in the synthesis has the potential to access a wide range of PBD dimer analogues in the future. The work also includes investigations into the Vilsmeier-Haack formylation of the tetralactam system and subsequent side chain elaboration through Wittig olefination chemistry.

Five PBD dimers were evaluated for their *in vitro* cytotoxicity and were found to be highly potent exhibiting nano-molar and sub-nanomolar activity against the Human Chronic Myeloid Leukaemia cell line assay K_{562}. Interestingly, the unsubstituted C2-aryl dimer **227** was the most potent exhibiting an IC_{50} value of 0.28 nM, closely followed by the *para*-methyl substituted compound **229** (IC_{50} = 0.38 nM). The more highly branched analogues were generally less cytotoxic with activity in the 10-30 nanomolar range. There results appear to indicate that steric volume is an important factor in determining the cytotoxicity of the compounds and the results support the hypothesis that a good fit within the minor groove of DNA is critical for potent biological activity.

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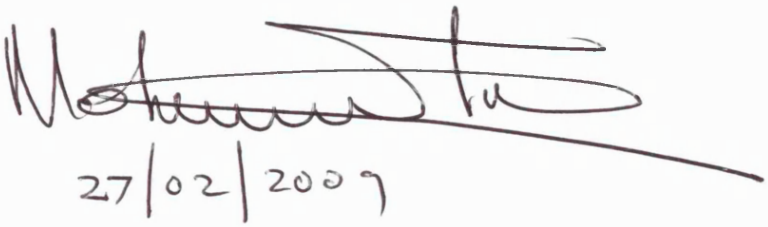
I Mohammed Tirech declare that the work presented in this thesis is my own



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ABBREVIATIONS

AcOH — Acetic acid

Ac_2O — Acetic anhydride

All — Allyl

Alloc — Allyloxycarbonyl

BAIB — Iodobenzene Diacetate

Bn — Benzyl

BOC — tert-butyloxycarbonyl

BOC_2O — di-*tert*-butyl dicarbonate

CBz — Benzyloxycarbonyl

CPI — cyclopropylpyrroloindole

CR-UK— Cancer Research UK

CT-DNA — Calf Thymus DNA

DBU — 1,8-Diazobicyclo[5.4.0]undec-7-ene

DCC — Dicyclohexyl Carbodiimide

DCM — Dichloromethane

DEAD — Diethyl Azodicarboxylate

DHP — Dihydropyran

DIAD — Diisopropyl Azodicarboxylate

DIPEA — Diisopropylethylamine

DMAP — 4-Dimethylaminopyridine

DMF — *N*,*N*-Dimethylformamide

DMSO — Dimethylsulfoxide

DNA — Deoxyribonucleic Acid

ES — Electrospray

EDCI — 1-Ethyl-3-(3-Dimethyl-Aminopropyl) Carbodiimide

FAB — Fast Atom Bombardment

Fmoc — 9-Fluorenylmethyloxycarbonyl

HOBt — 1-Hydroxybenzotriazole Hydrate

HPLC — High Performance Liquid Chromatography

HRMS — High Resolution Mass Spectrometry

IR — Infrared

KOtBu — Potassium *tert*-butoxide

LDA — Lithium Diisopropylamide

LiHMDS — Lithium Bis(trimethylsilyl)amide

MeOH — Methanol

MEM — Methoxyethoxymethyl

MOM — Methoxymethyl

MS — Mass Spectrometry/Molecular Sieves

MTT — 3-(4,5-Dimethylthiazolyl)-2,5-diphenyltetrazolium Bromide

NCI — National Cancer Institute

NMO — *N*-Methylmorpholine *N*-Oxide

NMR — Nuclear Magnetic Resonance

PBD — Pyrrolobenzodiazepine

PDC — Pyridinium Dichromate

PTSA — *para*-Toluenesulfonic acid

Py — Pyridine

pH — Potential of Hydrogen

pKa — Negative Logarithm of the Acid Ionization Constant (pK_a)

QCS — Quinolinium Camphosulfonate

RNA — Ribonucleic acid

SEM — Trimethylsilylethoxymethyl

TBAF — Tetrabutylammonium Fluoride

TBDMS — *tert*-Butyl-dimethylsilyl

TBS — *tert*-Butyl-dimethylsilyl

TEA — Triethylamine

TEMPO — 2,2,6,6-Tetramethyl-1-piperidinyloxy (free radical)

Teoc — Trimethylsilylethoxycarbonyl

TFA — Trifluoroacetic Acid

TFO — Triple helix forming oligonucleotides

THF — Tetrahydrofuran

THP — Tetrahydropyran

TIPS — Tri-isopropylsilyl

TPAP — Tetra-n-propylammonium Perruthenate

TPP — Triphenylphosphine

Troc — 2,2,2-Trichloroethoxycarbonyl

UCL — University College London

1 THE PYROLLO[2,1-*c*][1,4]BENZODIAZEPINES (PBDs)

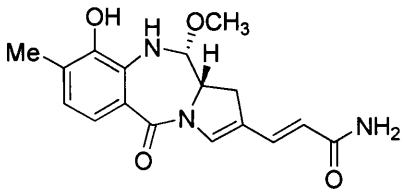
1.1 Introduction

There has been a growing interest in discovering and developing small molecules that are capable of interacting with nucleic acids in a sequence selective manner. Such DNA interactive agents have the potential to allow single gene or set of genes to be up or down regulated, thus offering potential new therapies for genetic-based diseases, such as in cancer (e.g. down regulate oncogenes in cancer).

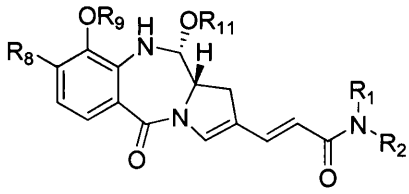
Pyrrolobenzodiazepines (PBDs) have been investigated because of their drug like properties and potent antitumour activity. Following their discovery, there has been a sustained effort to synthesise both natural and unnatural PBDs (Thurston and Bose, 1994; Kamal *et al.*, 2001). Much of the interest in PBDs stems from their ability to bind covalently to DNA in a sequence selective manner (e.g. AGA motifs), which could lead to the synthesis of molecules capable of regulating gene expression (Thurston *et al.*, 1999a). For example a PBD dimer SJG-136 with sequence selectivity for Pu-GATC-Py motifs has been chosen for Phase I clinical trials with CR-UK in Britain and with the NCI in USA (Alley *et al.*, 2004).

1.2 PBD Natural Product Family

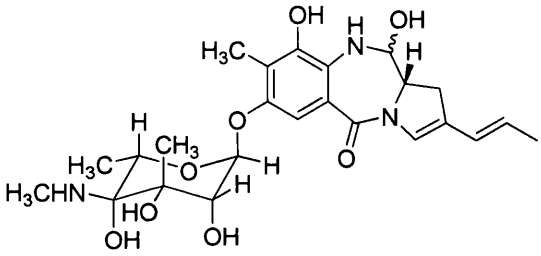
PBDs are a family of biosynthetically derived compounds produced by various actinomycetes (Hurley *et al.*, 1977). The first PBD to be isolated was anthramycin, which was obtained from the fermentation broth of a *Streptomyces refuineus* species in 1963 by Tendler and Korman (Tendler and Korman, 1963). The isolated anthramycin was found to have potent antitumour, antibiotic and antiprotozoal activity. The active compound was originally called 'Refuin' (Hebrew for health) but was later isolated as a pure crystalline antibiotic by Leimgruber in 1965 (Leimgruber *et al.*, 1965a) and assigned the name anthramycin. Since then 12 naturally occurring PBDs have been discovered **(Figure 1.2a)** and isolated from various *Streptomyces* species, all exhibiting different substitution patterns in the PBD ring system (Thurston *et al.*, 1999a).



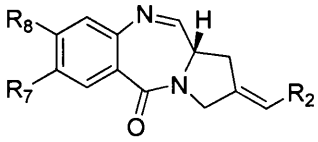
1 Anthramycin



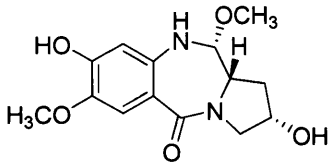
2 Porothramycin A: $R_{11} = R_8 = H$, $R_9 = R_1 = R_2 = CH_3$
3 Porothramycin B: $R_8 = H$, $R_{11} = R_9 = R_1 = R_2 = CH_3$
4 Mazethramycin A: $R_{11} = R_8 = R_1 = CH_3$, $R_9 = R_2 = H$



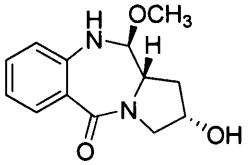
5 Sibiromycin



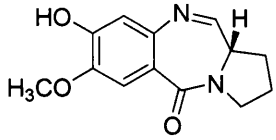
6 Tomaymycin: $R_7 = CH_3O$; $R_8 = OH$, $R_2 = CH_3$
7 Prothracarcin: $R_7 = R_8 = H$, $R_2 = CH_3$
8 Sibanomicin: R_7 = O-Pyranose as in 5, $R_8 = H$. $R_2 = C_2H_5$



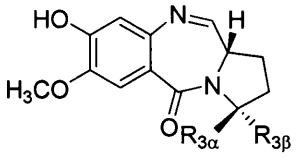
9 Chicamycin



10 Abbeymycin



11 DC-81



12 Neothramycin A: $R_{3\alpha} = H$; $R_{3\beta} = OH$
13 Neothramycin B: $R_{3\alpha} = OH$; $R_{3\beta} = H$

Figure 1.2a: Naturally Occurring PBDs.

1.2.1 PBD Ring System and Numbering

All PBDs posses a general basic structure of a tricyclic backbone, comprising a benzene A-ring, a diazepine B-ring and a pyrrolo C-ring (**Figure 1.2b**). PBDs differ in the type, number and position of substituents in the aromatic A-ring and the pyrrolo C-ring with the latter having different degrees of unsaturation, either fully saturated (as in DC-81, neothramycin and chicamycin), *endo*-unsaturated at C2-C3 (as in anthramycin, sibiromycin and porothramaycin) or *exo*-unsaturated at C2 (as in tomaymycin, porothracarcin). Another very important feature in all naturally occurring PBDs is (*S*)-stereochemistry at the C11a position (Thurston, 1993). It is worth noting that PBDs possessing *endo-exo*-unsaturation such as anthramycin etc, are the most potent naturally occurring PBDs.

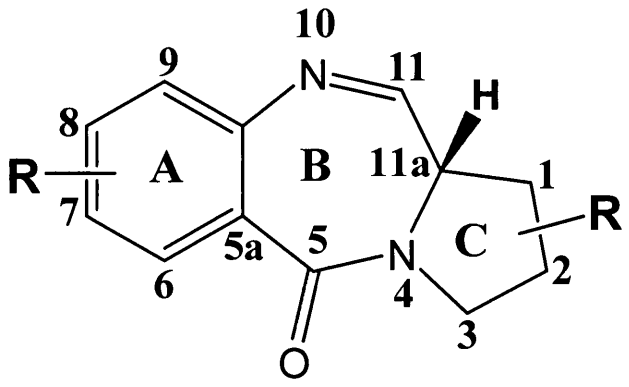


Figure 1.2b: PBD Ring System and Numbering.

1.2.2 PBD Interconvertable Forms

Another very important feature of PBDs is the N10-C11 moiety which can either exist as the imine, the hydrated carbinolamine or carbinolamine methyl ether depending on the precise structure of the compound and the method of isolation or synthetic workup. The imine and methyl ether forms can be interconverted through a dynamic equilibrium by dissolution of the imine in methanol, or by several cycles of refluxing the methyl ether in chloroform, followed by evaporation *in vacuo* (Leimgruber *et al.*, 1965b, Thurston and Langley, 1986).

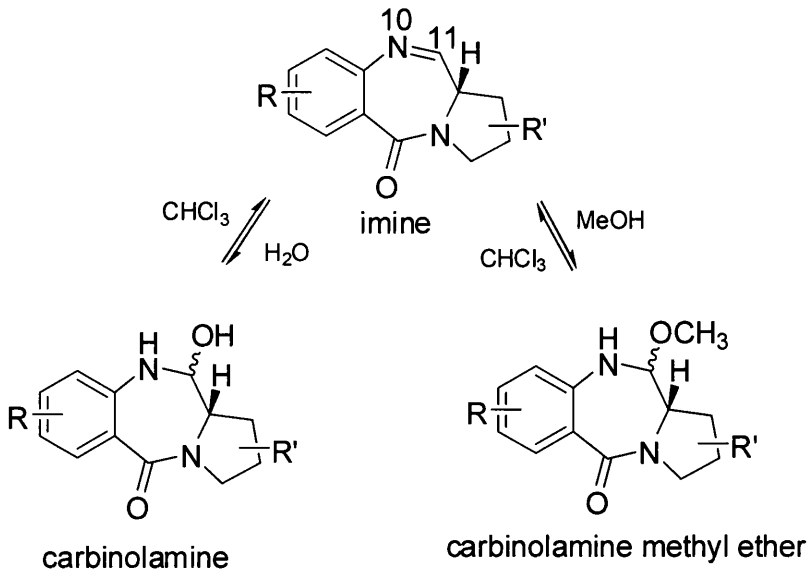


Figure 1.2c: PBD Interconvertable Forms.

1.3 Mode of Action of PBDs

PBDs are alkylating agents and they exert their biological activity by forming PBD-DNA adducts within the minor groove of DNA, thus interfering with DNA function. The structure of the PBD-DNA adduct has been investigated by many research groups using various biochemical (e.g. PBD-DNA radiolabelled experiments, footprinting studies) and analytical techniques (e.g. NMR, molecular modelling and fluorescence spectroscopy; Thurston, 1993) and it has now been established that after insertion in the minor groove of DNA, a covalent aminal bond is formed through a nucleophilic attack by the N2 of guanine on the N10-C11 electrophilic imine.

1.3.1 Alkylation Mechanism of PBDs

Despite the lack of experimental evidence (Thurston, 1993) as to the precise structure of the N10-C11 electrophilic species responsible for alkylating DNA, it is believed that the imine/iminium based mechanism (**Figure 1.3a**) is the most likely with the imine being protonated at physiological pH, thus increasing its electrophilicity towards nucleophillic attack by NH_2 of guanine which is consistent with an acid-catalysis mechanism. Carbinolamine and carbinolamine methyl ether forms, prior to alkylation can undergo reversible elimination of water or MeOH to form the iminium species.

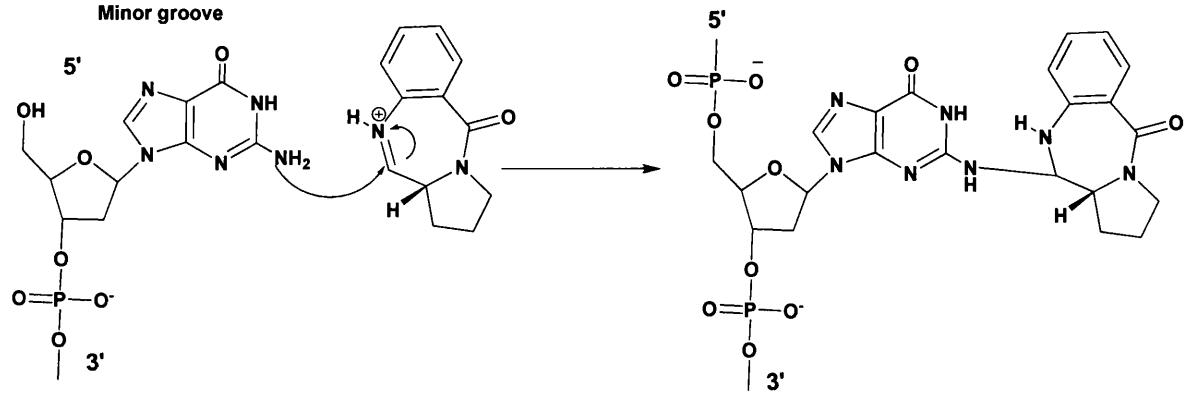


Figure 1.3a: Reaction of PBDs with DNA via iminium based mechanism of alkylation.

NMR experiments and molecular mechanics calculations have confirmed that the orientation of the PBD bound to DNA in the minor groove, is such that the aromatic A-ring is pointing towards the 3'end of the strand to which the drug is covalently bound (Kopka *et al.*, 1994). The (*S*)-conformation at the C11a of the PBD molecule gives the tricyclic structure a right handed twist which makes it fit more snugly into the minor groove of a right handed DNA helix. An (*R*)-configuration at C11a would make the PBD molecule twist in the opposite way (Kopka *et al.*, 1994) and would clash with the walls of minor groove of a right handed helix resulting in poor DNA binding and biological activity. Significantly, a synthetic PBD with an (*R*)-configuration at C11a was shown to be devoid of both DNA binding affinity and *in vivo* cytotoxicity (Hurley and Boyd, 1998).

1.3.2 Sequence Selectivity

DNA footprinting assays and NMR experiments have demonstrated that PBDs bind to DNA in a sequence selective manner, spanning three base pairs with a strong preference for 5'-Pu-G-Pu-3' motifs (Pu = Purine, G = Guanine and Py = Pyrimidine) with a rank order of Pu-G-Pu > Pu-G-Py ~ Py-G-Pu >Py-G-Py. This sequence selectivity for PBDs covalently bound to guanine flank by purine bases arises from base stacking and the inherently small twist at purine-purine steps in DNA (Kopka *et al.*, 1994).

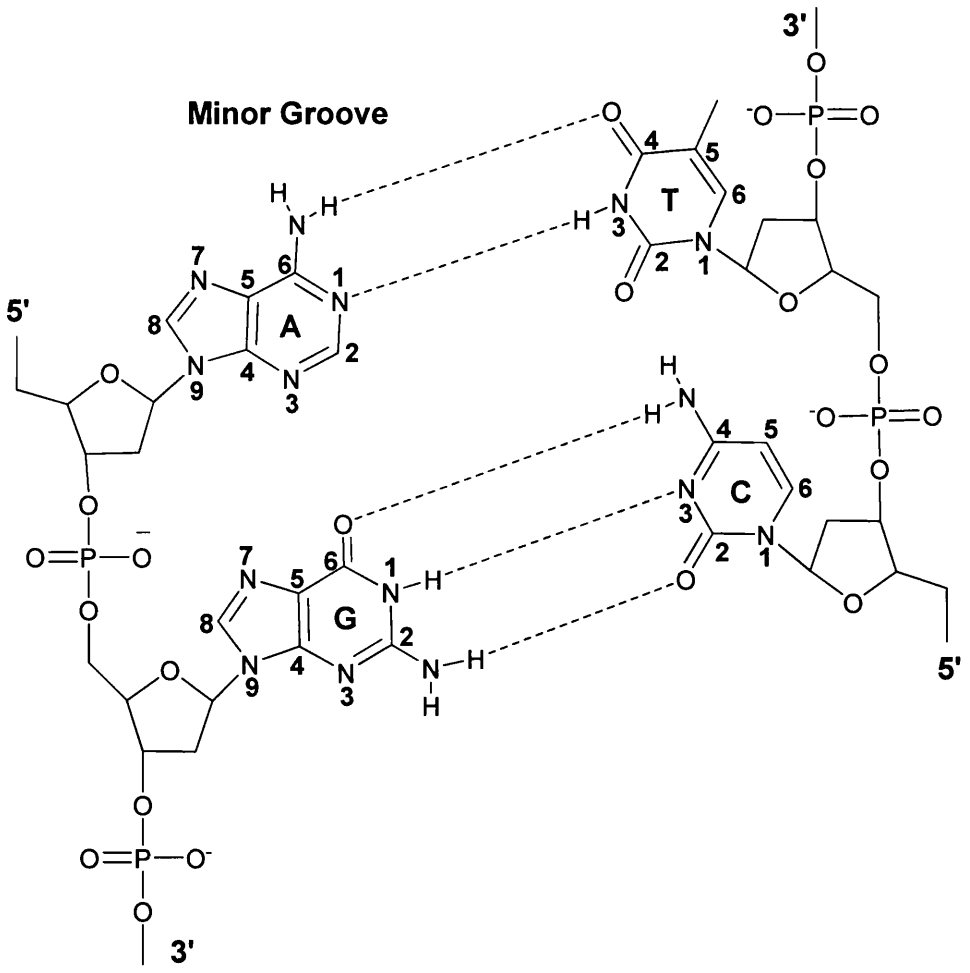


Figure 1.3b: Structure of DNA bases with thier unique hydrogen bonding pairs.

A model has been proposed to rationalise the sequence selectivity of PBDs for 5'-PuGA-3' as shown in **Figure 1.3c**. It shows additional stabilization by hydrogen bonding between the N10-H and the 3'-adenine N3 with the preferred orientation of the PBD A-ring pointing towards the 3' end of the alkylated modified DNA strand. It has been shown (Kopka *et al.*, 1994) that hydrogen bonding may not be responsible for sequence selectivity, but may be important in further stabilizing the PBD-DNA complex and increasing its DNA binding affinity and biological activity. For example, anthramycin is stabilized by hydrogen bonds to DNA from the C9-OH and the NH_2 on the acrylamide side chain but compared with tomaymycin the OH group is moved to the C8 position where it cannot interact with DNA and the acrylamide tail is absent which forms hydrogen bonds. Therefore one would expect anthramycin to bind more snugly to DNA than tomaymycin, which it does (Kopka *et al.*, 1994; Hurley and Boyd, 1988).

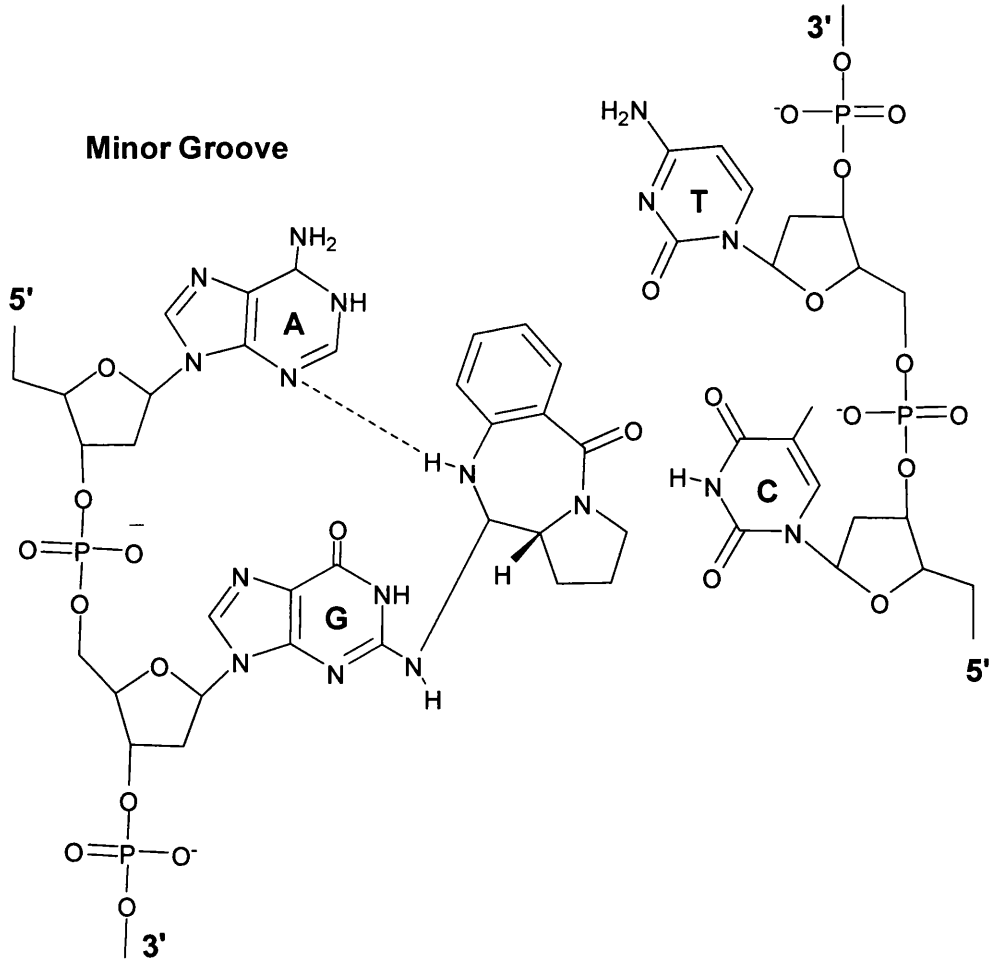


Figure 1.3c: Proposed model to rationalise the sequence selectivity of PBDs for 5'-PuGA-3'.

This PBD-DNA adduct has been demonstrated to inhibit both endonuclease activity (Puvvada *et al.*, 1993) and *in vivo* transcription (Puvvada *et al.*, 1997), presumably as a result of the PBD covalently binding DNA, thus preventing transcription and replication of DNA and RNA which ultimately leads to cell death (Kohn, 1975). In addition, persistent excision-dependent single and double strands breaks were shown to occur in cellular DNA, however failure to remove the PBD adduct is more serious event then formation of persistent single and double strand breaks (Petrusek *et al.*, 1982). Finally Tang *et al.* have studied the role of the uvrA, uvrB and uvrC proteins in the repair of PBD-DNA adducts and it was concluded both uvrA and uvrB proteins work together to excise the PBD-guanine adduct, thus producing lethal double strands breaks (Tang *et al.*, 1991).

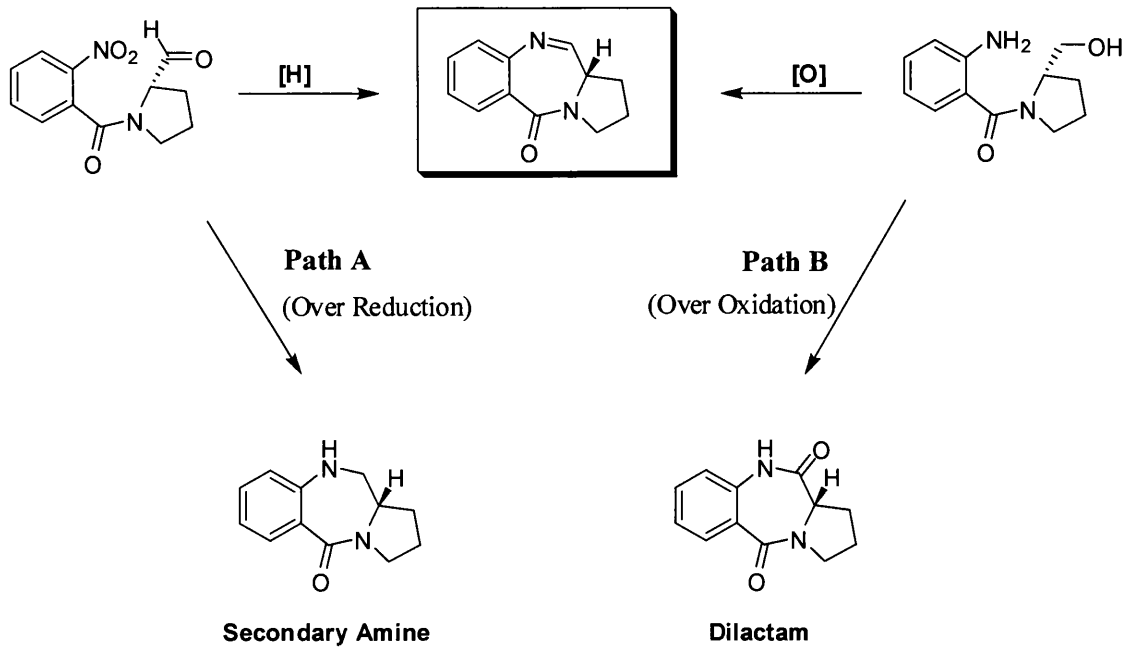
It has been established that a number of oncogenes, including c-Ha-ras contain highly GC-rich areas (Mattes *et al.*, 1988), and many known DNA cross-linking agents with significant antitumour activity bind selectivity to GC-rich regions, which may contribute to their potency. On this basis, it is believed that PBDs may prove successful drug candidates for cancer chemotheraphy as they are GC-specific and covalently bind to DNA, which may explain their potent *in vivo* antitumour activity.

1.4 Synthesis of PBDs

1.4.1 Challenges in PBD Synthesis

The synthesis of PBDs can be challenging, as introducing an imine or carbinolamine in at the N10-C11 position is complicated by the lability of these functional groups under numerous synthetic conditions (Thurston and Bose, 1994). Straightforward reduction/oxidation of the appropriate precursor (**Scheme 1.4a**) to PBD imines is very difficult as this often leads to secondary amines (**path A**) or dilactams (**path B**). For these reasons the formation of the sensitive N10-C11 imine functionality is usually generated during the final synthetic step under the mildest possible conditions.

In addition, reactions conditions capable of causing racemization at the C11a position must be avoided in order to maintain the correct 3-dimensional shape of the molecule, which is essential for biological activity. A further challenge to PBD synthesis is introduction of reactive substituents to the molecule, in particular unsaturated side chains to the C-ring.

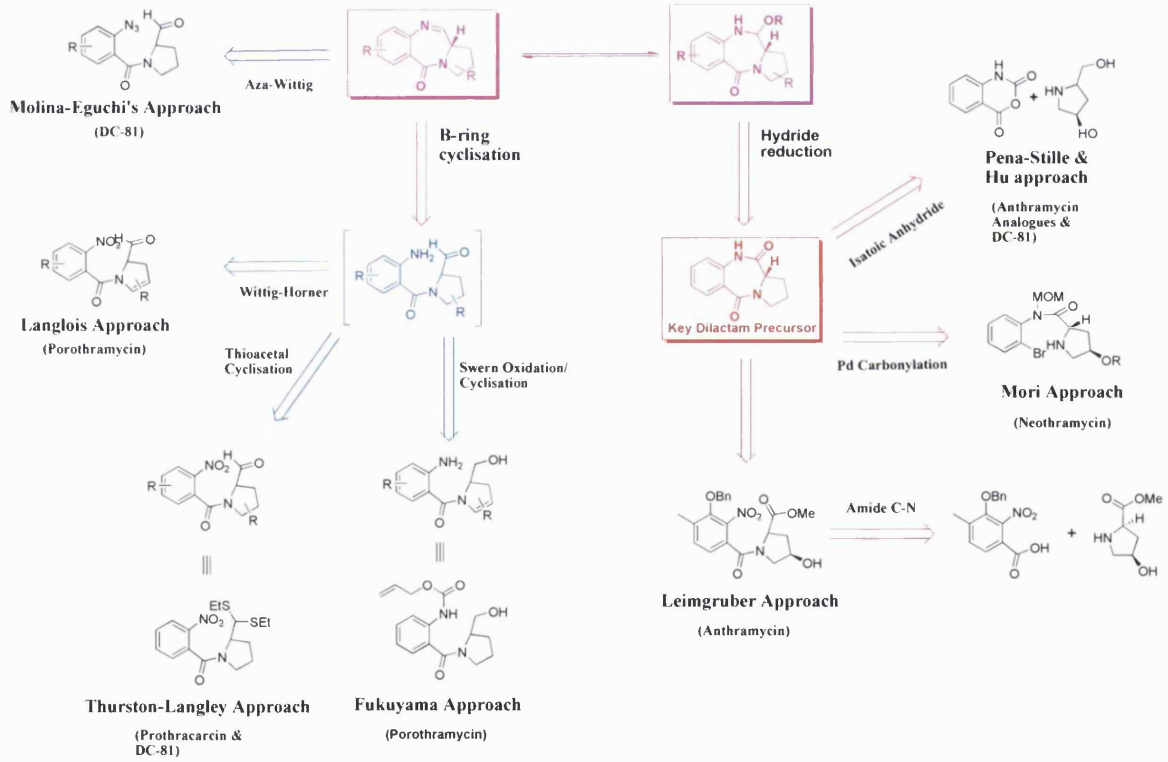


Scheme 1.4a: Reduction/Oxidation methods to form N10-C11 imine containing PBDs.

1.4.2 Retrosynthetic Analysis

Since the first total synthesis of anthramycin was reported in 1968, there have been many synthetic approaches that have been developed and published, but only a few have shown sufficient versatility and efficiency. In this section, some of the most important synthetic routes to PBDs will be reviewed below.

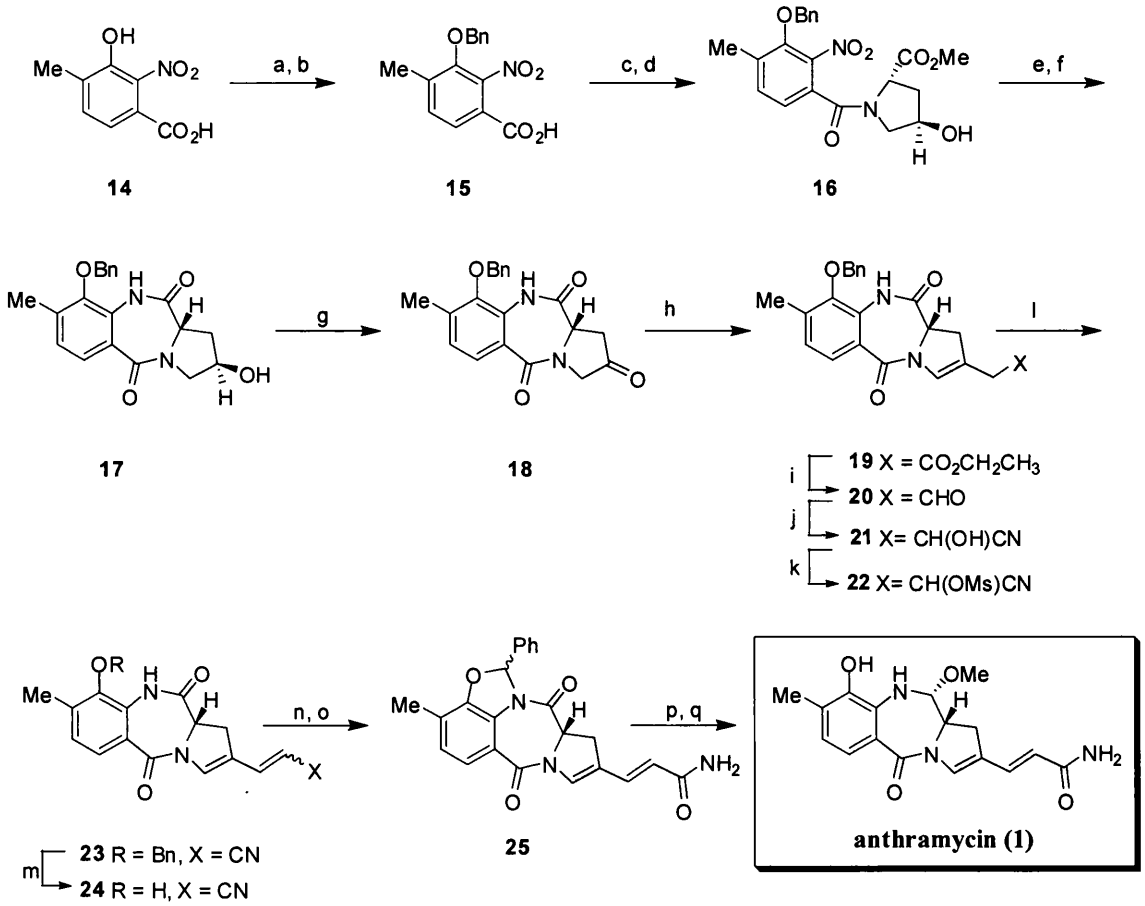
The retrosynthetic analysis shown below **(Scheme 1.4b)** identifies the most important strategies for PBD synthesis. N10-C11 bond was identified as the most suitable initial disconnection by many authors as PBD imines can be converted to the equivalent carbinolamines via the dynamic equilibrium that exists between them. The retrosynthetic analysis is divided into two major strategies. One strategy shown in red involves hydride reduction of dilactams to N10-C11 imime/carbinolaime moieties (Leimgruber, Peña-Stille, Mori and Hu approaches) the other shown in blue involves B-ring cyclization methods to generate the N10-C11 moiety (Thurston, Fukuyama, Langlois and Molina-Eguchi approaches). Some of the above strategies use elaborate methods to introduce unsaturation in the C-ring, such as palladium-catalysed Heck and Suzuki couplings and Wittig olefinations.



Scheme 1.4b: Retrosynthetic analysis of the most important synthetic strategies to PBD synthesis.

1.4.3 The Leimgruber Approach

In 1968, Leimgruber *et al.* reported the total synthesis of anthramycin **Scheme 1.4c**. The key step was the sodium borohydride mediated reduction of the corresponding O9,N10-benzal protected pyrrolo[2,1-*c*][1,4]benzodiazepines-5,11-dione (or dilactam) to the carbinolamine (Leimgruber *et al.*, 1968).



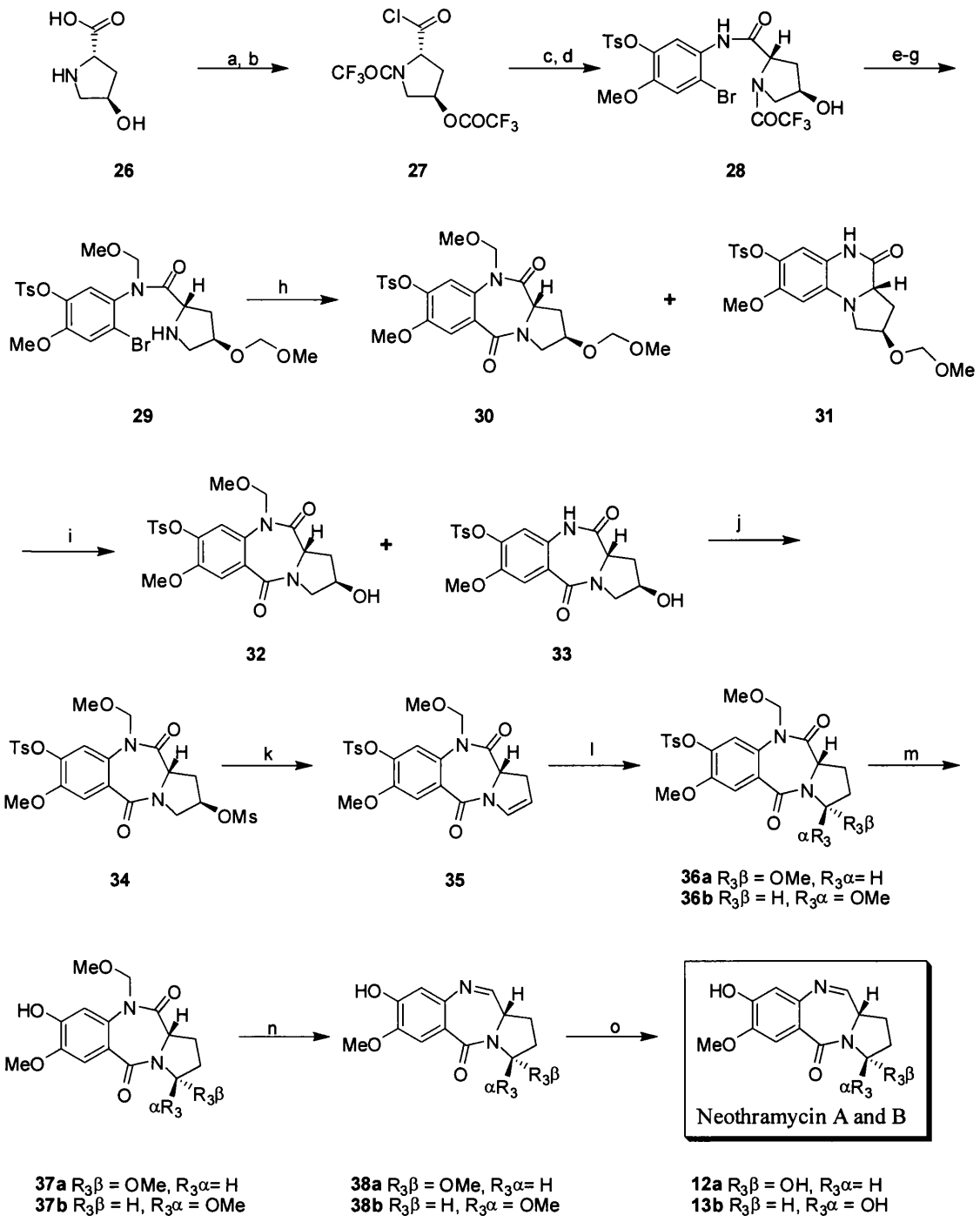
Scheme 1.4c: Leimgruber's total synthesis of anthramycin

a) K_2CO_3, DMF, $PhCH_2Br$; **b**) KOH, H_2O, THF; **c**) $(COCl)_2$, CH_2Cl_2; **d**) methyl-*L*-hydroxyproline hydrochloride, THF, TEA; **e**) $Na_2S_2O_4$, THF, water; **f**) H_2O/HCl, THF; **g**) CrO_3/H_2SO_4/Acetone; **h**) sodium triethylphosphonoacetate, THF; **i**) DIBAL, toluene, – 60 °C, **j**) 1. $NaHSO_3$. 2. KCN; **k**) MsCl, pyridine, 5 °C; **l**) TEA, benzene, reflux; **m**) TFA, $BF_3.Et_2O$, r.t., HCl, MeOH; **n**) $PhCH(OMe)_2$, H_2O/HCl, THF; **o**) PPA (aqueous workup); **p**) $NaBH_4$, MeOH; **q**) 0.01 M HCl, MeOH.

The amide **16** was prepared by coupling of the acid chloride of **15** with the amino acid derivative L-hydroxyproline methyl ester in the presence of triethylamine base. Reduction of the nitro group with sodium dithionate produced an aniline, which on treatment with aqueous hydrochloric acid cyclised to the dilactam **17**. Following Jones oxidation, the ketone **18** was subjected to the Wadsworth-Emmons Olefination, providing the β,α-unsaturated ester **19** in 74% yield. Reduction of the ester with diisobutylaluminium hydride (DIBAL-H) in toluene afforded the labile aldehyde **20**, which converted *in situ* to the sodium bisulphite adduct. Reaction with potassium cyanide then produced the mixture of epimeric cyanohydrins **21**. The epimeric mesylate **22** was prepared by treatment with methanesulphonyl chloride and was then subjected to elimination in the presence of triethylamine to afford a *trans* mixture of conjugated nitriles **23** in a ratio 4:1. The benzyl group was removed using TFA in the presence of boron trifluoride etherate to produce the phenol **24** which was converted to the benzal derivative by condensation with benzaldehyde dimethylacetal followed by hydrolysis to the amide **25** with hot polyphosphoric acid. The dilactam was reduced to the protected carbinolamine with sodium borohydride in methanol, and the benzal protecting group was cleaved with aqueous HCl in methanol, to provide anthramycin methyl ether **1** which was identical to an authentic sample of the natural product.

1.4.4 The Mori Approach

In 1986, Mori *et al.* reported the total synthesis of neothramycin (**Scheme 1.4d**) via palladium catalyzed carbonylation (Mori *et al.*, 1986a). The same author later reported the total synthesis of *E*- and *Z*- isomers of the tomaymycin natural product by successfully applying the same methodology (Mori *et al.*, 1986b).



Scheme 1.4d: Mori's total synthesis of neothramycin A and B.

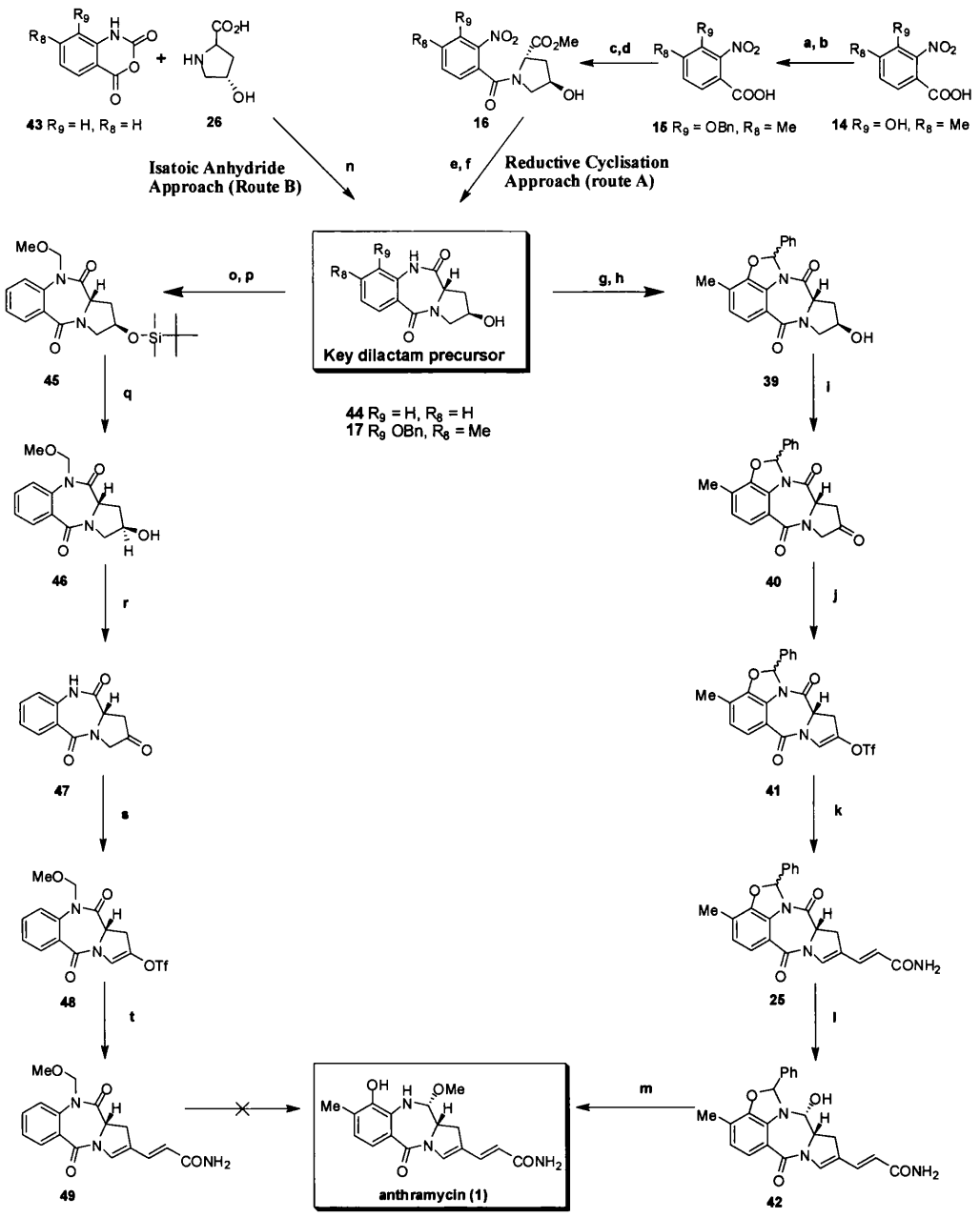
a)$(CF_3CO)_2$; **b**) PCl_5; **c**)2-bromo-4-methoxy-5-tosyloxy-aniline; **d**) aq.$NaHSO_3$; **e**) MOM-Cl, i-Pr_2NEt; **f**) MOM-Cl, NaH; **g**) NH_3-MeOH; **h**) $Pd(PPh_3)_4$, CO (10 atm.), *n*-Bu_3N, $PhCH_3$; i) aq.HCl, MeOH; **j**) MsCl; **k**) DBU; **l**) MeOH, CSA, 50 °C, 4 days; **m**) aq. KOH; **n**) 1. $NaBH_4$ 2. Silica gel; **o**) 0.01 M HCl, dioxane, 0 °C, 20 min.

The amide **28** was prepared by coupling the acid chloride of *N*-trifluoroacetyl-protected *trans*-hydroxy-L-proline **27** to the aniline. Methoxymethylation of both the amide and the hydroxy-functionalities were achieved followed by cleavage of the *N*-trifluoroacetyl group to furnish the secondary amine **29**. Insertion of carbon monoxide under 10 atm pressure in the presence of $Pd(PPh_3)_4$ resulted in cyclisation to the dilactam **30** in 69% yield. In addition, formation of the quinoxaline derivative **31** was observed in 23% yield. Removal of the *O*-methoxymethyl group was achieved using aqueous HCl in methanol to give the free secondary alcohol **32** in 68% yield. The inevitable loss of both MOM groups was observed in the reaction to produce **33** in 27% yield. Olefin **35** was prepared in 90% yield by methanesulphonation of compound **33** followed by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Methoxylated compounds (**36a**) and (**36b**) (ratio 1:1) were prepared by allowing olefin **35** to stand in methanol for several days at 50 °C with catalytic amounts of camphorsulphonic acid (CSA). Hydrolysis of the tosyl group of (**36a**) and (**36b**) furnished (**37a**) and (**37b**) in quantitative yield. Selective reduction of the amide group at the C11 position was achieved by reaction with $LiAlH_4$, followed by treatment with silica gel. The melting points and optical activity of the resulting methyl neothramycin **38a** and **38b** compared favorably with those in the literature. Neothramycin A (**12a**) and (**13b**) B were obtained by treating the corresponding methyl neothramycin with 0.01 M HCl

1.4.5 The Peña-Stille Approach

In 1987, Peña and Stille reported the attachment of the anthramycin acrylamide side chain by the palladium-catalysed coupling reaction of vinyl triflates (Peña and Stille 1987). The dilactam precursor (**Scheme 1.4e, Route B**) was formed by condensation with an isatoic anhydride with *L*-hydroxyproline. Interestingly, the authors did not report the conversion of the N10-MOM protected dilactam to the carbinolamine methyl ether to produce anthramycin.

However, in 1989, the same workers (Peña and Stille 1989) applied similar methodology to a total synthesis of anthramycin except they employed the procedure previously reported by Leimgruber to generate the key dilactam precursor by reductive cyclization of the nitro-ester amide and the selective hydride reduction of O9, N10-benzal protected dilactam to give the anthramycin methyl ether (**Scheme 1.4e, Route A**).



Scheme 1.4e: Peña and Stille total synthesis of anthramycin

Reductive cyclisation route (A): **a**) K_2CO_3, DMF, $PhCH_2Br$; **b**) KOH, H_2O, THF; **c**) $(COCl)_2$, CH_2Cl_2; **d**) methyl-L-hydroxyproline hydrochloride, THF, TEA; **e**) $Na_2S_2O_4$, THF, water; **f**) H_2O/HCl, THF; **g**) Pd/C, H_2, MeOH; **h**) $PhCH(OMe)_2$, H_2O/HCl, THF; **i**) DMSO, $(COCl)_2$, TEA, CH_2Cl_2; **j**) Pyridine, Tf_2O; **k**) CH_2=$CHCONH_2$, $(CH_3CN)_3PdCl_2$, DABCO, MeOH; **l**) $NaBH_4$, MeOH; **m**) 0.01 M HCl, MeOH. **Isatoic anhydride route (B)**: **n**) DMSO, 115-120 °C; **o**) TBDMSCl, imidazole, DMF; **p**) NaH, THF, CH_3OCH_2Cl; **q**) BF_3.OEt, THF, H_2O; **r**) $(COCl)_2$, CH_2Cl_2, TEA; **s**) Pyridine, Tf_2O; **t**) CH_2=$CHCONH_2$, $(CH_3CN)_2PdCl_2$, DABCO, MeOH;

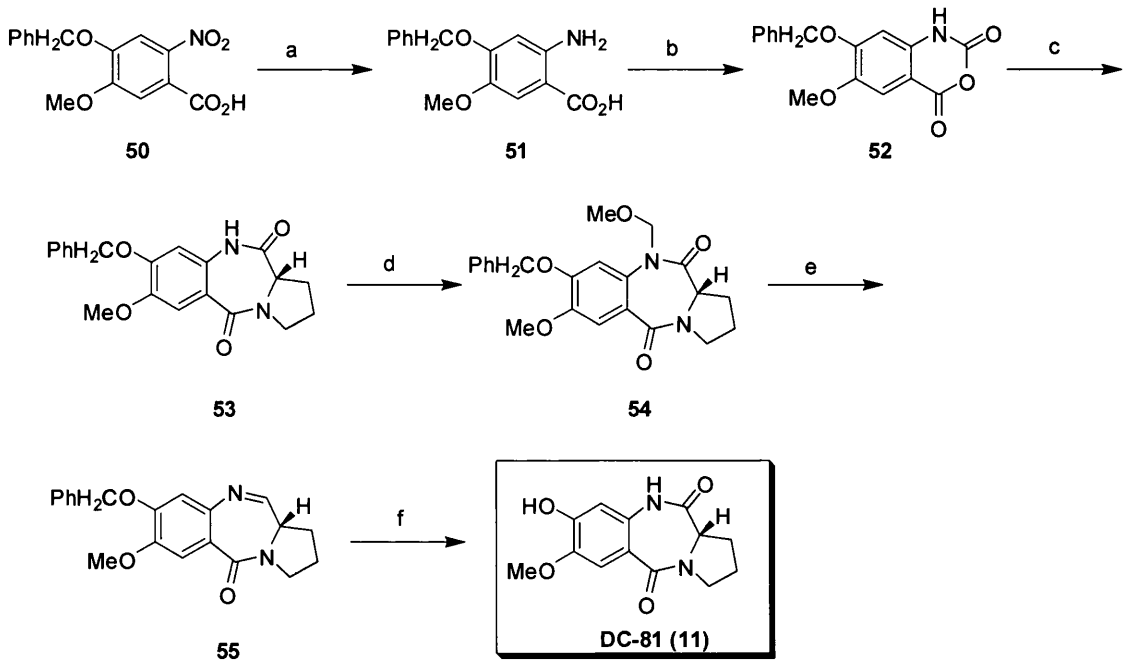
Following route B (**Scheme 1.4e, Route B**), the dilactam precursor **44** was prepared from the condensation with *N*-methyl isatoic anhydride **43** with L-hydroxyproline (**26**). The C2-hydroxy group was protected as TBS ether followed by methoxymethylation of the N10 amide functionality under strong basic conditions to provide **45**. The C2-TBS ether was removed with TBAF in quantitative yield. Swern oxidation provided the ketone **47** in 60% yield which was converted to the vinyl triflate using pyridine and triflic anhydride (**48**) in 70% yield. The vinyl triflate **48** underwent heck-type coupling reactions with acrylamide stannanes to provide anthramycin precursor **49** in 35% yield.

Following route A (**Scheme 1.4e, Route A**), the dilactam precursor **17** was synthesized using the same procedure as previously reported by Leimgruber. Following hydrogenolysis of the benzyl group, both the phenol and the amide nitrogen was simultaneously protected by reaction of the free phenol with benzaldehyde dimethyl acetal to provide **39.** Swern oxidation of the dilactam alcohol afforded the ketone **40**, which was then converted to the vinyl triflate **41**. The acrylamide side chain was attached via palladium catalyzed Heck-coupling with the vinyl triflate to provide the diene **25**. Selective reduction of the dilactam **25** with sodium borohydride provided the carbinolamine **42**. The stereochemistry of **42** was assigned on the basis of an observed lack of ^{1}H NMR coupling between protons at C11 and C11a, the dihedral angel between the protons being ~90 °C. Cleavage of the benzal protecting group was achieved by using alcoholic hydrochloric acid to give anthramycin methyl ether (**1**), with identical ^{1}H NMR spectrum to that of the natural product.

1.4.6 The Hu Approach

In 2001, Hu *et al.* reported a very short and efficient synthesis (**Scheme 1.4f**) of the PBD analogue, DC81 (Hu *et al.*, 2001). The synthetic approach used a key dilactam precursor which was made from an isatoic anhydride route, with the critical step of the synthesis being hydride reduction of MOM-protected dilactam to the PBD imine (first reported by Mori *et al.*, 1986a).

The amine **51** was prepared in 92% yield from reduction of a substituted 2-nitrobenzoic acid **50** with stannous chloride. Reaction of the resulting amine **51** with triphosgene under reflux generated the isatoic anhydride **52** in 98% yield. The key dilactam **53** was prepared from the coupling of L-proline to the anhydride **52** in DMSO at 120 °C, followed by methoxymethylation of the N10 amide functionality **54** in high yield. The key step in this synthesis was the hydride reduction of MOM-protected dilactam **54**, which was achieved by treating the dilactam **54** with $LiBH_4$ for 9 h to furnish the benzyl protected DC-81 PBD imine **55**. Finally the benzyl group was cleaved by treatment with 10% Pd-C in cyclohexadiene to afford DC-81 **11** in 90% yield.



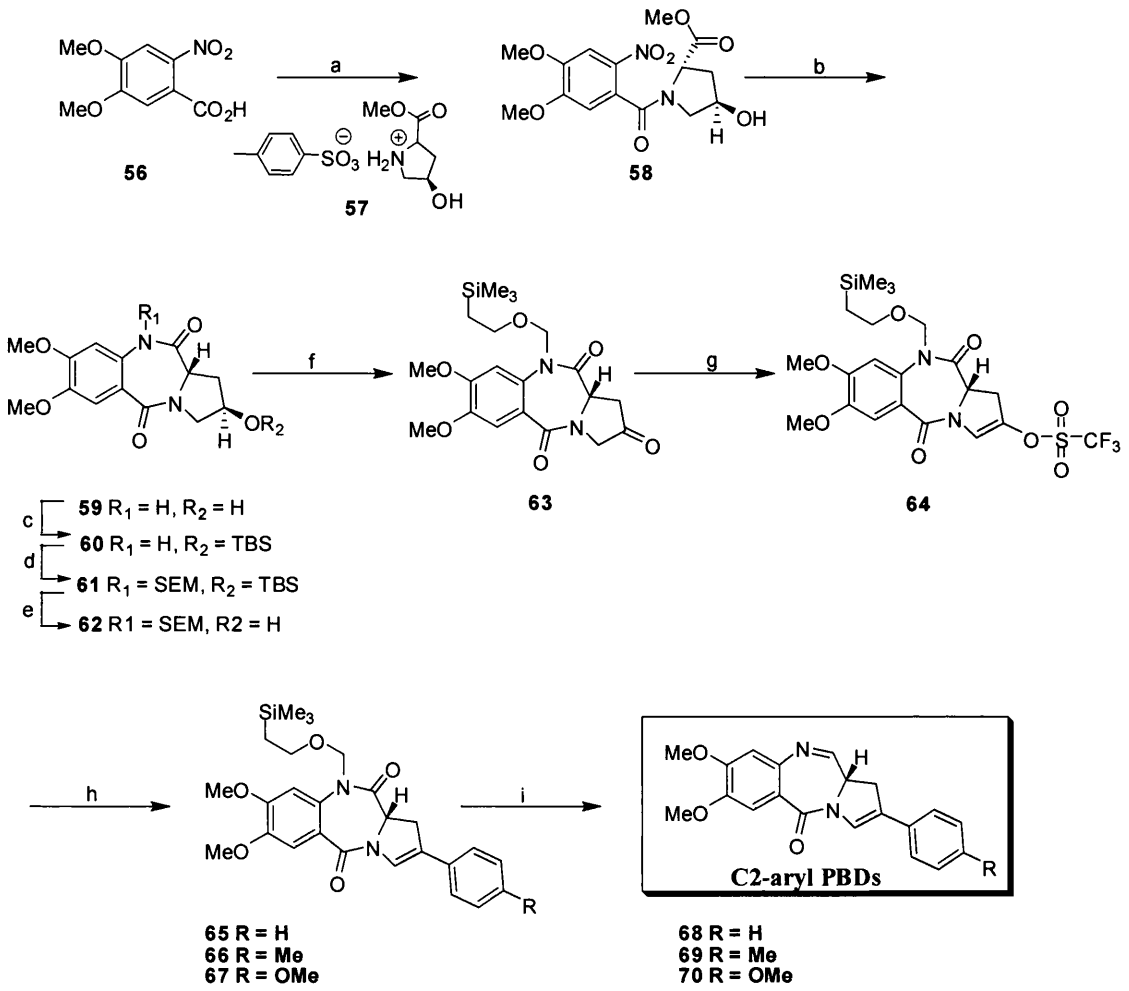
Scheme 1.4f: Hu's total synthesis of DC-81.

a) $SnCl_2.2H_2O$, MeOH; **b)** Triphosgene, THF, Δ; **c)** L-proline, DMSO, 120 °C; **d)** NaH, THF, CH_3OCH_2Cl; **e)** $LiBH_4$, THF; **f)** 10% Pd/C cyclohexadiene.

1.4.7 The Howard-Thurston Approach

In 2002, N. Cooper *et al.* reported the synthesis of C2-aryl substituted PBDs (Cooper *et al.*, 2002). Although the synthetic strategy was initially based on the classic Leimgruber approach, the novel use of a hemiaminal protecting group (N10-SEM) was used to promote the C11-lactam reduction which was analogous to the Mori and coworkers. In addition, the novel application of the Suzuki reaction was used to introduce aryl substituents at the C2 position of PBDs **(Scheme 1.4g)**.

The A-C-ring backbone **58** was prepared from the coupling of the pre-formed C-ring **57** to the commercially available 6-nitroaveratric acid (**56**). Following reductive/cyclisation by catalytic hydrogenation to give the dilactam **59**, the C2-alcohol was protected as a TBS ether **60**. The N10 position was SEM protected with SEM-Cl under basic conditions to provide **61**, which was then followed with the removal of the TBS ether at the C2 position with TBAF at room temperature without affecting the N10-SEM protecting group to provide the alcohol **62**. Swern oxidation furnished the ketone **63**, which was converted to the C2-C3 enol-trilfate **64** using trifluoromethanesulfonic anhydride in the presence of pyridine. Three different Suzuki reactions were performed on separate batches of enol triflate **64** using commercially available boronic acids, resulting in the synthesis if **65-67**. Reduction of the SEM-protected dilactams using sodium borohydride provided the three novel C2-C3 *endo* unsaturated C2-aryl PBDs **68-70**.



Scheme 1.4g: Howard-Thurston's synthesis of C2-aryl C2-C3 unsaturated PBDs.

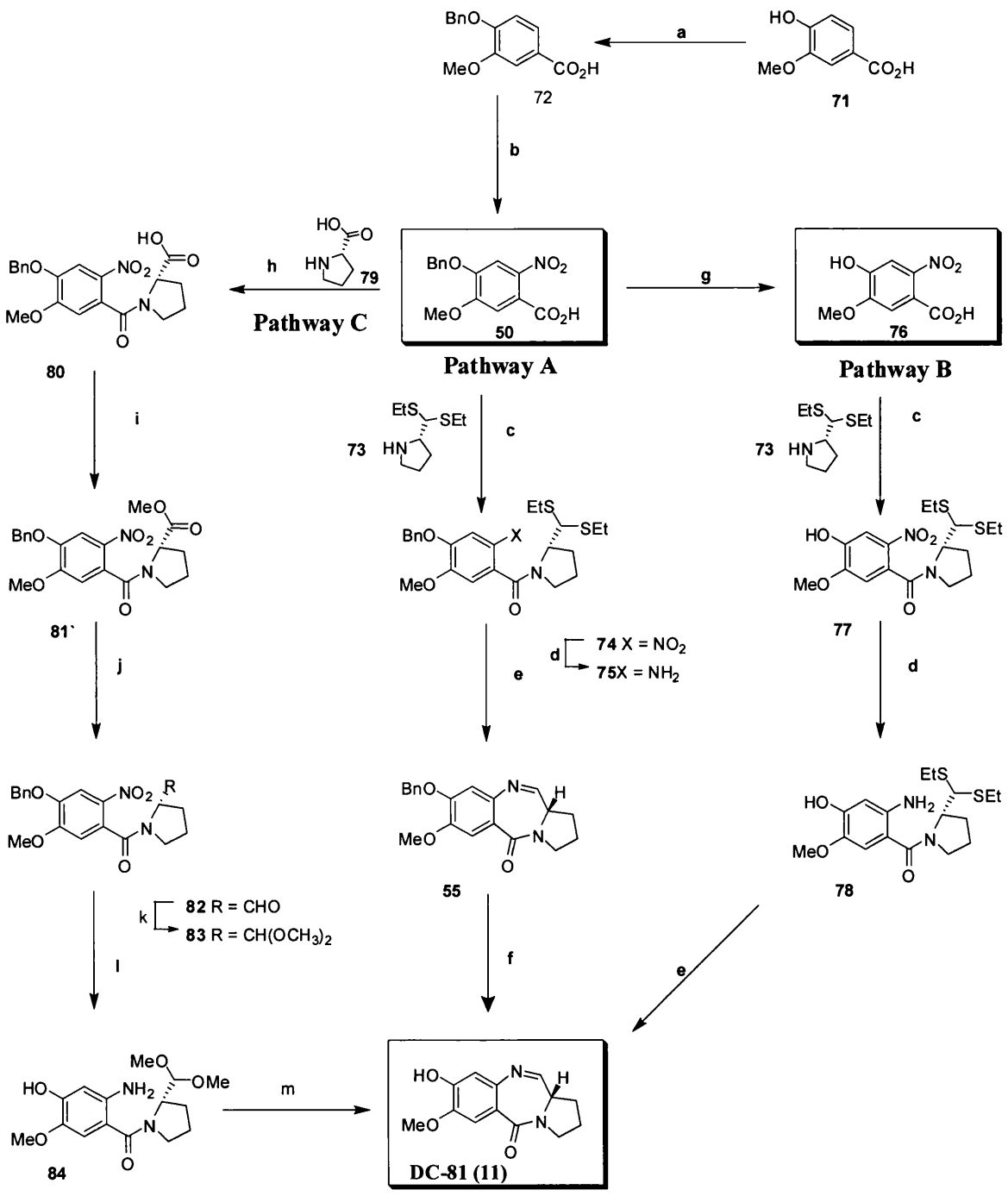
a) $(COCl)_2$, DMF, TEA, CH_2Cl_2, 57%; **b)** 10% Pd/C, H_2, EtOH, 75%; **c)** TBS-Cl, imidazole, DMF, 95%; **d)** SEM-Cl, NaH, DMF, 0 °C, 77%; **e)** TBAF, THF, 70%; **f)** $(COCl)_2$, DMSO, TEA, CH_2Cl_2, 0 °C, 49%; **g)** triflic anhydride, pyridine, CH_2Cl_2, 60%; **h)** $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, EtOH, benzene, 78% (**65**); $MePhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, EtOH, benzene, 66% (**66**); $MeOPhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, EtOH, benzene, 90% (67); **i)** $NaBH_4$, Silica gel, EtOH, THF, 74% (**68**), 38% (**69**), 72% (**70**).

1.4.8 The Thurston-Langley Approach

In 1987, Langley and Thurston reported the use of mercuric chloride mediated cyclisation of *N*-(2-aminobenzoyl)pyrrolidine-2-carboxaldehyde diethyl thioacetal for the total synthesis of prothracarcin (Langley and Thurston, 1987).

In 1990, Thurston and co-workers applied the same mercuric chloride mediated cyclisaton of amino dithioacetals procedure to synthesise DC-81 (Thurston *et al.*, 1990a). The final synthetic step in the method required a final deprotection of the *O*-benzyl protected DC-81 in the presence of cyclohexadiene and Pd-C (**Scheme 1.4h, Pathway A**). This step represented a major drawback in the synthesis as this step was preformed in the presence of the very sensitive N10-C11 imine; as a result, a secondary amine was obtained as a by-product. In 1992, the same group (Bose *et al.*, 1992a) reported two other pathways to DC-81 (**Scheme 1.4h, Pathway B & C**) showing that 9-OBn deprotection can be carried out prior to B-ring cyclisation thus avoiding the formation of undesirable secondary amine by product.

The synthesis began with vanillic acid (**71**), which was benzylated to afford 4-benzyloxy-3-methoxybenzoic acid (**72**) in 76% yield. The tin (IV) chloride mediated nitration proceeded smoothly to provide the nitro-acid **50** in 83% yield. Starting from this common intermediate **50,** the amide **74** (**Scheme 1.4h, pathway A**) was prepared in 68% yield from the coupling of pyrrolidine-2-carbaldehyde diethyl thioacetal (**73**), prepared from L-proline via a literature method to the acid chloride of **50**. The amine **75** was prepared by reduction of the nitro group with stannous chloride, cleavage of the thioacetal with mercuric chloride furnished the benzyl-protected DC-81 (**55**) in 68% yield. Deprotection of the *O*-benzyl group occurred in the presence of 10% Pd-C in cyclohexadiene to afford DC-81 (**11**) in 89% yield. ^{1}H NMR spectra of the debenzylated product was identical to the natural product DC-81.



Scheme 1.4h: Thurston-Langley's synthesis of DC-81 via three reaction pathway **(A, B and C).**

a) $PhCH_2Cl$, THF, NaOH, H_2O, Δ; **b**) $SnCl_4$, HNO_3, CH_2Cl_2, –25 °C; **c**) 1. $(COCl)_2$, THF; 2. THF, TEA, H_2O; **d**) $SnCl_2.H_2O$, MeOH, Δ; **e**) $HgCl_2$, $CaCO_3$, CH_3CN, H_2O; **f**) 10% Pd-C, cyclohexadiene, EtOH, 20 °C; **g**) BF_3.OEt-EtSH; **h**) $(COCl)_2$, THF; **i**) $(COCl)_2$, MeOH; **j**) DIBAL-H, $PhCH_3$, 20 °C **k**) $(CH_3O)_3CH$. Dowex 50X resin, CH_2Cl_2; **l**) 10% Pd-C, H_2, EtOH; m) Amberlite IR-120(H^+), CH_3CN, H_2O.

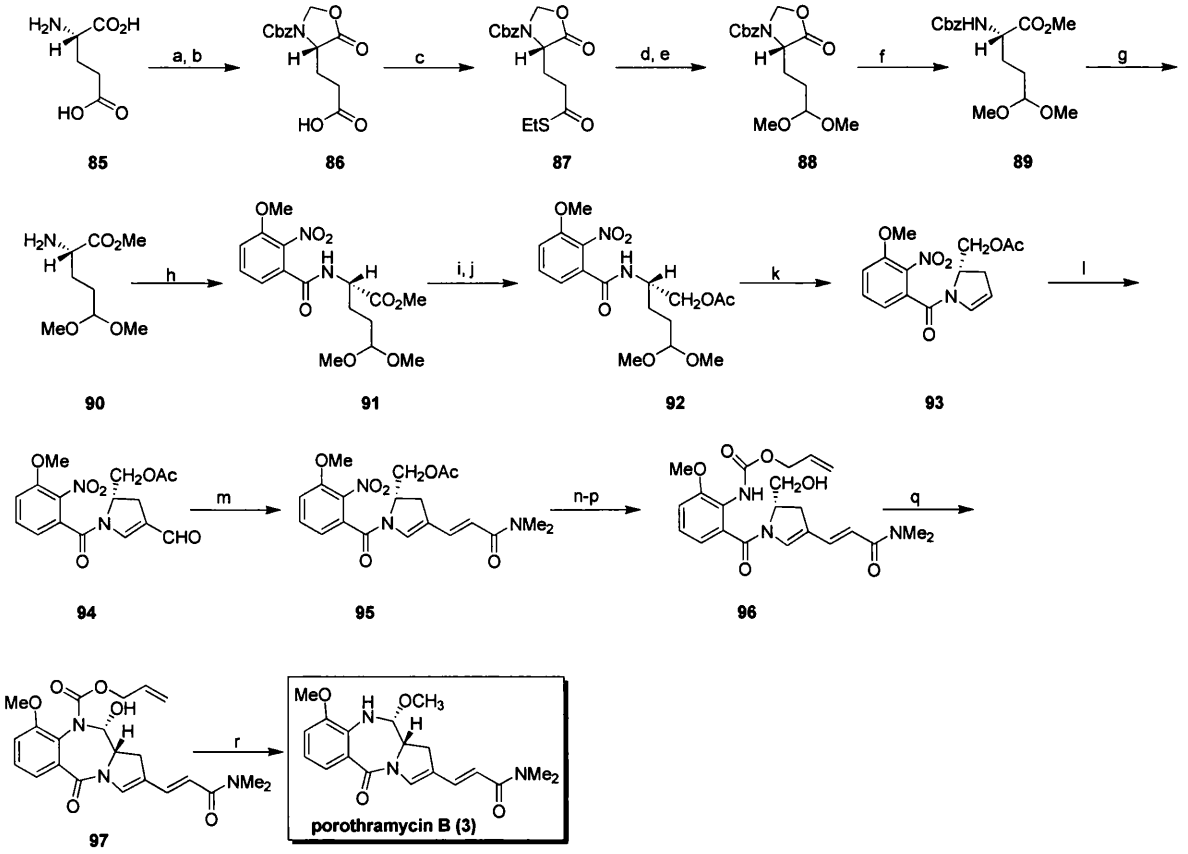
In pathway B (**Scheme 1.4h**) *O*-debenzylation of the nitro-benzoic acid **50** was achieved using $BF_3.OEt_2$-EtSH to provide **76** in 90% yield. The amino thioacetal **77** was synthesized using the same procedure as followed in **pathway A**, which cyclised upon treatment with mercuric chloride in CH_3/H_2O to afford DC-81 (**11**) in 80% yield.

In pathway C (**Scheme 1.4h**), the common intermediate **50** was coupled to L-proline (**79**) via the acid chloride to afford amide **80,** which was esterified to give **81** in 95% yield. The aldehyde **82** was prepared by reduction of the ester **81** with DIBAL-H, which was protected as the dimethyl oxygen acetal **83**. Reduction of the nitro group and simultaneous deprotection of the benzyl ether by catalytic hydrogenation afforded **84** in 52% yield. Removal of the oxygen acetal functionality with acidic amberlite resin caused spontaneous cyclisation to DC-81 (**11**) in 47% yield. DC-81 prepared via this route appeared to be racemic, thus affecting the DNA binding potential.

1.4.9 The Fukuyama Approach

In 1993, Fukuyama *et al.* reported the first total synthesis of porothramycin B via a novel cyclisation technique based on the oxidation and deprotection of an *N*-(allyloxycarbonyl)-protected amino alcohol (Fukuyma *et al.*, 1993). L-Glutamic amino acid **85** was converted to the known oxazolidine **87** via benzyl carbamate *N*-protection followed by reaction with paraformaldehyde (**Scheme 1.4i**). The free carboxylic acid **86** was converted to the thioethyl ester **87** in 87% yield according to Steglich's procedure (Neises and Steglich, 1978). The thiol ester was reduced to the aldehyde upon treatment with triethylsilane, which was then immediately protected as the dimethylacetal **88**. Subsequent treatment with sodium methoxide provided the *N*-Cbz-amino ester **89** in 70% yield. Removal of the benzyl carbamate group by hydrogenolysis, provided the amino ester **90** in quantitative yield. The amide **91** was obtained in 95% yield by coupling with 3-methoxy-2-nitrobenzoyl chloride to amine **90**. The ester group was then reduced to the primary alcohol and acetylated to afford **92**. The enamide **93** was prepared by subjecting the acetal **92** to a facile cyclisation-elimination reaction by treatment with quinolinium camphorshulphonate (QCS), followed by Vilsmeier-Haack formylation reaction to provide aldehyde **94**. After acetylation of the partially deacetylated alcohol, the introduction of the *N*-*N*-dimethylacrylamide C2-side chain was achieved by employing the Wittig reaction with a stablised ylid to give the conjugated *trans*-amide **95** in 74% yield. Reduction of the nitro group (zinc/acetic acid/CH_2Cl_2), hydrolysis of the acetate functionality

and protection of the aniline as the allyl carbamate furnished **96**. Swern oxidation of **96** resulted in spontaneous cyclisation in a stereospecific manner to afford protected porothramycin A in 72% yield (**97**), which was converted to the unstable porothramycin A when treated with $Pd(Ph_3)_4$/pyrrolidine according to Deiziel's procedure (Deiziel, 1987). Crystallisation from EtOAc/MeOH provided pure porothramycin B (**3**): the structure was confirmed by comparison of ^1H NMR spectra to the natural product porothramycin B.



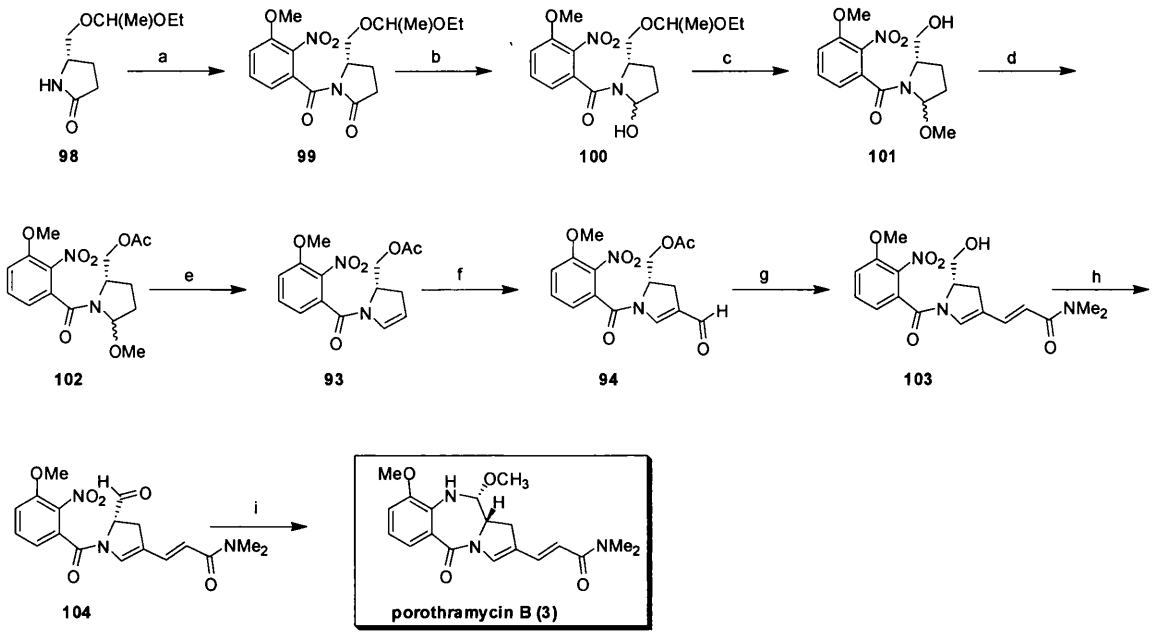
Scheme 1.4i: Fukuyama's total synthesis of porothramycin B.

a) CBzCl, NaOH; **b**) $(CH_2O)_n$, *p*-TsOH, benzene; **c**) EtSH, DCC, DMAP, CH_3CN; **d**) Et_3SiH, 10% Pd-C, acetone; **e**) CSA, $CH(OMe)_3$, MeOH; **f**) NaOMe, MeOH; **g**) H_2, 10% Pd-C, EtOH; **h**) 3-methoxy-2-nitrobenzoyl chloride, satd. $NaHCO_3$, CH_2Cl_2; **i**) $LiBH_4$, THF, $LiBEt_3H$. **j**) Ac_2O, pyridine. **k**) CSA, quinoline, benzene; **l**) 1. $POCl_3$, DMF; 2. NaOAc, H_2O; **m**) $Ph_3P{=}CHCON(Me)_2$, benzene; **n**) Zn, AcOH, CH_2Cl_2; **o**) satd. Na_2CO_3, MeOH. **p**) $ClCO_2CH_2CH{=}CH_2$, pyridine, DCM. **q**) $(COCl)_2$, DMSO, TEA, CH_2Cl_2. **r**) $Pd(PPh_3)_4$, pyrrolidine.

1.4.10 The Langlois Approach

In 1993, Langlois *et al.* also reported the total synthesis of porothramycin B (Langlois *et al.*, 1993) using the methodology first exemplified in their synthesis of the neothramycins (Andriamialisoa and Langlois, 1986).

(5*S*)-5-Ethoxymethyl-2-pyrrolidine **98** was coupled to 3-methoxy-2-nitrobenzoyl chloride to provide imide **99**, followed by reduction with DIBAL-H to give the carbinolamine **100** **(Scheme 1.4j)**. Deprotection, methylation and acetylation of **100** gave **102**, which was eliminated by heating a toluene solution in the presence of quinolinium camphorsulphonate (QCS) to provide the enamide **93** in 87% yield. The enamide was subjected to a Vilsmeier-Haack formylation to furnish the key enamidoaldehyde intermediate **94** in 87% yield. Introduction of the C2 *N*-*N*-dimethylacrylamide side chain was achieved by using a Wittig-Horner Olefination with a suitably functionalised diethylphosphonate ylid. Saponification of the product with $Ba(OH)_2$ furnished the alcohol **103** in 78% yield. Swern oxidation of the primary alcohol required the use of DIPEA as a base to avoid racemisation of the aldehyde **104**. The use of excess Raney nickel at room temperature led to the reduction of the aromatic nitro group, which then spontaneous cyclised. Subsequent treatment with a very dilute TFA solution provided porothramycin B (**3**) in 45% yield.

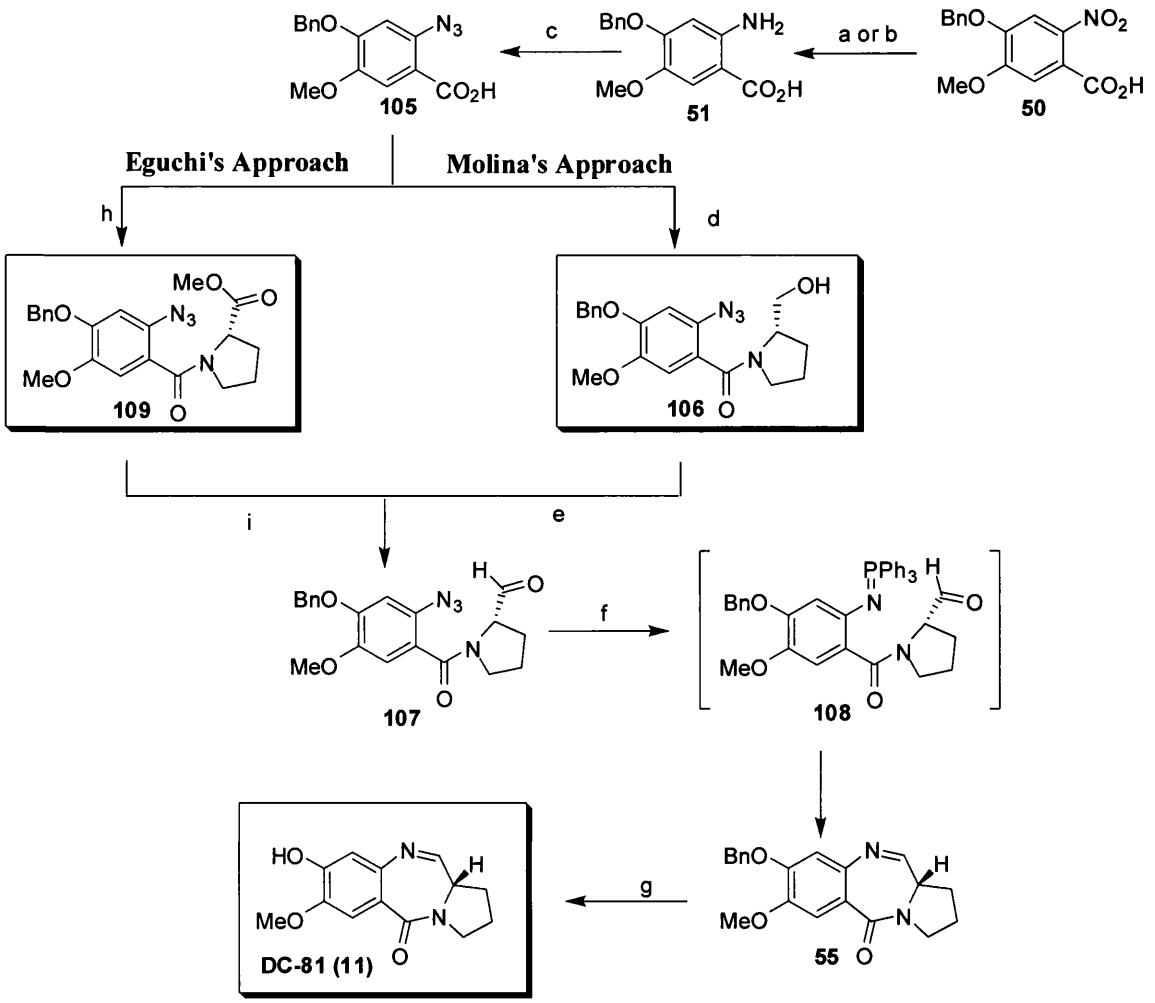


Scheme 1.4j: Langlois's total synthesis of porothramycin B.

a) 1. NaH, THF, KI; 2. 3-methoxy-2-nitrobenzoyl; **b**) DIBAL-H, THF; **c**) TsOH, THF, H_2O, MeOH; **d**) Ac_2O, pyridine; **e**) CSA, quinoline, $PhCH_3$; **f**) $POCl_3$, DMF; **g**) 1. $(EtO)_2P(O)CH_2CONMe_2$; 2. 1N $Ba(OH)_2$; **h**) $(COCl)_2$, DMSO, DIPEA, DCM; **i**) 1. Raney-Ni; 2. TFA (0.002%) DCM, MeOH (9:1).

1.4.11 The Molina and Eguchi Approach

In 1995, two groups working independently (Molina *et al.*, 1995 and Eguchi *et al.*, 1995) reported the synthesis of DC-81 via an intramolecular aza-Wittig reaction. The two approaches differ slightly in their routes to the synthesis of the azide, but the key reaction for both approaches is the intramolecular aza-Wittig cyclisation in the presence of triphenylphoshine (TPP). Interestingly the entire synthetic route was also repeated with the phenolic A-ring hydroxyl precursor to avoid the problematic 9-OBn deprotection step (Molina *et al.*, 1995).



Scheme 1.4k: Molina's and Eguchi's aza-Wittig approach to the synthesis of DC-81.

a) Fe, HCl, EtOH/AcOH/water; **b**) $SnCl_2.2H_2O$, MeOH; **c**) 1. $NaNO_2$, H_3O^+; 2. NaN_3;**d**) 1. $SOCl_2$, benzene, reflux; 2. L-prolinol, CH_2Cl_2/H_2O, $NaCO_3$; **e**) PCC, CH_2Cl_2; **f**) TPP, CH_2Cl_2 or Toluene; **g**) H_2, Pd/C cyclohexadiene; **h**) 1. $SOCl_2$, reflux; 2. THF L-proline methyl ester hydrochloride, TEA, DMAP; **i**) DIBAL-H, CH_2Cl_2.

Molina's approach (**Scheme 1.4k**) involved reduction with iron in hydrochloric acid of the 4-benzyloxy-5-methoxy-2-nitrobenzoic acid **50** to the *O*-aminobenzoic acid derivative **51**. Diazotisation of the aniline provided the azidobenzoic acid derivative **105** in 69% yield, which was coupled with L-prolinol via the acid chloride to furnish the amide **106**. The primary alcohol was converted to the aldehyde (**107**) by oxidation with PCC in dichloromethane

(DCM), which underwent an intramolecuar aza-Wittig cyclisation reaction in the presence of TPP and DCM to furnish the benzyl protected-DC-81 (**55**) in 92% yield (The cyclisation step proceeds via the iminophosphorane intermediate **108**). Cleavage of the 9-OBn group was achieved by catalytic hydrogenation to provide DC-81 (**11**).

Eguchi's approach was analogous to that of Molina, with a few minor differences (**Scheme 1.4k**). Reduction of the 4-benzyloxy-5-methoxy-2-nitrobenzoic acid **50** to the *O*-aminobenzoic acid derivative **51** was achieved with sodium borohydridenickel (II) chloride system in 53% yield. The amide **109** was obtained by coupling with L-proline methyl ester with the azidobenzoyl chloride, which was then reduced using DIBAL-H to give the aldehyde **107**. An intramolecular aza-Wittig reaction was carried out in toluene instead of DCM to afford the benzyl protected DC-81 in 98% yield, followed by deprotection of the O-benzyl group through catalytic hydrogenolysis to provide DC-81.

1.4.12 Discussion of Synthetic Approaches to PBDs.

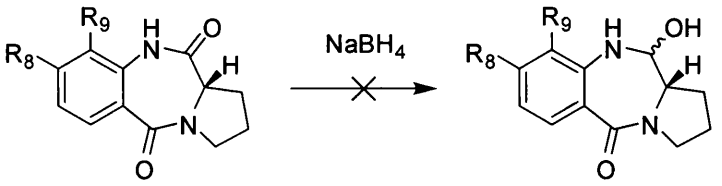
The synthetic approaches to PBDs described above met with varying degrees of success. Each method had to take into account the nature and position of the substituents in the target natural products, as well as the need to retain (*S*)-stereochemistry at the C11a position and produce the sensitive N10-C11 eletrophilic moiety under mild conditions.

In the Leimgruber and Pena-Stille approaches anthramycin was successfully synthesized by employing hydride reduction of the O9,N10-benzal protected dilactam to give the corresponding carbinolamines. This method has great potential as PBD dilactams are extremely stable and are relatively straightforward to obtain, as robust and high yielding routes to PBD dilactams are well established.

However the success of the regioselective reduction of the C11-carbonyl for the synthesis of anthramycin analogues with $NaBH_4$ depends on a number of factors, including A- and C-ring substitution patterns and the requirement for N10-amide protection. As a result, optimisation of PBD dilactam reduction conditions is extremely important in PBD synthesis.

1.4.12.1 Reduction of N10-C11 dilactams

Thurston and co-workers (Thurston *et al.*, 1984) performed model studies to investigate the role of the phenoxazoline ring system in limiting the reduction of dilactams to carbinolamines. They prepared a number of dilactam analogues with different substituents both in the aromatic ring and the N10 position. Dilactams lacking substitution at the N10 position failed to reduce, with $NaBH_4$ to the corresponding carbinolamine intermediate (**Scheme 1.4l**). This was attributed to the initial reaction of the hydride reducing agent with the amidic N10 proton (and C-9 hydroxyl proton in **110b**) followed by precipitation of an insoluble non-reducible complex.



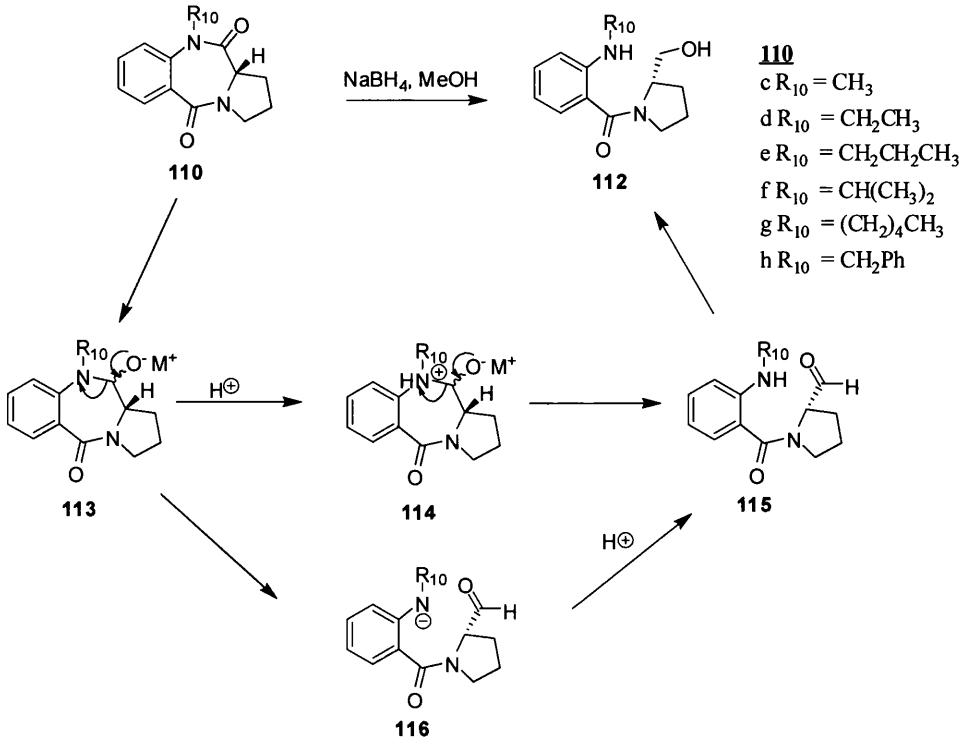
110 **111**

a R_8 = H, R_9 = H
b R_8 = Me, R_9 = OH

Scheme 1.4l: Unsubstituted dilactams **110a** and **110b** failed to reduce with $NaBH_4$.

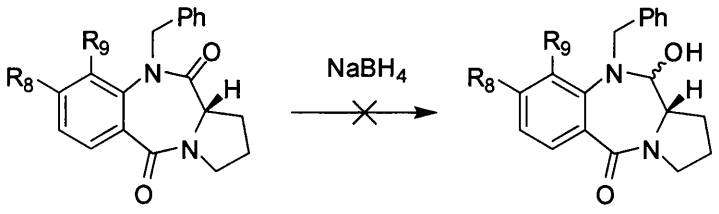
Dilactams with increasing steric bulk at the N10-position underwent overreduction to the corresponding ring opened amino alcohols of type **112**. A mechanism was proposed (**Scheme 1.4m**) by Thurston and co-workers to rationalize the overreduction of dilactams of type **110**. It was suggested that ring opening of the initial carbinolamine complex **113** to amino aldehyde **115** occurs, which is further reduced to the amino alcohol **112**. Since dissociation of the carbinolamine to the amino aldehyde is the critical step that commits the reaction to proceed to overreduction, two factors were considered that might favor this process. 1) Transient protonation of the N10-nitrogen by MeOH (structure **114**), when the nitrogen has sufficient basicity, might facilitate ring opening to the amino aldehyde (**Scheme 1.4m**). 2) Dissociation could result in a negative charged N10-nitrogen which should be favored if resonance stabilization can occur through the aromatic ring (structure **116**).

Interestingly, there appear to be many examples in related lactam systems where stabilization of a nitrogen anion by ring substituents favors overreduction (Thurston *et al.*, 1984).



Scheme 1.4m: N10-Substituted dilactams (**110c-h**) with increasing steric bulk facilitated overreduction to the corresponding ring opened amino alcohols of type **112**.

In order to investigate whether N10-substituted dilactams **110c-h** allowed extension of resonance from the N10-nitrogen, N-benzylated derivatives **110i** and **110j** with electron donating groups (EDG) in the aromatic ring were prepared (**Scheme 1.4n**). It was predicted (Thurston *et al.*, 1984) that the increased electron density of the aromatic rings might prevent stabilization of the intermediate **116** (see **Scheme 1.4m**). However these dilactams failed to be reduced at all with $NaBH_4$, which was attributed to the relatively higher electron density on the N10-nitrogen due to decreased donation into the ring. Higher electron density at N10 would facilitate interaction of the nitrogen lone pair with the adjacent carbonyl, decreasing its electrophilicity towards incoming hydrides.

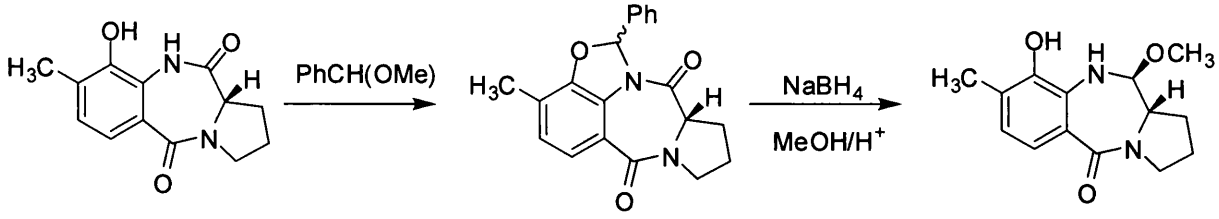


110 111

i R_8 = H, R_9 = OCH_3
j R_8 = Me, R_9 = OCH_2Ph

Scheme 1.4n Substituted dilactams with electron donating groups in the aromatic ring **110i** and **110j.**

Intriguingly, incorporating the N10-nitrogen and O9-oxygen substituents into a benzoxazoline ring system as in **117**, resulted in smooth conversion to the carbinolamine **118** in high yield.



110b **117** **118**

Scheme 1.4o The Successful $NaBH_4$ reduction of hydroxyl dilactam **110b** by utilizing the phenyloxazoline ring system employed by Leimgruber.

The success of this reduction using the phenyloxazoline ring system was rationalized by the authors, by considering nitrogen protonation being more important than the alternative mechanism involving resonance extension from the nitrogen. Consequently, the authors suggested that inclusion of the N10-nitrogen into a phenyloxazoline ring lowers the electron density on the nitrogen (base weakening) through inductive transmission via the benzal carbon, therefore reducing the resonance effect of the C9-oxygen.

The nitrogen is less easily protonated, reducing the possibility of dissociation to the amino aldehyde **115** (Thurston *et al.*, 1984). They concluded that $NaBH_4$ reduction of dilactams is only useful for C9 hydroxyl substituted analogues, formed *via* hydride reduction of their phenoxazoline precursors.

Although the above explanation advanced by Thurston *et al.* suggests the N10-nitrogen basicity is reduced by the inductive transmission effect via the benzal carbon, therefore preventing ring opened products, it still does not fully explain how the resonance effect of the C9-oxygen is reduced, thus allowing smooth reduction at the C11-carbonyl, which does not occur when the C9-oxygen is not part of the phenyloxazoline ring system as observed with dilactam 110i and j possessing EDG (**Scheme 1.4n**).

A complementary explanation takes into account the effect of the conformation of the phenoxazoline ring on mesomeric donation of the oxygen lone pairs in the A-ring. Modelling studies suggest that the oxygen lone pairs cannot fully overlap with the A-ring π-system when the oxygen is constrained in the phenoxazoline ring (see **Figure 1.4p and q**). However, when the oxygen is part of a 9-OH or 9-OMe group the lone pairs can easily overlap with the A-ring π-system. Thus 9-OH/9-OMe dilactams resist C11-reductions, whilst phenoxazoline protected dilactams are reduced to carbinolamines.

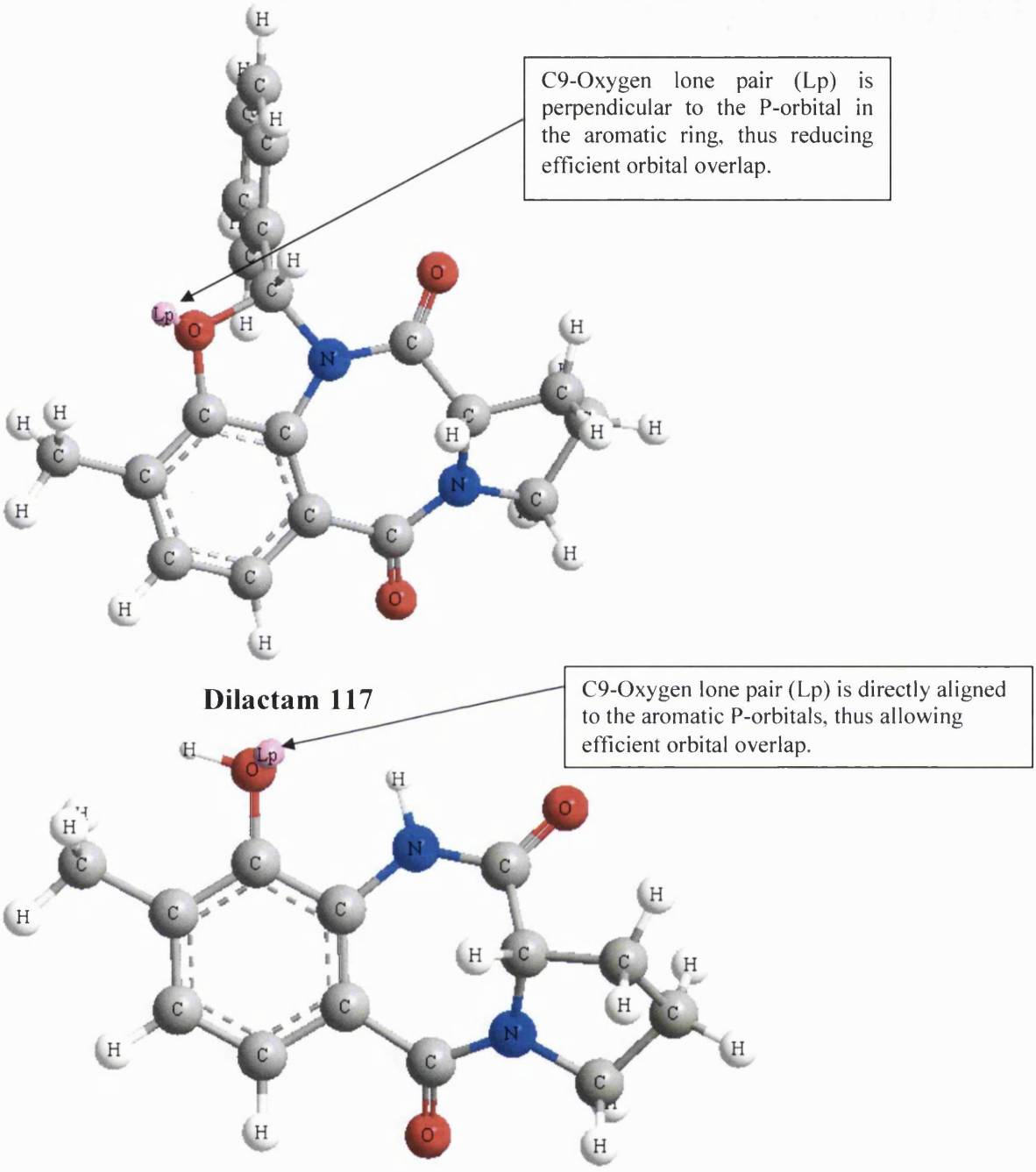
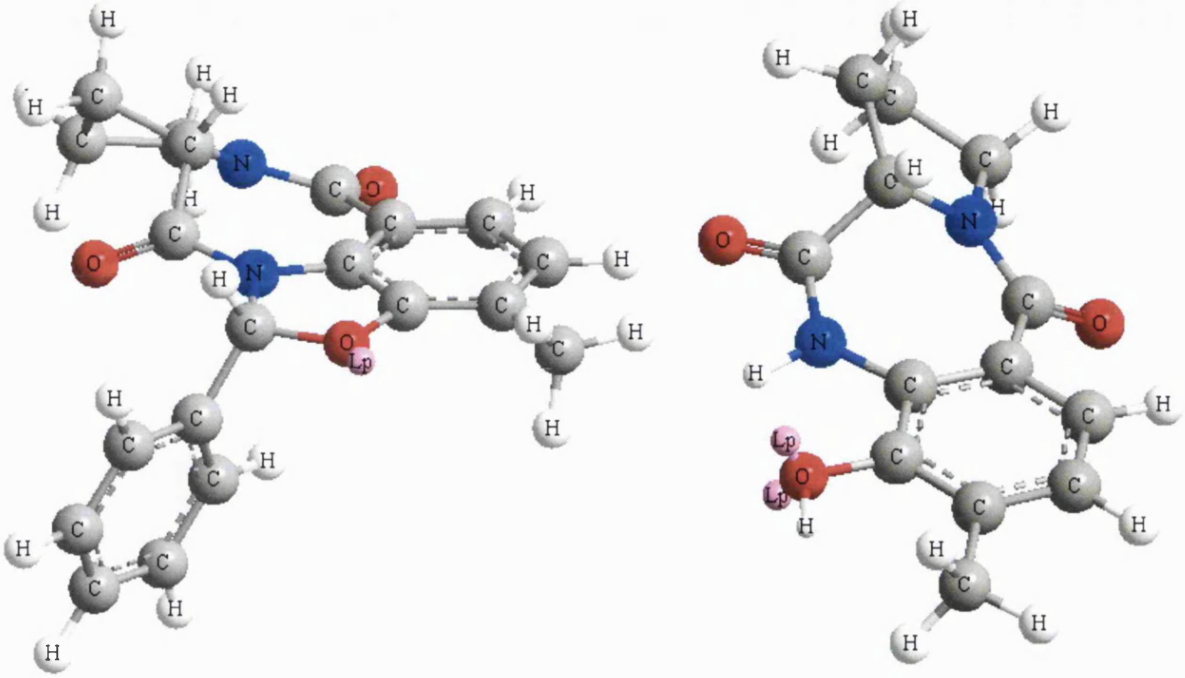


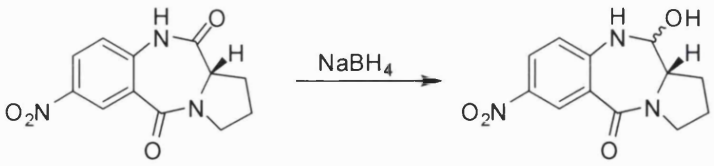
Figure 1.4p: Energy minimized molecular model of phenyloxazoline protected dilactam **117** and the free C9-oxygen dilactam **110i**, illustrating the orientation of the C9-Oxygen lone pairs shown in Pink (Lp).



Dilactam 117 **Dilactam 110i**

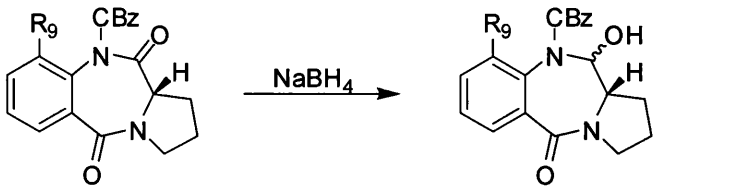
Figure 1.4q: Side on view Energy Minimized molecular model of dilactam **110i** and **117** illustrating the orientation of the C9-oxygen lone pairs shown in Pink (Lp).

In 1985, Suggs and co-workers supported the hypothesis that the presence of electron withdrawing groups on the A-ring of PBD promotes C11-reduction, by reporting the succesful reduction of the 7-nitro substituted dilactams (Suggs *et al.*, 1985; **Scheme 1.4r**).



Scheme 1.4r: Suggs's hydride reduction 7-nitro substituted dilactams.

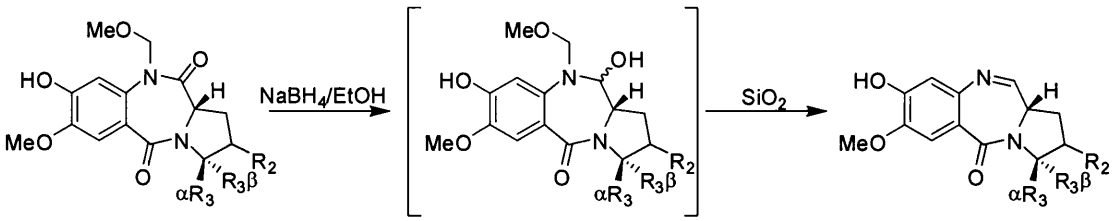
In 1989, Nagasaka *et al.* reported the use of involving a N10-benzyloxycarbonyl (CBz) group to selectively reduce down a dilactam (Nagasaka *et al.*, 1989) with $NaBH_4$ to the carbinolamine in 69-76% yield **(Scheme 1.4s).** The presence of the additional carbamate carbonyl converts the amide to an imide moiety, reducing electron density at C11 position.



R_9 = H (69%) or OBn (76%)

Scheme 1.4s: Nagasaka's N10-CBz dilactam reductions with $NaBH_4$.

In 1986, Mori *et al.* reported that introduction of N10-methoxymethyl (MOM) substituents allows selective $NaBH_4$ reduction of PBD dilactams to carbinolamines. Neothramycin (Mori *et al.*, 1986a, 85% yield) and tomaymycin (Mori *et al.*, 1986b, 89% yield) both possessing EDG in their aromatic rings but significantly lacking C9 hydroxyl groups were all successfully synthesized by this reduction route **(Scheme 1.4t)**. This demonstrated that C9-unsubstituted PBD dilactams with EDG can be efficiently reduced by $NaBH_4$.



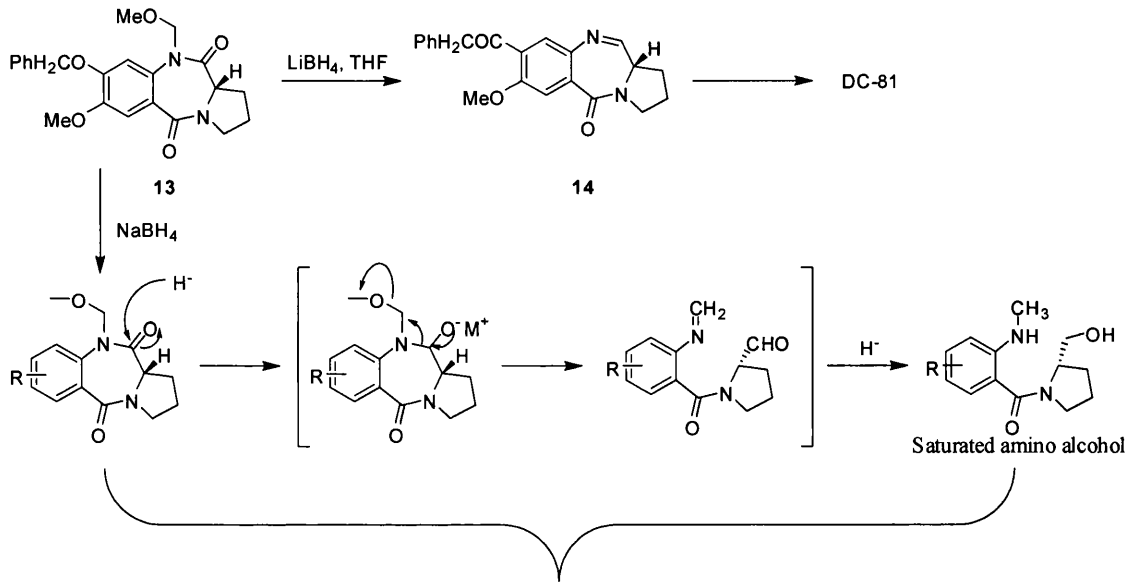
Neothramycin A; **R3α** = OMe, **R3β** = H, R_2 = H

Neothramycin B; R3**a** =H, R_3**b** = OMe, R_2 = H

Tomaymycin E/Z; **R3α** = H, **R3β** = H, R_2 = =CH-CH_3

Scheme 1.4t: Mori's synthesis of neothramycin and tomaymycin utilizing MOM-protected dilactams in C11-hydride reductions.

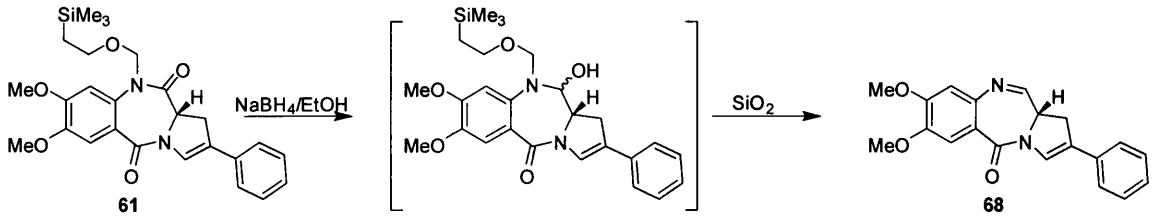
Nonetheless, other workers have been unable to reduce variously substituted N10-MOM protected dilactams in their laboratories using Mori's reported or similar conditions (Langley and Thurston, 1987). However, in 2001, Hu *et al.* reported the successful hydride reduction of MOM-protected dilactams to synthesize DC-81. Their initial attempts to reduce MOM-protected dilactam **96** to form the N10-C11 imine moiety failed in their hands using either the method reported by Mori and co-workers or using a number of reaction condition variations (Hu *et al.*, 2001). Instead, ring opened products were obtained via 3-aza-Grob fragmentation (**Scheme 1.4u**). Eventually the MOM-protected dilactam was successfully reduced using $LiBH_4$ (1 molar Equivalent) in THF at – 10 °C (see section **1.4.6, Synthesis of PBDs)**



Ring opened products via 3-aza-Grob fragmentation

Scheme 1.4u: Hu's total synthesis of DC-81 via $LiBH_4$ reduction of MOM-protected dilactams (also showing 3-aza-Grob fragmentation with $NaBH_4$).

In 2002, Cooper *et al.* reported the synthesis of C2-C3 *endo* unsaturated C2-aryl PBD monomers (Cooper *et al.*, 2002) which were prepared from N10-SEM protected dilactams intermediates type **61** (**Scheme 1.4v**), and subjected to $NaBH_4$ reduction to the transient SEM-protected carbinolamine, which gave the desired imines upon exposure to wet silica gel (**68**, 74%).

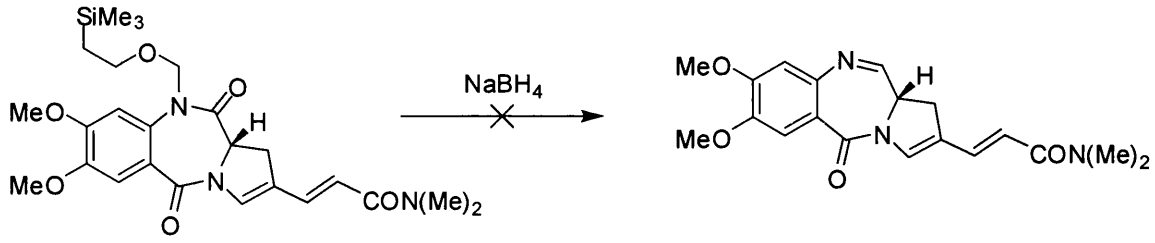


Scheme 1.4v: Cooper's synthesis of C2-aryl PBDs via N10-SEM protected dilactams.

Although N10-SEM or MOM protected dilactams intermediates can be used to generate the N10-C11 imine/carbinolamine moiety for a number of PBD substrates, their general applicability for the synthesis of anthramycin type analogues (C2-acrylamide side chains) has yet to be fully demonstrated.

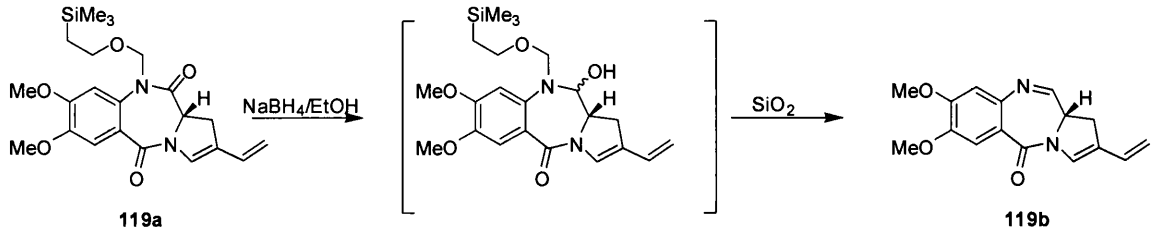
For example, in 1989, Pena and Stille reported the total synthesis of naturally occurring PBD, anthramycin (Peña and Stille, 1989). Initially they followed the strategy of Mori *et al.*, but interestingly, they did not report the conversion of the N10-MOM protected dilactam to anthramycin, but reverted instead to the O9,N10-benzal protecting stratey of Leimgruber. These same workers also described a number of C2-alkynic and vinylic N10-MOM protected anthramycin PBD precursors prepared via Stille coupling, but did not report their conversion to the N10-C11-imine moiety final product (Peña and Stille, 1987).

More recently, in 2004, Chen *et al.* reported the application of Heck coupling reactions to install C2-acrylyl side chains to PBDs (Chen *et al.*, 2004). They reported that attempts to reduce C2-*N,N*'-dimethylacrylamide dilactams to *endo-exo* unsaturated PBDs via N10-SEM protected dilactams first reported by cooper *et al.* failed in their hands (see **Scheme 1.4w**). Instead, another route was subsequently developed to obtain these PBDs via a N10-Troc protected PBD C11 carbinolamine intermediates.



Scheme 1.4w: Chen's attempted synthesis of C2-C3 *endo* unsaturated PBDs via N10-SEM protected dilactams.

In 2004, in a complementary study, Tiberghien *et al.* reported the application of the Stille coupling reaction to the synthesis of C2-substituted *endo-exo* unsaturated PBD (Tiberghien *et al.*, 2004) which were prepared according to the method of Cooper *et al.* approach in the synthesis of C2-aryl PBDs from N10-SEM protected dilactams intermediates of type **119a** (see **Scheme 1.4x**). However, they reported that the yields of the final reduction/deprotection step of the C2-vinylic and acetylenic PBDs were very disappointing (~ 25%) which was attributed to collateral reduction of the exo double dond.



Scheme 1.4x: Tiberghien's synthesis of C2-C3 *endo* unsaturated PBDs via N10-SEM protected dilactams.

Much work has been performed in order to develop efficient hydride reductions methods for protected dilactams, particularly the N10-acetal protected dilactams (e.g. SEM/MOM). However, this method has been shown to have limitations, notably in its application to the synthesis of anthramycin type analogues containing *endo/exo* unsaturation, where lack of C11-reduction or possible collateral reduction of *exo* double bonds under these reductive conditions have been observed. Nevertheless, the use of dilactam intermediates can offer a versatile approach to the synthesis of N10-C11 imine/carbinolamine containing PBDs, especially since the known methods of dilactam synthesis appear relatively straightforward in providing high yielding pathways to key intermediates. For example, the cyclisation of *N*-(2-nitrobenzoyl)pyrrolidine-2-carboxylic acid esters (Leimgruber, Peña-Stille and Howard-Thurston approach), the condensation of isatoic anhydride with substituted prolines (Peña-stille approach) and the palladium-catalysed carbonylation of proline-2-haloanilides have been

successfully employed to synthesise numerous synthetic and natural PBDs **(see Table 1.4a below)**.

Table 1.4a: Comparison of hydride reduction approaches to PBDs.

Hydride reduction approaches of PBD dilactams			
Approaches	**Key synthetic step**	**Advantages**	**Disadvantage**
Leimgruber	- Reduction of O9,N10-benzal protected dilactams.	- Ideal for 9-OH PBDs.	Highly dependent on A-ring structure, thus lacks general applicability.
Mori	- Reduction of N10-MOM protected dilactams. - Pd carbonylation step to form dilactam.	Not dependent on A-ring structure, thus does not lack applicability.	- MOM –highly carcinogenic. - Other groups have failed to reduce N10-MOM dilactams.
Peña-Stille	- Isatoic anhydride coupling to form dilactam. - Heck coupling reaction to introduce C2-side chain	- Efficient and versatile side chain introduction method.	MOM–highly carcinogenic. - Other groups have failed to reduceN10-MOM dilactams.
Hu	- Reduction of N10-MOM protected dilactams	- Efficient and versatile route to PBD imines	- MOM –highly carcinogenic. – Over reduction of B-ring
Howard-Thurston	- Reduction of N10-SEM protected dilactams. - Suzuki coupling to incorporate C2-side chains.	- A versatile dilactam reduction method to PBD imines. – Efficient and versatile side chain introduction.	- B-ring over reduction and reduction of unsaturated side chains.

1.4.12.2 Non-dilactam approaches to PBD synthesis

The Thurston-Langley approach (**Scheme 1.4h**), employing the cyclisation of amino dithioacetals cyclisation strategy to the synthesis of prothracarcin and DC-81 provides a mild and versatile approach to N10-C11 carbinolamines formation. The limitation with this approach is that mercury salts are used during the cyclisation steps which need to be exhaustively removed by column chromatography leading to low yields as well as the inevitable risk of contamination of mercury salts in the final target molecule, which could be a major problem for drug safety as mercury is extremely toxic. In addition, containment can be an issue as ethanethiol used in the protection step is extremely pungent and can be used to odorise natural gas. As a result, working on a large scale with this reagent can be a major issue for laboratories.

The Fukuyama approach (**Scheme 1.4i**) involves a non-hydrogenolytic B-ring cyclisation technique, which offers a mild and high yielding approach to N10-protected C11 carbinolamine PBDs. The drawback with this spontaneous oxidation/ring closure reaction can be formation of dilactams due to over oxidation of the primary alcohol, as reported by Gregson *et al.* for the synthesis of SJG-136 PBD dimer (Gregson *et al.*, 2001a). In addition, a number of synthetic steps are requred to establish the C-ring component for C2-substituted PBDs.

The Molina-Eguchi's aza-Wittig approach (**Scheme 1.4k**) applied to the synthesis of DC-81 has provided another mild and efficient B-ring cyclisation technique. However, accessibility of the potentially explosive azide can be problematic, requiring extra synthetic steps from readily avalible amines or nitro starting materials. Its general applicability has not been investigated, in particular whether unsaturated side chains to the C-ring can be introduced.

The Langlois approach (**Scheme 1.4j**) employs the use of Raney nickel as a cyclisation catalyst to form the PBD B-ring appears promising. The potential drawback with this technique is that it may be prone to over-reduction depending on the quality of Raney nickel and also the possibility of reducing other double bonds in the molecule, in particular *exo-endo* unsaturation in the C-ring when dienes are not conjugated to an amidic electron sink.

The Howard-Thurstons approach (**Scheme 1.4g**) utilizes dilactam intermediates which are N10-SEM-protected to generate a series of C2-aryl PBDs monomers that represent a structural sub-class not observed in nature. The ease and preparation of the dilactams intermediates offer a real potential to the synthesis PBDs analogues. The simplicity of the Suzuki reaction coupled with the diverse range of commercially available boronic acids suggests the possibility of generating libraries of analogues through parallel combinatorial methodologies (cooper *et al.*, 2002). The limitation with this approach, as with all approaches that utilizes dilactam intermediates, is that it may suffer from over-reduction to secondary amines.

The presence of olefinic substituents at the C2-position greatly enhances cytotoxicity and DNA binding affinity of PBDs (Thurston, 1993). The introduction of unsaturated C2-substituents in PBDs such as those found in anthramycin is challenging. Therefore approaches to the incorporation of unsaturation in the C-ring are highly desirable in PBD synthesis. Some of the approaches described above have developed elaborate methods to solve this problem. The Peña-Stille approach demonstrated that enol triflates can be coupled to either olefins (Heck) or vinyl stannanes (Stille) via palladium-catalysed reactions to install the acrylamide side chain in the synthesis of anthramycin (**Scheme 1.4e**). This approach has offered a versatile alternative to the Leimgruber approach, as Pena-Stille's introduction of the C2-acrylamide side chain was required in three steps compared to Leimgruber's six steps (**Scheme 1.4c**).

Both Fukuyama (**Scheme 1.4i**) and Langlois (**Scheme 1.4j**) approaches to porothramycin involved introduction of the acrylamide side chain via a Vilsmeier-Haack reaction. This approach demonstrates the potential to transform aldehydes into a variety of PBD analogues with interesting C2-olefinic side chains via Wittig type reactions. Below is a table summarizing all the advantages and disadvantages of all the B-ring cyclisation approaches to PBDs (**Table 1.4b**).

Table 1.4b: Comparison of B-ring cyclisation approaches to PBDs.

B-ring cyclisation approaches of PBDs			
Approaches	**Key synthetic step**	**Advantages**	**Disadvantage**
Thurston-Langley's	- Cyclisation of amino dithioacetals to form PBD imine.	- Mild and versatile cyclisation method to form N10-C11 imine moiety.	- Mercury salts formed are extremely difficult to remove and contaminates target molecule
Molina-Eguchi's aza-Wittig	- Intramolecular aza-Wittig cyclisation reaction	- Mild and efficient cyclisation technique.	- Accessibility to azide can be problematic and lengthy. – Applicability of C-ring introduction not investigated.
Fukuyama	- Swern oxidation/cyclisation to form PBD N10-C11 carbinolamine moiety.	- Mild and Efficient cyclisation method.	- Lengthy C-ring synthesis. – Risk of over oxidation to dilactam.
Langlois	- Raney nickel reduction/cyclisation to form PBD N10-C11 moiety	- Efficient cyclisation method	- Risk of B-ring over reduction. -Reduction of unsaturated C-ring side chains.

1.5 Structure-Activity-Relationships (SARs)

The synthesis of a large number of PBD analogues has helped pave the way in providing information for determining the essential structural features that govern biological activity of PBDs.

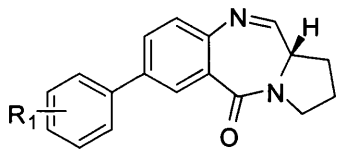
1.5.1 Essential Features

Any design of a novel PBD compound must obey the following essential features which are critical for the PBD binding affinity with DNA. These include having an electrophilic functionality at the N10-C11 position which is responsible in forming an irreversible aminal covalent bond with the N2 of guanine.

An (*S*)-configuration at the C11a position provides the molecule with a right handed twist when viewed from the C-ring towards the A-ring, which allows it to fit snugly within the minor groove of DNA.

1.5.2 A-Ring SAR

The type and position of functional groups in the A-ring can influence the cytotoxicity of PBDs. This influence is considered to be dominated by electronic factors, for example Guiotto *et al.* synthesised a series of C7-aryl substituted PBDs (**Figure 1.5a)** and evaluated their biological activity (Guiotto *et al.*, 1998). They found that electron withdrawing 3'-nitro group (**120b**) reduces activity by approximately 30 fold in the A2780 human ovarian carcinoma cell line, where as electron donating 4'-methoxy group (or hydroxyl) enhances the activity (**120a**).



120

120a $\mathbf{R_1}$ = 4'-OMe IC_{50} (μM): A2780 = 0.56

120b $\mathbf{R_1}$ = 3'-NO_2 IC_{50} (μM): A2780 = 34.5

Figure 1.5a: C7-aryl substituted PBDs.

Electron donating groups in the A-ring enhance the biological activity of PBDs (Hurley *et al.*, 1988; Guiotto *et al.*, 1998) and are consistent with the acid-catalysed iminium alkylation mechanism proposed in section **1.3.1**. For example, Substituents such as methoxy and hydroxy groups from the A-ring donate electrons mesomerically to the N10-nitrogen by resonance stabilization. This results in the N10-nitrogen becoming more basic due to it increasing electron density (i.e. large pKa), raising its likelihood of it being protonated as the activated iminium species, increasing its electrophilicity towards guanine residues. Interestingly, electron donating groups can promote stability of the imine form of PBDs, whereas mesomeric electron withdrawing groups such as C7-nitro can stabilise the carbinolamine form (Thurston *et al.*, 1999b).

The same group (Thurston *et al.*, 1999b) synthesized pyridine (**121**), pyrazine (**122**) and pyrimidine (**123**) A-ring analogues of PBDs and their cytotoxicity were evaluated (**Figure 1.5b**). Changing the benzoid A-ring to these electron deficient rings actually reduced cytotoxicity.

In 1997, O'Neil *et al.* reported the synthesis and cytotoxicity evaluation of halogen-substituted PBDs (**Figure 1.5b, 124**). None of them exhibited any higher activity in the A2780 human ovarian carcinoma cell line than their non-halogenated parent analogues (O'Neil *et al.*, 1997).

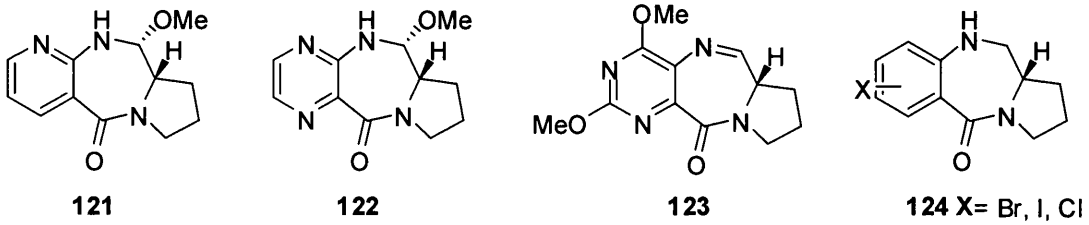


Figure 1.5b: A-ring modification of PBDs.

1.5.2 B-Ring SAR

As previously discussed, possession of an (*S*)-stereochemistry at the C11a position is essential for the biological activity of PBDs (Thurston, 1993), as this provides the molecule with the correct 3-dimensional shape to fit snugly within the walls of the minor groove.

1.5.2.1 Non-Covalent Interactions of PBDs.

In 1985, Kaneko *et al.* first reported that some PBD dilactams which lack the N10-C11 imine/carbinolamine moiety responsible for covalent binding to DNA possess significant antitumour activity (Kaneko *et al.*, 1985).

These findings suggested that other features on the PBD molecule, such as the A- and C-ring susbstituents, may be involved in non-covalent interactions with DNA bases, which may explain their significant DNA binding and antitumour activities.

In order to obtain an insight into non-covalent interactions of PBDs with DNA, Jones *et al.* synthesized fifteen PBDs dilactams analogues which do not possess the N10-C11 imine moiety. Two members in the series **125** and **126** **(Figure 1.5d)** were shown to elevate the melting point of calf-thymus (CT) DNA by a significant 3 °C (Jones *et al.*, 1990). This showed for the first time that non-covalent interactions play an important role in DNA binding. It also showed a strict requirement for the presence of hydroxyl or acetoxy functionalities at both the C2 and C8 positions with a further requirement for an (*R*)-configuration at C2 (Jones *et al.*, 1990). In order to rationalize the non-covalent interactions of the dilactams with DNA, molecular modeling studies were performed by Jones *et al.* and one model was suggested **(Figure 1.5c)** in which the dilactam amide face (N10-C11) was bound to DNA and C2-and C8-hydroxyl groups hydrogen bonded to DNA bases.

In 1996, Foloppe *et al.* reported the synthesis of eleven N10-C11 amidine PBD monomers to investigate the role played in non-covalent interactions (Foloppe *et al.*, 1996). These derivatives (**127**, **Figure 1.5d**) slightly elevated the DNA melting temperature by 0.7 °C which was comparable to DC-81 (**11**) binding affinity (ΔT_m 0.7 °C).

More recently, Kamal *et al.* (2002) reported that mixed imine-dilactam dimers (**128**) exhibited significant DNA bind affinity by elevating the melting temperature of CT-DNA by 17.0 °C after incubation for 18 h at 37 °C (Kamal *et al.*, 2002). Under similar conditions DSB-120 dimer (**129**), which has two imino functionalities elevate the melting of CT-DNA by 15.4 °C. This demonstrated the importance of non-covalent interactions in enhancing DNA binding affinity and antitumour activity (**Figure 1.5c**).

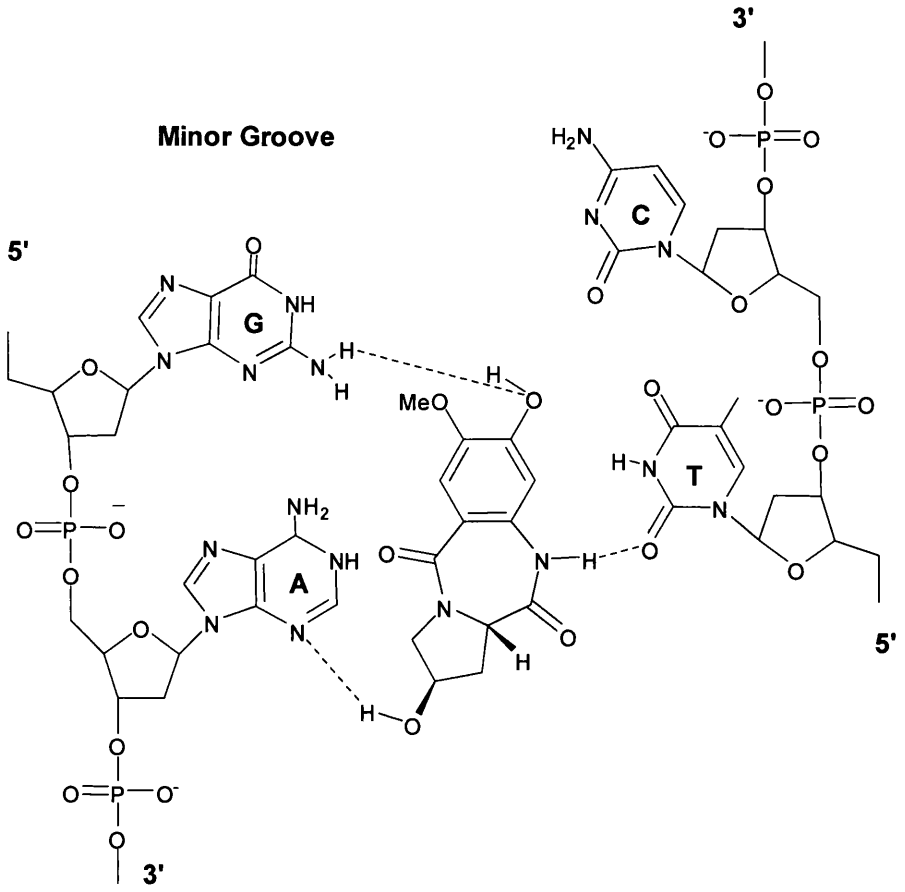
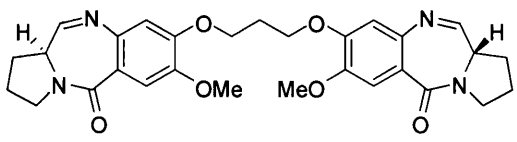
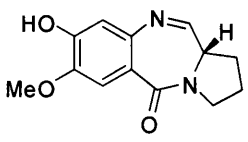


Figure 1.5c: proposed model to rationalize non-covalent interactions of dilactam **125** with DNA.

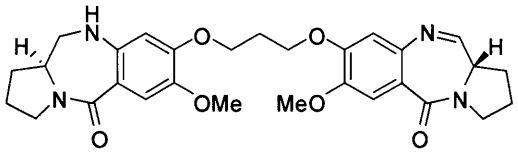
In 2004, the same group (Kamal *et al.*, 2004) synthesized a mixed imine-amine dimer (**130**. **Figure 1.5d**) which was found to elevate CT-DNA by up to 11 °C, which is less then the mixed imine-dilactam dimer (ΔT_m 17.0 °C). This could indicate the importance of the carbonyl group in non-covalent interactions.



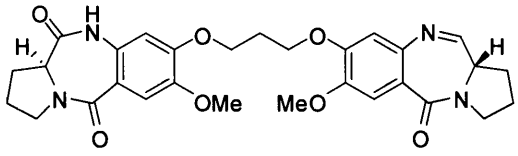
DSB 120 (129) , ΔT_m = 15.4 °C



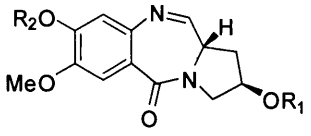
DC-81 (11), ΔT_m = 0.7 °C



130, ΔT_m = 11.0 °C



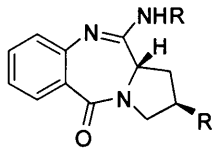
128 ΔT_m = 17.0 °C



ΔT_m = 3.0 °C

125 R_1 = OH or OH

126 R_2 = OAc or OAc



127 ΔT_m = 0.7 °C

Figure 1.5d: The effect of N10-C11 modification on thermal denaturation (ΔT_m) with CT-DNA (ΔT_m is a measure of DNA binding affinity).

1.5.3 C-Ring SAR

1.5.3.1 Effects of Substitution in the C-ring

A number of naturally occurring PBDs namely anthramycin, tomaymycin and sibiromycin, which are C2 functionalised are reported as the most potent compounds in the PBD natural product family. They have shown remarkable binding affinity and exhibit greater cytotoxicity compared to their unsubstituted counterparts. In order to confirm this observation, Howard, Thurston and coworkers have synthesised a range of unatural C2-substituted PBDs.

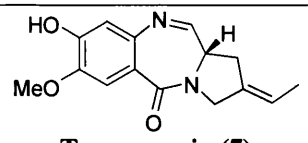
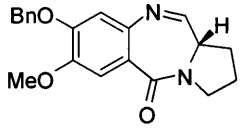
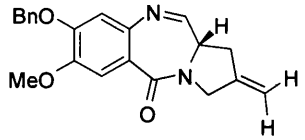
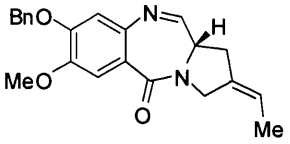
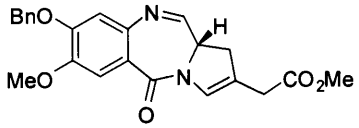
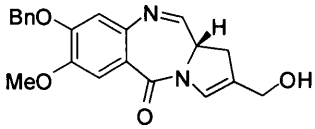
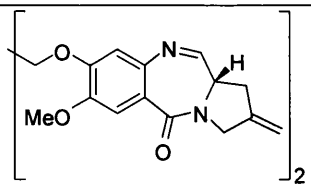
1.5.3.2 Effects of C2-*Exo* Unsaturation

Tomaymycin (**7**) has been found to be significantly more potent then DC-81(**11**) (Thurston 1993; Puvvada *et al.*, 1993; Thurston *et al.*, 1999a). The difference in activity has been attributed to the presence of C2-*exo* unsaturation, as the only difference between DC-81 and tomaymycin, is that tomaymycin possesses C2-*exo* double bond. In order to confirm this significance of C2-*exo* unsaturation, Howard and Thurston synthesised a series of C2-*exo* analogues of tomaymycin (Gregson *et al.*, 2000a). Cytotoxicity and thermal denaturation data of the PBD analogues confirmed that C2-*exo* unsaturation enhances both cytotoxic potency and DNA binding affinity. The cytotoxicity data is summarised in **Table 1.5a**, where two examples from the series clearly show that the C2-methylene (**132**) and C2-ethylidene (**133**) analogues were more potent then their C2-unsubstituted C8-benzyl-DC-81 (**131**) counterpart. It is interesting to note that tomaymycin, which differs in structure in having a C8-OH rather than C8-OMe/Bn substituents is 270-fold more cytotoxic in human ovarian carcinoma cell line CH1. This establishes the importance of a free hydroxyl group at the C8 position in maximising DNA binding affinity.

1.5.3.3 Effects of C2/C2'-*Exo* Unsaturated PBD Dimers

In 1999, Gregson *et al.* reported the synthesis of a second generation of C8/C8'-linked PBD dimers containing C2/C2'-*exo* unsaturation (SJG-136, **136**) which exhibited one of the lowest IC_{50} values for any synthetic PBD monomer or dimer (Gregson *et al.*, 2001a). SJG-136 was evaluated for its *in vitro* cytotoxicity in a number of human ovarian carcinoma cell lines In the CH1 cell line SJG-136 was 1500-fold more potent then the clinically used cisplatin antitumor agents (see **Table 1.5a**). The details for SJG-136 are described in section **1.7; PBD Dimers** of this chapter.

Table 1.5a: Effects of C-ring modification on cytotoxicity in human ovarian carcinoma cell line CH1.

Compounds	CH1 Cytotoxicity IC_{50} (μM)[a]	Reference
 **Tomaymycin (7)**	0.00013	
 **C8-benzylated DC-81(131)**	0.145	(Gregson *et al.*, 2000a)
 **132**	0.017	
 **133**	0.031	
 **134**	0.016	
 **135**	0.09	(Gregson *et al.*, 2000b)
 **SJG-136 (136)**	0.00012	(Gregson *et al.*, 2001a)

[a]IC_{50}: Dose that inhibits 50% of cell growth.

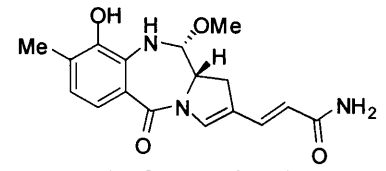
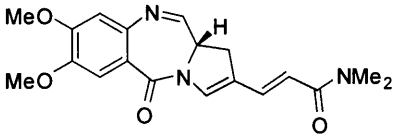
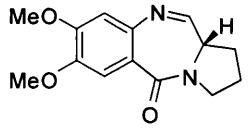
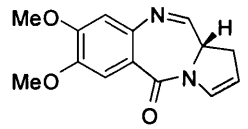
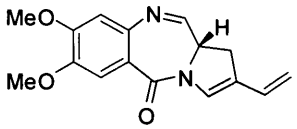
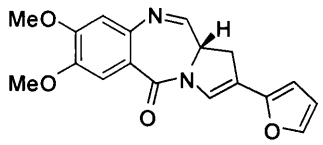
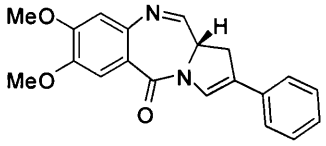
1.5.3.4 Effects of C2/C3-*Endo* Unsaturation.

Anthramycin binds more efficiently to DNA than tomaymycin (Puvvada *et al.,* 1993), a characteristic thought to be associated with the C2/C3-*endo* unsaturation of anthramycin. To elaborate on this feature Gregson *et al.* synthesised a series of C2/C3 *endo* unsaturated PBD analogues. Two examples from the series, the homoallylic ester (**134**) and alcohol (**135**) PBD are the most potent compounds, exhibiting sub-micromolar IC_{50} values in the human ovarian carcinoma cell line CH1, when compared to their parent C2-unsubstituted C8-benzyl-DC-81 (**131**) counterpart (see **Table 1.5a**). This study clearly shows that introduction of C2/C3 *endo* unsaturation enhances both DNA binding affinity and cytotoxicity; illustrating the significance effect that *sp*2 carbon centre in the C-ring has on biological activity (Gregson *et al.*, 2000b).

Chen *et al.* reported the synthesis of C2/C3 *endo* unsaturated PBDs with conjugated acrylyl C2-substituents (Chen *et al.*, 2004). All the analogues possessed significant cytotoxicity according to the NCI 60 cell line screen with **137** surpassing anthramycin (**1**) in potency. This data is summarised in **Table 1.5b,** clearly demonstrating that 9-OH group was not essential for activity.

In a complementary study, Tiberghien *et al.* successfully synthesized a series of C2-substituted *endo* unsaturated PBDs as part of an ongoing SAR investigation of PBDs (Tiberghien *et al.*, 2004). The entire set of novel PBD analogues was evaluated in the NCI 60 cell line screen together with the methyl ether of DC-81 (**138,** GI_{50} = 2.39 μM) as a control. Introduction of a C2/C3 *endo* unsaturation as in **139** (GI_{50} = 1.28 μM) results in a modest increase in cytotoxicity, but inclusion of C2-vinyl substituents in conjugation with C2/C3 double bond (**140,** GI_{50} = 0.123 μM) further increases the activity. Cytotoxicity is further potentiated by a 10-fold order of magnitude (**141,** GI_{50} = 0.01 μM) when a heteroaryl group such as furan is incorporated. The result for the furylphenyl analogues **141** is similar in potency to the reported C2-aryl substituted PBDs (Cooper *et al.*, 2002, **see section 1.5.3.5**).

Table 1.5b: Effects of C-ring modification on cytotoxicity in the NCI 60 cell line screen

Compounds	Average GI_{50} (μM)[a]	Reference
 **Anthramycin (1)**	0.029	(Chen *et al.*, 2004)
 **(137)**	0.012	
 **138**	2.39	(Tiberghien *et al.*, 2004)
 **139**	1.28	
 **140**	0.123	
 **141**	<0.01	
 **C2-Aryl (68)**	0.01	(Cooper *et al.*, 2002)

[a] GI_{50}: Dose that inhibits 50% of cell growth.

1.5.3.5 Effects of C2/C3-*Endo* Unsaturated C2-Aryl PBDs.

Cooper *et al.* reported the synthesis of C2/C3 *endo* unsaturated C2-aryl PBDs which represented a new structural PBD subclass that has not been observed in nature (Cooper *et al.*, 2002). These C2-aryl analogues have remarkable activity against a number of cancer cell lines, especially (subnanomolar-picomolar activity at GI_{50} level) towards melanoma and ovarian cell lines (**68**, see **Table 1.5b**).

This gain of activity of C2-unsaturated PBDs has been attributed to a combination of factors;

- **Geometry of molecule-** Introducing a *sp*2 carbon centre in the C-ring results in flattening of the C-ring causing a change in shape between the C-ring and the B-ring, allowing the PBD to fit deeply and snugly within the walls of the minor groove. It is thought that this cause the PBD/DNA adduct to remain undetected by DNA repair enzymes for a longer length of time (S. J. Gregson *et al.*, 2001a).

- **Orientation of C2-substituents-** having the *sp*2 carbon centre in the C-ring in conjugation with C2-substituents results in the C2-group being projected along the minor groove, in an optimal position to pick favorable interactions such as hydrogen bonding, electrostatic or van der Waals' forces.

- **Reducing reactivity of cellular thiol nucleophiles-** C2-*exo* unsaturation can lead to lower electrophilicity at the N10-C11 position (Morris *et al.*, 1990) reducing the vulnerability to attack of cellular nucleophiles, such as thiols, at this position (implicated in the inactivation of DSB-120), allowing greater availability of the agent at tumour site.

It's interesting to note that C2 unsaturated PBDs have a unique characteristic in which the double bond at the C2 position has a tendency to migrate into the C-ring (*endo*) to benefit from the conjugation with nitrogen at the 4-position through overlapping of molecular orbitals. This characteristic of PBDs was strategically utilized in Leimgruber's pioneering synthesis of anthramycin (Leimgruber *et al.*, 1968) in which the product of the Horner-Emmons Wittig olefination results in formation of the double bond (**142**) outside the C-ring, but through proton

abstraction under basic conditions the *exo* double bond migrated into the C-ring (**143**) giving rise to the C2/C3-*endo* unsaturation of anthramycin (**Figure 1.5e**).

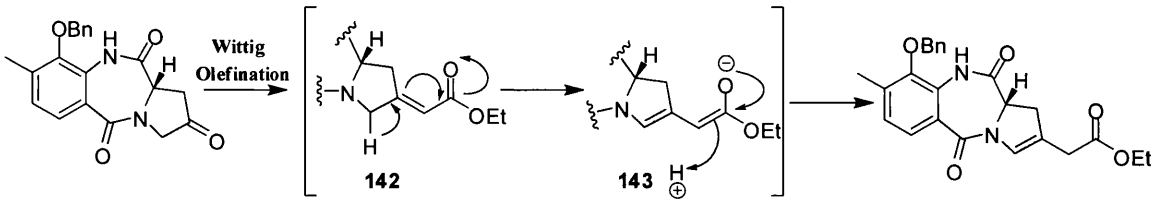
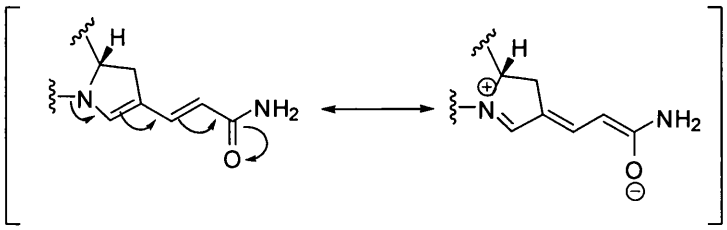


Figure 1.5e: Proposed mechanism for double migration during the Wittig-olefination reaction in Leimgruber's synthesis of anthramycin.

This characteristic feature is reinforced if there is a possibility of further resonance stabilization from C2-substituents (e.g. **Figure 1.5f**; anthramaycin acrylamide chain and C2-aryl groups).



C2-acrylamide resonance stablisation

Figure 1.5f: Resonance stabilization effect of *Endo-Exo* unsaturated PBDs.

1.5.3.6 Summary of SAR

A summary of the SAR of PBDs is shown below in **Figure 1.5g** (after Thurston *et al.*, 1993).

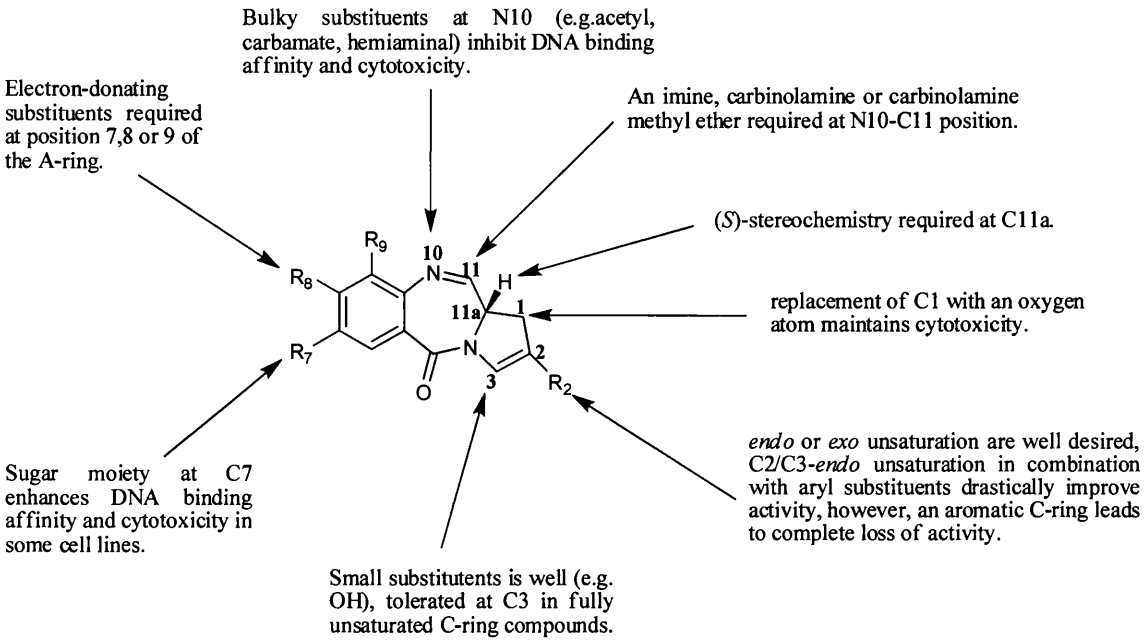


Figure 1.5g: Structure-Activity relationships of PBD ring system.

1.6 Clinical Applications of PBDs

1.6.1 Anthramycin and Sibiromycin.

Both anthramycin (**1**) and sibiromycin (**5**) have been used in clinical studies for the treatment of cancers but were withdrawn from clinical trials due to dose-limiting cardiotoxicity effects (Thurston and Hurley, 1983). Both these molecules possess C9-OH group in the aromatic ring and it is believed that the 9-hydroxy group is responsible for the cardiotoxic effects, especially

since that both neothramycin and tomaymycin, both lacking the 9-OH group are reported to be devoid of cardiotoxicity activity (Hurley, 1977; Fiyita *et al.*, 1982). The cardiotoxic effects of anthramycin and sibromycin closely mimic those of anthracyclines anticancer agents, Adriamycin and Daunomycin, which feature a para quinone ring. Anthramycin can undergo oxidation or tautomerization to the quinoneimines (**Figure 1.6a).** The quinine moieties present in the structure of **144** and **146** can generate superoxide and hydroxyl free radicals which can cause damage to a variety of cellular membranes, causing cell death. The heart is thought to be particularly susceptible to this damage because it has a large number of mitochondria, which are a site of free radical generation, and because it has low levels of antioxidant enzymes (J. Doroshow *et al.*, 1980). It's interesting to note that Co-enzyme Q10, which has a benzoquinone structure, is used as an antioxidant to provide specific protection against free radical damage to the heart caused by anthracyclines (Iarussi *et al.*, 1994).

Fortunately, even though the 9-hydroxy group found in anthramycin is involved in forming stable hydrogen bonds with DNA as well the acute cardiotoxicity effects, it is not essential for antitumour activity which was clearly demonstrated by SAR studies on anthramycin analogues devoid of the 9-OH group and which retained a significant *in vivo* cytotoxic potency of the parent anthramaycin (Chen *et al.*, 2004).

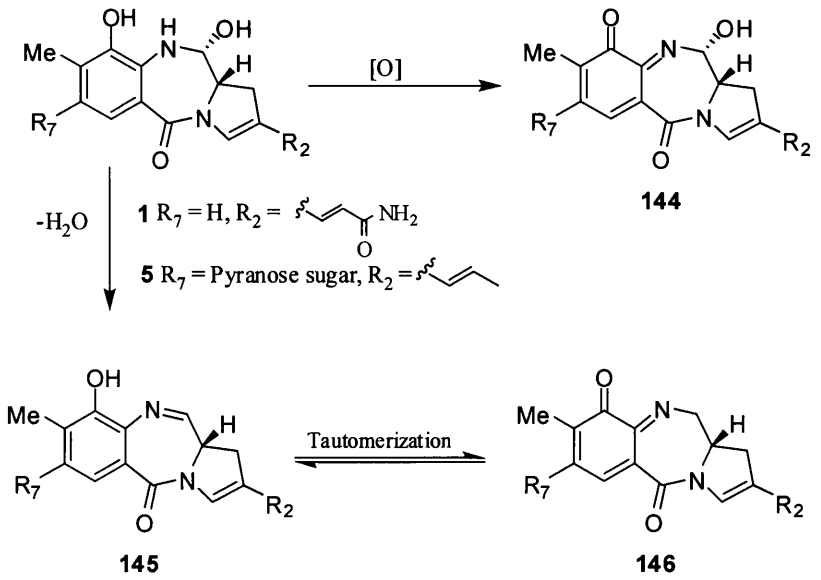


Figure 1.6a: Proposed mechanism for the conversion anthramycin (**1**) or sibiromycin (**5**) to the cardiotoxic quinoneimines via oxidation or tautomerization routes.

1.6.2 Neothramaycin and SJG-136 (PBD dimer).

Neothramaycin (**12**) has undergone Phase I clinical trials (Tsugaya *et al.*, 1986; Krugh *et al.*, 1989) and was highly effective for superficial carcinoma of the bladder, with four out of the eleven patients (36%) having the tumours completely eradicated, while 6 patients having partial disappearance (50%). Unfortunately due to dose-limiting nausea and vomiting, as well as overall lack of efficacy this PBD was withdrawn from clinical trials.

A synthetic PBD dimer SJG-136 has shown remarkable *in vivo* antitumour activity and exhibits no cardiotoxicity, suggesting it should be well tolerated in man (Alley *et al.*, 2004). Due to its excellent *in vivo* profile it has been selected for Phase I clinical trials with CR-UK and the NCI, with results suggesting that it should enter Phase II clinical trials very soon (detailed information for SJG-136 (**136**) is in section **1.7** of this chapter).

1.6.3 Water-soluble PBDs (Bisulphite adducts).

Water-soluble PBDs are highly desirable as they can potentially enhance the *in vivo* biological activity and bioavailability of the PBDs. Converting PBDs to bisulphite adducts makes them a lot more water-soluble. Some PBD bisulphite analogues have entered clinical trials and are described below.

In 1985, Keneko *et al.* reported the first PBD-bisulphite analogue (**147**, **Figure 1.6b**) which displayed significant *in vivo* antitumour profile against P388 leukaemia models (Keneko *et al.*, 1985). In 2001, Langlois *et al.* reported the synthesis of anthramycin bisulphite derivative (**148**, **Figure 1.6b**) which retained a significant degree of the potency of the parent anthramycin in various cell lines (Langlois *et al.*, 2001).

Keneko *et al.* also reported spadicomycin (**149**, **Figure 1.6b**), an anthramycin sodium bisulphite adduct, which was developed by Mitsubishi Pharma, which entered into clinical trials in the mid 1980's in Japan. Spadicomycin did reach phase III clinical trials, but unfortunately it was withdrawn due to dose-limiting hepatic and renal dysfunction toxicity. Interestingly the molecule was much less cardiotoxic than the parent PBD anthramycin.

Due to SJG-136 success in reaching Phase I clinical trials, and with the high probability of it entering Phase II clinical trials, it has prompted the development of follow up molecules of this type in our laboratory at the School of Pharmacy. Spirogen chemists at the School of Pharmacy, have synthesized the PBD dimer ZC-423 (**150, Figure 1.6b**) which differs in structure from SJG-136 by having a 4-methoxyphenyl substituent at the C2 position along with C2/C3 *endo* unsaturation and is prepared as a bisulphite adduct. It has shown outstanding and excellent *in vivo* antitumour profile, surpassing even SJG-136 *in vivo* performance. These outstanding results warrant progression of ZC-423 into pre-clinical development (Howard *et al.*, 2005).

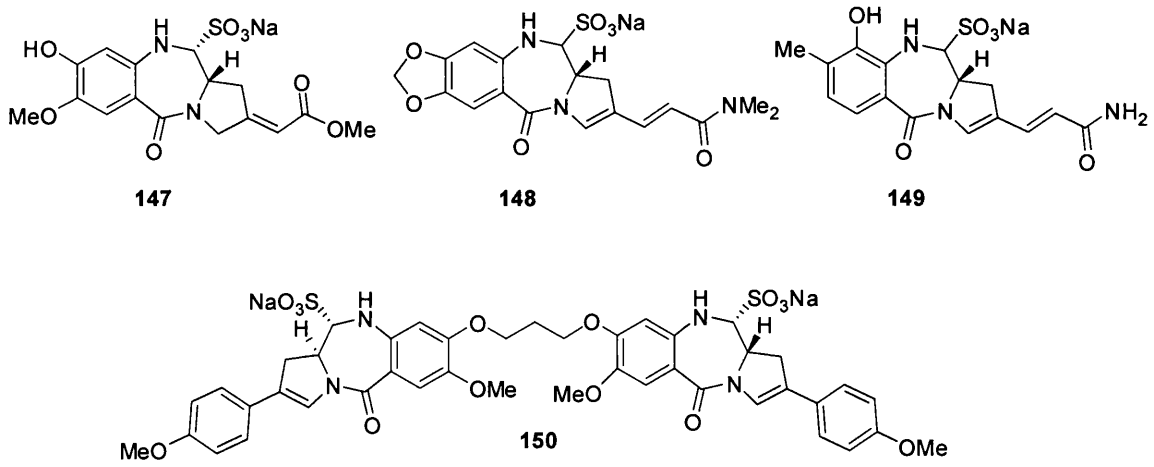
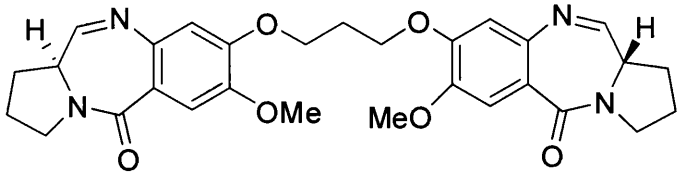


Figure 1.6b: Water-soluble PBD bisulphite adducts.

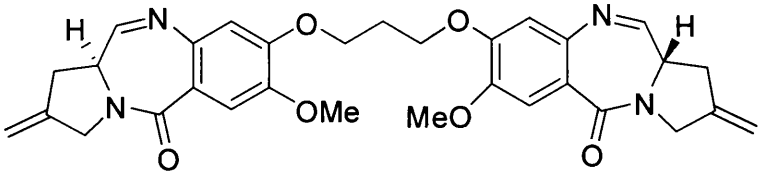
1.7 PBD Dimers

1.7.1 Rationale Behind their Synthesis

As previously mentioned, PBDs are monoalkylating agents which covalently bind to the exocyclic N2 of guanine in the minor groove of DNA via their electrophilic N10-C11 imine/carbinolamine moieties, with a preferred sequence selectivity for 5'-PuGPu motifs. On this basis, a rational design of novel PBD analogues was embarked upon where two PBD units were joined together through their C8 positions via a flexible 1,3-propanediyldioxy ether linkage to form bis-alkylating agents capable of forming interstrand DNA crosslinks by covalently binding to guanine bases on opposite strands. It was predicted that such a bis-alkylating agent would extend the number of base pairs (bp) spanned (3 bp for monomers), thus enhancing DNA sequence selectivity (Bose *et al.*, 1992b). Many clinically useful anticancer agents such as nitrogen mustards (i.e. chlorambucil) work by forming interstrand crosslinks with DNA which are known to disrupt DNA replication in rapidly dividing cells.



DSB-120 (**129**)



SJG-136 (**136**)

Figure 1.7a: DSB-120 and SJG-136 PBD dimers

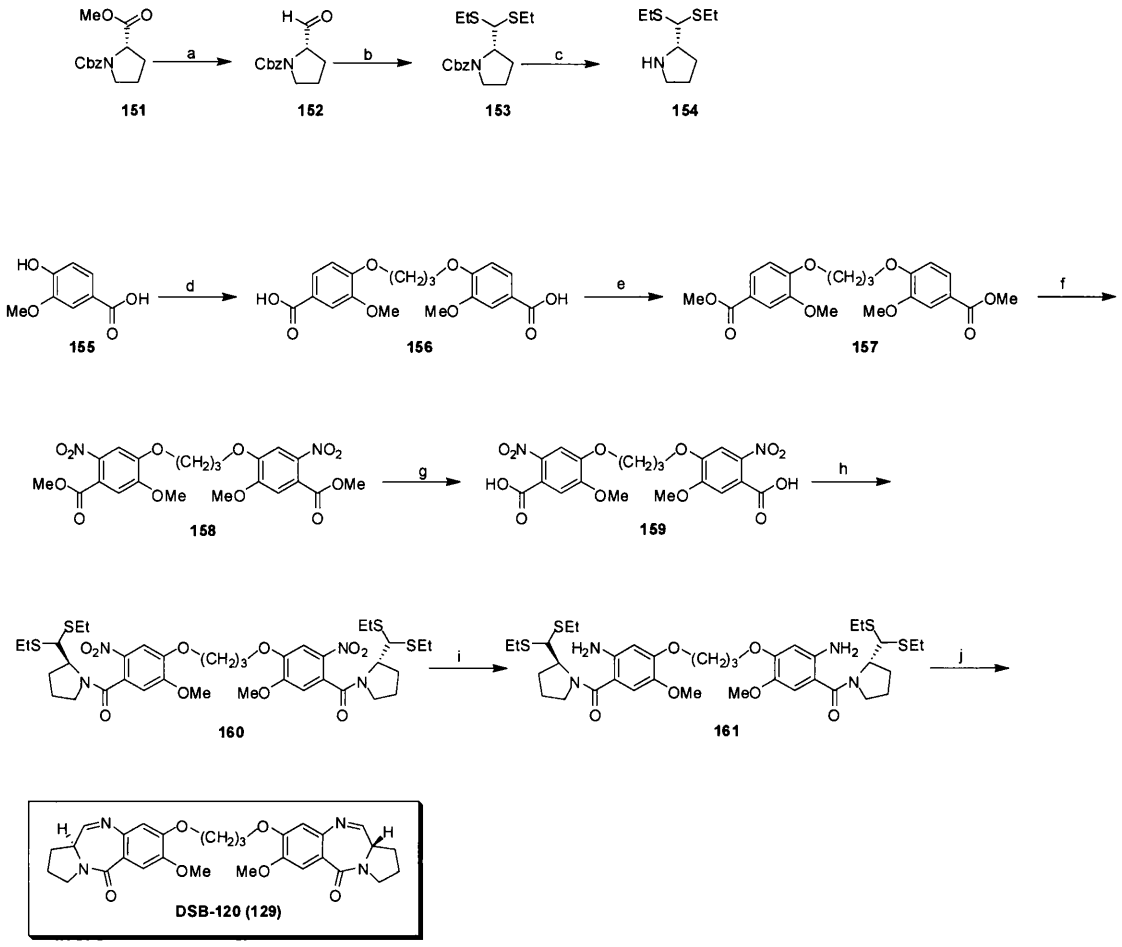
1.7.2 PBD Dimer- DSB-120

This concept of joining two PBD units together was first reported in 1988 by Suggs *et al.* who joined two DC-81 monomers via their C7 positions (Farmer *et al.*, 1988). This PBD dimer showed moderate DNA binding, but was found to be vulnerable to pH changes, as reversibility of the cross linkage in DNA was observed at pH 4-10 However, molecular models indicated that C8 linked dimers would have greater isohelicity within the minor groove, leading to greater DNA binding affinity compared to C7 linked dimers reported by Suggs *et al.* As a result, in 1992, D.S. Bose and co workers (Bose *et al.*, 1992b & c) reported the first example of C8-linked PBD dimer (**Figure 1.7a**, DSB-120) based on the DC-81 natural product monomer using the previously established thioacetal cyclization procedure. DSB-120 PBD dimer was shown to have remarkable DNA binding affinity which was observed through thermal denaturation studies with CT-DNA by elevating the DNA melting temperature (ΔT_m) by a significant 17.0 °C. In the same experiment the DC-81 parent monomer gives only a small increase in ΔT_m (0.7 °C). These results are consistent that PBD dimers form very stable interstrand crosslinks (Thurston *et al.*, 1996). The dimer was also shown, through investigations using an agarose gel electrophoresis assay, to be highly efficient crosslinking agents. The results revealed in the same assay, that the DSB-120 dimer was 50 and 300-fold more efficient than the major groove crosslinking agent's cisplatin and melphalan, respectively (Bose *et al.*, 1992b & c). *In vitro* cytotoxicity showed the dimer to be hundred to a thousand times more active in cytotoxicity than the monomer DC-81. Molecular modeling studies and NMR revealed that the dimer spanned six base pairs, actively recognizing 5'-PuGATCPy sequences. Unfortunately, DSB-120 did not progress through the initial *in vivo* studies due to the molecule's poor *in vivo* profile in the NCI xenograph assays. The low efficacy of DSB-120 was attributed to its high reactivity with thiol-containing molecules and protein alkylation prior to reaching the tumor site (Walton *et al.*, 1996).

1.7.2.1 Synthesis of DSB-120

DSB-120 was synthesized (Bose *et al.*, 1992b & c) using the previously established thioacetal cyclization procedure (Thurston *et al.*, 1990). The C-ring building block was synthesized (**Scheme 1.7b**) by utilizing the commercially available *N*-CBz protected methyl ester proline C-ring (**151**) which was reduced to the aldehyde (**152**) with DIBAL-H. The *N*-CBz protected dithioacetal **153** was synthesized by treatment with ethanethiol (HSEt) and TMSCl, followed by CBz removal using iodotrimethylsilane (TMS-I) in DCM to furnish the (2*S*)-pyrrolidine-2-carboxaldehyde dithioacetal C-ring (**154**).

The dimer acid **156** was synthesized (**Scheme 1.7b**) by heating two vanillic acid (**155**) units with diiodopropane in the presence of NaOH for 48 h. Following conversion to the methyl ester **157**, nitration was achieved with $SnCl_4$-*f*-HNO_3 to afford the nitro esters **158** in high yield. Hydrolysis of the esters groups to the corresponding bis-nitro acid **159** in quantitative yield was achieved using aqueous NaOH in THF at room temperature. The bis-nitrobenzoic acid was converted to its acid chloride and coupled with (2*S*)-pyrrolidine-2-carboxaldehyde dithioacetal to furnish the bis amide **160** in 65% yield. Reduction of the nitro group to the bis-amino thioacetal **161** with $SnCl_2.2H_2O$ in refluxing methanol, followed by cyclisation with $HgCl_2$-$CaCO_3$ to afford the target C8-linked dimers in good yield (**129**, DSB-120).



Scheme 1.7b: Bose's synthesis of DSB-120 dimer.

a) DIBAL-H, THF; **b**) EtSH, TMS-Cl, CH_2Cl_2; **c**) TMS-I, CH_2Cl_2; **d**) $I(CH_2)_3I$, DMF, $NaOH_{aq}$, THF, Δ; **e**) dimethyl sulphate, K_2CO_3, acetone, Δ; **f**) *f*-HNO_3, $SnCl_4$, CH_2Cl_2, –20 °C; **g**) $NaOH_{aq}$, THF; **h**) 1. $(COCl)_2$, DMF, THF; 2. (2*S*)-pyrrolidine-2-carboxaldehyde dithioacetal (**154**), TEA, H_2O, THF; **i**) $SnCl_2.2H_2O$, MeOH, Δ; **j**) $HgCl_2$, $CaCO_3$, CH_3CN, H_2O.

1.7.3 PBD Dimer- SJG-136

In 1999, S. J. Gregson and co workers (S. J. Gregson *et al*,. 1999) described the design and synthesis of a second generation C8/C8'-PBD dimer containing a C2/C2'-*exo* unsaturation (**Figure 1.7a**, SJG-136). The rational behind this design was based on the observation that C2-*exo* unsaturated PBDs, such as tomaymycin are less vulnerable to attack by sulphur nucleophiles such as glutathione, which may allow greater availability of the agent at the target DNA site (Morris *et al.*, 1990).

1.7.3.1 Cross-Linking and Molecular Modelling Studies

The DNA cross-linking efficiency of SJG-136 (S. J. Gregson *et al.*, 2001a) was investigated using a gel electrophoresis based assay developed by Hartley and coworkers (Hartley *et al.*, 1991).

The assay involves using a linear double-stranded DNA derived from the plasmid pBR322 (4632 bp, linearised with *Hind* III followed by ^{32}P-end-labelling). Following complete denaturation to the single-stranded DNA form, the presence of SJG-136 interstrand cross-links results in reannealing to double stranded DNA during electrophoresis on an agarose gel. Quantitation of the autoradiography by densitometry allowed calculation of the concentration required to effect 50% cross-linking. The results indicate that SJG-136 is a remarkably efficient cross-linking agent, being 440-fold more efficient than the major groove interstrand linking agent melphalan.

The formation of DNA interstrand cross-links by SJG-136 has been studied by computer-based molecular modeling studies **(Figure 1.7c)**. It has been established that SJG-136 binds within the minor groove of DNA in a symmetrical fashion, with both sides of the molecule chemically bonded to guanine residues on opposite strands of DNA. Introduction of an sp^2 carbon centre in the C2 position results in flattening of the C-ring which leads to a more planar arrangement of the molecule, enabling it to be immersed more deeply in the minor groove of DNA. As a consequence SJG-136 is well accommodated in DNA, so much so that very little distortion of DNA occurs with very little of the molecule being exposed outside the walls of duplex DNA **(Figure 1.7c)**. It is thought that this causes the SJG-136/DNA adducts to remain undetected for a longer length of time (S. J. Gregson *et al.*, 2001a & b). This is extremely interesting as much resistance to anti-cancer drugs which alkylate DNA, such as Cisplatin, is a result of DNA repair enzymes recognizing distortions or perturbations in the DNA and removing them, for example cisplatin causes a 90 °C bend in DNA when bound.

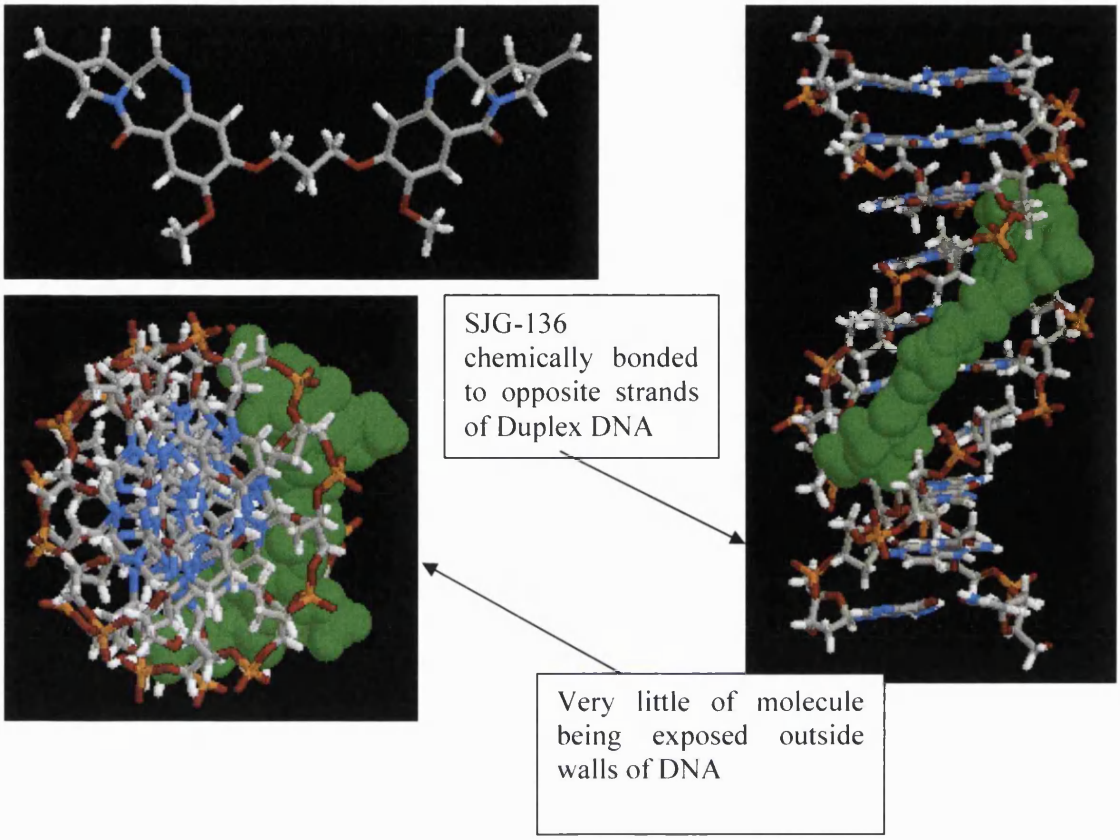


Figure 1.7c: Energy minimized molecular model of interstrand cross-link formation for SJG-136 bound to duplex DNA.

1.7.3.2 Sequence Selectivity of SJG-136

A combination of NMR, molecular modeling and DNA footprinting studies with SJG-136 has confirmed that it spans 6 base pairs and would preferentially crosslink PuGATCPy sequences (Martin *et al.*, 2005, **Figure 1.7d**).

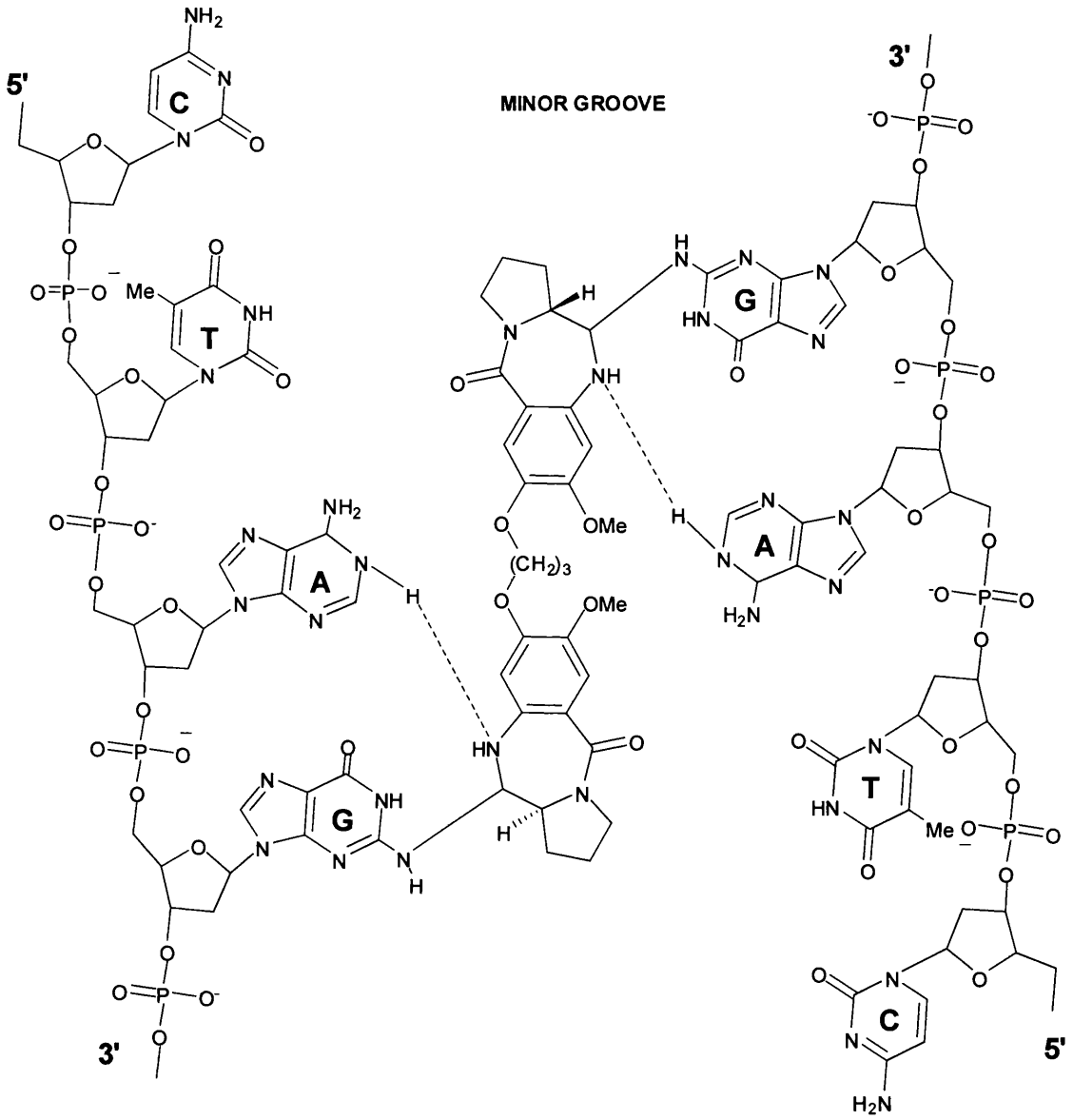


Figure 1.7d: Interstrand cross-linking and DNA sequence selectivity of PBD dimers.

As previously discussed, a number of oncogenes, including c-Ha-ras contain highly GC-rich regions in their sequences and PBDs are GC-site specific, which may contribute to their potency as antitumour agents. In 1994, S. Neidle *et al.* attempted to correlate the sequence selectivity of DSB-120 (or SJG-136) with its biological activity. The authors screened a number of oncogene sequences from the EMBL database for the target 5'-PuGATCPy sequences.

Interestingly, the sequences were not as randomly spread as expected. In fact several oncogenes (for example, v-src and bcl-3) have higher actual occurrences of these PuGACTPy sequences then would be predicted statistically. In others oncogenes such as abl, v-abl, bcl-2 and two ras genes the PuGATCPy sequences is significantly underrepresented and indeed several genes lack the DSB-120 binding sequence completely (S. Neidle *et al.*, 1994*)*. These results may suggest that these PBD dimers will have a greater affect on tumours that have elevated frequencies of the GATC sequence compared to ones which don't. It would be now be interesting to verify if tumours that are sensitive to SJG-136 contain oncogenes which have an overexpression of GATC regions in their sequences.

1.7.3.3 *In Vitro* and *In Vivo* Biological Evaluation of SJG-136

SJG-136 was found to be significantly more potent than DSB-120, which lacks substitution/unsaturation at the C2 position, and was shown to have remarkable DNA binding affinity and enhanced cytotoxicity and crosslinking efficiency, illustrating the benefit on biological activity of introducing C2-*exo*-unsaturation.

Table 1.7a: *In Vitro* cytotoxicity and thermal denaturation data for SJG-136, DSB-120 and cisplatin (adapted from S. J. Gregson *et al.*, 2001a).

$\Delta T_m/C$ [a] after incubation at 37 °C		IC $_{50}/\mu M$ [b]		
Compounds	**18 h**	**A2780**	**A2780cisR**	**RF[c]**
SJG-136	33.6	0.0000225	0.000024	1.1
DSB-120	15.1	0.0072	0.21	29.2
Cisplatin	-	0.265	8.4	32

[a]For CT-DNA at pH 7.00 ± 0.01, ΔTm = 67.83 ± 0.06 °C (mean value from 30 separate determinations). All ΔTm values ± 0.1-0.2 °C. For a 1:5 molar ratio of ligand-DNA, calf thymus DNA concentration = 100 μM and ligand concentration = 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01]. The ΔTm for SJG-136 at a [ligand]:[DNAp] molar ratio of 1:5 increases to a value of 34.4 °C after 72 h incubation. [b]Cells were incubated with compounds for 96 h at 37 °C and cell number assessed using Sulforhodamine B; [c]***RF*** **=Ration of IC_{50} resistant/parent.**

SJG-136 *in vitro* cyctoxicity data in human ovarian carcinomas cell lines A2780 and its Cisplatin-resistant counterpart A2780cisR clearly (see **Table 1.7a**) show that this compound is extremely cytotoxic, exhibiting one of the lowest IC_{50} values for any synthetic PBD analogue (S. J. Gregson *et al*,. 2001a). The most significant results were obtained in the Cisplatin-resistant A2780cisR cell line in which SJG-136 has high cytotoxicity in the picomolar region (IC_{50} = 0.000024 μM) and is approximatley 9000-fold more potent than DSB-120 (IC_{50} = 0.21 μM). In addition, SJG-136 suffers very little cross resistance (RF = 1.1, see **table 1.7a**) whilst with DSB-120 experiencing a 30-fold increase in resistance (RF = 29.21). Further more, SJG-136 raises the melting temperature of calf thymus DNA by a huge value of 33.6 °C after 18 h incubation at a [PBD]: [DNA] ration of 1:5 (see **Table 1.7a** below for details). SJG-136 has shown outstanding *in vivo* antitumour activity, as demonstrated by the high score of SJG-136 in the National Cancer Institute (NCI) hollow fiber assay. In fact SJG-136 is ranked as one the top five most active compounds tested in this assay to date (Alley *et al.*, 2004).

More significantly, SJG-136 demonstrated outstanding *in vivo* antitumour activity in ovarian cancer xenograft models. For example, in the cisplatin-sensitive CHI human ovarian tumour xenografts model, cisplatin at 4 mg/kg and SJG-136 at 0.2 mg/kg gave comparable levels of tumour growth delay (Hartley *et al.*, 2004) on the schedule testing (**see below**, **Figure 1.7e**). In comparison, in the cisplatin-resistant CH1cisR tumour, cisplatin was ineffective at 4 mg/kg, whereas SJG-136 produced a significant growth delay at 0.2 mg/kg (**see below**, **Figure 1.7f;** Hartley *et al.*, 2004). As a result of SJG-136 encouraging antitumour profile, it has entered Phase I clinical trials in the United Kingdom with CR-UK and United States with NCI, and preliminary results suggest Phase II evaluation is imminent. Consequently, this has prompted development of follow-up molecules of this type in our laboratories.

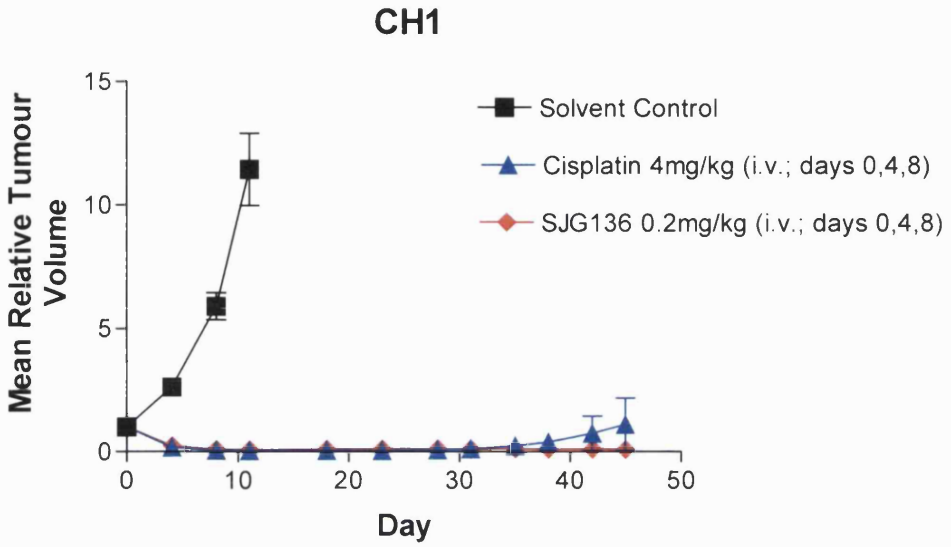


Figure 1.7e: *In vivo* activity of SJG-136 *versus* cisplatin against the CH1 human ovarian tumor carcinoma xenografts. (Hartley *et al.*, 2004; Vehicle control (1% DMSO/saline; n=10) or cisplatin (4mg/kg in saline; n = 6) or SJG-136 (0.2mg/kg in 1% DMSO/saline; n = 6) administered by intravenous injection into the tail vein on days 0, 4 and 8).

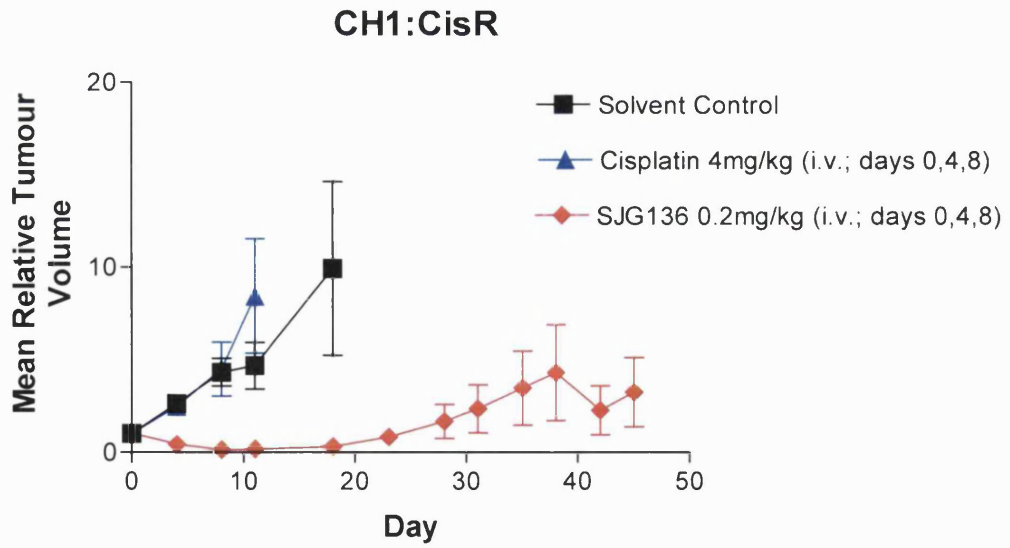


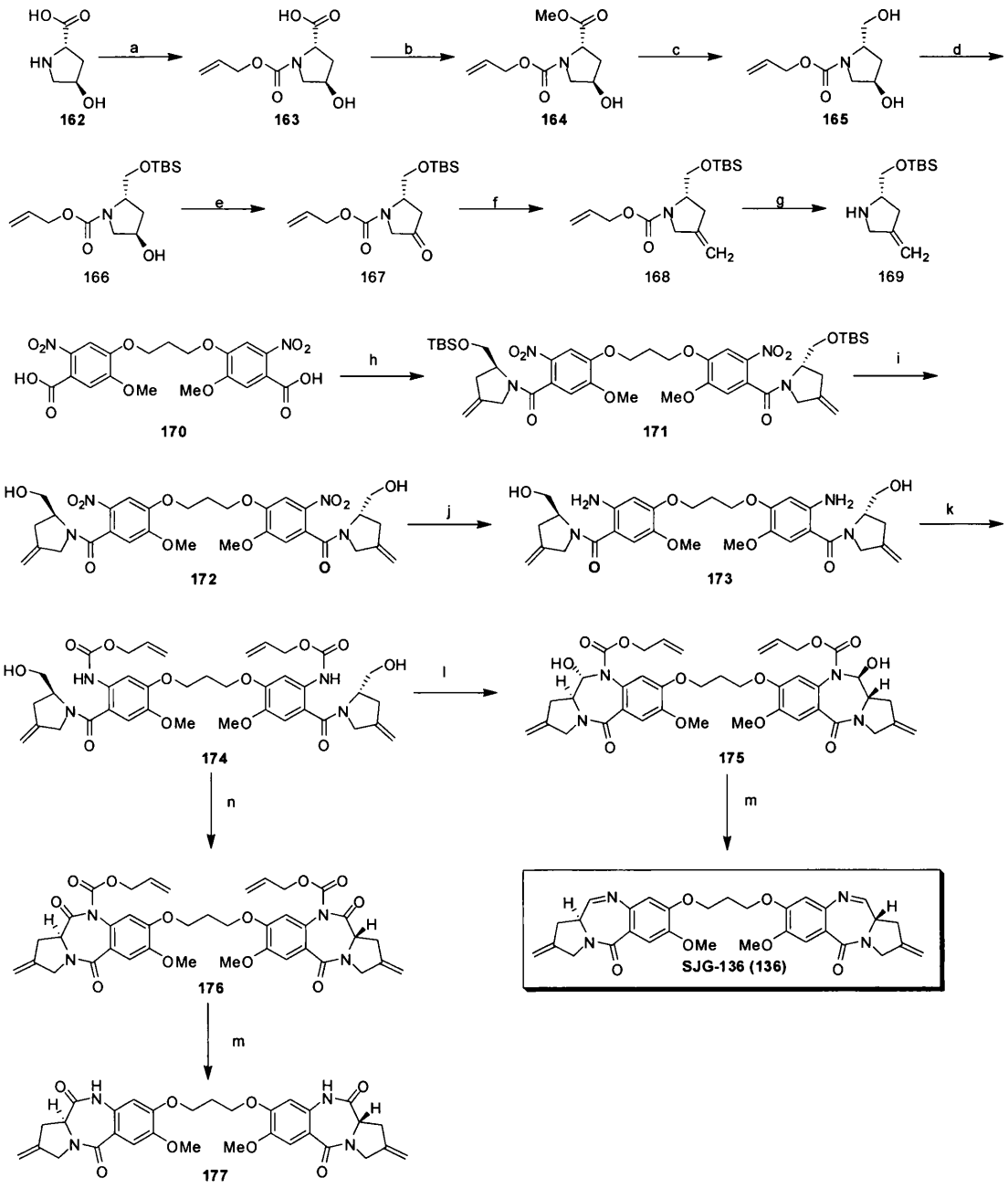
Figure 1.7f: *In vivo* activity of SJG-136 *versus* cisplatin against the acquired resistant line CH1cisR human ovarian tumour carcinoma xenografts (Hartley *et al.*, 2004).

1.7.3.4 Synthesis of SJG-136

SJG-136 was synthesized by employing the B-ring cyclisation strategy of the Fukuyama approach (Fukuyama *et al.*, 1993). The key step in the synthesis is the Swern oxidation of the primary alcohol which provokes spontaneous B-ring cyclisation. Finally, palladium-mediated Alloc cleavage furnished SJG-136 (S. J. Gregson *et al.*, 2001a).

Initially, commercially available trans-4-hydroxy-*L*-proline was *N*-protected as the allyl carbamate **163** in 87% yield (**Scheme 1.7g**). The acid was converted to the methyl ester group **164** in modest yield (43%) with catalytic H_2SO_4 in refluxing methanol. The methyl ester was reduced to the diol **165** in quantitative yield using $LiBH_4$. Chemoselective silylation of the primary alcohol over the secondary alcohol was achieved by employing DBU as silyl transfer agents to provide the mono-TBS ether **166** in 52% yield. Oxidation to the ketone **167** in quantitative yield was achieved using either Swern conditions or TPAP in the presence of NMO and 4 Å molecular sieves. Introduction of the C4 unsaturation was achieved by applying the Wittig reaction to ketone **167** to provide the olefin **168** in 87% yield. Cleavage of the Alloc group was achieved by using Palladium-catalyzed hydrostannolysis with tributyltin hydride to furnish amine **169** in 77% yield.

The known dimer core **170** (Thurston *et al.*, 1996) was converted to the bis-benzoic acid chloride with oxalyl chloride and subsequently coupled to **169** to provide the bis-nitrobenzyl amide **171** in 74% yield The bis-nitrobenzyl alcohol **172** was obtained in 94% yield by deprotecting the TBS groups under mild conditions with TBAF in THF. Reduction of the nitro groups was achieved by employing $SnCl_2.2H_2O$ in refluxing methanol to provide bis-aniline **173** in 61% yield. Following Alloc protection at the N10 positions to provide **174** 50% yield, **174** was subjected to Swern oxidation to obtain the protected carbinolamine **175**. Unfortunately, **174** were prone to overoxidation to the tetralactam **176** under these conditions. Instead **175** were obtained in modest yield (32%) by treatment of **174** with TPAP/NMO/4 Å molecular sieves. Finally deprotection of the Alloc group with $Pd(PPh_3)_4/PPh_3$/pyrrolidine afforded the novel PBD dimer (SJG-136) **136** in 43% yield. Cleavage of the N10/N10'-Alloc group from **176** under identical conditions furnished tetralactam **177** in 43% yield.



Scheme 1.7g: Synthesis of SJG-136 PBD dimer.

a) Alloc-Cl, aq. NaOH, THF, 0 °C; **b**) MeOH, H_2SO_4, Δ; **c**) $LiBH_4$, THF, 0 °C; **d**) TBS-Cl, TEA, DBU, CH_2Cl_2; **e**) TPAP, NMO, 4 Å sieves, CH_2Cl_2-CH_3CN; **f**) Ph_3PCH_3Br, KO^{t}Bu, THF, 0 °C; **g**) Bu_3SnH, $Pd(PPh_3)_2Cl_2$, H_2O, CH_2Cl_2; (**h**) 1. $(COCl)_2$, DMF, 2. then **169**, TEA, H_2O, THF, 0 °C; **i**) TBAF, THF, 0 °C; **j**) $SnCl_2.2H_2O$, MeOH, Δ; **k**) Alloc-Cl, pyridine, DCM, 0 °C; **l**) TPAP, NMO, 4 Å sieves, CH_2Cl_2, CH_3CN; **m**) $Pd(PPh_3)_4$, PPh_3, pyrrolidine, CH_2Cl_2, CH_3CN, 0 °C; **n**) $(COCl)_2$, DMSO, TEA, DCM, -45 °C.

2 AIMS

The PBD ring system has generated a considerable amount of interest due to SJG-136 entering Phase I clinical trials in the UK and USA (Alley *et al.*, 2004). SJG-136 success has prompted development of follow up molecules of this type in our laboratories.

2.1 Design and Synthesis

Synthetic introduction of an additional aryl substituent at the C2-position has dramatically enhanced the *in vivo* activity of PBD monomers (Cooper *et al.*, 2002). This dramatic gain in activity has been partially attributed to C2/C3 *endo* unsaturation in conjugation to C2-aryl fragment which causes the geometry of the molecule to change enabling it to fit deeply and snugly in the minor groove of DNA as well as providing C2-aryl group to project directly along the walls of the minor grove, in an optimal position to pick up favourable electrostatic and hydrogen bonding or van der waals' interactions. Upon dimerisation, PBDs not only efficiently cross-link DNA but are also known to enhance sequence selectivity and antitumour activity. Therefore, the principal objective was to synthesise novel PBD dimers through the introduction of various C2-aryl groups via a new, improved and concise methodology (**Figure 2.1a**).

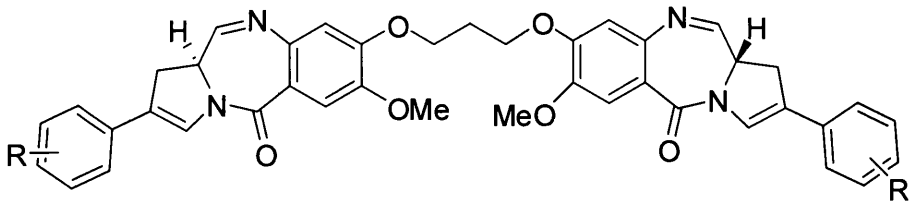
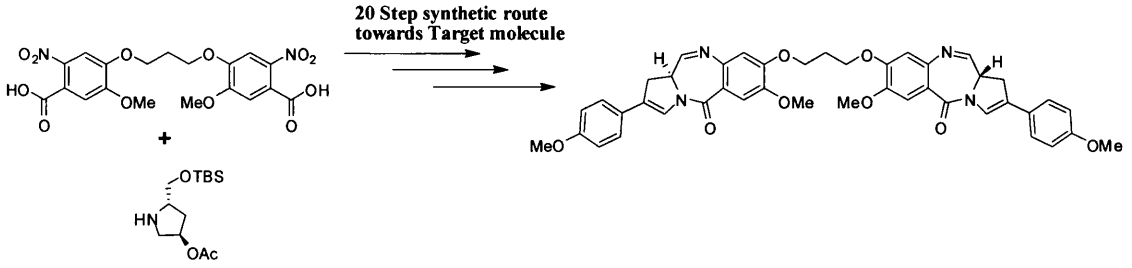


Figure 2.1a: Target C2-aryl PBD dimers.

The synthesis of a number of novel C2-aryl PBD dimers analogues would not only provide potentially highly potent antitumour agents with improved cytotoxicity but would also provide further SAR information on the role played by the additional aryl fragment in the biological activity of PBDs, as this is not fully understood.

PBD dimer synthesis can be cumbersome, often involving a large number of synthetic steps. For example, Chen *et al.* reported the synthesis of C2-aryl PBD dimer imine via an N10-protected PBD C11-carbinolamine route where the C2-aryl methoxy substituent was added by a Suzuki-cross coupling reaction. Nonetheless, the synthetic strategy involves six steps to synthesize the C-ring starting material with the total number of synthetic steps being twenty to reach the target C2-aryl PBD imine dimer (**Scheme 2.1b;** Chen *et al.*, PhD Thesis 2004).



Scheme 2.1b: Twenty step synthetic route to the synthesis of C2-aryl methoxy PBD dimer imines via N10-protected PBD C11-carbinolamine.

Leimgruber's dilactam strategy offers the possibility of directly obtaining the PBD ring system from commercially avalable proline building blocks and readily avalible *O*-nitrobenzoic acids. This strategy would be applied initially to the synthesis of the C-ring unsubstituted PBD dimer, DSB-210 and then to the more complex C2-aryl PBD dimers targets via the triflate/Suzuki approach reported by cooper *et al.*

2.2 Biological Evaluation

The resulting PBD dimers would be assessed for their cytotoxicity in human tumour cell lines assays. All PBD dimers would also be subjected to a gel electrophoresis assay to measure their DNA cross-linking efficiency.

3 RESULTS AND DISCUSSION

3.1 Synthetic Strategy

The synthetic strategy for the synthesis of novel C2-aryl PBD dimers combines the strengths of Howard and Thurston's approach to C2-aryl monomers (Cooper *et al.*, 2002) with Hu's approach to the synthesis of DC-81 natural product PBD monomer (Hu *et al.*, 2001).

Howard-Thurston Approach- The synthetic strategy was initially based on the classic Leimgruber and Pena and Stille approach to anthramycin (**see sections 1.4.2 and 1.4.4**), but a hemiaminal protecting group (N10-SEM) was utilised to promote the $NaBH_4$ mediated N10/C11-lactam reduction (**see section 1.4.6**) which was analogous to Mori's MOM-protected dilactams reduction. The C2-aryl substituents were obtained through Suzuki coupling chemistry via a key enol-triflate intermediate, which is similar to the enol-triflate dilactams reported by Peña and Stille (1989) in their synthesis of anthramycin.

Hu's Approach- Hu and co-workers developed a short and efficient synthesis (**see section 1.4.5**) of DC-81 incorporating the use of an improved hydride reduction method involving $LiBH_4$.

The key Aspects in the Synthetic Strategy are;

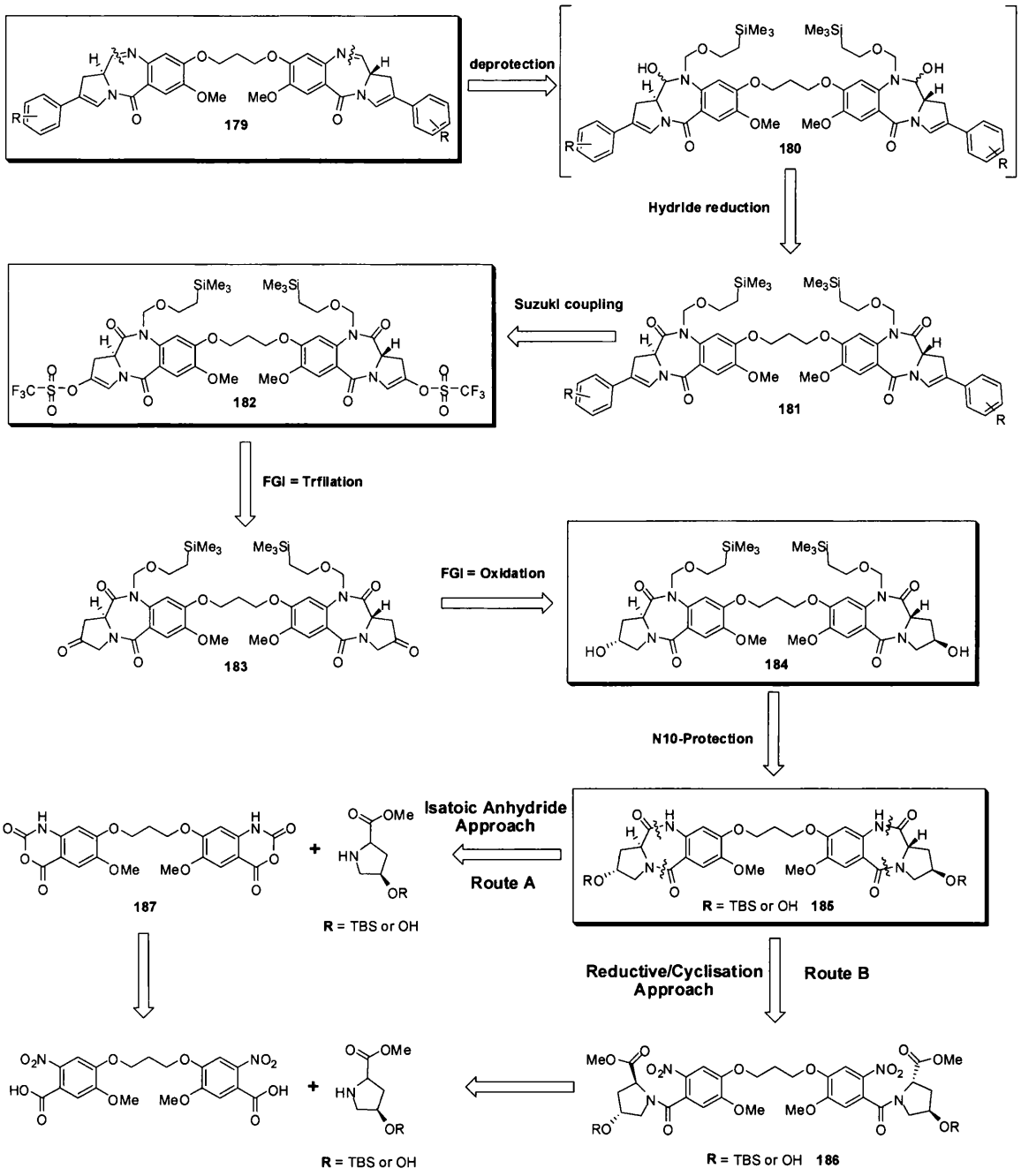
- The use of dilactam intermediates which are extremely stable and are relatively straight forward to obtain.
- The use of C2-C3 enol triflates which can be subjected to versatile palladium catalysed cross-coupling reactions; in particular Suzuki type reactions which can generate a diverse array of analogues from commercially available arylboronic acids.
- The formation of the critical N10-C11 imine moiety via N10/C11-lactam hydride reduction of protected dilactams followed by N10-SEM deprotection under mild conditions. All the known methods of hydride reduction of protected dilactams preserve the required (*S*)-stereochemistry at C11a position.

3.1.1 Retrosynthetic Analysis of the Target C2-Aryl PBD Dimer's

The key chemical steps and intermediates in the synthetic strategy are illustrated in the retrosynthetic analysis shown below (**Scheme 3.1a**). The first disconnection of the target PBD dimer **179** begins with the disconnection of the N10-C11 bond to give the transient N10-SEM protected carbinolamine **180**, which can be obtained by selective N10/C11-lactam reduction with $NaBH_4$ of **181**. The C2/C3-*endo* unsaturated C2-aryl PBD intermediate **181** can be derived by subjecting the enol-triflate **182** to Suzuki reactions with arylboronic acids. It was anticipated that the enol-triflate would be obtained from the C2-ketone **183** which would in turn be formed by oxidation of the C2-alcohol **184**. The key N10-SEM protected C2-alcohol tetralactam intermediate **184** could be acquired from the versatile key tetralactam precursor **185** through a series of protection and deprotection steps.

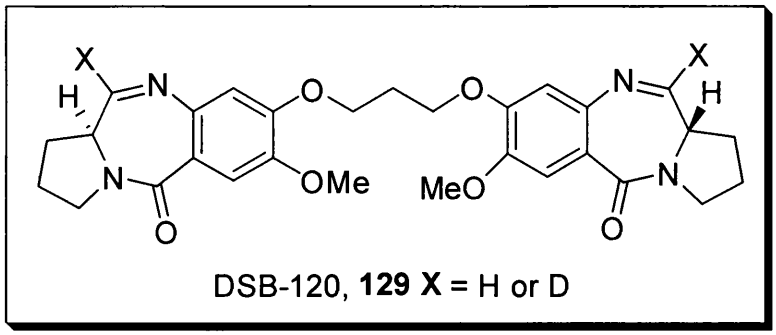
The key tetralactam precursor **185** could conceivably be produced via two synthetic approaches;

- **Isatoic Anhydride Approach (Route A)** - involved coupling of isatoic anhydride intermediate **187** to pre-formed secondary amine **188**. Intermediate **187** could be prepared through a triphosgene reaction with the well known bis-nitrobenzoic acid dimer core **159**.
- **Reductive/Cyclisation Approach (Route B)** - involved reductive/cyclisation of the bis-nitrobenzamide **186**, which could originate from amide coupling with the well known bis-nitro-acid dimer core **159** to the secondary amine C-ring **188**.



Scheme 3.1a: Retrosynthetic analysis of the target C2-aryl PBD dimers.

3.2 A Novel and Concise Synthesis of DSB-120- Via Tetralactam Hydride Reduction and N10-SEM Deprotection



DSB-120, **129 X** = H or D

The initial objectives were to set up model studies to investigate the two approaches towards the synthesis of C2-aryl PBD dimers via tetralactam intermediates. In 1992, D.S. Bose *et al.* reported the synthesis of a C8/C8' linked PBD dimer, DSB-120 (**129**), and was found to be a highly potent interstrand crosslinking agent with enhanced cytotoxicity (Bose *et al.*, 1992b & c, **see section 1.7 PBD dimers** for details). DSB-120 was selected as a model compound to perform these studies, as synthesis of C-ring unsubstituted PBDs required fewer chemical transformations, since no installation of C2-substituents was required.

3.2.1 Retrosynthetic Analysis of the DSB-120.

The key objective of these model studies was to investigate the feasibility of utilizing the isatoic anhydride approach (**Route A**) compared to the reductive/cyclisation approach (**route B**) for generating the key tetralactam precursor **192 (Scheme 3.2a).** In addition, the model studies would be used to optimise the final reduction/deprotection step involving the N10-SEM protected dilactam. The retrosynthetic analysis of DSB-120 illustrated below in **Figure 3.2a** is analogous to the retrosynthetic analysis of the target C2-aryl dimer shown in **Figure 3.1a**, except no intricate C-ring modifications occur.

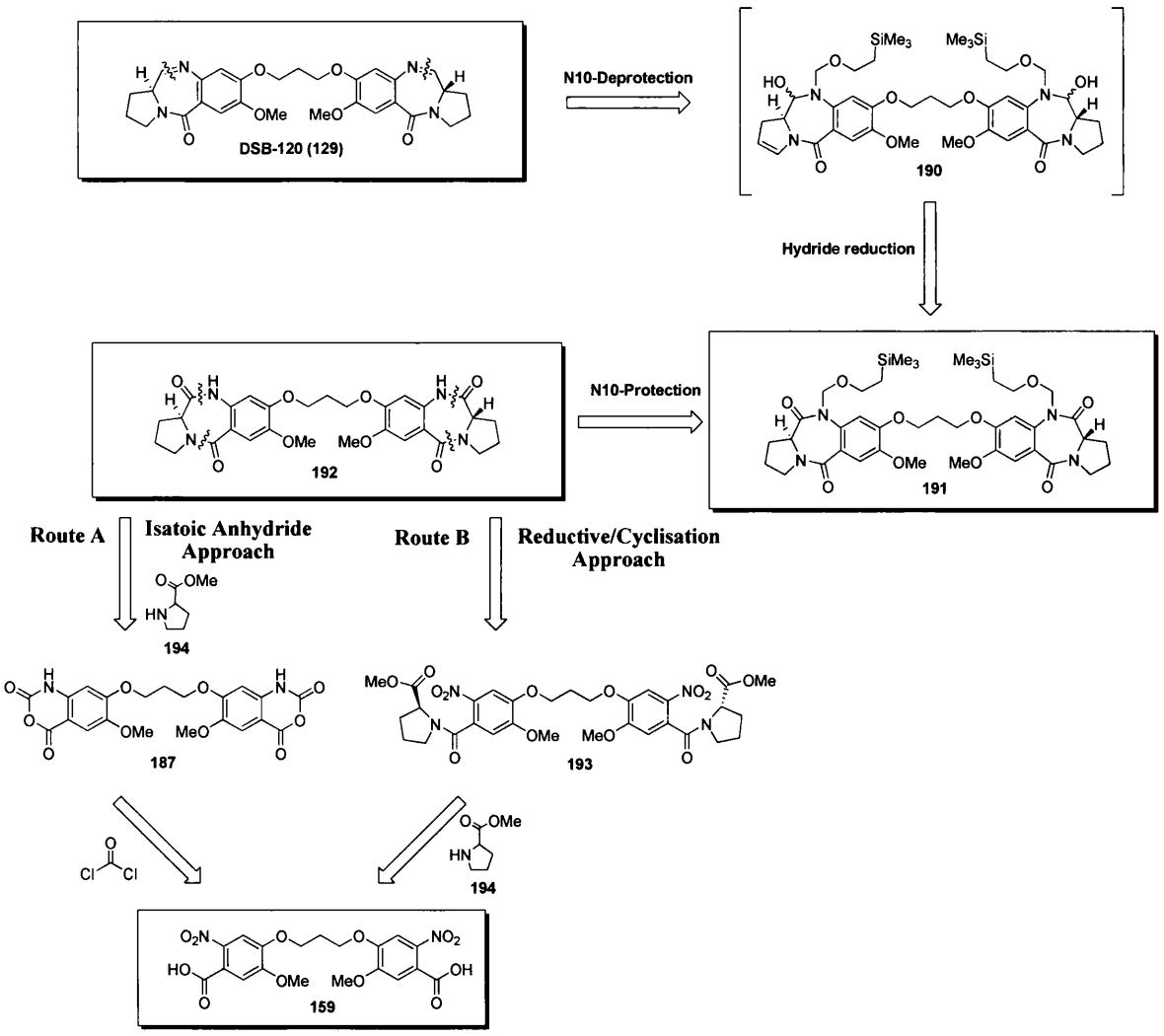


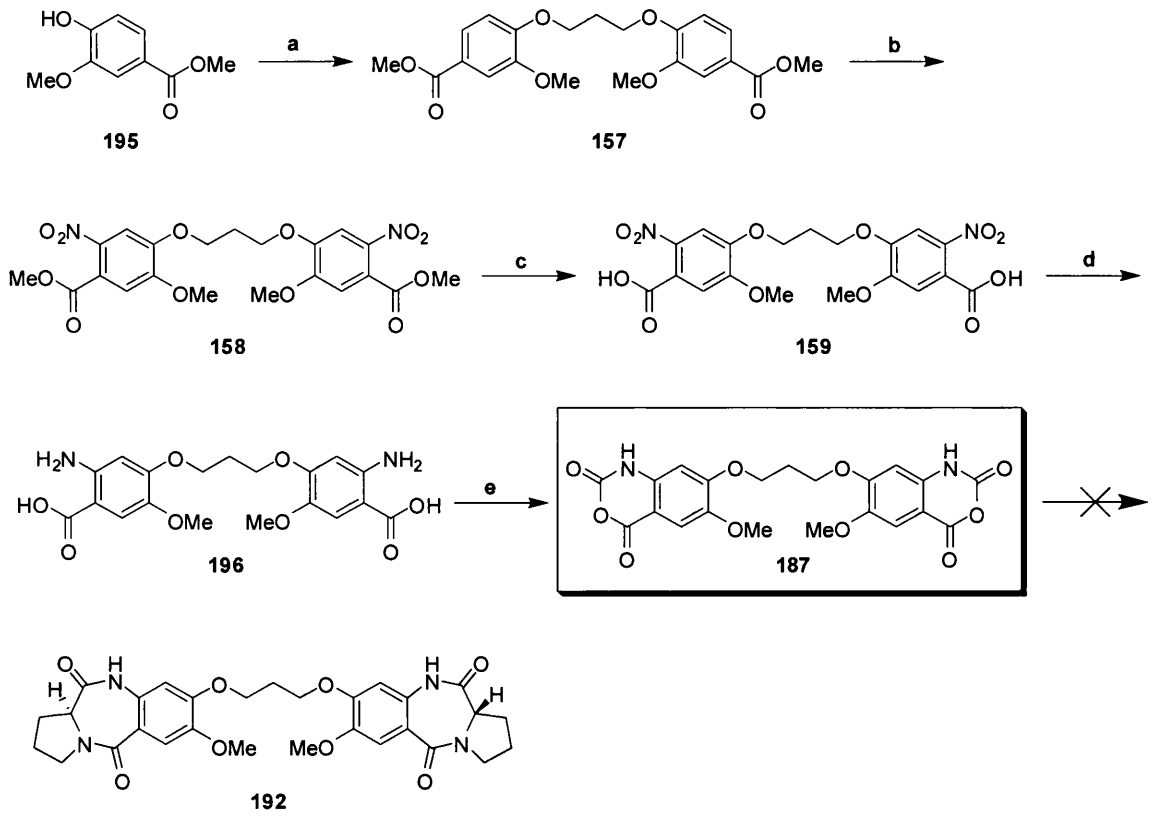
Figure 3.2a: Retrosynthetic analysis for the synthesis DSB-120- Model studies to investigate approaches.

3.2.2 Attempted Synthesis of Key Tetralactam Precursor 192- via Isatoic Anhydride Approach.

As previously discussed, the synthesis of the target C2-aryl PBD dimers is based entirely upon versatile tetralactam intermediates. Therefore, generating an efficient route for the synthesis of key tetralactam precursor **192** is critical. The first approach explored was the isatoic anhydride strategy (**route A**) which involves coupling the isatoic anhydride intermediate **189** to the commercially available C-ring to potentially generate the key tetralactam precursor **192**.

3.2.2.1 Synthetic Route

The methodology adopted was base upon Hu and co-worker's synthesis of the natural product PBD monomer DC-81 (Hu *et al.*, 2001).



Scheme 3.2b: Attempted synthesis of key tetralactam precursor **192** via isatoic anhydride approach.

a) $HO(CH_2)_3OH$, Ph_3P, Diisopropylazodicarboxylate, THF, 0 °C, 89%; b) $Cu(NO_3)_2$, Ac_2O, 0 °C, 79%; **c**) 1 M NaOH aq, THF, r.t., 88%; d) Ammonium formate, EtOH, r.t., 92%; **e**) Triphosgene, THF, Δ, 70%.

The synthesis began (**Scheme 3.6b**) with the preparation of the well known bis-nitrobenzoic acid **159** reported previously by Gregson *et al.* (2001a & b). The dimer core **159** was obtained by Mitsunobu reaction with commercially available methyl vanillate **195** to give the bis-benzoester **157** in 89% yield. Subsequent nitration using copper nitrate hydrate afforded the bis-nitrobenzoate **158** which was converted to the bis-nitrobenzoic acid **159** by ester hydrolysis using 1 M NaOH aq in 88% yield.

The next step was to reduce the nitro groups to the corresponding Bis-amine **196**. The first method investigated was $SnCl_2.2H_2O$ in refluxing methanol reported by Hu *et al.* in their synthesis of DC-81. Unfortunately this resulted in only partial reduction of the nitro groups as well as formation of unwanted side products, as observed by LC-MS analysis of the reaction mixture. The reaction was repeated under a variety of different conditions all of which resulted in either partial reduction and/or formation of unidentified and unwanted side products. As a result an alternative nitro reduction method was explored.

Catalytic hydrogenation with Pd-C was investigated (Cooper *et al.*, 2001) and attempts to dissolve the insoluble bis-nitrobenzoic acid dimer core **159** in a suitable hydrogenation solvent proved problematic, the best hydrogenating solvents, ethanol>EtOAc>DMF, failed to sufficiently dissolve the compound. However, after performing careful solubility tests, a mixture of ethanol:THF (90:10 v/v) was found to dissolve the bis-nitrobenzoic acid and the solution was subjected to hydrogenation with Pd-C. Despite using a suitable solvent system, partial reduction and unwanted side products were observed by LC-MS analysis and TLC. Optimizing the different conditions of stoichiometry and pressure did not solve this problem. Another reduction method was attempted using catalytic transfer hydrogenation, in the presence of Pd-C and 1,4-cyclohexadiene (Thurston *et al.*, 1990; Felix *et al.*, 1978). Reaction monitoring by TLC and LC-MS revealed a mixture of products, including large amounts of starting material, partial reduction and numerous side products. However despite varying the reactions conditions (including stoichiometry, temperature and reaction time) no improvement in the reaction occurred. Finally, the successful reduction of both nitro groups was achieved using an ammonium formate method (Ram and Ehrenkaufer, 1984) to provide the bis-amine **196** in 92% yield. The presence of the bis-amine compound **196** was supported by LC/MS 1.53 min (ES+) m/z (relative intensity) 465 ($[M + H]^{+\cdot}$, 70) 487 ($[M + Na]^{+\cdot}$, 35) compared to bis-nitro acid starting material LC/MS 1.25 min (ES+) m/z (relative intensity) 405 ($[M + H]^{+\cdot}$, 100). In addition, ^1H NMR data revealed a characteristic up field shift in aromatic protons in the bis-amine compound **196** compared to Bis-nitro compound **159**, which is consistent with amine formation (**Figure 3.2c**).

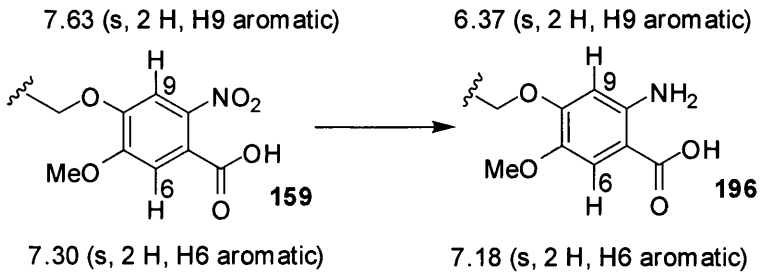


Figure 3.2c: ^{1}H NMR chemical shift (δ ppm) for **159** and **196.**

The bis-amine **196** was then treated with triphosgene in refluxing THF (Hu *et al.*, 2001) to give the bis-isatoic anhydride intermediate **187** in 92% yield. Again LC/MS confirmed the formation of product **187** 3.65 min (ES-) m/z (relative intensity) 457 ($[M - H]^{+\cdot}$, 87), 479 ($[M + Na]^{+\cdot}$, 60) compared to bis-amine **196** LC/MS 1.53 min (ES+) m/z (relative intensity) 465 ($[M + H]^{+\cdot}$, 100). ^{1}H NMR exhibited a down field shift of 9-H signal consistent with the change from the more polar bis-amine compound **196** to the less polar isatoic anhydride **187** (δ 7.18 & 6.37 ⟶ δ 7.28 & 6.80 ppm).

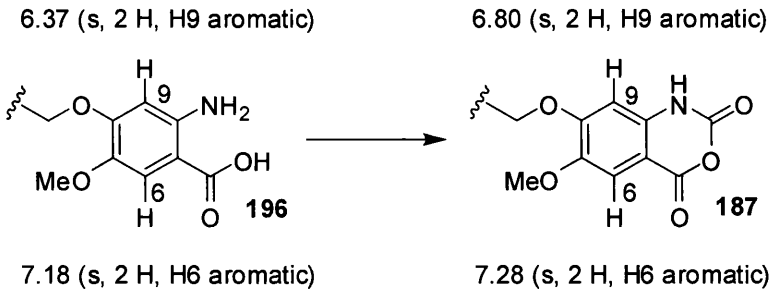
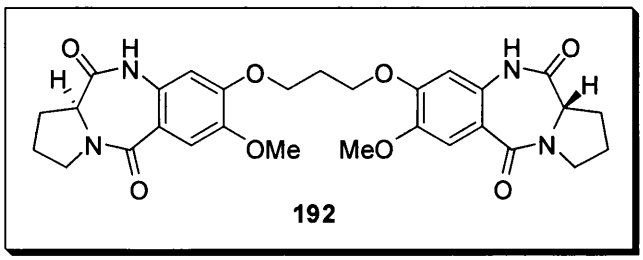


Figure 3.2d: ^{1}H NMR chemical shift (δ ppm) for **196** and **187.**

The critical step of the synthesis was coupling of the commercially available methyl ester proline C-ring **194** with the isatoic anhydride intermediate **187.** Following Hu and co-worker's reported coupling conditions (Δ in DMSO at 120 °C) failed to obtain the desired tetralactam product **196**. Instead, the reaction mixture appeared to have decomposed to a dark brown tar which was believed to be caused by refluxing in DMSO at high temperatures for long periods of time. Therefore, microwave chemistry was investigated to reach the required coupling temperature more rapidly.

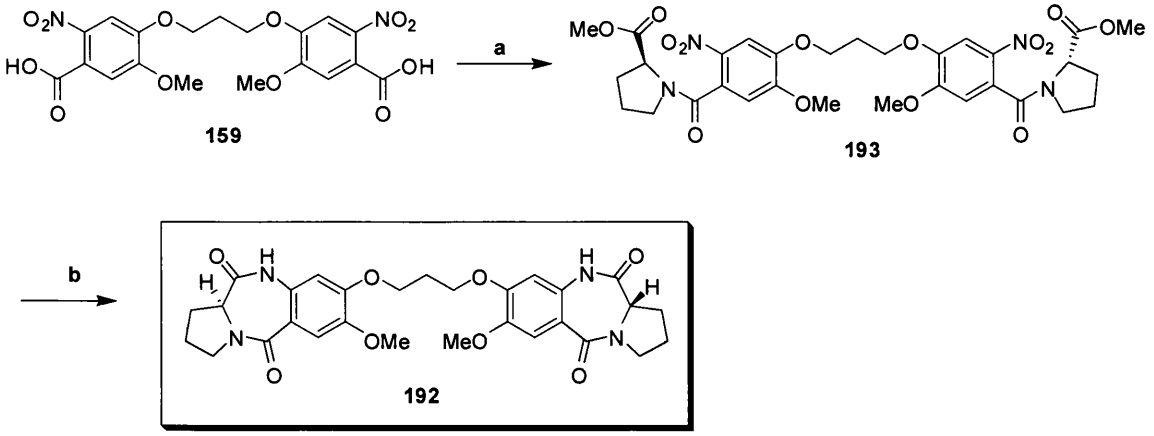
However, despite varying the reactions conditions (including stoichiometry, temperature, solvents and time) no improvement could be made, with only trace amounts of the desired product **196** being observed by LC/MS analysis (trace at 1.28 min (ES+) m/z (relative intensity) 565 ($[M + H]^{+\cdot}$, 54)]). Due to these disappointing results the isatoic anhydride strategy was not pursued any further and the approach was abandoned.

3.2.2 Successful Synthesis of Key Tetralactam Precursor 192-Via Reductive/Cyclisation Approach.



3.2.2.1 Synthetic Route

The methodology adopted was initially based upon Peña and Stille synthesis of anthramycin (Peña and Stille, 1986).



Scheme 3.2e: Synthesis of key tetralactam precursor **192** via reductive/cyclisation approach.

a) 1. $(COCl)_2$, DMF, CH_2Cl_2, r.t.; 2. **194** C-ring, TEA, THF, – 60 °C, 95%. b) 1. 10% Pd-C, EtOH:EtOAc, H_2 (50 psi); 2. HCl_{conc}/H_2O/THF (0.03:10:1 v/v), r.t., 90%.

The synthesis commenced (**Scheme 3.2e**) with the well known bis-nitrobenzoic acid dimer core **159** which was converted to the corresponding acid chloride by treatment with oxalyl chloride followed by catalytic amounts of DMF. Subsequent coupling to the commercially available methyl ester proline C-ring **194** provided the bis-nitrobenzoamide **195** in 95% yield. The structure was supported by LC/MS 5.82 min (ES+) m/z (relative intensity) 689 ($[M + H]^{+}$, 100) 711 ($[M + Na]^{+}$, 35). In addition, the ^{1}H and ^{13}C NMR spectra of **195** exhibited signals expected for the bis-nitrobenzoamide (**Figure 3.2f**), including an additional methoxy signal corresponding to the methyl ester proline C-ring at δ 3.79 (s, 6 H, CO_2Me x 2) and methyl signal at 52.3 ppm respectively. In addition, a doublet of doublets (dd) signal was observed corresponding to the H2 proton of the C-ring at δ 4.72 (dd, 2 H, J = 8.63, 4.41 Hz, H2) and methine single at 60.6 ppm respectively.

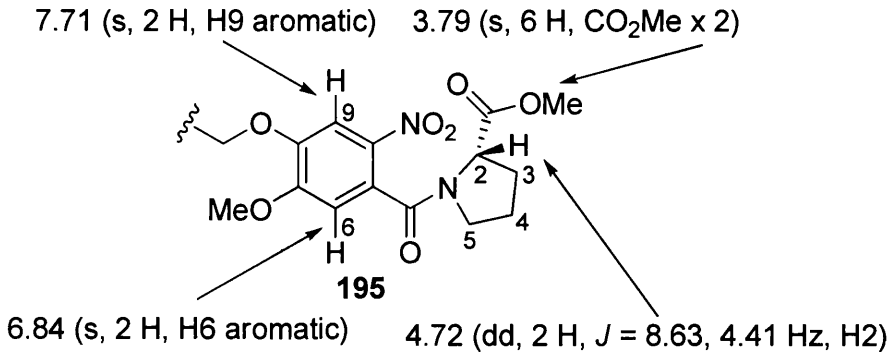


Figure 3.2f: ^{1}H NMR chemical shift (δ ppm) for **195**.

B-ring cyclisation (**Figure 3.2g**) was expected to take place after reduction of the nitro group through catalytic hydrogenation with Pd-C, with subsequent acid treatment (Conc.HCl) with H_2O/THF as previously reported by Pena and Stille (1989).

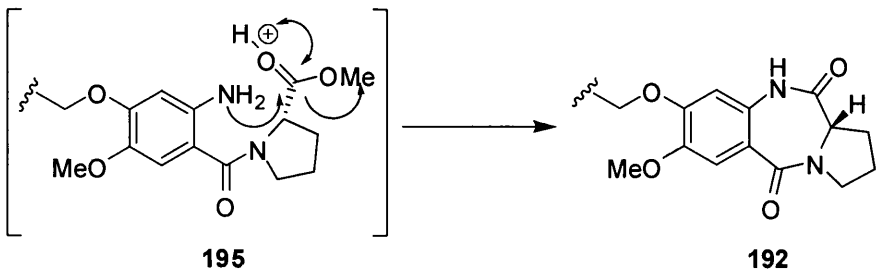


Figure 3.2g: Mechanism for B-ring cyclisation to form tetralactam precursor **192.**

LC-MS analysis After 3 h of hydrogenation of the bis-aniline **195** at 45 Psi in ethanol revealed the incomplete consumption of starting material. After adding additional 50 mL of EtOAc to the reaction mixture it was allowed to hydrogenate for further 3 h at 50 Psi, when LC-MS revealed incomplete consumption of starting material and the formation of number of transient intermediates (**Figure 3.2h)** towards the formation of the key tetralactam intermediate **192**.

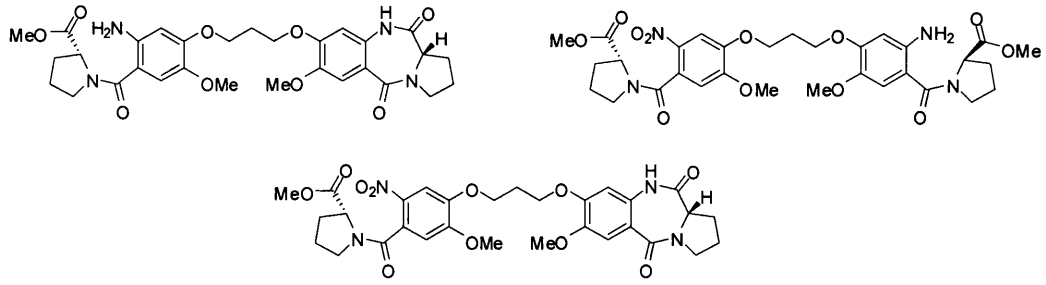


Figure 3.2h: Transient intermediates observed during 6 h hydrogenation.

The reaction mixture was allowed to hydrogenate overnight after which time LC-MS analysis revealed complete consumption of starting material. This reaction mixture was immediately worked-up and treated with THF:water (Conc. HCl) and allowed to stir for 3 h when LC-MS revealed formation of the tetralactam precursor **192** in 90% yield with no subsequent purification step needed (LC/MS 4.88 min (ES+) m/z (relative intensity) 565 ($[M + H]^{+\cdot}$, 100) 588 ($[M + Na]^{+\cdot}$, 20)). Acid treatment steps in PBD synthesis are a major cause of concern, as racemisation at the C11a position has been known to occur under acidic conditions. For example, Thurston and co-workers reported the synthesis of DC-81 using an acidic amberlite resin step to promote B-ring cyclisation via an oxygen acetal route, which resulted in racemisation of DC-81 (Bose *et al.*, 1992a). The racemic DC-81 was found to have to have reduced activity against tumour cells. As a result, Optical rotation analysis of this compound was measured and was found to be $[\alpha]_D^{23} = +397°$ ($c = 0.5$, DMSO) indicating that an optically active PBD was produced in this reaction. The successful B-ring cyclisation was confirmed by the characteristic downfield signal at δ10.19 ppm in ^1H NMR spectra (**Figure 3.2i**) corresponding to the N10 amidic proton. In addition, there was a marked upfield shift in resonance of the H9 aromatic proton from δ 7.71 → δ 6.74 which is consistent in changes from nitro → amide or amine functional groups (**Figure 3.2j**).

To conclude the reductive/cyclisation approach was successful in generating the key tetralactam intermediate **192** in quantitative yield, and this methodology could be applied to the synthesis of C2-aryl PBDs.

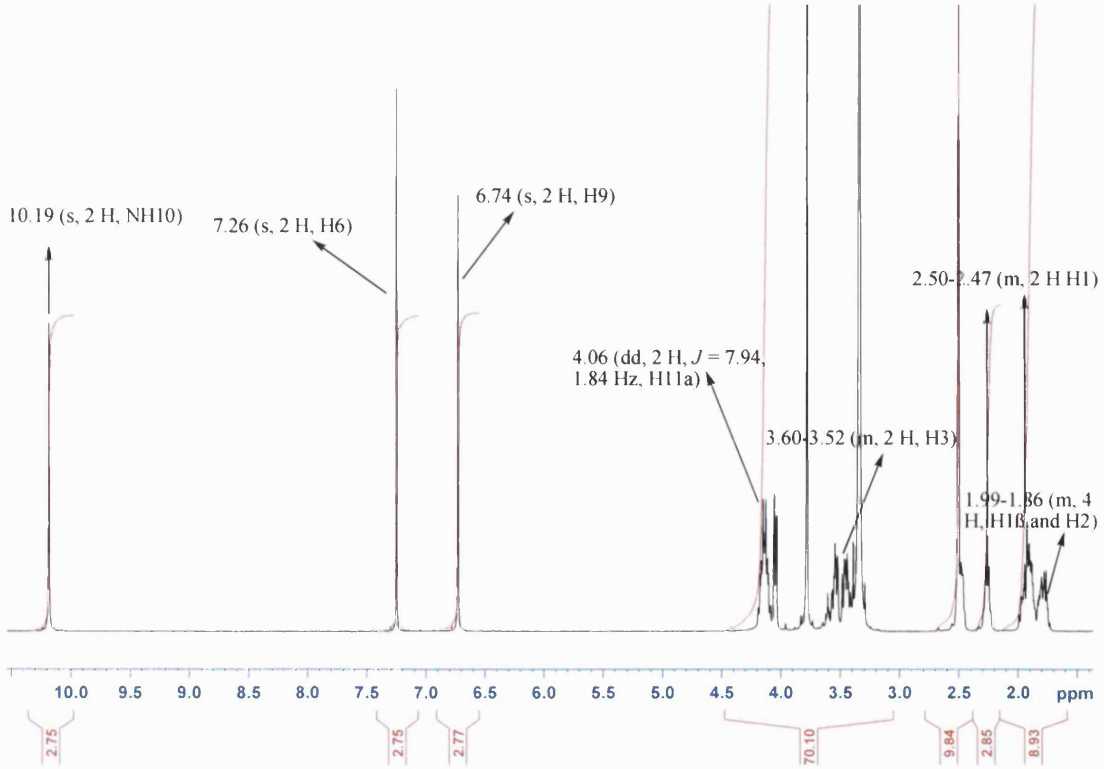


Figure 3.2i: ^{1}H NMR spectra of Key tetralactam precursor **192**.

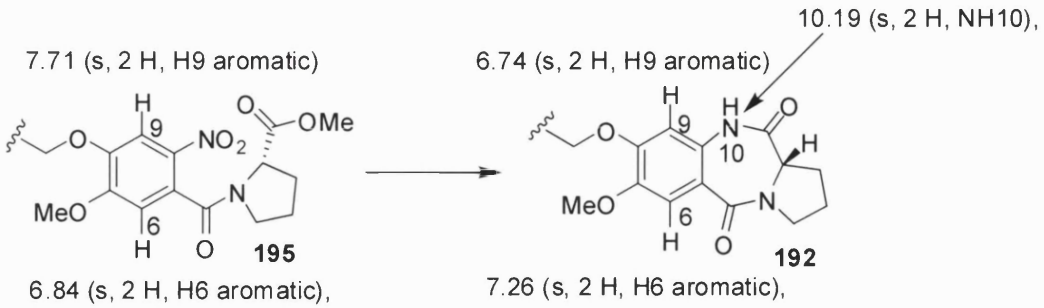
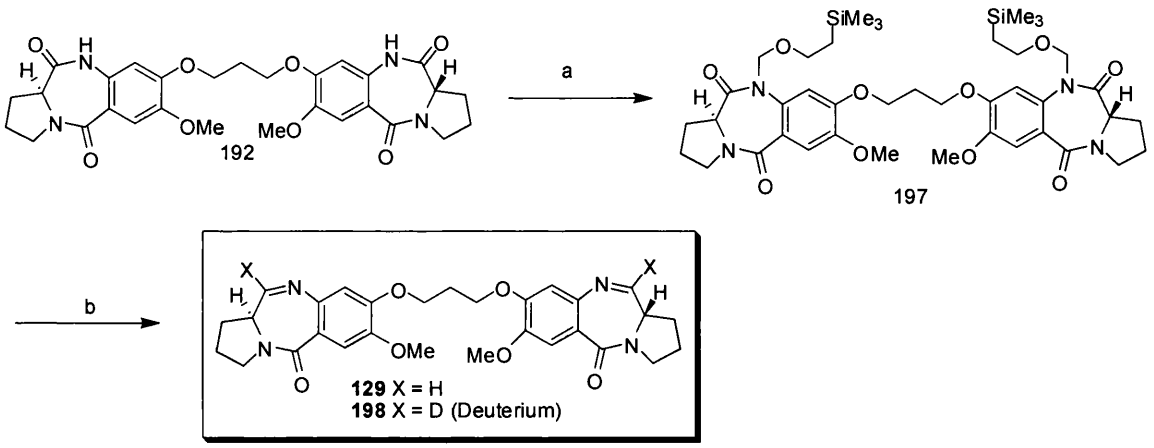


Figure 3.2j: ^{1}H NMR chemical shift (δ ppm) for **195** and **192.**

3.2.3 Successful Synthesis of DSB-120 and its Deuteriated Analogue 198

DSB-120 PBD dimer and its deuterated analogue **198** were successfully synthesised by employing a regioselective hydride reduction of the N10-SEM protected tetralactam **197**, with subsequent N10-SEM deprotection to the sensitive N10-C11 imine moiety, critical for biological activity. In addition, the model system was used as an opportunity to optimise the critical hydride reduction/deprotection step.

3.2.3.1 Synthetic Route



Scheme 3.2k: Synthesis of DSB-120 (**129**) and its deuterium analogue **198**

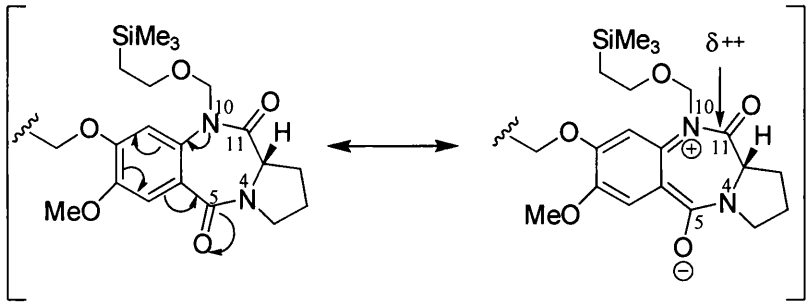
a) 1. n-BuLi, THF, – 40 °C; 2. SEM-Cl, – 40 °C to r.t., 52%; b) 1. XBH_4 X = Na or Li and $NaDH_4$, r.t.; 2. SiO_2, EtOH:Water, r.t., 55%.

The first step was to protect the amidic N10 nitrogen to generate the N10-SEM protected tetralactam **197 (Scheme 3.2k)**. Protection was necessary for two reasons; 1) to prevent deprotonation of the N10-proton by incoming hydride ions (Thurston *et al.*, 1984) 2) and avoid over-reduction of the N10-C11 imine to the biologically in-active secondary amine (Thurston and Bose, 1994).

Tetralactam **192** was initially treated with NaH in DMF, followed by SEM-Cl, but it was found that better results were obtained when n-BuLi was used instead of NaH. After subsequent stirring overnight, LC-MS revealed complete consumption of starting material, formation of large quantities of the desired product **197** (7.85 min (ES+) m/z (relative intensity) 825 ([M + H]$^{+}$, 80)), as well as small amounts of mono-N10-SEM protected tetralactam. Despite varying the stoichiometry of both base and reagents, conversion of the mono protected product to **197** did not occur. Purification by flash chromatography isolated the desired di-N10-SEM protected product **197** in 52% yield, plus small amounts of mono-protected product which were analyzed by ^{1}H and ^{13}C NMR to confirm their structures.

The structure of **197** was confirmed by the presence of two distinct doublets corresponding to geminal protons in SEM-N$\underline{CH_2}$O at δ 5.46 (d, 2 H, J = 10.0 Hz) and δ 4.69 (d, 2 H, J = 10.0 Hz) in ^{1}H NMR and methylene signal in ^{13}C NMR spectra at 79.2 ppm respectively. All of the proton integrations supported the evidence that both sides of molecule were protected. In addition, the appearance of the TMS signals in ^{1}H NMR at 0.002 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$) and methyl signal at 0.0 ppm in ^{13}C NMR spectra, as well as the complete absence of the amidic N10-proton, all confirmed the success of the N10-SEM protection. Consistent optical rotation values of $[\alpha]_D^{24}$ = +218° (c = 0.5, $CHCl_3$) suggested that C11a stereochemistry had been maintained during the reaction, despite using a relatively strong base.

The critical step of the synthesis was C11-lactam reduction using $NaBH_4$, with subsequent N10-SEM deprotection upon exposure to silica gel and EtOH:Water. As was previously discussed (See section **1.4.11, Discussion of synthetic approaches to PBDs)**, the success of the hydride reduction step is dependent upon A-ring substituents, N10-amide protection and C-ring substitution pattern. However the regioselective reduction of the C11-carbonyl is dominated by stereoelectronic factors. The N10-C11 amide functional group is in fact part of a vinylogous imide, where the loan pair on the nitrogen atom is delocalised into the benzene ring and the carbonyl group of the N4-C5 amide (**Figure 3.2l**). This vinylogous behaviour makes the C11-carbonyl more electrophilic and the C5-carbonyl less eletrophilic.



Scheme 3.2l: Vinylogous behavior of PBDs.

Initially, the methodology adopted was based upon the hydride reduction method procedure reported by Cooper *at al.* (2002). This required the use of 20 equivalents of $NaBH_4$ in THF:EtOH, with stirring for 16 h at room temperature to form the transient carbinolamine intermediate **199** (see **scheme 3.2o**) which was 'isolated' and treated with silica gel in EtOH:Water and allowed to stir for a further 16 h at room temperature. The LC/MS and TLC of this reaction mixture revealed formation of an unknown single product which was isolated by flash chromatography in 60% yield. Analysis by ^1H NMR spectroscopy (**Figure 3.2m**) discovered that the reaction had resulted in complete overreduction of the N10-C11-imine functional group to furnish the biologically in-active N10-C11 secondary amine product **200**. This was confirmed by appearance of multiplets at δ 3.65-3.51 ppm which corresponded in the 2D-Cosy ^1H NMR spectra as pair of geminal protons coupling to each other, plus a perfect correlation with the H11a proton (**Figure 3.2n**). Also, an additional methylene signal was observed in the ^{13}C NMR spectra at 67.2 ppm, which correlated with the geminal protons at δ 3.65 - 3.51 ppm in the 2D-cosy ^{13}C vs ^1H NMR.

A mechanism has been proposed for the conversion of the N10-SEM tetralactam **192** to the N10-C11 imine moiety (**192**) and the secondary amine **200** which is illustrated in **Scheme 3.2o**. The mechanism shows that complete over-reduction to the secondary amine can occur from a direct result of the N10-SEM removal during the hydride reduction step, allowing another hydride ion to attack the imine functional group resulting in formation of the secondary amine. Therefore, it was envisaged that reducing the reaction time could prevent the SEM group removal, and this observation was taken into account when optimization of the reaction was investigated. Although formation of the secondary amine was not desirable in this context, Howard and co workers have reported that a PBD secondary amine can if needed be converted

to its imine by the use of an oxidizing agent such as TPAP with *N*-methylmorpholine-*N*-oxide (NMO) as a co-oxidant (Kamal *et al.*, 1997).

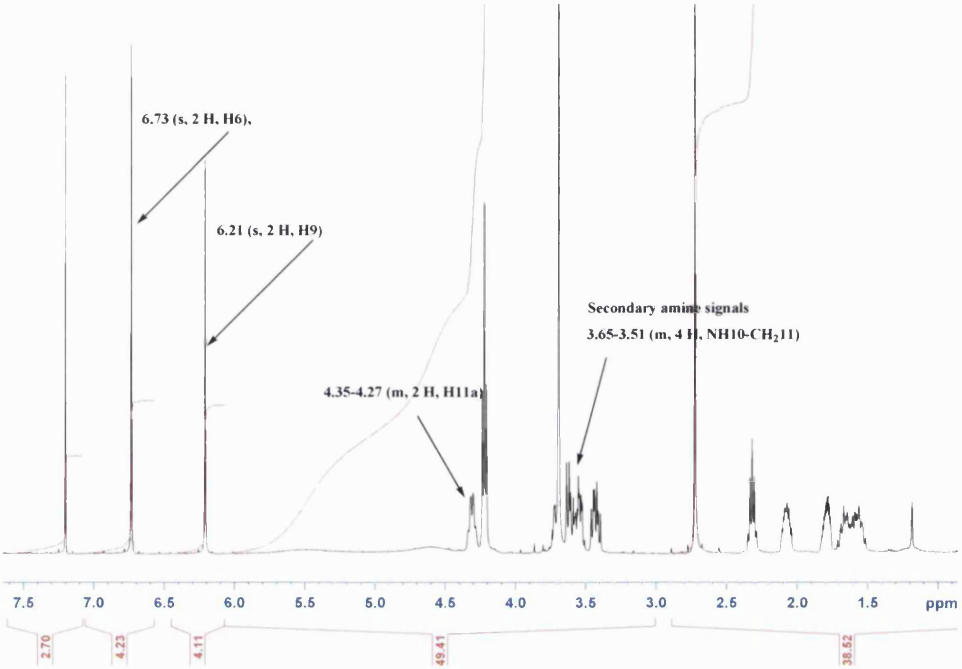


Figure 3.2m: ^{1}H NMR Coupling in Secondary Amine product **200**

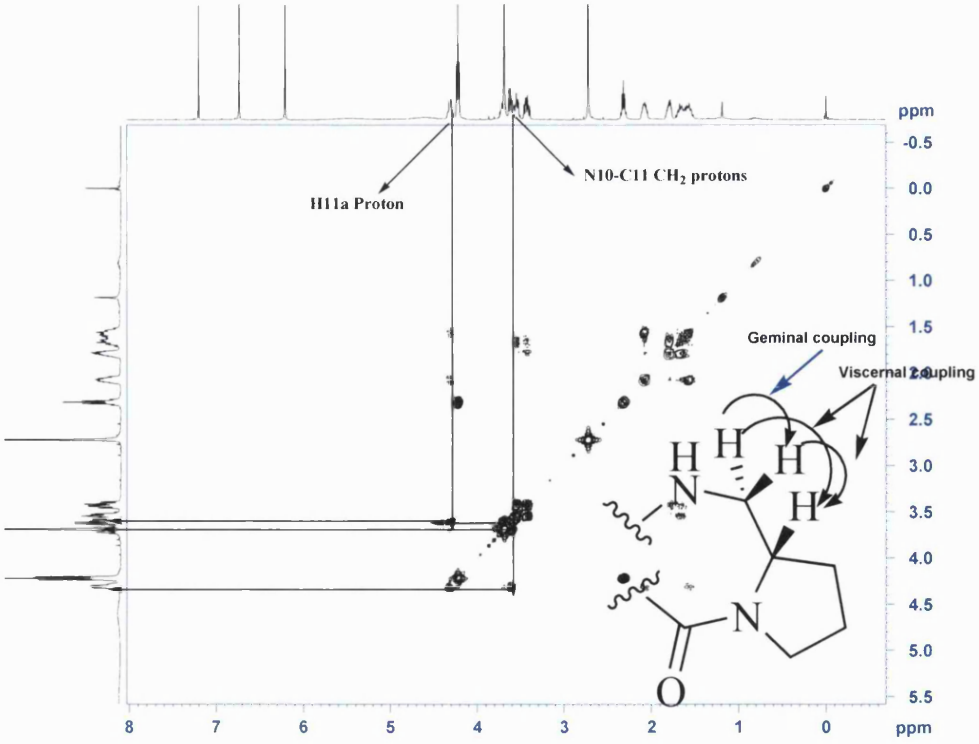
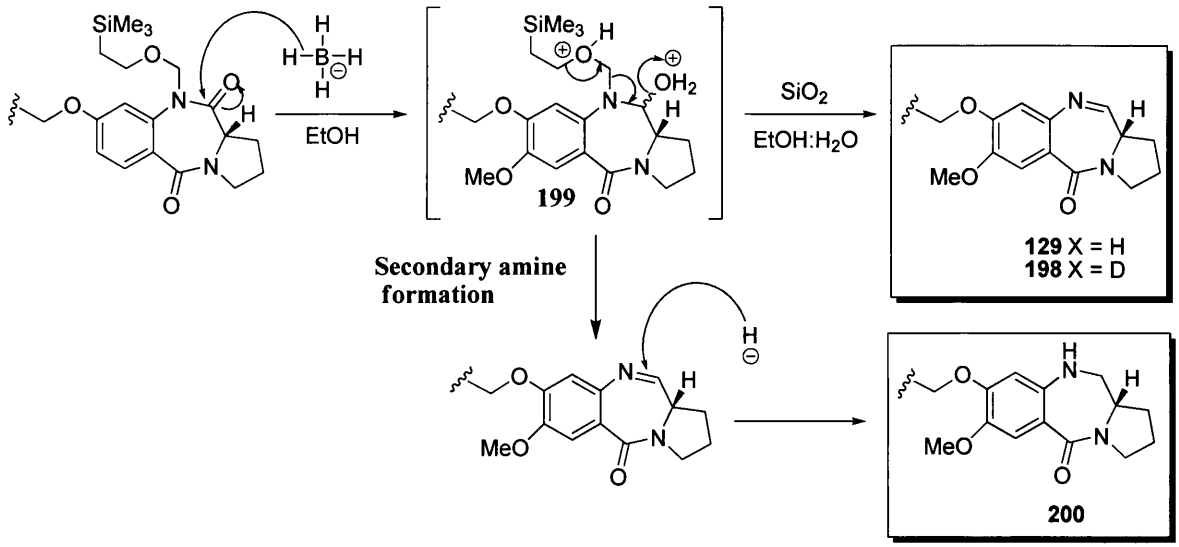


Figure 3.2n: 2D-Cosy NMR (^{1}H vs ^{1}H) for Secondary Amine product **200**.



Scheme 3.2o: Proposed Mechanism for the formation of the N10-C11 imine functional group and the secondary amine product **200**.

The successful reductive/cyclisation approach paved the way for the preparation of the N10-SEM tetralactam intermediate **192** on a large scale, thus facilitating the optimization of the hydride reduction step. The reduction step could be readily monitored by LC-MS, as the acidic elution conditions promoted loss of the N10-SEM unit to afford the imine. The reaction was repeated several times under a variety of stoichiometry, reducing agent and reaction time, and the results are summarized in **Table 3.2b**.

Twenty equivalents (20 eq) of $NaBH_4$ (or $NaBD_4$) were required for optimal reduction. It is believed that the high stoichiometry allows rapid reduction of the C11-carbonyl group before any loss of the SEM group can occur. Purification by flash chromatography resulted in 20% and 25 % yields for the DSB-120 and its deuteriated analogue **198** respectively (see **table 3.2b**).

Optimal conditions were acheived when twenty equivalents of $LiBH_4$ were used with 5 h reaction time, resulted in 45% isolated yields for DSB-120 after column purification with no secondary amine contamination.

Table 3.2b: Summary of conditions tested for the optimization of the hydride reduction step.

Reducing agent	Equivalents (eq)	Reaction Time (h)	Yields[a] (%)	$[\alpha]_D^{T\,b}$	Comments
$NaBH_4$	10	8	-	-	No reaction
$NaBH_4$	10	16	-	-	In complete consumption of starting material
$NaBH_4$	20	16	60	-	Complete over-reduction to secondary amine
$NaBH_4$	20	8	20	+828°	Formation of N10-C11 imine moiety.
$NaBD_4$	20	16	55	-	Complete over-reduction to secondary amine
$NaBD_4$	20	8	25	+830°	Formation of N10-C11 imine moiety.
$LiBH_4$	3	8	-	-	LC-MS conversion (%)[c] SM:P = 90:10
$LiBH_4$	10	8	-	-	SM:P = 40:60
$LiBH_4$	20	5	45	+835°	Formation of N10-C11 imine

[a] = All yields are for pure, isolated compounds. [b] = Optical rotations measure in HPLC $CHCl_3$ (see experimental sections for concentrations and temperature [T] details). [c] = % conversion rates based on LC-MS area under the curve (AUV, %) SM = Starting material, P = Product, N10-C11 imine formation.

Interestingly, the measured optical rotations values (See **table 2.3b**) of the various DSB-120 compounds ($[\alpha]_D$ = +830°, +828°, +835°) were much higher than the literature values for DSB-120 previously reported ($[\alpha]_D$ = +330°; Thurston *et al.*, 1996). High optical rotations values were also reported by Tercel *et al.* in their synthesis of DSB-120 via a modified Fukuyma oxidative cyclisation approach (Tercel *et al.*, 2003).

They reported that the $[\alpha]_D$ values previously reported in the literature for DSB-120 used $CHCl_3$ solvent which contains 1-2% EtOH as a stabilizer. All their measured $[\alpha]_D$ values were recorded using dried and fractionally distilled $CHCl_3$, and they found that adding 2% EtOH to the $CHCl_3$ immediately reduced the measure $[\alpha]_D$ values by more than half. In fact all the optical rotations shown in **Table 3.2b** are carried out in HPLC grade $CHCl_3$ which contains no such stabilizers, which explains such high optical rotations, similar to those reported by Tercel *et al.*

All the DSB-120 compounds synthesized were analyzed by LC-MS and gave single peaks. The ^1H and ^{13}C NMR spectra provided evidence for the successful conversion to the N10-C11 imine functionalities which showed the appearance of the characteristic doublet corresponding to the H11-imine signal at ~ δ 7.87, plus a methine signal at ~165 ppm respectively, as well as complete loss of SEM signals (see ^1H NMR spectra shown below, **Figure 3.2p**). Further confirmation was obtained from ES spectroscopy where the molecular ions were observed as the base peak (MS (ESI) *m/z* (relative intensity): 533 ($[M + H]^{+}$, 100%)) plus high resolution accurate mass analysis (HRMS: $[M + H]^{+}$ Theoretical, $C_{29}H_{32}O_6N_4$ m/z 533.2394, found (ES^+) m/z 533.2415).

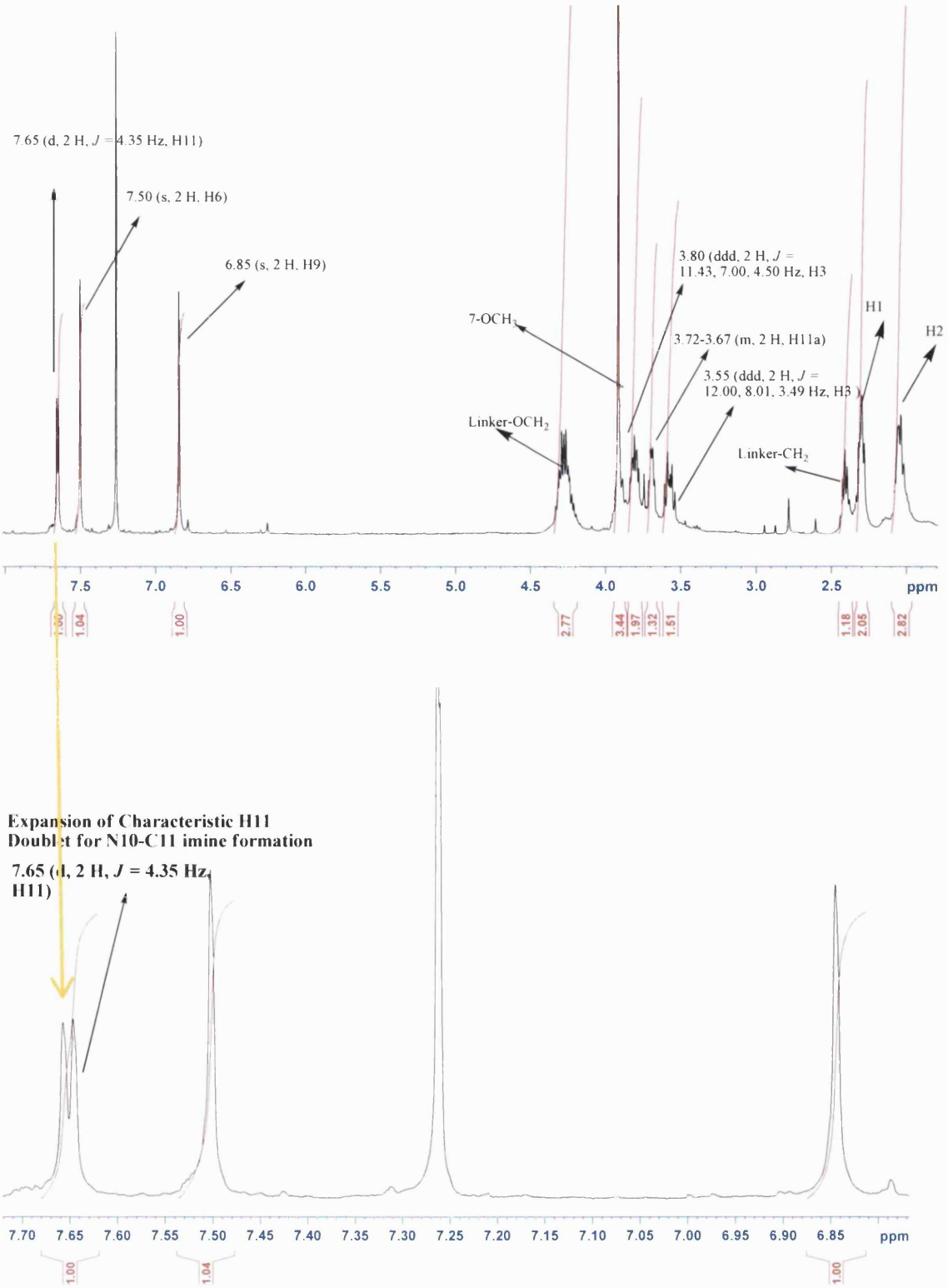


Figure 3.2p: ^{1}H NMR of DSB-120 Synthesized by $LiBH_4$ reduction method with expansion of H11 doublet signal.

3.2.3.2 Explanation of ^{1}H NMR Spectra of the Deuterated DSB-120 Analogue **198**.

The characteristic H11 doublet at ~ δ 7.89 ppm in ^{1}H NMR spectra of DSB-120 confirms the formation of the N10-C11 imine moiety. However, in the case of deuteration at the C11 position, there is, as expected, an absence of the deuterium H11 doublet signal (^{2}H) in the ^{1}H NMR spectra (**Figure 3.2q**).

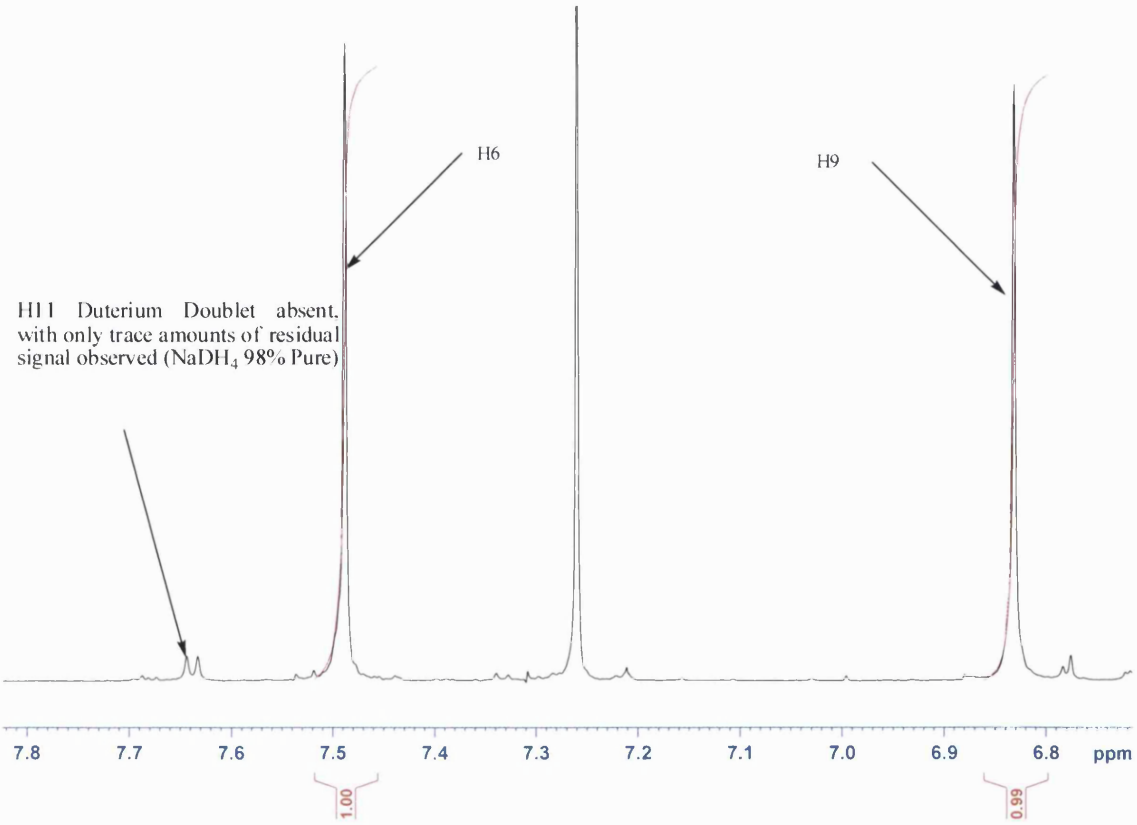


Figure 3.2q: expansion on the absent H11 deutrium signal in the ^{1}H NMR spectra of **198**.

This is because, although deuterium (^{2}H) possesses an angular momentum or spin (**I** = 1), and therefore a magnetic moment, the strength of this magnetic moment is very small. In fact the magnetic moment of ^{2}H is only 4.11 $\gamma/10^{7}$ T^{-1} S^{-1} (gyromagnetic ratio = γ), therefore it interacts very weakly with the applied magnetic field. This causes the energy differences between the permitted energy levels or orientations of ^{2}H to be very small (ΔE, **Figure 3.2r**). As a consequence very little radio wave energy is needed to 'flip' the nuclei from ground to excited

states, resulting in a small net absorption of energy, which is directly proportional to the intensity of the spectroscopic transition. As a result, only very weak signals are observed.

In contrast the ^{1}H proton has a very large magnetic moment of 26.75 $\gamma/10^{7}$ T^{-1} S^{-1} compared to ^{2}H (γ = 4.11 $\gamma/10^{7}$ T^{-1} S^{-1}) and therefore has a much greater interaction with the applied magnetic field. This causes a greater difference in population of the permitted energy levels of ^{1}H (ΔE, **Figure 3.2r**) resulting in a very large net absorption of energy, hence a very strong spectroscopic transition and strong signals in spectra. This stronger intensity exhibited by ^{1}H is totally eclipses the signal produced by the ^{2}H and therefore the deuterium signal is not observed in ^{1}H NMR spectra. Interestingly, if a proton decoupler is used, then the deuterium signal can be seen in the ^{1}H NMR spectra.

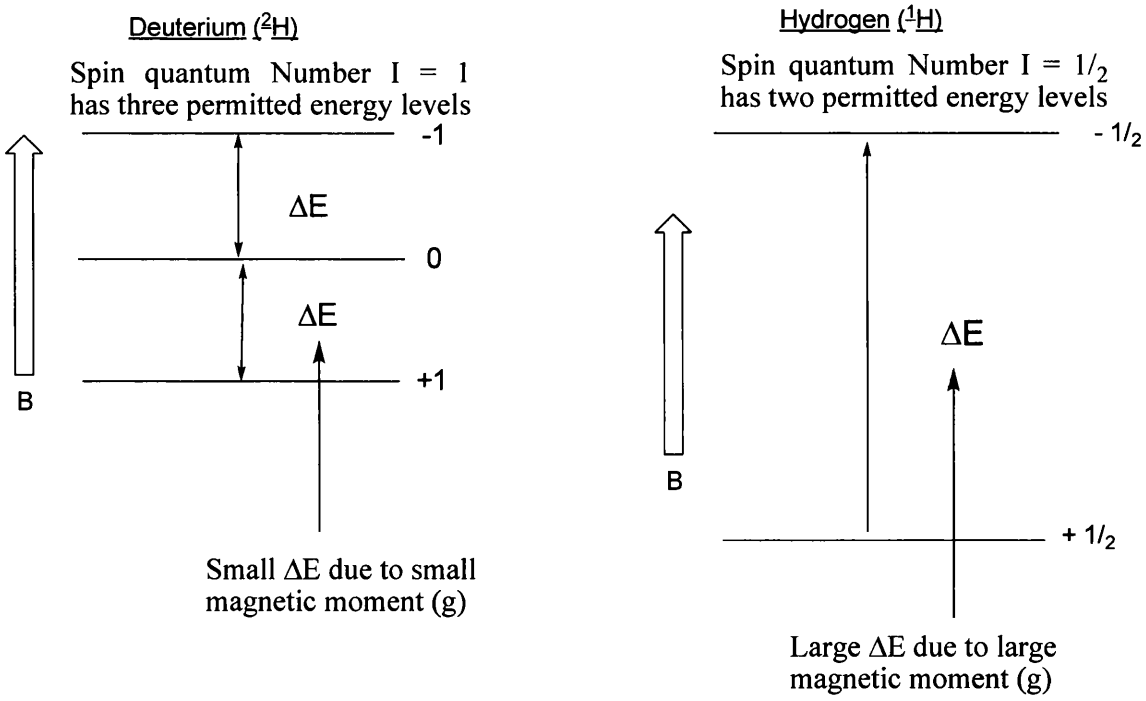
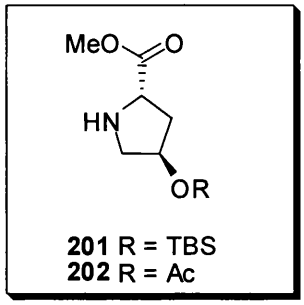


Figure 3.2r: Energy levels difference (ΔE) for hydrogen (^{1}H) and deuterium (^{2}H) in a magnetic field B. Note the effects of the different gyromagnetic ratios (γ) of the two nuclei (adopted from OCP Nuclear Magnetic Resonance, P.J. Hore).

3.3 Synthesis of Protected PBD C-Ring Precursor.



The next challenge was to apply the chemistry used for the synthesis of DSB-120 to the synthesis of C2-aryl PBD dimers. The retrosynthetic analysis shown below (previously discussed in section **3.1.1**, **Figure 3.1a)** identifies the key C2-hydroxy tetralactam precursor **185 (Figure 3.3a)**, which can be obtained from amide coupling of the known bis-acid **159** (previously discussed in section **3.2.2.1**) with the secondary amine (C-ring) **188**.

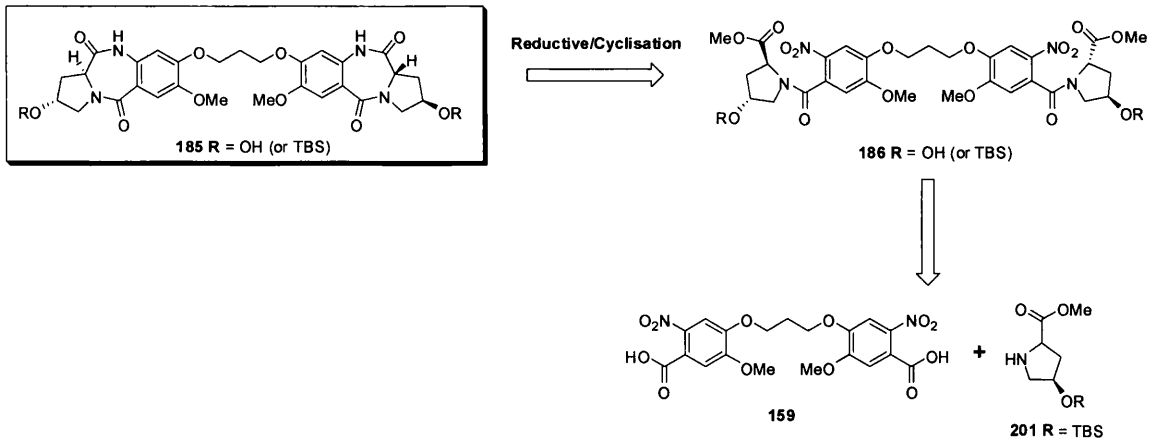


Figure 3.3a: Retrosynthetic analysis of the Key tetralactam precursor **185.**

3.3.1 C-Ring Protection Strategy.

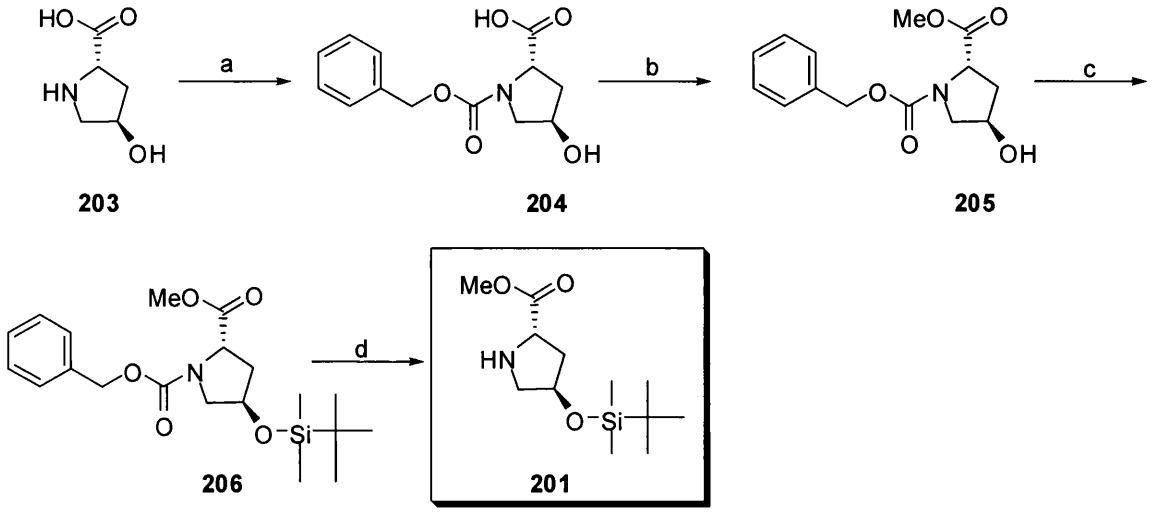
C-ring protection strategies have been successfully applied to the synthesis of a number of PBD analogues (Gregson *et al.*, 2000a & b and 2004; Cooper *et al.*, 2002; Z.Chen *et al.*, 2004).

Protection of the C2-hydroxy substituent of the PBD C-ring was necessary for two reasons; 1) it would prevent SEM protection of the C2-OH during the N10-SEM protection step; 2) avoids side reactions occurring during the amide coupling step resulting in higher yields (Z.Chen *et al.*, 2004 PhD thesis).

3.3.2 Synthesis of *O*-TBDMS Protected C-Ring

A TBDMS ether protecting group was selected for C2-OH protection, as this was known to be orthogonal to the N10-SEM protective group as well as being stable to strong bases such as sodium hydride or butyllithium employed later on during the N10-SEM protection step.

3.3.2.1 Synthetic Route.



203 204 205 206 201

Scheme 3.3b: Synthesis of *O*-TBS C-ring **201**.

a) $PhCH_2CO_2Cl$, $NaHCO_3$, toluene, 94%; **b**) MeOH, H_2SO_4, Δ, 99%; **c**) TBDMS-Cl, imidazole, DMF, r.t., 95%; **d**) 10% Pd-C, H_2, EtOH, 50 Psi, 77%.

The C-ring building block **201** was readily prepared in four steps (Cooper *et al.*, 2002, **Scheme 3.3b**). The synthesis commenced with commercially available *trans*-4-hydroxy-*L*-proline (**203**) which was *N*-protected as the benzyl-carbamate **204** using benzyl chloroformate in 94% yield. Esterification with concentrated sulphuric acid in refluxing methanol, afforded the methyl ester **205** in quantitative yield. Treatment with TBS-Cl, imidazole and DMF gave the *O*-TBS protected C-ring **206** in 95% yield. Finally the removal of the *N*-benzyl carbamate (CBz) protecting group was achieved using catalytic hydrogenation using 10% Pd-C in EtOH to generate the secondary amine *O*-TBS protected C-ring **201** in 77% yield. The structure of **201** was confirmed by LC/MS which showed a single peak at 2.13 min (ES+) m/z (relative intensity) 597 ($[M + H]^+$, 50), 619 ($[M + Na]^+$, 20), as well as ^1H and ^{13}C NMR spectra revealing no signals between 7.23-7.51 ppm and 120-130 ppm respectively where signals arising from the aromatic benzyl moiety of the CBz would normally be observed, confirming the success of the CBz removal reaction (**Figure 3.3c**).

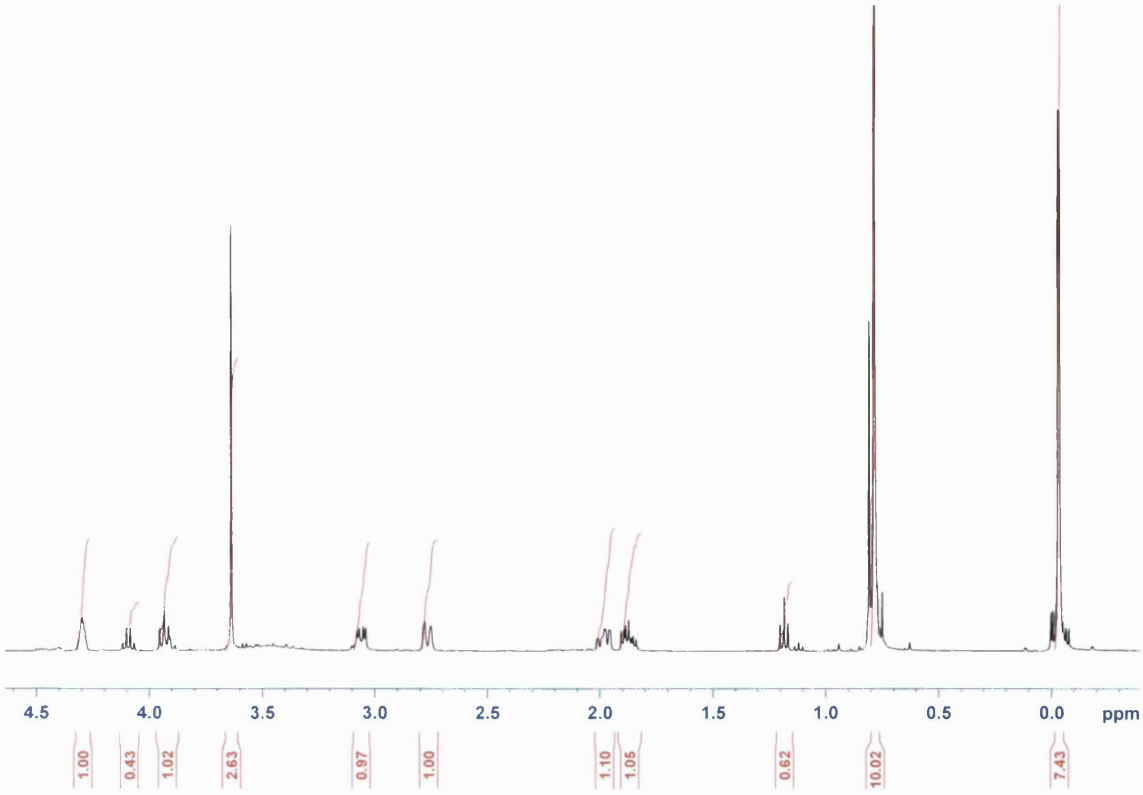


Figure 3.3c: ^1H NMR Spectra of *O*-TBS C-ring fragment after CBz deprotection **201**.

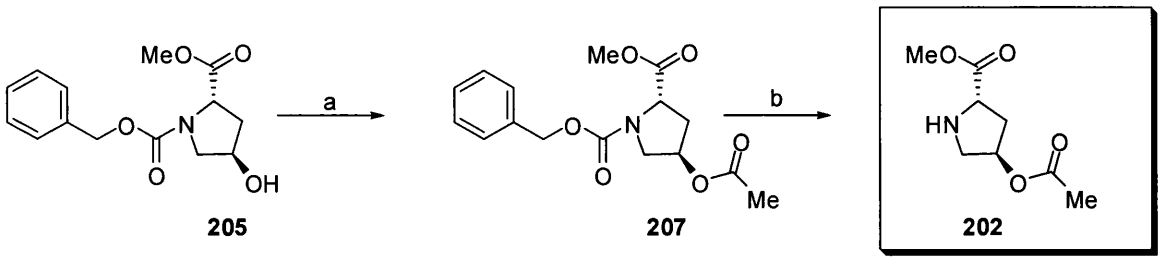
3.3.2.2 Problematic CBz Removal on a Large Scale.

Major problems were encountered during the CBz removal step on a large scale. The 1g scale reaction successfully underwent hydrogenolysis to remove the CBz group. However, when the reaction was preformed on a scale of greater than one gram, it was found that no removal of the CBz (benzyl signals still present at δ 7.25 ppm, m, 5H) was observed. Even after careful variations of reaction conditions (reagents, reaction time and pressure), it was found that the hydrogenolysis reaction was limited to 2-3 g at most.

3.3.3 Synthesis of *O*-Acetoxy Protected C-Ring

As an alternative to the *O*-TBS C-ring, the acetate protected C-ring was synthesised, this approach has been reported for the synthesis of *endo/exo* substituted PBD dimers (Z.Chen *et al.*, 2004 PhD thesis).

3.3.3.1 Synthetic Route



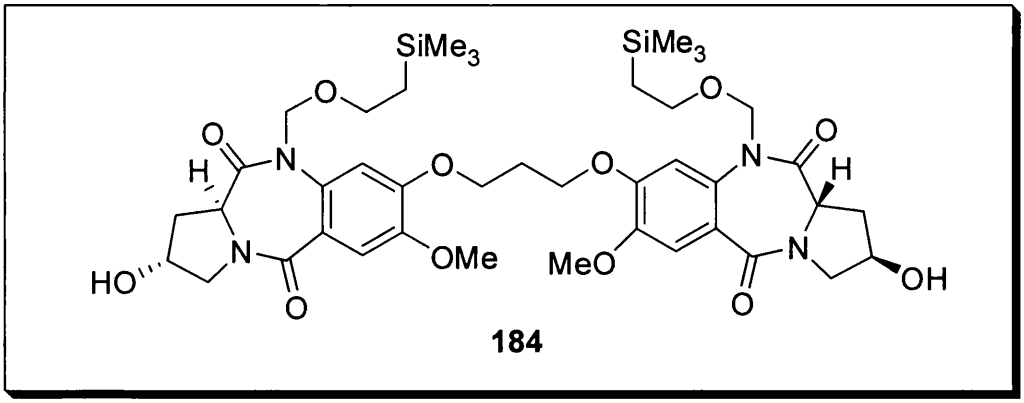
Scheme 3.3d: Synthesis of *O*-acetoxy C-ring **202**.

a) AcCl, TEA, CH_2Cl_2, r.t., 94%; **b**) 10% Pd-C, H_2, EtOH, 50 Psi, 98%.

Acetate protection of the hydroxyl group in the C-ring was carried out just before removal of the CB z group (**Scheme 3.3d**). The alcohol **205** was treated with acetyl chloride, TEA and DCM to give the *O*-acetoxy C-ring **207** in 94% yield.

Characteristic acetyl signals were observed in the ^{1}H NMR at δ 2.02 ppm. In addition, LC/MS 2.13 min (ES+) m/z (relative intensity) 597 ($[M + H]^{+\cdot}$, 50), 619 ($[M + Na]^{+\cdot}$, 20), revealed the required mass for **207.** Removal of the CBz protective group by catalytic hydrogenation provided the acetate protected secondary amine C-ring **202** in quantitative yield. Significantly, the CBz deprotection could be carried out on a 5-10 g scale (c.f. with TBS C-ring). Once again confirmation of the successful CBz removal was provided by characteristic absence of the aromatic signals in ^{1}H and ^{13}C NMR spectra as well as single peak in LC/MS at 2.13 min (ES+) m/z (relative intensity) 597 ($[M + H]^{+\cdot}$, 50), 619 ($[M + Na]^{+\cdot}$, 20), further confirming the structure of **202**. The successful synthesis of the protected C-ring fragment could now be applied to the synthesis of C2-aryl substituted PBD dimers.

3.4 Synthesis of Key N10-SEM Tetralactam Intermediate 184



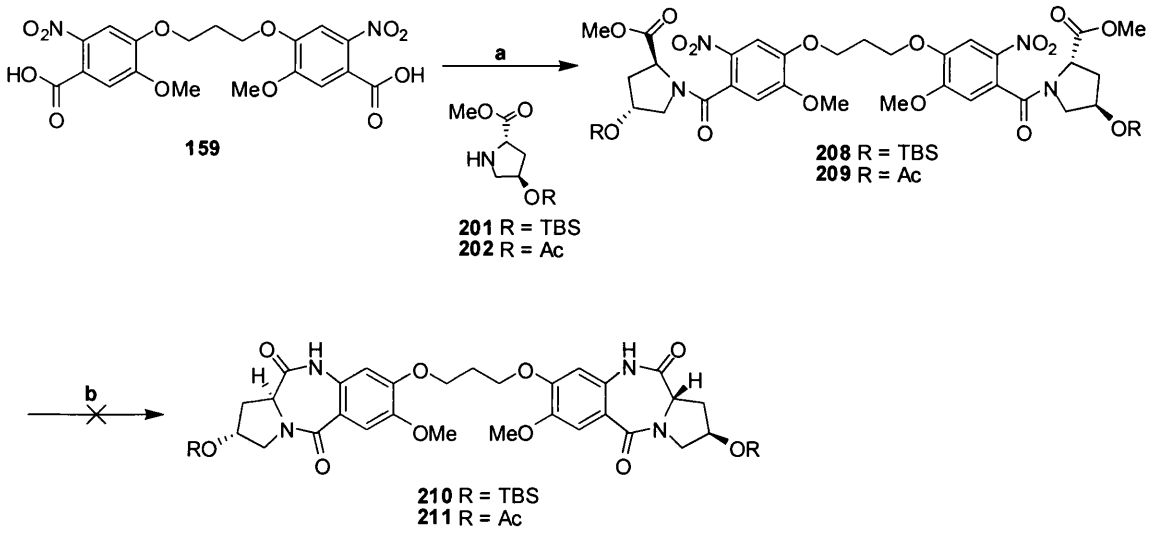
184

The next synthetic challenge was to apply the synthetic strategy described for the synthesis of DSB-120 (see section **3.3.1)** and C-ring fragment prepared in section **3.2.2** to the synthesis of the key tetralactam intermediate **184**.

3.4.1 Attempted Synthesis of Key Tetralactam Intermediate 184.

The initial strategy employed to generate the key tetralactam intermediate **184** was analogous to that described for the synthesis of DSB-120. The key steps in the synthesis were amide coupling of the bis-nitro acid **159** to the preformed protected C-ring fragment **202** (or **201**) to obtain the bis-nitrobenzamide **188** (or **208** R = TBS). B-ring cyclisation to afford the tetralactam precursor **185** (or **210** R = TBS) was expected to take place (see section **3.2.2 for DSB-120 synthesis)** after reduction of the nitro group using catalytic hydrogenation with Pd-C, with subsequent acid treatment (Conc.HCl) with H_2O/THF (Peña and Stille, 1989). The tetralactam intermediate would then undergo N10-SEM protection followed by TBS deprotection to obtain the key intermediate **184**.

3.4.1.1 Synthetic Route



Scheme 3.4a: Attempted Synthesis of key tetralactam precursor **210/211** via reductive/cyclisation approach using catalytic hydrogenation and acid treatment.

a) 1. $(COCl)_2$, DMF, CH_2Cl_2, r.t.; 2. TEA, THF, – 60 °C, then **201** (55%, **208**) and **202** (65%, **209**); **b**) 10% Pd-C, EtOH:EtOAc, H_2 (55 psi); 2. HCl_{conc}/H_2O/THF (0.03:10:1 v/v), r.t.

The synthesis commenced (**Scheme 3.4a**) with the activation of the known bis-nitrobenzoic acid **159** to its acid chloride with subsequent coupling to the preformed protected C-rings **201** and **202** to give the bis-nitrobenzoamides **208** (50%) and **209** (58%) respectively. The ^{1}H and ^{13}C NMR both confirmed the amides **208** and **209** which characteristic signals for amide coupling (**Figure 3.4b**).

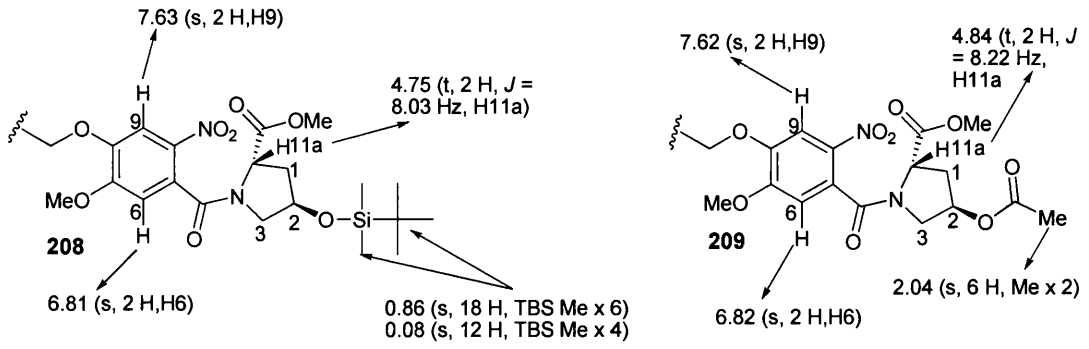


Figure 3.4b: Key ^{1}H NMR signals (δ ppm) for amides **208** and **209**.

The next step was to reduce the nitro groups to the transient bis-aminobenzamide by catalytic hydrogenation (10% Pd-C. H_2), and then promote B-ring cyclisation by treatment with acid (HCl_{conc}/H_2O/THF) to generate the key tetralactam intermediate **210/211**.

Amide **208** was hydrogenated for 3 h, after which time multiple products were observed by LC/MS and TLC. After changing the reaction stoichiometry, time and reagents no further improvement was obtained with numerous spots/peaks still being observed. Attempted purification of the multi-spot mixture by flash column chromatography was extremely difficult, and no isolation of the desired product could be achieved.

Some success was achieved with the amide **209**, which was subjected to hydrogenation for 16 hours and afforded partial and fully ring cyclised tetralactam product **211** as well as other identified impurities. The crude mixture was treated with Conc.HCl/THF:H_2O and allowed to stir for 3 h to encourage any remaining free amine and partial ring cyclised product to cyclise. TLC and LC-MS revealed the reaction had provided a mixture of the desired tetralactam precursor **211** as well as numerous impurities and identified side products.

Purification by flash column chromatography allowed the isolation of the desired tetralactam precursor **211 (see Figure 3.4c** below for key ^{1}H NMR signals), but disappointingly the yields was very low (8%). Despite trying to vary the reaction conditions no improvement was made and it was concluded that the hydrogenation reaction did not favour formation of the tetralactam intermediate **184** and consequently, an alternative reductive/cyclisation method was explored.

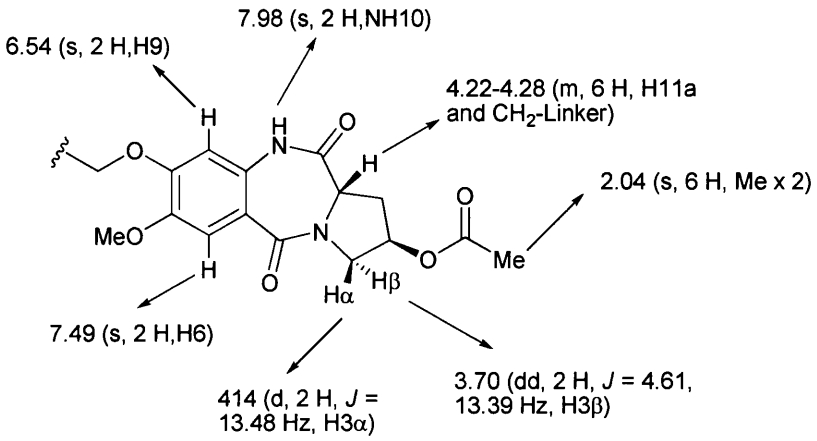


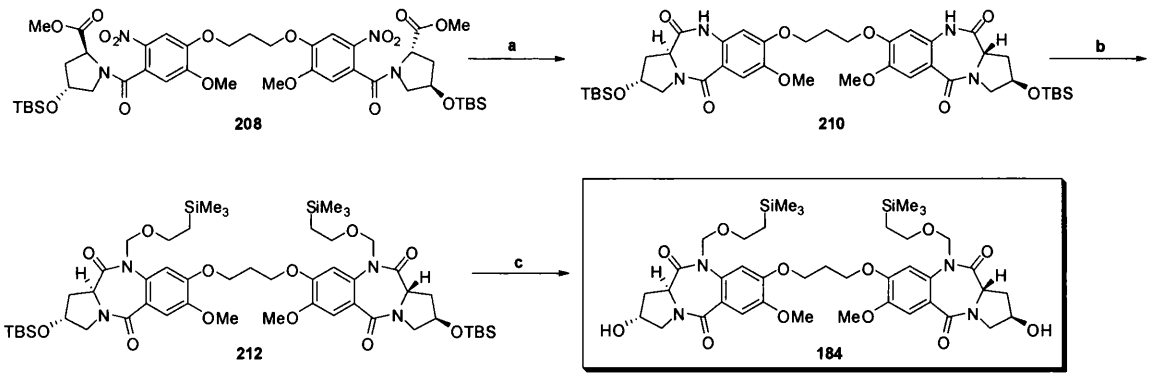
Figure 3.4c: Key ^{1}H NMR signals (δ ppm) for isolated tetralactam **211**.

3.4.2 Successful Synthesis of Key Tetralactam Intermediate 184- via Raney nickel and hydrazine Reductive/Cyclisation reaction

In 1982, Yuste *et al.* reported a method of selectively reducing aromatic nitro groups in the presence of benzyl ethers using Raney nickel and hydrazine in refluxing methanol (Yuste *et al.*, 1982). This method appeared promising in promoting B-ring cyclisation of the bis-nitrobenzamide. Interestingly, Langlois and co-workers successfully employed Raney nickel as a catalyst in the presence of acid to reduce aromatic nitro-carboxaldehydes to promote B-ring cyclisation of a number of carbinolamine PBD analogues (Andriamialisoa and Langlois, 1986; Langlois *et al.*, 1993).

Applying a modified version of Yuste *et al.* method, reductive/cyclisation to the key tetralactam precursor **210** was achieved by adding the bis-nitrobenzamide **208** (R = TBS) in methanol dropwise to a slurry of Raney nickel. Upon reaching reflux, hydrazine was added dropwise to

the reaction mixture which was allowed to reflux at this temperature for 1 h, after which time LC/MS 7.98 min (ES+) m/z (relative intensity) 825 ($[M + H]^{+\cdot}$, 100) revealed a single peak corresponding to the cyclised key tetralactam precursor **210 (Scheme 3.4d)**. After cooling, the excess Raney nickel was filtered through celite and the solvent evaporated down *in vacuo*, where formation of a white solid was observed. Ether was added to this and the product precipitated out of solution which was collected by vacuum filtration and dried to give a white solid in 55% yield. Problems arose when the reaction was carried out on a large scale (10-15g), and the product precipitated out of solution before removal of the excess Raney nickel could be performed. Consequently, the desired product had to be washed out with hot methanol from the excess Raney nickel. This problem was solved if the filtration took place immediately after reaction completion and while the reaction mixture was still hot. **The only disadvantage with this method is that up most care must be taken when performing this reaction due to the pyrophoric nature of the Raney nickel combined with refluxing methanol.**



Scheme 3.4d: Successful Synthesis of Key tetralactam intermediate **184** via Reductive/Cyclisation using Raney nickel and hydrazine.

a) Raney nickel, hydrazine, MeOH, Δ, 55%; **b**) 1. *n*-BuLi, THF, – 40 °C; 2. SEM-Cl, 52%; **c**) TBAF, THF, r.t., 98%.

The success of the reductive/cyclisation reaction was confirmed by the characteristic downfield signal at δ 8.33 (s, 2 H, NH10) in ^{1}H NMR spectra corresponding to the N10 amidic proton. In addition, there was a marked upfield shift in resonance of the H9 aromatic proton from δ **7.63** → δ **7.26** which is consistent with the change from an electron withdrawing nitro group to an electron donating acetamide. In addition, TBS signals arising at δ 0.86 (s, 18 H, TBS CH_3 x 6), δ 0.08 (s, 12 H, TBS CH_3 x 4) respectively in ^{1}H NMR spectra (**see Figure 3.4e** below) were observed indicating the successful incorporation of the C-ring. Optical rotation analysis was found to be $[\alpha]_D^{24}$ = + 325° (*c* = 1, MeOH) indicated that an optically active PBD was obtained in this reaction.

The next step was to treat the tetralactam precursor **210** with SEM-Cl under strongly basic conditions (*n*-BuLi) to provide the N10-SEM protected tetralactam **212** in 52% yield to promote C11-lactam reduction. The structure of **212** was confirmed by the presence of two distinct doublets corresponding to geminal protons highlighted in SEM-N$\underline{CH_2}$O at δ 5.46 (d, 2 H, *J* = 10.0 Hz) and δ 4.69 ppm (d, 2 H, *J* = 10.0 Hz) in ^{1}H NMR and methylene signal in ^{13}C NMR spectra at 79.2 ppm respectively (**Figure 3.4e**). All these signals correlated perfectly with each other in the 2D-COSY NMR with all the proton integrations supported the evidence that both sides of molecule were protected. In addition, a single peak was observed in the LC/MS analysis at 4.75 min (ES+) m/z (relative intensity) 1085 ($[M + H]^{+\cdot}$, 85) confirming the success of the N10-SEM protection. Consistent optical rotation values $[\alpha]_D^{25}$ = + 199° (*c* = 1, $CHCl_3$) also showed that C11a stereochemistry had been maintained during the reaction.

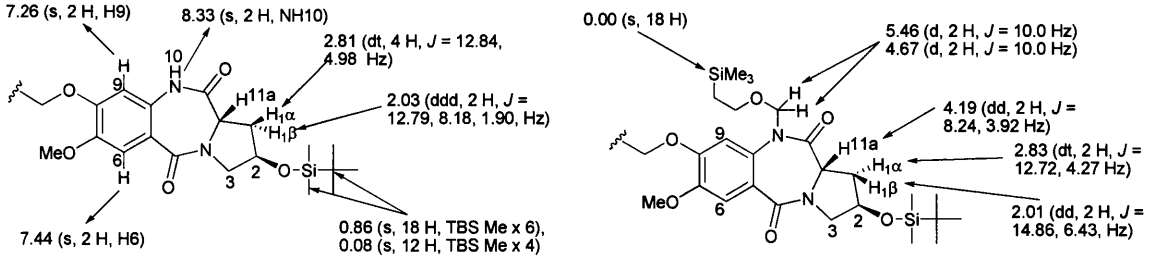


Figure 3.4e: Key ^{1}H NMR signals (δ ppm) for **210** and **212**.

Having effectively derivatized the N10 position, the C2-TBS ether was selectively cleaved with TBAF at room temperature without affecting the orthogonal N10-SEM protecting group to give the key tetralactam intermediate **184** in 98% yield. The success of the C2-TBS deprotection was confirmed by the ^{1}H and ^{13}C NMR spectra where the TBS signals were no longer observed (**see ^{1}H NMR in Figure 3.4f**, shown below). It was decided not to cyclise the **209** bis-nitrobenzamide (R = Ac), as the acetate protecting group would not be stable to the strong basic conditions used to install the orthogonal N10-SEM group.

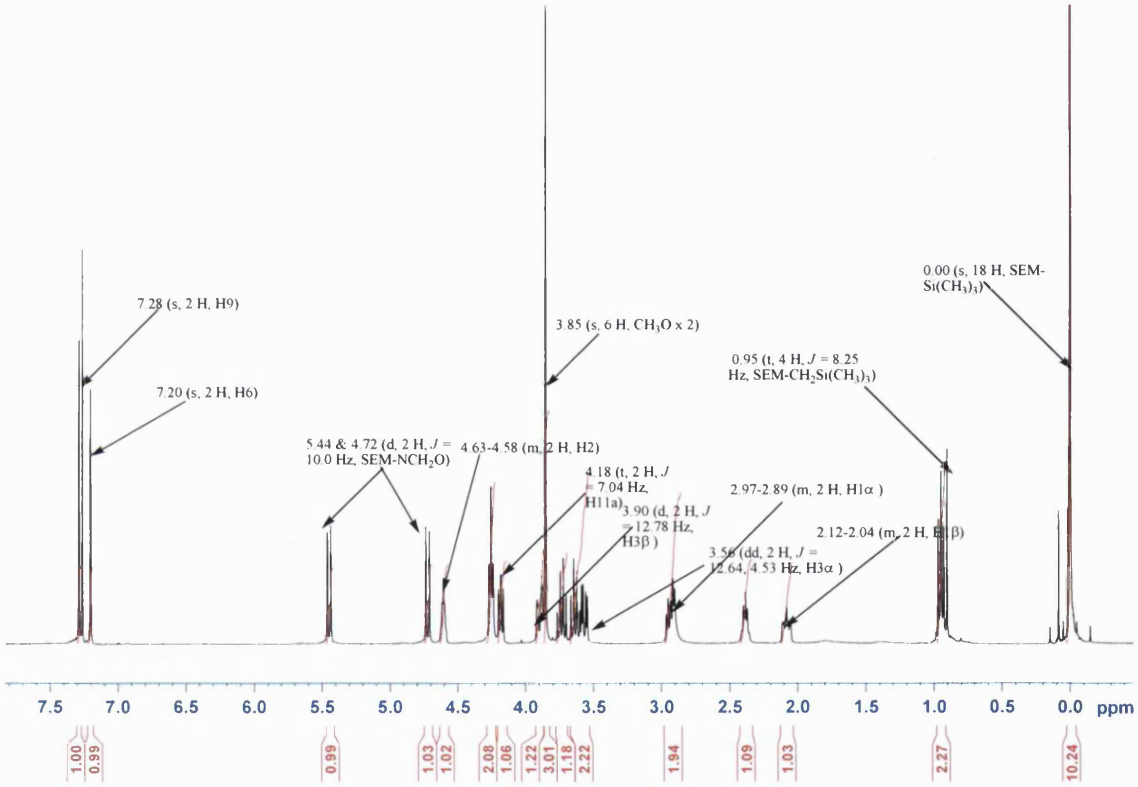


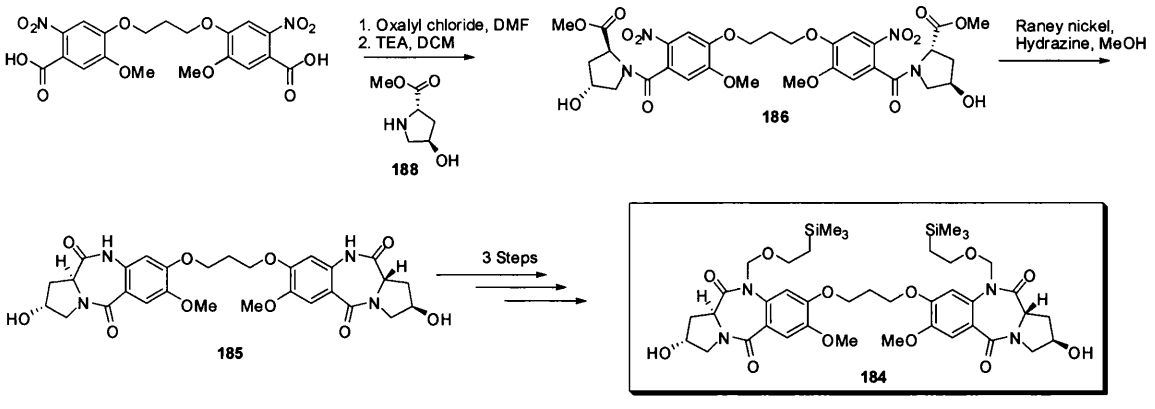
Figure 3.4f: ^{1}H NMR spectra of Key tetralactam intermediate **184**.

The success of the reductive/cyclisation method in generating this critical tetralactam intermediate helped pave the way for synthesizing a number of C2-aryl PBD dimers. In addition, this tetralactam system was exploited to investigate the formylation of this intermediate and subsequent side chain elaboration through Wittig olefination chemistry in attempts to generate a whole new type of novel C2-substituted PBD dimers.

3.4.3 Optimised Amide Coupling Method.

As discussed previously, protection of the C-ring alcohol was necessary to prevent any side reactions occurring during the amide coupling step. Fortunatley, by this stage in the synthesis collegues working on a different target (project) has developed conditions which avoid *O*-silylation.

Application of these conditions to the coupling to the bis-nitrobenzoic acid dimer core afforded **186** in 98% yield. Reductive cyclisation of the bis-nitrobenzamide **186** with Raney nickel and hydrazine afforded the key tetralactam precursor C2-hydroxy **185** in 52% yield. The alcohol **185** was subsequently protected as the C2-OTBS ether with TBS-Cl, imidazole and DMF, followed by N10-SEM protection then finally C2-OTBS deprotection using TBAF to afford the critical tetralactam precursor **184** in 99% yield. The amide coupling yield was significantly higher than the coupling yield obtained from using the protected C-ring (~55%). The indentities of the intermediates were confirmed by ^{1}H and ^{13}C NMR spectra which revealed similar key signals to those observed for the analogues intermediates in the earlier C-ring approach (**see experimental section 5.5.1 for details**). Satisfyingly, this optimised method meant that the synthesis of the key tetralactam intermediate **184** could be achieved in five steps compared to 8 steps, circumventing the need for the tedious synthesis of the protected C-ring which took 4 steps, as well as avoiding the problematic CBz removal step.



Scheme 3.4g: Optimised amide coupling step to generate the key tetralactam intermediate **184**.

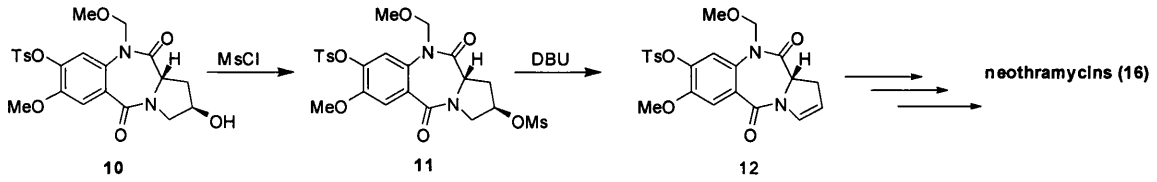
3.5 Exploiting the key Tetralactam Intermediate 184

The synthesis of the key tetralactam intermediate **184** was critical to the synthesis of novel C2-aryl PBD dimers. However there was an opportunity to exploit this tetralactam intermediate in order to investigate the formylation of this tetralactam system and subsequent side chain elaboration through Wittig olefination chemistry. This would in effect generate a novel C2-C3 *endo/exo* unsaturated PBD dimers not observed in nature.

3.5.1 Literature Precedent

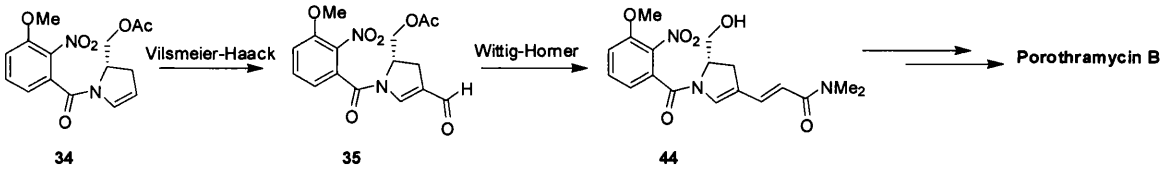
The synthetic strategy employed for the synthesis of C2-C3-*endo/exo* unsaturated PBD dimers was based upon Mori's approach to the synthesis of neothramycin isomers (Mori *et al.*, 1986a) and Langlois' approach to the total synthesis of porothramycin B (Langlois *et al.*, 1993).

In Mori and co-worker's synthesis of neothramycins, the key intermediate **10** (**Scheme 3.5a**), which was prepared via a palladium catalysed carbonylation reaction (Mori *et al.*, 1986a), was methylsulphonated, followed by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford the enamide **12** (**Scheme 3.5a**). Through a series of synthetic steps the target neothramycins isomers (**16**) were obtained, the critical step being the selective C11-lactam reduction with $LiAlH_4$ followed by treatment with silica gel to generate the N10-C11 imine moiety (**see section 1.4.3**). However, compounds **10** and **12** can also be employed as key intermediates for the synthesis of C2-C3 *endo/exo* unsaturated PBD dimers.



Scheme 3.5a: Mori's synthesis of Neothramycins.

Langlois and co-worker's total synthesis of porothramycin B included a key enamide intermediate **34** (**Figure 3.5b**) which was prepared by elimination of an alkoxy group with QCS, which was then transformed into the enealdehyde derivative **35** by performing a Vilsmeier-Haack reaction (DMF-$POCl_5$). The acrylamide C2-side chain was then introduced by using a Wittig-Horner reaction with a stabilised ylid (Langlois *et al.*, 1993, **see section 1.4.8**). This strategy demonstrated the potential to transform aldehydes of type **35** into a variety of PBD analogs with interesting C2/C3 *endo/exo* unsaturated C2-side chains via Wittig olefination chemistry. In principle, Vilsmeier-Haack and olefinations reactions could be applied to enamides of type **12** to afford C2-susbtituted PBD dilactams.



Scheme 3.5b: Langlois' synthesis of porothramycin B.

3.5.2 Retrosynthetic Analysis

Below is a retrosynthetic analysis (**Figure 3.5c**) revealing the key steps in the synthesis of C2-C3 *end/exo* unsaturated PBD dimers, which combines aspects of both the Mori and Langlois approaches, with the key enamide **213** and formyl **214** highlighted. The critical step of the synthesis would be the selective hydride reduction of C11-lactam to the carbinolamine intermediate, followed by treatment with silica gel to provide the N10-C11 imine moiety.

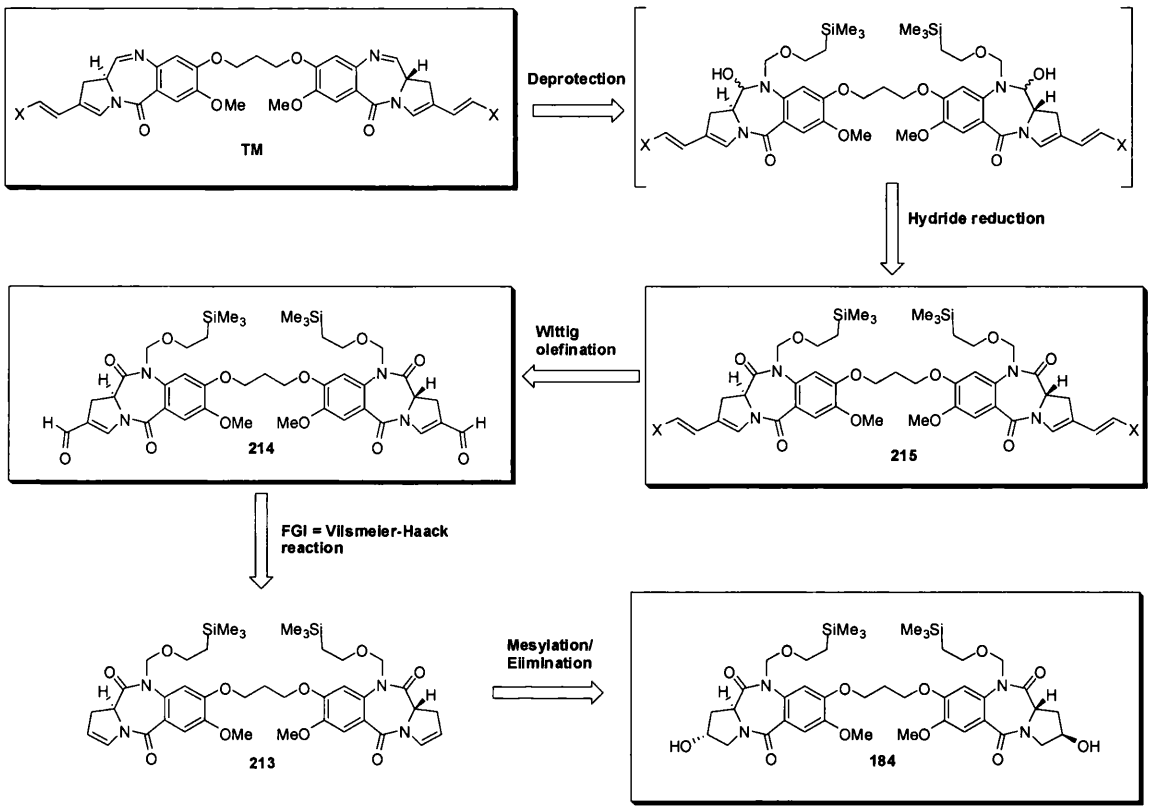
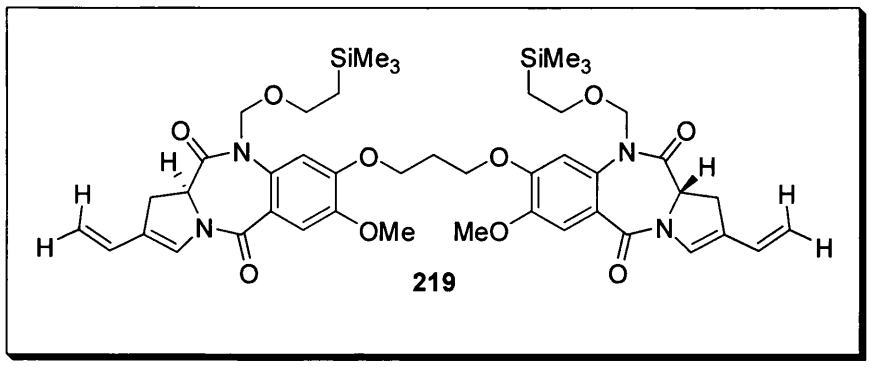


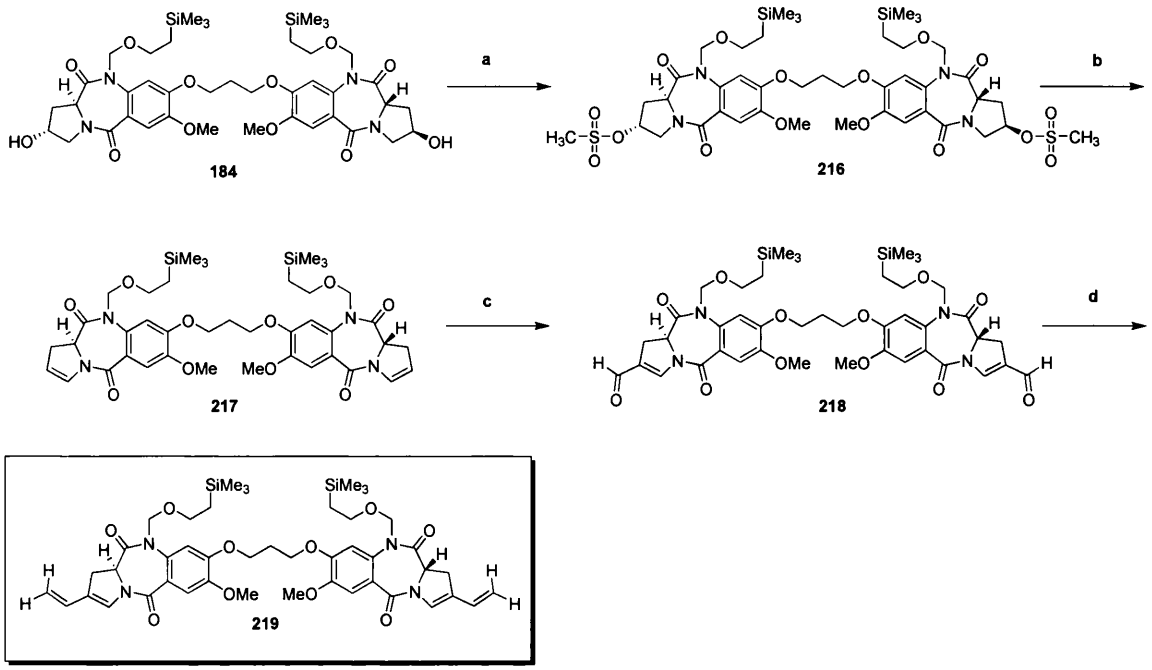
Figure 3.5c: Retrosynthetic analysis towards the synthesis of C2-C3 *endo/exo* unsaturated PBD dimers.

3.5.3 Successful Synthesis of C2-Vinyl Intermediate 219

The strategy outlined above was applied to the synthesis of C2-vinyl substituted PBD **219** (**see section 3.5.1**).



3.5.3.1 Synthetic Route.



Scheme 3.5d: Synthetic route for the synthesis of C2-vinyl intermediate **219**.

a) Methylsulphonyl Chloride, TEA, CH_2Cl_2, 0 °C, 98%; **b**) DBU, anhydrous acentonitrile, microwave (190 °C, 195 Psi), 65%; **c**) Vilsmeier reagent, anhydrous CH_2Cl_2, 0 °C, 30%; **d**) Ph_3PCH_3Br, KO^tBu, anhydrous THF, 0 °C, 52%.

The synthesis began (**Scheme 3.5d**) with the methylsulphonation of the key C2-hydroxy tetralactam **184** with MsCl, TEA to give **216** in quantitative yield. Elimination with DBU afforded the crude enamide precursor **217**. Initially minor impurities present were difficult to remove by column chromatography, but after systematic optimization of the elution system (100% Et_2O to 80:20 v/v Et_2O/AcCN) column chromatography gave the pure bis-enamide **217** as a yellow foam in 65% yield.

The ^1H and ^{13}C NMR spectra (**Figure 3.5e**) supported the structure of the enamide **217** which showed the disappearance of the H3α and H3β signals, which were replaced by a characteristic downfield singlet signal at δ 6.90 ppm and δ 128.4 ppm respectively. In addition, the H2 signals changed from a multiplet to a singlet in the ^1H NMR spectra, plus a dramatic shift in the

position of the C2 signal in the ^{13}C NMR spectra from 53.9 ppm → 113.9 ppm. Further confirmation came from LC/MS analysis which showed a single peak at 7.92 min (ES+) m/z (relative intensity) 822 ($[M + H]^{+\cdot}$, 20), 844 ($[M + Na]^{+\cdot}$, 25). Optical rotation values $[\alpha]_D^{27}$ = + 129° (c = 0.85, $CHCl_3$) also showed that complete racemisation had not occurred during the synthetic step. The ^1H NMR spectrum is shown with the signals from the bis-enamide compound assigned (**Figure 3.5f**).

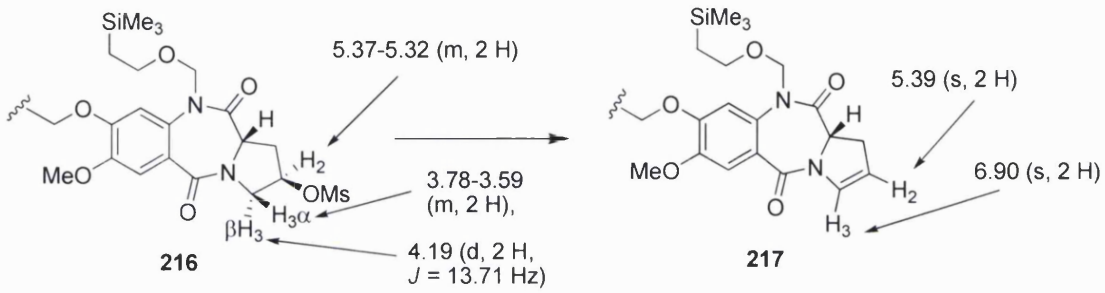


Figure 3.5e: Key ^1H NMR Chemical Shifts (δ ppm) for **216** and **217**.

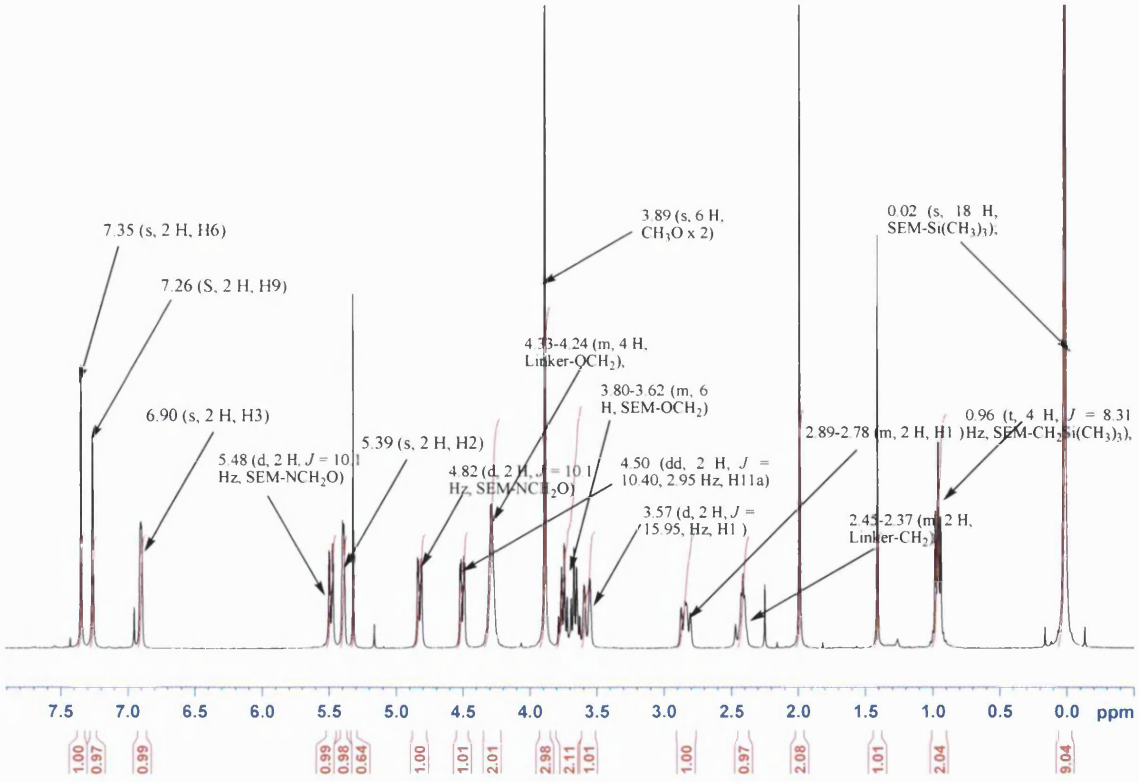


Figure 3.5f: ^1H NMR Spectral assignment of the bis-enamide **217.**

The next step was to introduce a formyl group at the C2 position through a Vilsmeier-Haack reaction, which was achieved by treating the bis-enamide **217** with the commercially available Vilsmeier reagent in DCM at 0 °C. After stirring for 3 h, TLC revealed a mixture of desired product **218** plus number of impurities. Isolation of the desired product by flash chromatography furnished the required C2-formyl intermediate **217** in 30% yield. Measured optical rotations values dropped to zero after the Vilsmeier-Haack formylation reaction suggesting racemisation had occurred.

3.5.3.2 Discussion of Racemisation during Vilsmeier-Haack Reaction.

It was believed that the hydrochloric acid produced as a by-product during the Vilsmeier-Haack reaction was responsible for the racemisation at the C11a position. The only other possible cause, which was Na_2CO_3 (used for quenching the reaction) was promptly eliminated when less basic Sat. $NaHCO_3$ was used as a alternative to quench the reaction, and the product isolated from this reaction still displayed optical rotations values dropping to zero. As racemisation was believed to occur in the presence of the hydrochloric acid produced as a by-product, the following potential mechanism was proposed (**Scheme 3.5g**).

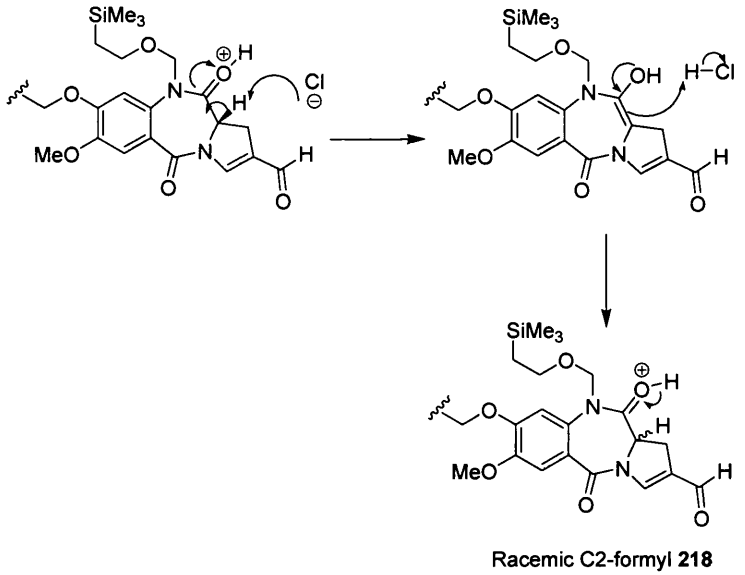


Figure 3.5g: Possible mechanism of racemisation driven by the presence of HCl gas.

Enolisation of the C11-amidic carbonyl by abstraction of the acidic H-11a proton by chloride ions (Cl^-) destroys the chirality at this critical position. Subsequent equilibration back to the carbonyl would afford the, now racemic, tetralactam intermediate **218**.

It's very interesting to note that racemisation did not occur during the Vilsmeier-Haack reactions carried out by the Langlois and Fukuyama approaches in the synthesis of porothramycin B, with both authors quoting optically active compounds from this reaction ($[\alpha]_D$ = - 219° (*c* = 1.0) and $[\alpha]_D$ = - 182° (*c* = 0.61)). One possible explanation for this observation is that all the Vilsmeier-Haack reactions carried out by Langlois and Fukuyama were performed on ring-opened PBD intermediates, but in this synthesis the Vilsmeier-Haack reaction was carried out on ring-closed tetralactam intermediates. In such ring-closed tetralactam systems, the H-11a proton is in close proximity to the C11 carbonyl which may increase its acidity, thus facilitating deprotonation under weak basic conditions. In fact, the C11 carbonyl is part of a vinylogous imide, increasing its electrophilicity and making the 11a proton more acidic. Although ring-opened systems described by Langlois and Fukuyama are still prone to racemisation at the C11a position, the ring closed tetralactam intermediates are lot more susceptible to epimerization at the C11a location due the vinylogous effect of the C-11 amide, which is not apparent in ring-opened systems.

The Vilsmeier-Haack reaction was repeated numerous times using a variety of reaction conditions. However, no improvements in optical rotations values or yields could be obtained. Therefore, it was decided to persist with this low yielding and racemic Vilsmeier-Haack reaction, and to continue with the next step of the synthesis. Characterisation was obtained for this racemic bis-formyl compound **218**, and ^1H NMR spectra (**Figure 3.5h**) confirmed the structure which exhibited characteristic downfield singlet corresponding to the CHO-formyl at δ 9.73 ppm (s, 2 H) as well as absence of the H2 signal which was normally present at δ 5.39 ppm (s, 2 H, H2).

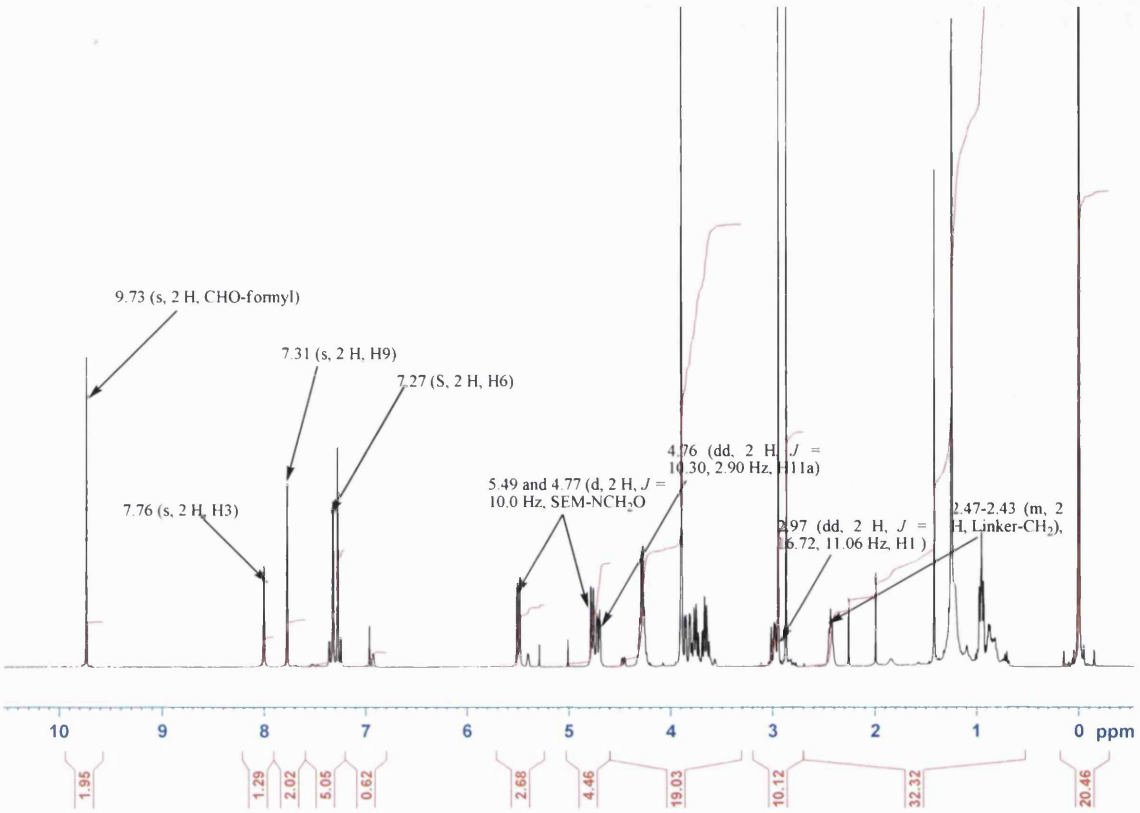


Figure 3.5h: ^{1}H NMR Spectral assignment of the bis-formyl **218**.

The next step was to introduce a C2-*exo* double bond using Wittig olefination chemistry. Langlois *et al*, reported that the C2-*N*,*N*-dimethylacrylamide side chain was introduced by performing a Horner-Wadsworth-Emmons olefination reaction on the aldehyde intermediate using a suitably functionalized stabilized ylid (Langlois *et al*., 1993). However, in this approach, a Wittig olefination reaction was carried out instead (Gregson. *et al*., 2004) using methyltriphenylphosphonium bromide in the presence of potassium *tert*-butoxide to generate the unstablised ylid. The ylid obtained was then condensed with the aldehyde **218** to afford the C2-vinyl product **219** in 52% yield.

The structure of **219** was confirmed by ^{1}H NMR spectra (**Figure 3.5i**) which revealed the appearance of new olefinic signals corresponding to the doublet of doublets signals (dd) at δ 6.51 ppm (H12) and 5.15 ppm (H13), along with methine signal at 131.1 (C12) and methylene signal at 116.4 ppm (C13) in the ^{13}C-NMR spectrum indicating the presence of the vinylic substituent.

In addition, the formation of a double bond was further confirmed by the coupling constant observed between the vinylic protons H12 and H13 (J = 17.3 and 10.6 Hz respectively) which are characteristic for alkene *trans* and *cis* couplings. The ^{1}H NMR spectra of the bis-vinylic intermediate **219** is shown in **Figure 3.5j** with the key olefinic signals highlighted.

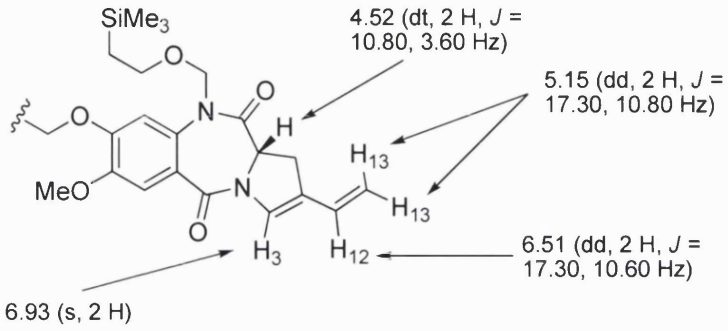


Figure 3.5i: Key ^{1}H NMR chemical shifts (δ ppm) for **219**.

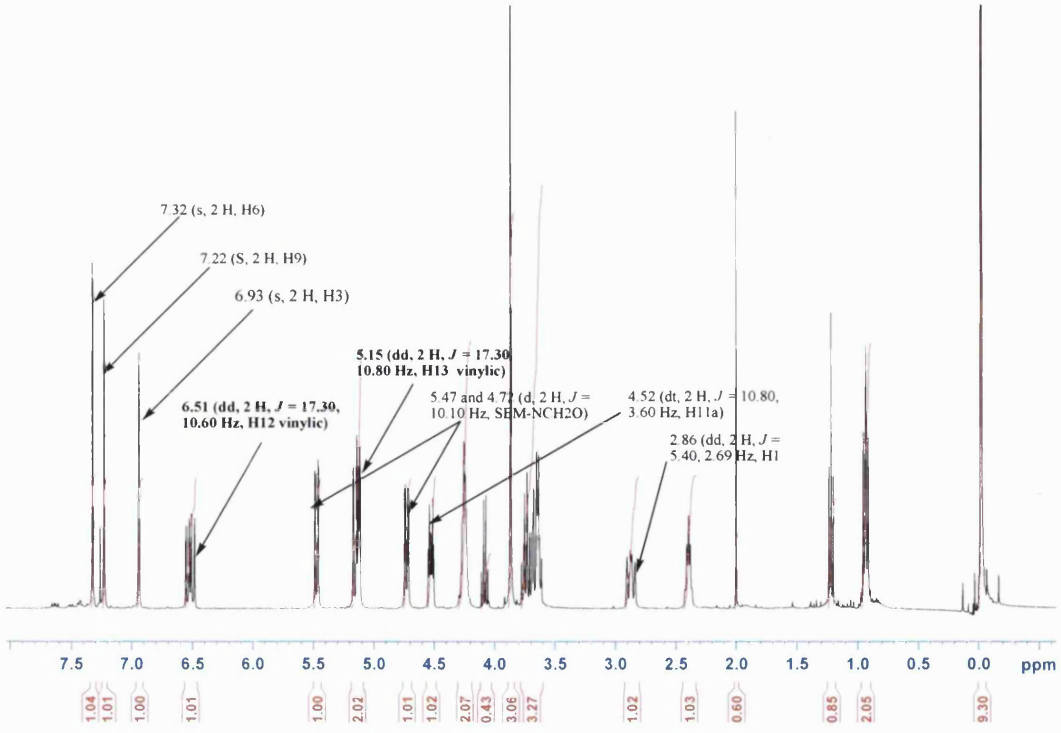


Figure 3.5j: ^{1}H NMR Spectrum of the Bis-Vinylic intermediate **219**

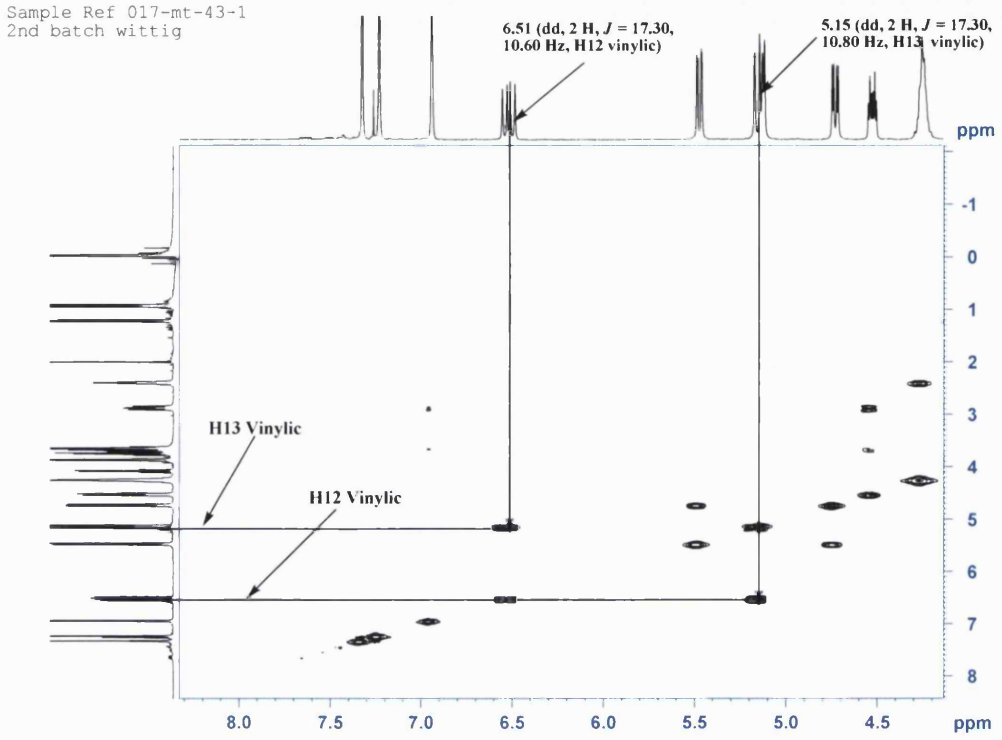
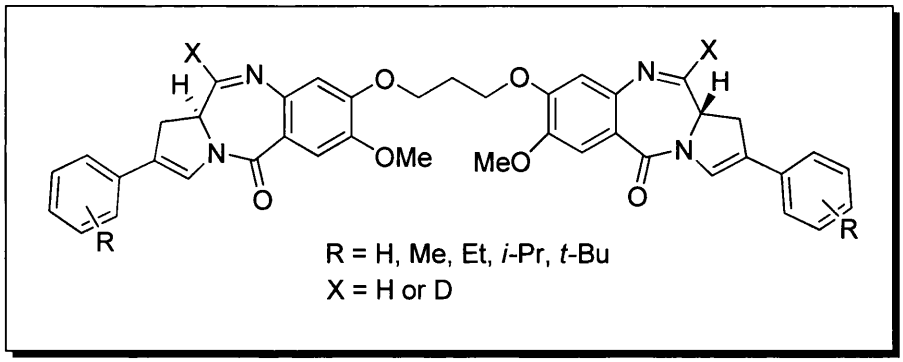


Figure 3.5k 2D-COSY ^{1}H NMR Spectrum of the bis-Vinylic intermediate **219**, showing the Vinylic protons (H12 & H13) coupling together.

As was expected, the measured optical rotation values of the bis-vinyl **219** intermediate were to zero which indicated the product was racemic. At this point of the synthesis it was decided not to proceed any further in this approach to generate the N10-C11 imine moiety. This was because the compounds obtained via this route would be racemic and therefore have reduced biological activity. Also quantities of the precious key tetralactam intermediate **184** were limited, and were therefore committed instead to the synthesis of novel C2-aryl PBD dimers, where the compounds should have potent biological activity, possessing enhanced antitumour properties (Cooper *et al.*, 2002).

3.6 Synthesis of Novel C2-Aryl PBD Dimers

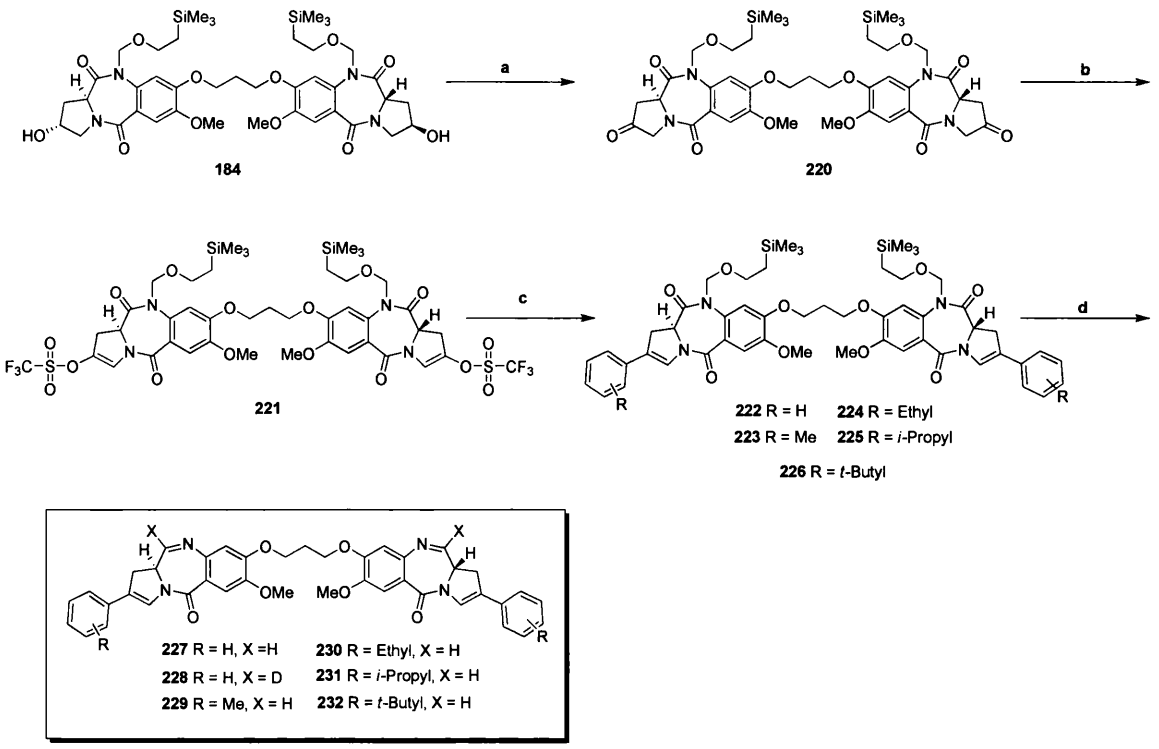


3.6.1 Synthetic strategy

A retrosynthetic analysis for the synthesis of novel C2-aryl PBD dimers have already been discussed (see **section 3.1.1). Scheme 3.6a** shows the forward synthetic route for a series of novel C2-aryl PBD dimers. The key steps are the transformation of the critical tetralactam intermediate **184** to the key C2-C3 enol triflate **221**. Suzuki cross coupling reactions performed on the enol triflate generated the novel C2-aryl PBD dimers, with the penultimate and critical step being reduction of the tetralactam with $LiBH_4$, followed by N10-SEM deprotection to form the N10-C11 imine moiety, critical for biological activity.

The methodology adopted was based upon Cooper and co-worker's synthesis of C2-aryl PBD monomers (Cooper *et al.*, 2002) and Hu and co-worker's synthesis of DC-81 natrual product (Hu *et al.*, 2002).

3.6.1.1 Successful Synthesis of Key C2-C3 Enoltriflate **221**



Scheme 3.6a: Synthesis of novel C2-aryl PBD dimers.

a) $(COCl)_2$, anhydrous DMSO, anhydrous DCM, TEA, -60 °C, 52%; **b**) anhydrous pyridine, anhydrous CH_2Cl_2, anhydrous triflic anhydride, r.t., 55%; **c**) benzeneboronic acid, toluene, EtOH, H_2O, Na_2CO_3, $Pd(PPh_3)_4$, r.t., 93% (**222**); methylbenzeneboronic acid, 50%, (**223**); ethylbenzeneboronic acid, 72%, (**224**); *iso*-propylphenylboronic acid, 80%, (**225**); *tert*-butylphenylboronic acid, 83% (**226**); **d**) 1. $NaBH_4$, EtOH, anhydrous THF; 2. Wet silica gel, EtOH, H_2O, r.t., 58% (**227**); $NaDH_4$ only, 38% (**228**); 52% (**229**); 77% (**230**); 54% (**231**); 55% (**232**).

The synthesis commenced (**Scheme 3.6a**) with oxidising the secondary alcohol **184** to the corresponding ketone **220** in 52% yield under Swern conditions (Cooper *at al.*, 2002; Mancuso and Swern, 1981). Successful oxidation was confirmed by the disappearance of the H2 methine signal in the ^1H NMR spectrum. In addition, the H1 signals changed from multiplets to a clearly visible doublet at δ 3.54 (d, 2 H, J = 19.25, Hz, H1α) and doublet of doublets at δ 2.76 (dd, 2 H, J = 18.90, 10.26 Hz, H1β) respectively.

The next step was to convert the bis-ketone **220** to the bis-enoltriflate **221**. Although N10-SEM PBD dilactams have been shown to be good substrates for triflation reactions (Cooper *et al.*, 2002), The triflation yield of N10-protected carbinolamines PBD intermediates can be significantly enhanced by employing a large excess of reagents (Antonow *et al.*, 2007a & b; Z.Chen *et al.*, PhD thesis 2004). Therefore, the initial protocol used to synthesize the enol triflate **221** employed a large excess of triflic anhydride (14 eq) and pyridine base (14 eq) at 0 °C for 3 h. (Antonow *et al.*, 2007b). The TLC revealed formation of large quantities of desired product. However, after isolation by column chromatography, the ^{1}H NMR spectrum showed an impurity which could not be removed by chromatography. Although the yield obtained for this batch of enoltriflate was 52%, the presence of an impurity in the ^{1}H NMR spectrum was deemed to be unsatisfactory for the Suzuki cross coupling reactions and this led to the use of the standard triflation method employed by Cooper and co-worker's approach to C2-aryl PBD monomers (Cooper *et al.*, 2002). The conditions involved utilizing just 2.2 eq of triflic anhydride and 2.4 eq of pyridine at room temperature for 3 h, thus avoiding the use of a large excess of reagents. After isolation by column chromatography, ^{1}H NMR spectra revealed a complete absence of the impurity initially observed when employing a large excess of reagents, with a respectable yield for this type of reaction of 50%. The use of these reaction conditions is a lot more economical as triflic anhydride is expensive, as well as lending itself to large scale workup. The structure of bis-C2-C3 enoltriflate **221** was confirmed by the diagnostic downfield H3 triplet at 7.13 ppm in the ^{1}H NMR spectrum, with the multiplicity arising from long range coupling with the H1 protons (**Figure 3.6b**). Additionally, the signals for H1α were observed to shift downfield from 3.54 to 3.93 ppm respectively.

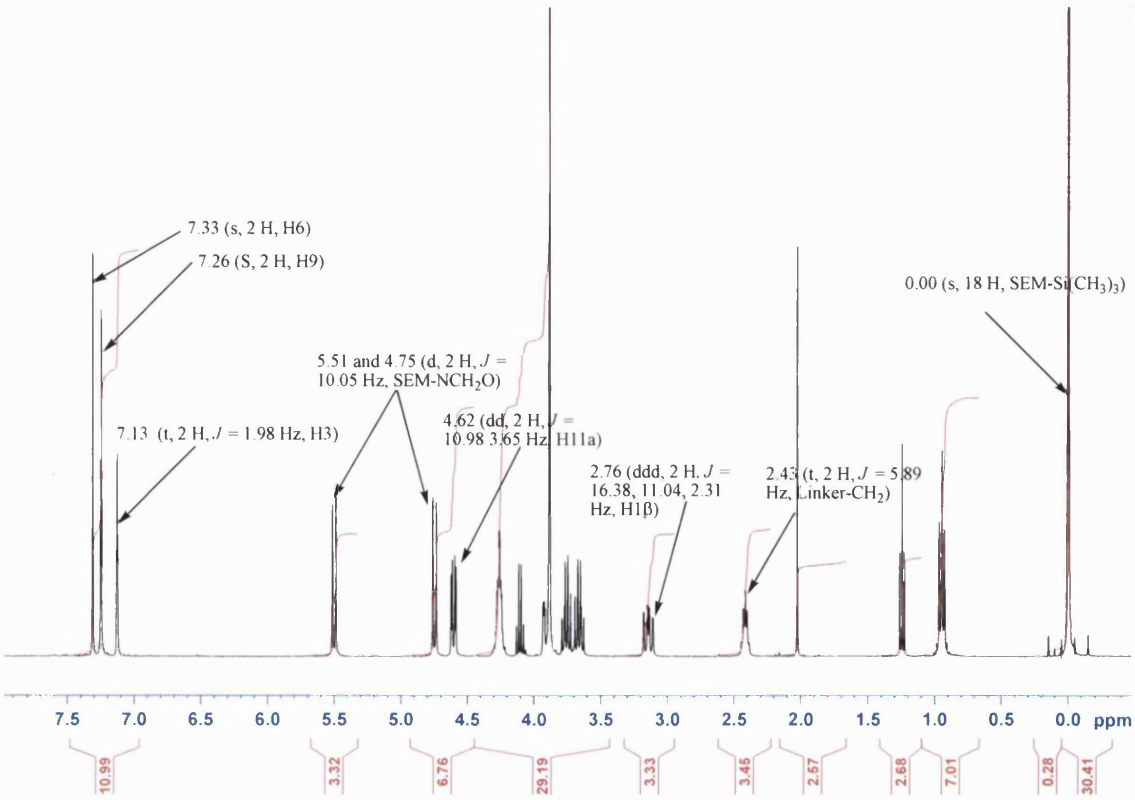


Figure 3.5b: ^{1}H NMR Spectrum assignment of the C2-C3 enol triflate **221**.

Interestingly, the H1β signals had clearly changed from double of doublets (dd) at δ 2.76 (2 H, J = 18.90, 10.26 Hz, H1β), to a double doublet of doublets (ddd) at δ 2.76 (2 H, J = 16.38, 11.04, 2.31 Hz, H1β) after the triflation step. Incorporation of a double bond in the C-ring during the triflation reaction, resulted in long range coupling between the H1β proton and the H3 proton (**Figure 3.6c**), as orbital overlap (or electronic transmission) is highly efficient in unsaturated systems, especially when bonds involved are antiperiplanar, which occurs in the C2-C3 unsaturated enol triflate **221**.

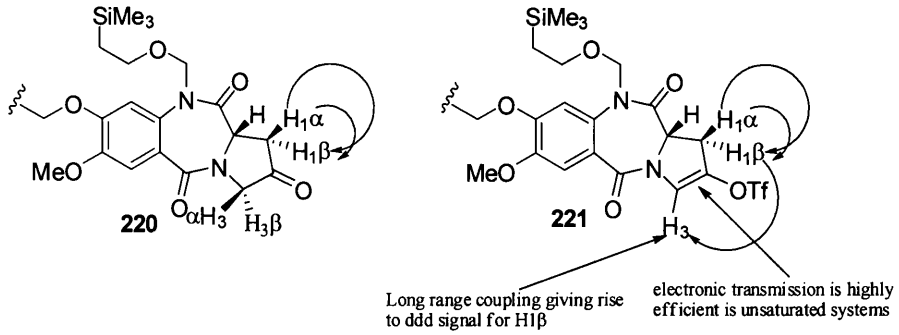
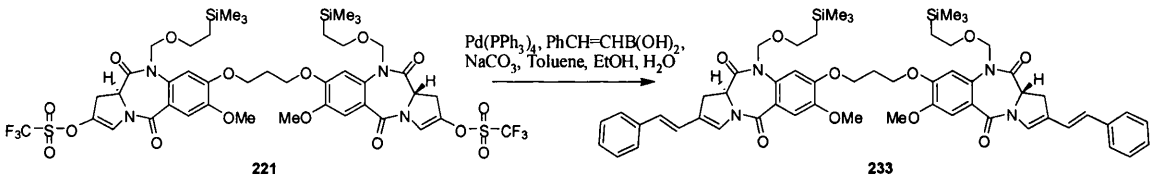


Figure 3.6c: Illustrating the effects of unsaturation in the C-ring in long range coupling between H1β and H3 protons in the bis-enol triflate **221**.

3.6.1.2 Synthesis of C2-Styryl Intermediate **233**



Scheme 3.6d: Synthesis of C2-styryl intermediate **233**.

The key enol triflate **221** was subjected to a series of Suzuki coupling reactions to introduce a variety of C2-aryl substituents at the C2-position of the molecule. An opportunity arose at this point in the synthesis to introduce styryl substituents using commercially available styryl boronic acids (**Scheme 3.6d**). Treatment of the bis-enol trfilate **221** with *trans*-styrylboronic acid and tetrakis ($Pd(PPh_3)_4$) provided a mixture of the desired product **233** as well as significant formation of a by-product. Separation by column chromatography furnished the bis-styryl **233** in a disappointing 20% yield, which was attributed to the formation of this unknown by-product. Isolation and analysis by ^1H NMR of the by-product revealed it to be an unsymmetrical PDB dimer with similar signals to the bis-styryl intermediate **233**. Confirmation that the compound was unsymmetrical came from two distinct H11a proton signals at 4.56 (dd, 2 H, J = 10.50, 3.30 Hz, H11a) and 4.56 (dd, 2 H, J = 10.50, 3.30 Hz, H11a) respectively.

Interpretation of the ^{1}H NMR spectra proved to be difficult to assign exactly the structure of the by-product that was formed, and attempts to limit or prevent its formation by changing the reactions conditions failed. As quantities of the bis-enol triflate were limited, it was decided to subject the enoltriflate **221** to Suzuki cross-coupling reactions with aryl substituents, rather than risk further attempts to optimize this reaction step.

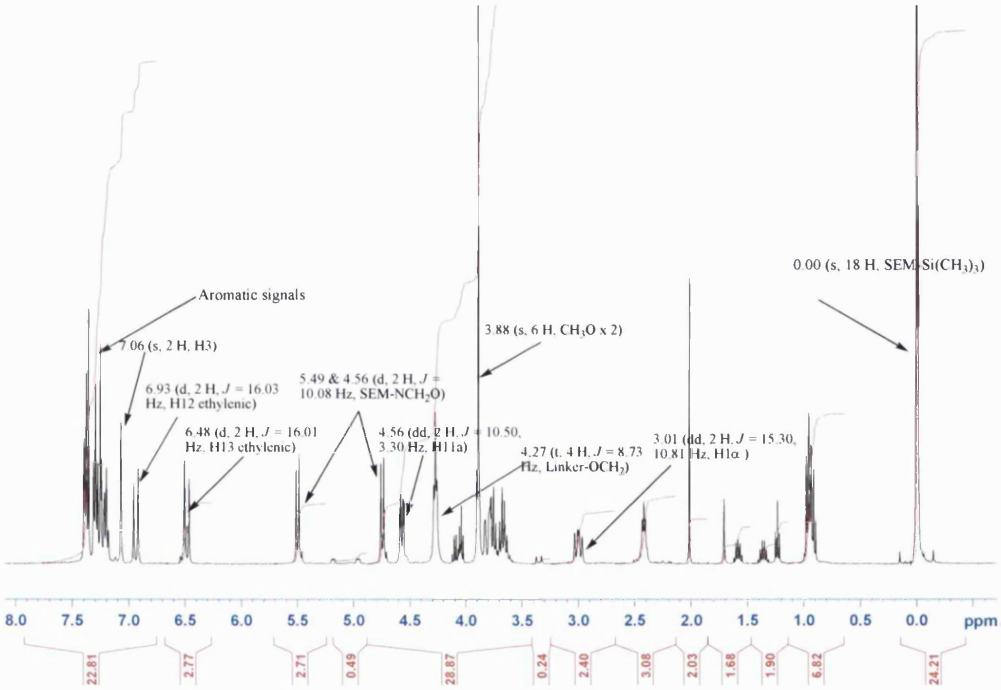
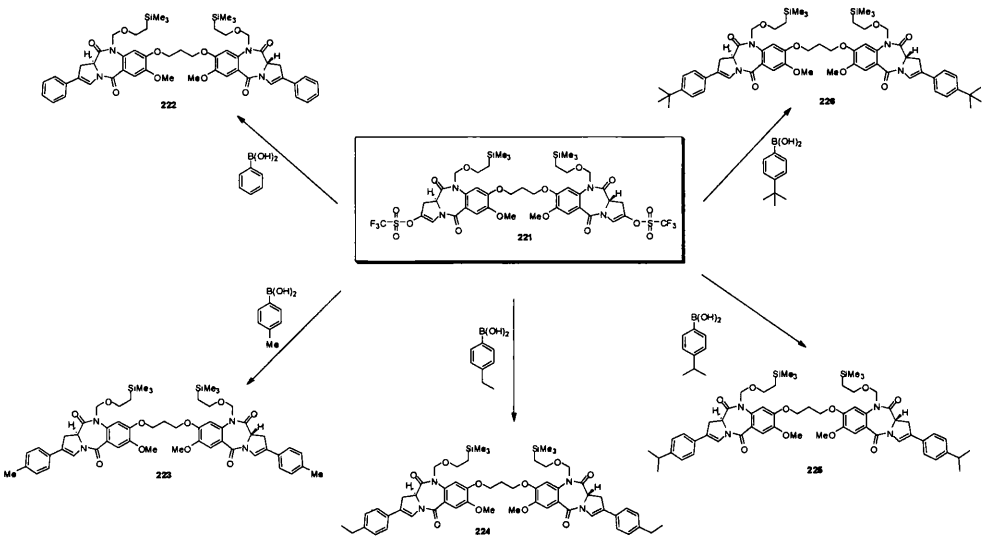


Figure 3.6e: ^{1}H NMR spectrum of the bis-styryl intermediate **233** with key Signals assigned

3.6.2 Successful Synthesis of Novel C2-Aryl PBD imine Dimers.

In contrast, the reactions with aryl boronic acids were very efficient with isolated yields after column chromatography ranging from 99% for the phenylboronic acid to 83% for the *t*-butylphenylboronic acid. The attachment of the aryl substituents were all confirmed by ^{1}H and ^{13}C NMR spectroscopy (see below, **Figure 3.6g).**



Scheme 3.6f: Suzuki coupling reactions with key enol triflate **221**.

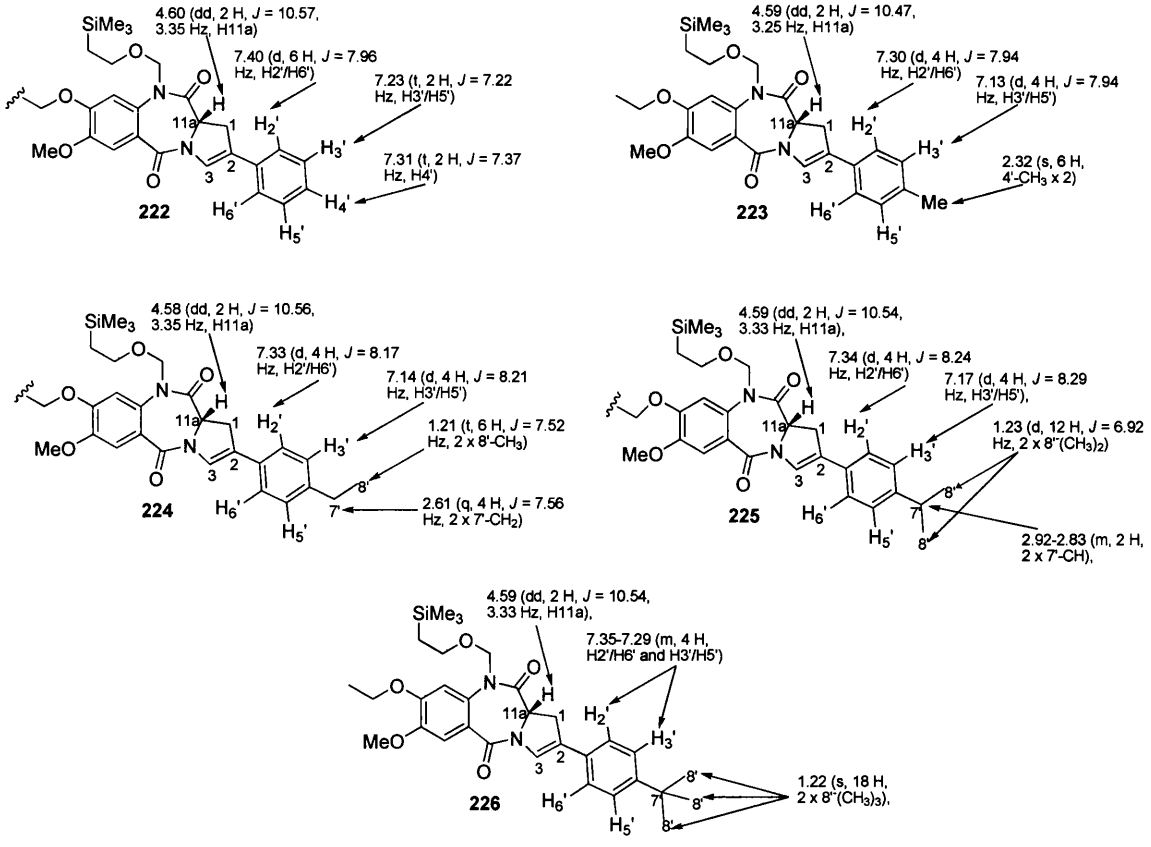
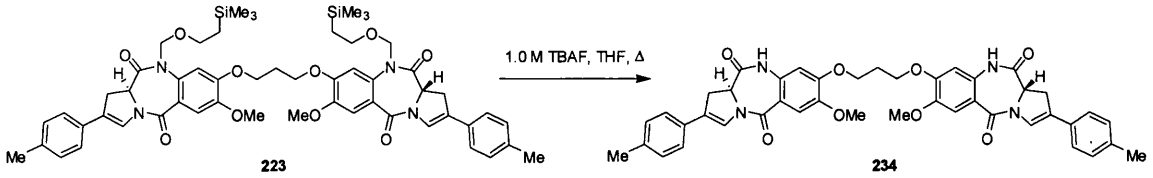


Figure 3.6g: Key ^{1}H NMR chemical shifts (δ ppm) for the novel C2-aryl intermediates **222, 223, 224, 225** and **226.**

For example, in the bis-C2-phenyl tetralactam **222**, an appearance of a downfield doublet at δ 7.40 (d, 4 H, J = 7.96 Hz, H2'/H6') as well as, two additional triplets in the aromatic region at 7.31 (t, 2 H, J = 7.37). Hz, H4') and 7.23 (t, 2 H, J = 7.22 Hz, H3'/H5') corresponding to the C2-aryl substituent, confirming the success of the Suzuki reaction.

3.6.2.1 Synthesis of Tetralactam C2-Methylbenzene Intermediate **234**



Scheme 3.6h: Synthesis of Tetralactam C2-aryl intermediate **234.**

Kaneko *et al.* first reported that some PBD dilactams which lack the N10-C11 imine/carbinolamine moiety responsible for covalent binding to DNA posses significant antitumour activity (Kaneko *et al.*, 1985). It has now been established that dilactams exert their biological activity by binding to DNA through a non-covalent interactions (see section **1.5.2.1, B-ring SAR for more details).** Therefore, attempts to synthesize PBD dilactam dimers were carried out by removing the N10-SEM protecting group to generate the N10-C11 lactam moiety. Such C2-aryl dilactam dimers could then be compared to C2-aryl imine dimers for their DNA binding affinity and cytotoxicity.

Removal of the SEM group has been reported to proceed with 1 M tetrabutylammonium fluoride solutions or with warming with dilute acid (Robertson, *Protecting Group Chemistry*, 2006; Whitten *et al.*, 1986). The use of such acidic conditions was a cause of concern, as this was suspected to cause racemisation in the Vilsmeier-Haack reaction. Therefore the bis-N10-SEM protected dimer **223** was treated with 1 M tetrabutylammonium fluoride in THF under reflux for 1 h (**Scheme 3.6h**), after which time a multispot mixture was observed by TLC, and the desired product **234** could not be isolated after flash chromatography. After repeating the reaction using different conditions of stoichiometry and reaction time, formation of the desired tetralactam C2-methylphenyl product **234** was observed by TLC, as well as multispot mixtures.

Isolation of the desired product was achieved using flash chromatography gradient elution, but in a disappointing yield of 7%. Despite the poor yields obtained from the SEM deprotection, the isolated bis-tetralactam C2-methylpheny **234** was analysed by ^{1}H NMR spectroscopy, which confirmed the structure by the presence of a characteristic large downfield signal at δ10.45 ppm corresponding to the N10 amidic proton, the complete loss of the SEM signals and the existence of aromatic signals indicating the C2-methylphenyl substituents remaining intact (see ^{1}H NMR below, **Figure 3.6p**). In addition LC/MS analysis revealed a single peak at 7.25 min (ES+) m/z (relative intensity) 741 ($[M + H]^{+}$, 100), 764 ($[M + Na]^{+}$, 10) confirming the mass of the structure **234**, with an optically active compound having been isolated $[\alpha]_D^{23}$ = +365° (*c* = 0.02, DMSO).

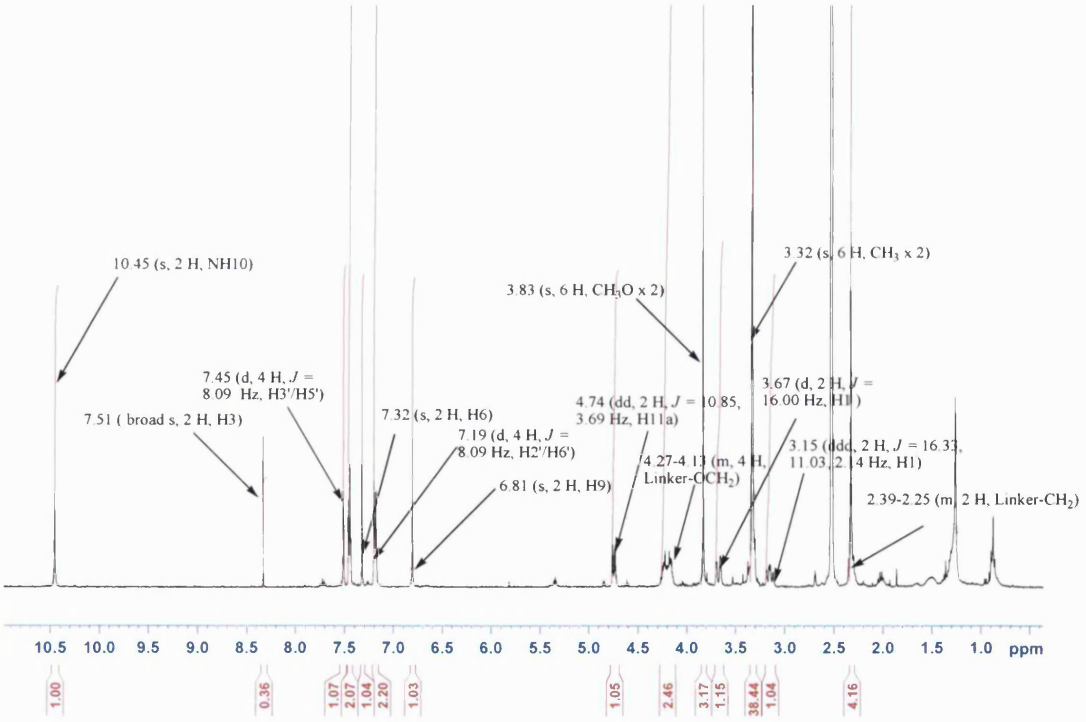


Figure 3.6p: ^{1}H NMR spectrum of the novel bis-tetralactam C2-methylphenyl **234**.

No further optimisation of this reaction was carried out as quantities of the bis-N10-SEM protected dimer **223** were limited, so rather than risk trying to optimise this reaction the material available was converted to the more biologically active N10-C11 imine moiety to generate the novel C2-methylphenyl dimer **229**.

The final synthetic step involved reduction/deprotection to generate the N10-C11 imine moiety vital for biological activity of PBDs. The reaction was performed using the optimized method developed for the synthesis of DSB-120 described in **section 3.2.3**.

All the bis-C2-aryl N10-SEM protected tetralactam intermediates were subjected to 20 equivalents of $LiBH_4$ for 3 h, to transiently afford the carbinolamine intermediate, which was converted to the PBD imines **227-232** upon exposure to moist silica gel. The isolated yields after column chromatography average a respectable ~57% yield, with all members of the C2-aryl PBD imines displaying consistent optical rotation values (see **Table 3.6a**), showing that the C11a-stereochemisty had been maintained during the reduction/deprotection step (a mechanism for this final step has been described earlier in **section 3.2.3**). In addition, the bis-C2-phenyl tetralactam **222** was treated with Sodium borodeuteride ($NaBD_4$, 98 atom % D) to generate the novel deuterated imine analogue **228** in an isolated yield of 43%, which is summarized in **Table 3.6a**.

The ^1H and ^{13}C NMR spectra provided evidence for the successful synthesis of the novel PBD imine dimers **227-232** which revealed a characteristic diagnostic H11-imine doublet signals ~ δ 7.86 ppm, as well as the complete loss of the SEM signals and the presence of the C2-aryl substituents. A representation ^1H NMR spectrum is shown in **Figure 3.6i**, for the bis-C2-ethylphenyl PDB imine **230**, with expansion of the H11 doublet signal.

Further confirmation was obtained from both ESI mass spectrometry where the molecular ions were observed as the base peak as well as high resolution mass spectrometry (HRMS) analysis confirming the mass of the molecules. The key ^1H and ^{13}C NMR shifts of these novel C2-aryl PBD imine dimers are summarized in **Table 3.6a**

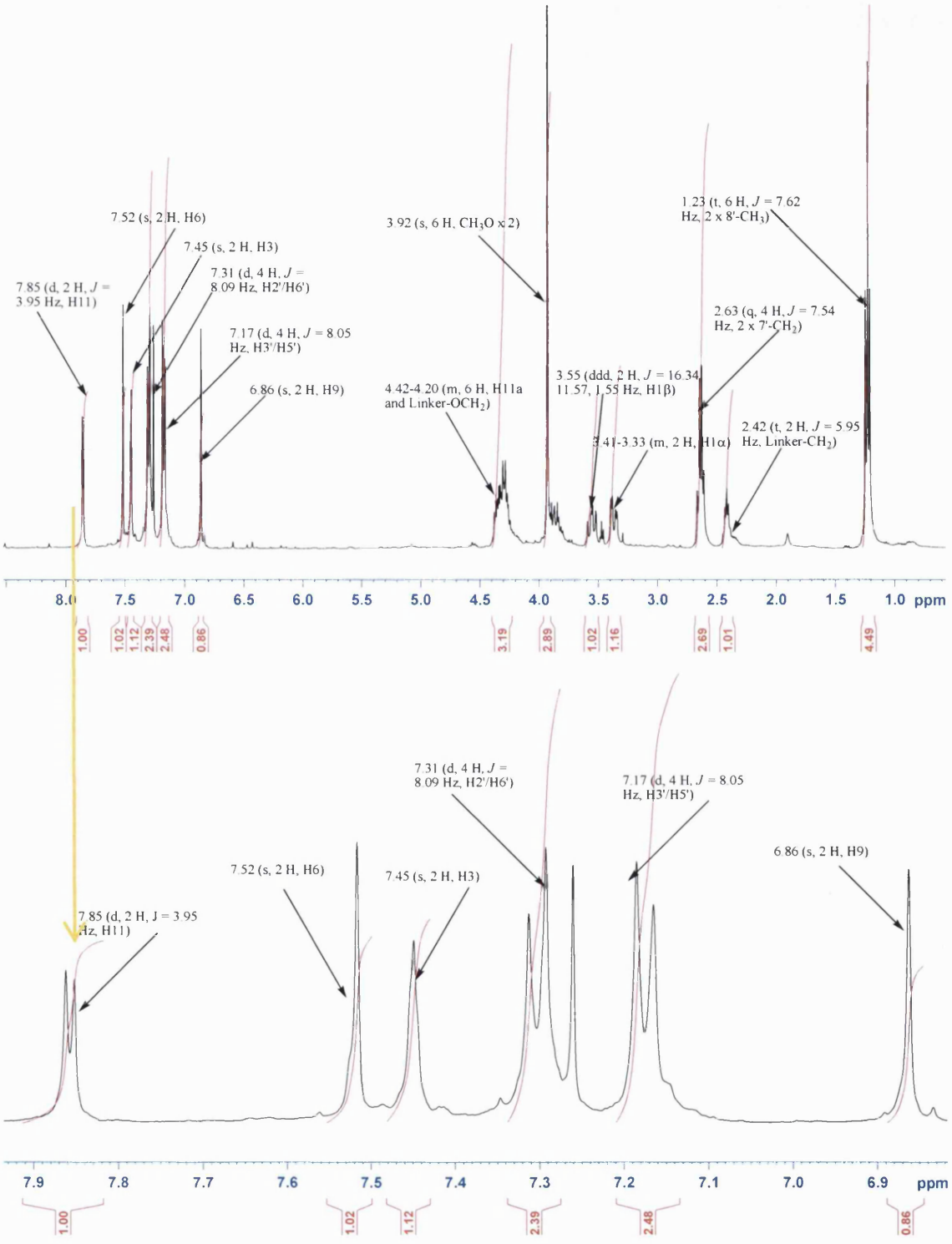
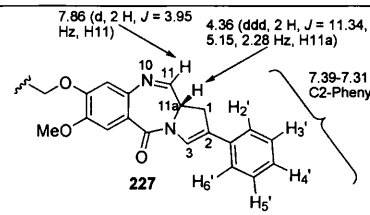
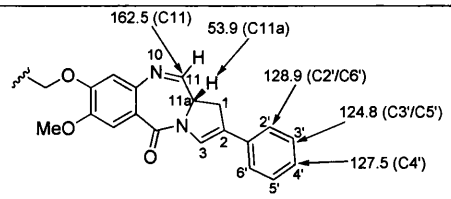
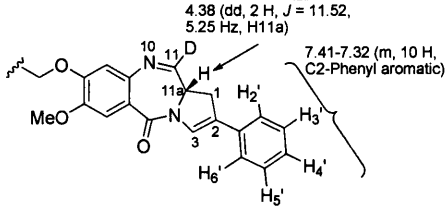
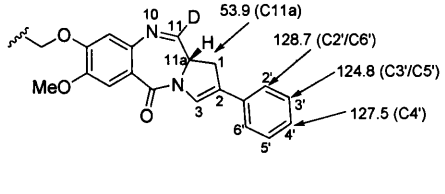
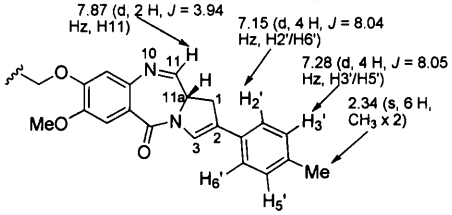
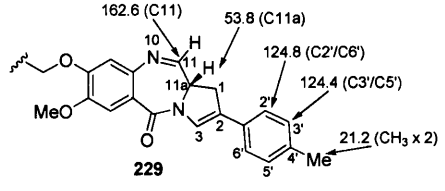
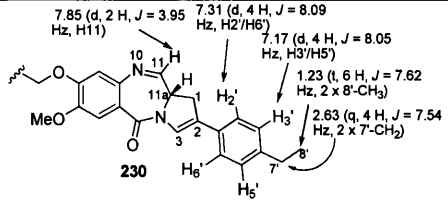
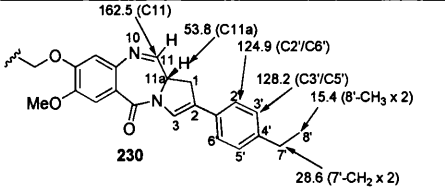
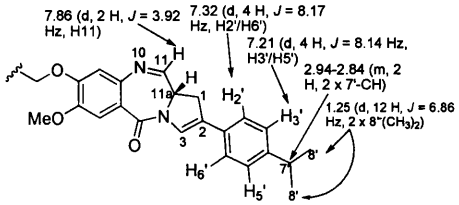
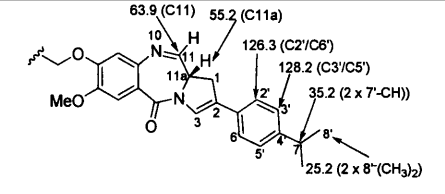
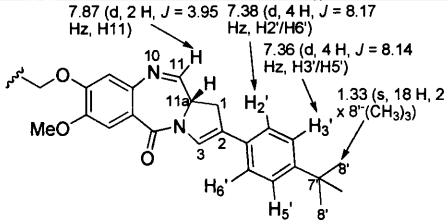
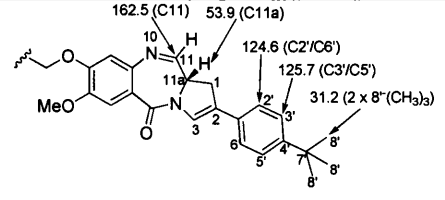


Figure 3.6i: ^{1}H NMR spectrum of bis-C2-ethylphenyl PBD imine **230**, with expansion of the H11-imine doublet and aromatic region.

Table 3.6a: Summary of Key ^{1}H and ^{13}C NMR Chemical Shifts (δ ppm), Yields (%) and Optical Rotations ($[\alpha]_D^T$) for the Novel C2-aryl PBD imine dimers **227-232.** [a] = All yields are for pure, isolated compounds. [b] = Optical rotations measured in HPLC $CHCl_3$ (see experimental sections for concentrations and temperature [T] details).

No:	^{1}H NMR	^{13}C NMR
227	 Yields[a] = 58%, $[\alpha]_D^{T,b}$ = +740°	
228	 Yields[a] = 38%, $[\alpha]_D^{T,b}$ = +700°	
229	 Yields[a] = 52%, $[\alpha]_D^{T,b}$ = +644°	
230	 Yields[a] = 77%, $[\alpha]_D^{T,b}$ = +620°	
231	 Yields[a] = 54%, $[\alpha]_D^{T,b}$ = +618°	
232	 Yields[a] = 55%, $[\alpha]_D^{T,b}$ = +625°	

3.7 Biological Evaluation of Novel C2-Aryl PBD Dimers

3.7.1 Introduction

The synthesis of the 5 novel C2-aryl dimers has allowed the role of the 4-substituent in the aryl fragment to be investigated in the molecule's cytotoxicity evaluation.

3.7.2 *In Vitro* Cytotoxicity studies

The five novel PBD dimers were evaluated for their *in vitro* cytotoxicity by Prof. John Hartley's group based at UCL Medical School by testing the ability of these novel PBD dimers to inhibit the growth of human chronic myeloid leukaemia K_{562} cells (IC_{50}) using the microculture tetrazolium (MTT) assay.

3.7.2.1 The Principle of the MTT Assay

The MTT assay is a colorimetric assay which measures the conversion or reduction of yellow MTT (a tetrazole) to purple formazan in the mitochondria of healthy living cells (see **Figure 3.7a).**

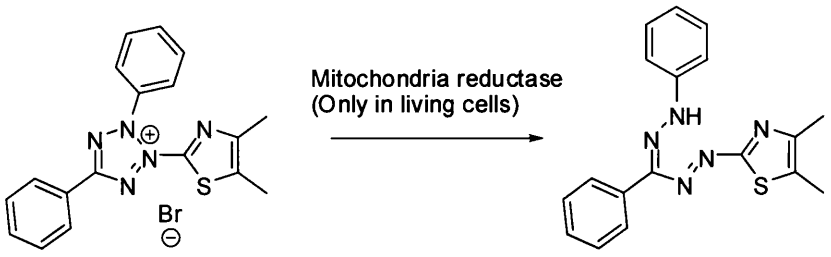


Figure 3.7a: Reduction of yellow MTT to purple formazan by mitochondria reductase enzymes in healthy living cells.

This reduction only takes place when mitochondria reductase enzymes are active or living, and therefore the conversion is used as a measure of viable living cells. When the amount of purple formazan produced by cells treated with a cytotoxic agent is compared with the amount of formazan produced by untreated controls cells, the effectiveness of the agent in causing cell

death can be determined. The purple solution absorbs at a particular wavelength (~500 nm) and can therefore be quantified by a spectrophotometer.

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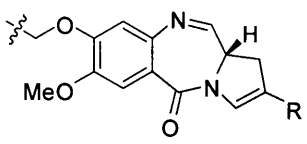
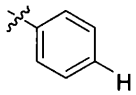
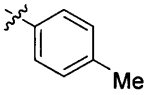
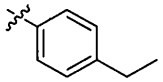
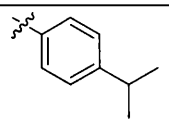
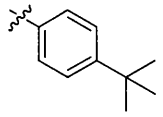
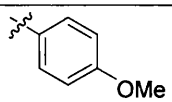
Table 3.7a: *In Vitro* cytotoxicity evaluation of novel C2-aryl PBD dimers in Chronic Myeloid Leukaemia K_{562} Cell lines.

No:	C2-aryl PBD Dimer  R =	IC_{50} (nM)[a] of PBD Dimers	Calculated LogP[b] of Dimers	IC_{50} (nM)[c] of parent PBD monomers
227		0.28	3.97	2.5
229		0.53	4.60	0.28
230		7.47	5.32	79.5
231		44.0	6.16	115.1
232		25.7	7.01	47.8
ZC-423		0.013[d]	3.76	4.9

[a] = Dose that inhibits 50% of cell growth in K_{562} cell lines (96 h incubation, **see experimental section 5.6.2 for details of Assay**).
[b] = Calculated LogP of carbinolamine PBD species at physiological conditions (LogP calculated from Organic chemistry Portal; http://www.organic-chemistry.org/prog/peo/)
c = K_{562} human chronic myeloid luekemia (Synthesised by Antonow *et al.*, 96 h incubation, **see Experimental section 5.6.1 for details of Assay**).
[d] = K_{562} human chronic myeloid luekemia (Synthesised by Chen *et al.*, 96 h incubation, **see Experimental section 5.6.1 for details of Assay**).

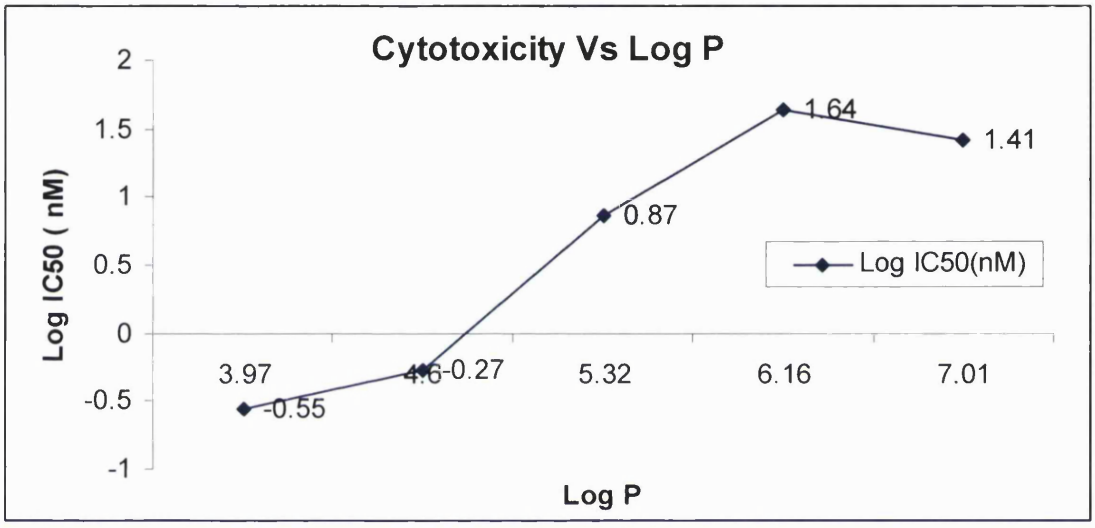
As was previously discussed the dramatic gain in biological activity of C2-aryl PBDs have been attributed to the C2-aryl fragment being able to project directly along the walls and floor of the minor groove in an optimal position to pick-up favorable van der Waals interactions within the floor and walls of the groove (hydrophobic interactions) as well as hydrogen bonding with base pairs and electrostatic contacts.

The cytotoxicity data clearly indicates that the five PBD dimers were highly potent exhibiting nano-molar and sub-nanomolar activity against the K_{562} cell line assay. Interestingly, the unsubstituted C2-aryl dimer **227** was the most potent exhibiting an IC_{50} value of 0.28 nM, closely followed by the *para*-methyl substituted compound **229** (IC_{50} = 0.38 nM). The more highly branched analogues were generally less cytotoxic with activity in the 10-30 nanomolar range.

These results appear to indicate that steric volume is an important factor in determining the cytotoxicity of the compounds. For example, the unsubstituted PBD dimer **227** is ~ 180 and 120 and more potent then the highly branched *iso*-propyl and *tert*-butyl substituted PBD dimers **231** (IC_{50} = 44.0 nM) and **232** (IC_{50} = 30.0 nM) respectively. This clearly shows that the proton and methyl groups appear to be well tolerated but the larger substituents are not. These results support the hypothesis that a good fit within the minor groove of DNA is critical for potent biological activity. A possible explanation for this observation could be attributed to steric clashes between the atomic van der Waals radii within the floor and walls of the groove as well as between base pairs. The reduced favourable van der Waals and hydrogen bonding interactions may not be enough to overcome the entropic cost of immobilizing the PBD within the groove upon binding, thus leading to an energetically unfavourable PBD-DNA adduct. This would result in poor DNA binding and reduced biological activity.

Another alternative explanation could be related to lipophilicity (or log P) of the C2-aryl groups. There is a clear correlation between the calculated LogP of the C2-aryl dimers and cytotoxicity (see below, **Graph 3.7a**), showing that as logP increases, significant loss in activity is observed. As the PBDs become more lipophilic (fatty) with the introduction of large hydrophobic alkyl groups it can be arrested in the lipid bilayer of the membrane, thus not being available for interaction with the DNA within the cell.

Interestingly, a C2-aryl PBD dimer which has a polar 4-methoxyphenyl substituent at the C2-position (**ZC-423**, Chen *et al.*, 2004 PhD Thesis) has been synthesised by Chen *et al.* and has been found to be extremely potent in K_{562} cell line exhibiting pico-molar potency (IC_{50} = 0.013 nM, **see table 3.7a**). It is highly hydrophilic then the C2-aryl PBD dimers **227-232** having a calculated LogP of 3.76, which clearly shows that having hydrophilic groups at the 4-position of the aryl fragment can enhance cytotoxicity of PBDs dimers.



Graph 3.7a: The correlation between cytotoxicity and calculated LogP within the series of C2-aryl PBD dimers **227-232**. (The graph is in a logarithmic scale and the IC_{50} values correspond to the K_{562} cell line on **table 3.7a**).

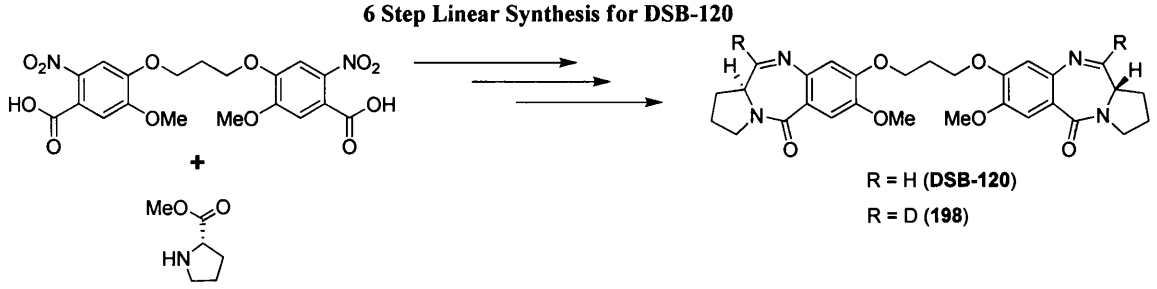
In addition, the IC_{50} values of the parent PBD monomers in K_{562} cell lines (See **table 3.7a**) were obtained (Antonow *et al.*, PhD thesis 2008) in order to attain a direct comparison of cytotoxicity of PBD dimers versus monomers. In general, all five PBD dimers displayed significant activity compared to their corresponding monomers. The unsubstituted C2-phenyl PBD dimer **227** and the substituted C2-ethyl PBD dimer **230** were both ~ 10 times more potent then their parent monomer, respectively. These results reinforce the concept of the importance of dimerization to enhance biological activity of PBDs.

4. CONCLUSION AND FUTURE WORK

4.1 Synthesis and Chemistry

The principal objective of the project was to synthesise novel PBD dimers with the introduction of various C2-aryl groups via a new, improved and concise methodology.

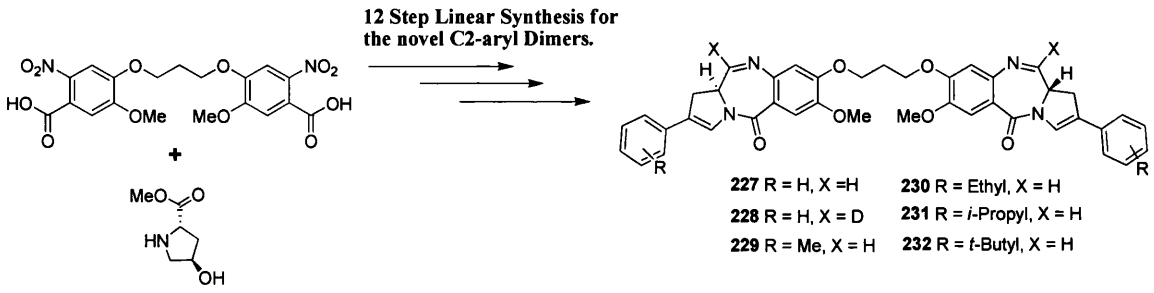
Initially, a model system was developed to investigate a synthetic strategy which incorporates tetralactam intermediates to synthesise novel PBD dimers. This investigation resulted in the successful synthesis of the known DNA interstrand cross-liking agent DSB-120 and its deuterated analogue **198** via a concise and potentially versatile synthetic route (**Scheme 4.1a**). This approach greatly reduced the number of synthetic steps of previously reported literature methods of DSB-120 (a total of 6 steps) as well as increasing the overall yields. For example, a widely used method developed by Thurston and co-workers involves the cyclisation of amino dithioacetals using mercuric chloride to provide DSB-120 in a ten step synthesis; six of which are used to synthesize the C-ring starting material from L-proline (Bose *et al.*, 1992a).



Scheme 4.1a: Synthesis of DSB-120 and its deuteriated analogue via a new and improved tetralactam approach developed from model studies.

In addition, the model system was used to optimize the final and critical reduction/deprotection step to generate the PBD imine from N10-SEM protected tetralactam intermediates. Initially, overreduction to the biologically inactive secondary amine occurred during the reaction, but after careful optimisation of the reaction conditions, a new and improved reduction method was subsequently developed.

This novel and concise tetralactam approach was then successfully applied to the synthesis of six novel C2-aryl PBD dimers. The key tetralactam intermediates were initially obtained from a reductive/cyclisation of bis-nitrobenzamide using Raney nickel and hydrazine. Introduction of the various C2-aryl fragments was achieved by utilizing palladium catalysed Suzuki cross-coupling reactions which were easy to perform, fast and high yielding. The new and improved reduction/deprotection step subsequently optimized during the investigation into the model studies to synthesize DSB-120, was successfully applied to the synthesis of the desired PBD imines (**227-232, Scheme 4.1b**). Again, this novel, concise and versatile tetralactam approach greatly reduced the number of synthetic steps of previously reported literature methods to obtain the target PBD imines. For example, the synthesis of C2-aryl PBD dimer **ZC-423** reported by Chen *et al.* involves using a twenty step linear convergent synthesis (Chen *et al.*, PhD Thesis 2004) and the synthesis of the C2-*exo* unsaturated PBD dimer SJG-136 reported by Gregson *et al.* involves a sixteen step convergent synthesis (S. J. Gregson *et al.*, 2001). Consequently, the concise and efficient tetralactam approach developed involves a twelve step linear synthesis which can be used to synthesize multi-gram quantities of various C2-aryl substituted PBD dimers.



Scheme 4.1b: Synthesis of novel C2-aryl PBD imine dimers (**227-232**) via a concise and versatile tetralactam approach.

Future work on the chemistry aspect of the project should focus on exploiting the synthetic methodology developed to prepare and design further PBD dimer analogues in conjunction with molecular modelling and information generated from SAR of existing compounds; in particular the SAR information gleaned from this research project which is explained further below in the biological evaluation section.

In addition, future work should also focus on exploiting the key tetralactam intermediate **184** via Vilsmeier-Haack Formylations and subsequent side chain elaboration through Wittig Olefination chemistry to generate novel C2-C3 *endo/exo* unsaturated PBD dimers not observed in nature. In particular, the work should concentrate on either developing new synthetic conditions to prevent racemisation during the Vilsmeier-Haack reaction or investigation into new synthetic methodologies to install the C2-formyl group.

4.2 Biological evaluation

All five PBD dimers were found to be highly potent exhibiting nano-molar and sub-nanomolar activity in the Human Chronic Myeloid Leukaemia K_{562} cell line assay. Interestingly, the unsubstituted C2-aryl dimer **227** was the most potent exhibiting an IC_{50} value of 0.28 nM, closely followed by the *para*-methyl substituted compound **229** (IC_{50} = 0.38 nM).

The results clearly indicate that steric volume is an important factor in determining the cytotoxicity of the compounds. The origin of this effect may reflect steric clashes between atomic van der Waals radii within the floor and walls of the minor groove. These results also support the hypothesis that a good fit within the minor groove of DNA is critical for potent biological activity. Therefore, future work in synthesising novel C2-aryl analogues should focus on susbstituents which have less steric volume in the C2-aryl fragment of PBDs. In addition, the most potent C2-aryl compounds in this series had calculated logP values below 5 (which obey one of the rules of Lipinski drug likeness criteria; i.e. logP less than 5). This reinforces the idea that large hydrophobic groups should be avoided in the future design of new PBD analogues, and that hydrophilic substituents should be installed at the C2-aryl position. Also, it is quite clear that calculated physical-chemical parameters can be used in QSAR studies to assist the synthesis and design of new PBD analogues. Additionally, it would be extremely interesting to have molecular modelling performed on the novel C2-aryl dimers to determine the exact orientation and interaction of the C2-aryl fragment within the minor groove and to confirm if there are indeed any steric clashes.

Moreover, the most potent analogue **229** from the C2-aryl series should be selected for further *in vivo* evaluation for its antitumour activity as it would be interesting to determine whether increased *in vitro* cytotoxicity translates into an improved antitumour agent.

Unfortunately, at the time of writing the thesis DNA cross-linking studies of the five novel PBD dimers performed by Prof. John Hartley group at UCL were not available to be added to the thesis due to delays in obtaining the results stemming from problems with contamination with radiolabelled DNA plasmids. It is anticipated that the results will be available in due course and will be presented at the day of the viva examination. In addition, It is quite clear that gel electrophoresis based methods of evaluating the DNA interstrand cross-linking ability used by Prof. Hartley at UCL have their limitations, notably low high throughput screening and the use of complex and long radiolabelled duplex DNA fragments. Therefore to overcome these limitations, Thurston and co-workers have developed a rapid high throughput method based upon ion pair reverse phase high performance liquid chromatography (HPLC) and spectroscopy technique (Narayanaswamy *et al.*, 2008) which can be used as an alternative for future work to evaluate the novel PBD dimers for their DNA interstrand cross-linking efficiency.

5 EXPERIMENTAL SECTION

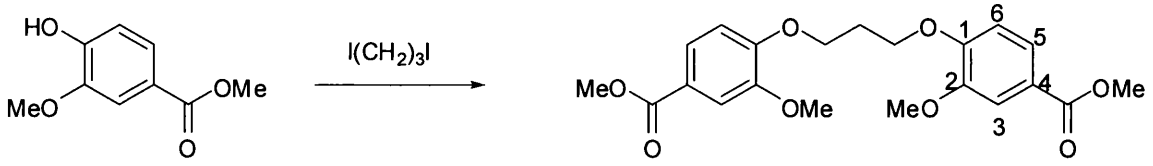
5.1 General Synthetic Methods

Optical rotations were measured on an ADP 220 polarimeter (Bellingham Stanley Ltd.) and concentrations (*c*) are given in g/100mL. IR spectra were recorded on a Perkin-Elmer Spectrum 1000 FT IR Spectrometer. ^{1}H and ^{13}C NMR spectra were acquired at 300 K using a Bruker Avance NMR spectrometer at 400 and 100 MHz, respectively. Chemical shifts are reported relative to TMS (δ = 0.0 ppm), and signals are designated as s (singlet), d (doublet), t (triplet), dt (double triplet), dd (doublet of doublets), ddd (double doublet of doublets) or m (multiplet), with coupling constants given in Hertz (Hz). A pro-PBD numbering system is used for carbon and proton assignments for synthetic intermediates (*i.e.*, based on the final tricyclic PBD ring system). Mass spectroscopy data were collected using a Waters Micromass ZQ instrument coupled to a Waters 2695 HPLC with a Waters 2996 PDA. Waters Micromass ZQ parameters used were: Capillary (kV), 3.38; Cone (V), 35; Extractor (V), 3.0; Source temperature (°C), 100; Desolvation Temperature (°C), 200; Cone flow rate (L/h), 50; De-solvation flow rate (L/h), 250. High-resolution mass spectroscopy data were recorded on a Waters Micromass QTOF Global in positive W-mode using metal-coated borosilicate glass tips to introduce the samples into the instrument. HPLC was performed using a Waters 2695 separator module and a waters OD column (25 cm x 4.6 mm) coupled to a Waters 2996 photodiode array detector. A mobile phase of hexane/*i*-PrOH (1:1 v/v) was used at a flow rate of 0.6 mL/min. Thin Layer Chromatography (TLC) was performed on silica gel aluminium plates (Merck 60, F_{254}), and flash chromatography utilised silica gel (Merck 60, 230-400 mesh ASTM). All other chemicals and solvents were purchased from Sigma-Aldrich or Fsiher chemicals.

5.2 A Novel and Concise Synthesis of DSB-120- Via Tetralactam Hydride Reduction and N10-SEM Deprotection

5.2.1 Attempted Synthesis of DSB-120 via Isatoic Anhydride Approach.

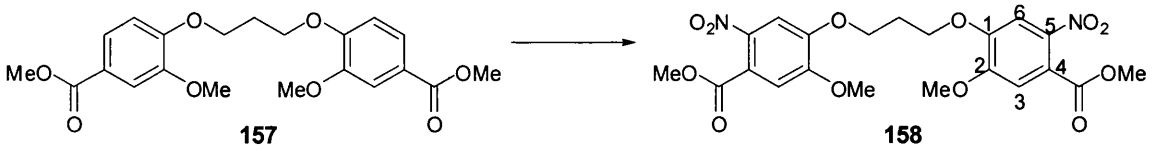
Synthesis of 1',3'-Bis[(4-methylester)-2-methoxyphenoxy]propane (157).



195 **157**

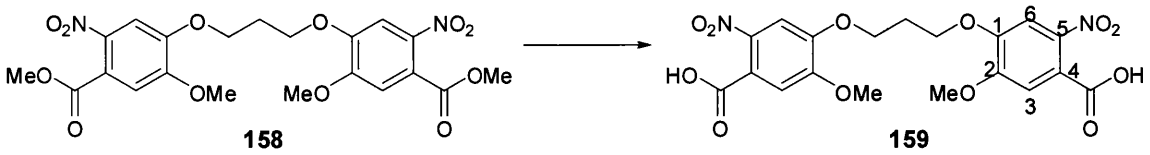
Methyl vanillate (20 g, 109.8 mmol; 1.0 equiv.) and triphenyl phosphine (43.2 g, 165 mmol; 1.5 equiv.) were dissolved in dry THF (400 mL) at 0 ^{0}C under a nitrogen atmosphere with mechanical stirring. Diethylazodicarboxylate (21.03 g or 22 mL, 120.8 mmol; 1.1 equiv.) was then added drop wise (20 min) and allowed to stir at 0 ^{0}C for 1 h at which time a solution of 1,3-propanediol (4.01 g or 3.81 mL, 53 mmol; 0.48 equiv.) in dry THF (5 mL) was added drop wise. The reaction mixture was allowed to stir at room temperature for additional 72 h after which time LC-MS revealed the reaction had gone to completion. The product was collected by vacuum filtration washed with THF and then air dried to constant weight (11.72 g). In addition, the filtrate was treated with methanol (600 mL) to give a second crop of product, which was collected by filtration (2.0g) and combined to afford the product (**157**) as a white solid (13.72 g, 64%); LC/MS 5.82 min (ES+) m/z (relative intensity) 405 ($[M + H]^{+\cdot}$, 92) 427 ($[M + Na]^{+\cdot}$, 45); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.64 (dd, 2 H, J = 8.40, 1.96 Hz, H2), 7.54 (d, 2 H, J = 1.89 Hz, H6), 6.93 (d, 2 H, J = 8.46, Hz, H3), 4.29 (t, 4 H, J = 6.12 Hz, Linker-O$\underline{CH_2}$), 3.96 (s, 6 H, CH_3O x 2), 3.89 (s, 6 H, CH_3CO_2 x 2), 2.40 (t, 2 H, J = 6.13 Hz, Linker-$\underline{CH_2}$); (Thurston *et al.*, *J.Org.Chem.* Vol. 61, No. 23, 1996); [Lit ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.64 (dd, 2 H, J = 8.59 Hz, H2), 7.54 (d, 2 H, J = 2.0 Hz, H6), 6.93 (d, 2 H, J = 8.50 Hz, H3), 4.30 (t, 4 H, J = 6.10 Hz, Linker-O$\underline{CH_2}$), 3.90 (s, 6 H, CH_3O x 2), 3.89 (s, 6 H, CH_3CO_2 x 2), 2.39-2.43 (m, 2 H, Linker-$\underline{CH_2}$).

Synthesis of 1',3'-Bis[(4-methylester)-2-methoxy-5-nitrophenoxy]propane (158).



Solid copper (II) nitrate trihydrate (20.50 g, 85 mmol; 2.5 equiv.) was added in portions to a suspension of the bis-ester **157** (13.72 g, 34 mmol; 1.0 equiv.) in acetic anhydride at 0 ^{0}C and was allowed to stir at this temperature for 1 h. After the reaction had reached room temperature it was poured into ice/water (600 mL) and allowed to stir for 20 h after which time a yellow precipitate had formed which was collected by vacuum filtration and dried to constant weight to afford the product **158** as a yellow solid (27.67 g, 90%); LC/MS 5.63 min (ES+) m/z (relative intensity) 495 ($[M + H]^{+\cdot}$, 92) 517 ($[M + Na]^{+\cdot}$, 45); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.52 (s, 2 H, H6), 7.08 (s, 2 H, H3), 4.29 (d, 4 H, J = 6.10 Hz, Linker-O$\underline{CH_2}$), 3.96 (s, 6 H, CH_3O x 2), 3.89 (s, 6 H, CH_3CO_2 x 2), 2.40 (t, 2 H, J = 6.13 Hz, Linker-$\underline{CH_2}$); (Thurston *et al.*, *J.Org.Chem.* Vol. 61, No. 23, 1996); [Lit ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.49 (s, 2 H, H6), 7.06 (s, 2 H, H3), 4.29 (t, 4 H, J = 6.0 Hz, Linker-O$\underline{CH_2}$), 3.95 (s, 6 H, CH_3O x 2), 3.90 (s, 6 H, CH_3CO_2 x 2), 2.43 (m, 2 H, Linker-$\underline{CH_2}$).

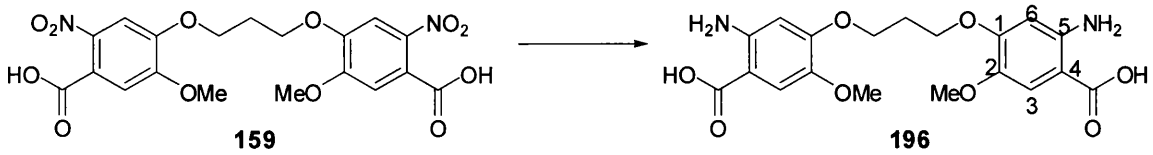
Synthesis of 1',3'-Bis[(4-carboxylic acid)-2-methoxy-5-nitrophenoxy]propane (159).



158 **159**

A suspension of the bis-nitro ester compound **158** (27.67 g, 56 mmol; 1.0 equiv.) in 1 M aqueous sodium hydroxide solution (400 mL) and THF (400 mL) was allowed to stir for 72 h at room temperature. The THF was removed by evaporation in *vacuo* and the remaining aqueous solution was adjusted to pH 1 with Conc.HCl. The resulting precipitate was collected by vacuum filtration and dried to afford the bis-nitro acid (**159**) as a yellow solid (14.40 g, 55%); LC/MS 5.22 min (ES+) m/z (relative intensity) 465 ($[M + H]^{+\cdot}$, 92) 488 ($[M + Na]^{+\cdot}$, 45); ^{1}H-NMR (d_6-DMSO, 400 MHz): δ 13.59 (bs, 2 H, CO_2H), 7.63 (s, 2 H, H6), 7.30 (s, 2 H, H3), 4.29 (t, 4 H, J = 6.08 Hz, Linker-O<u>CH_2</u>), 3.91 (s, 6 H, CH_3O x 2), 2.26 (t, 2 H, J = 6.07 Hz, Linker-<u>CH_2</u>); (Thurston *et al.*, *J.Org.Chem.* Vol. 61, No. 23, 1996); [Lit ^{1}H-NMR (d_6-DMSO, 400 MHz): δ 13.60 (bs, 2 H, CO_2H), 7.62 (s, 2 H, H6), 7.29 (s, 2 H, H3), 4.27 (t, 4 H, J = 5.9 Hz, Linker-O<u>CH_2</u>), 3.90 (s, 6 H, CH_3O x 2), 2.25 (t, 4 H, J = 5.9 Hz, Linker-<u>CH_2</u>).

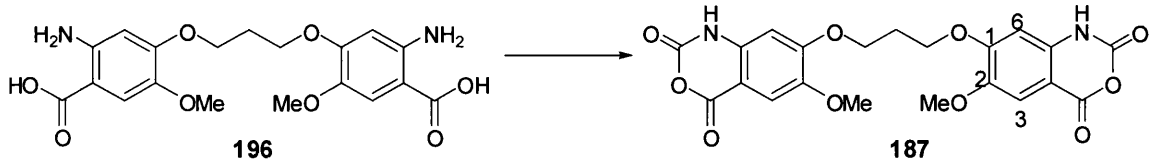
Synthesis of 1',3'-Bis[(4-carboxylic acid)-2-methoxy-5-aminophenoxy]propane (196).



159 **196**

The bis-nitro acid **159** (3.2 g, 6.86 mmol; 1.0 equiv.) was added in one portion to a suspension of 10% palladium on charcoal (0.96 g, 30% by wt) in ethanol (60 mL). Ammonium formate (4.32 g, 68.7 mmol; 10.0 equiv.) was then added in one portion to the mixture and was allowed to stir at room temperature for 1.5 h after which time LC-MS revealed the reaction had gone to completion. The reaction mixture was filtered through celite and evaporated in *vacuo* to afford **196** as a brown solid (3.42 g, 81.6%); LC/MS 5.34 min (ES+) m/z (relative intensity) 405 ([M + H]$^{+\cdot}$, 72) 427 ([M + Na]$^{+\cdot}$, 86); ^{1}H-NMR (d_6-DMSO, 400 MHz): δ 13.59 (bs, 2 H, CO_2H), 7.18 (s, 2 H, H6), 6.73 (s, 2 H, H3), 4.09 (t, 4 H, J = 6.14 Hz, Linker-O$\underline{CH_2}$), 3.65 (s, 6 H, CH_3O x 2), 2.23 (t, 2 H, J = 6.09 Hz, Linker-$\underline{CH_2}$).

Synthesis of 1',3'-Bis[(2-methoxyisatoicanhydride)]propane (187).

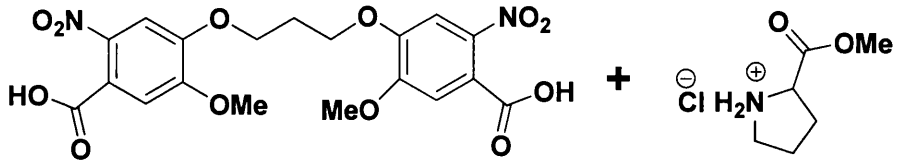


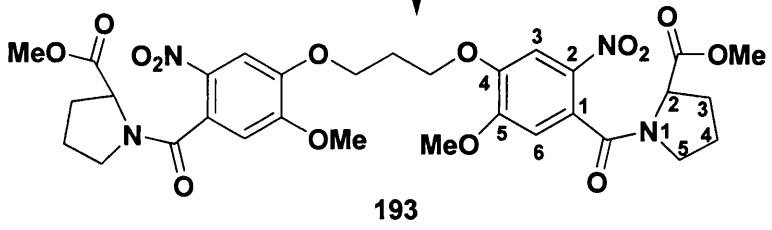
196 **187**

Triphosgene (3.50 g, 11.80 mmol; 1.4 equiv.) was added in one portion to a solution of bis-amine **196** (3.42 g, 8.42 mmol; 1.0 equiv.) in THF (80 mL) and heated at reflux for 3 h. After being cooled to room temperature, the solution was poured into ice/water and the resulting brown precipitate was collected by vacuum filtration and dried to constant weight to afford the bis-isatoic anhydride **187** as a brown solid (3.0g, 78%); LC/MS 6.89 min (ES+) m/z (relative intensity) 457 ([M + H]$^{+\cdot}$, 92) 479 ([M + Na]$^{+\cdot}$, 65); ^{1}H-NMR (d_6-DMSO, 400 MHz): δ 11.45 (bs, 2 H, NH), 7.28 (s, 2 H, H6), 6.80 (s, 2 H, H3), 4.24 (t, 4 H, J = 6.08 Hz, Linker-O$\underline{CH_2}$), 3.85 (s, 6 H, CH_3O x 2), 2.34 (t, 2 H, J = 6.22 Hz, Linker-$\underline{CH_2}$).

5.2.2 Successful Synthesis of DSB-120 and its Deuteriated Analogue 198- via Reductive/Cyclisation Approach

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(2*S*)-(5-methoxy-2-nitro-1,4-phenylene)carbonyl]]bis[pyrrolidine-2-carboxylic acid methyl ester]] (193).

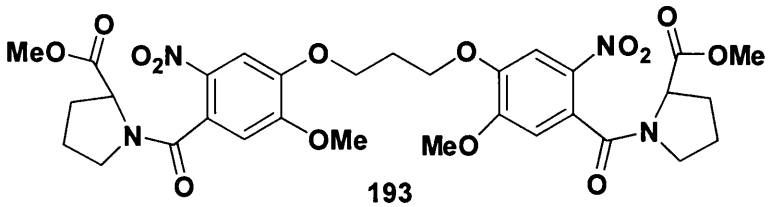




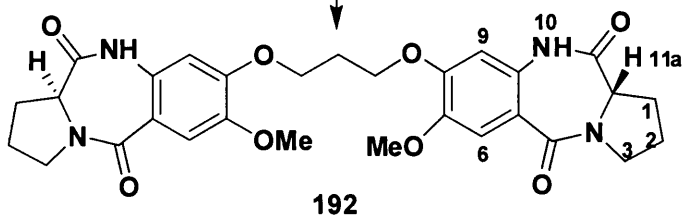
Oxalyl chloride (12.26 g or 8.4 mL, 96.57 mmol; 3.0 equiv.) and DMF (1 mL) were added slowly to a suspension of bis-nitro acid **159** (15.0 g, 32.19 mmol; 1.0 equiv.) in dry DCM (150 mL). The mixture was allowed to stir at room temperature for 20 h. DCM and excess oxalyl chloride was removed under vacuum until viscous yellow oil was obtained. The oil was triturated with diethyl ether resulting in a yellow precipitate which was then filtered and dried in vacuum oven for 1 h. The yellow solid was then added portion wise over period of 0.25 h to a solution of 2*S*-4-hydroxy-2-proline methyl ester **194** (12.0 g, 72.43 mmol; 2.25 equiv.) and Et_3N (22.4 mL, 160.95 mmol; 5.0 equiv.) in dry DCM (150 mL) at – 40 °C. LC/MS confirmed the reaction had gone to completion and the mixture was diluted with DCM (150 mL) and washed with saturated $NaHCO_3$ (1 × 300 mL), 1M HCl (1 × 300 mL), brine (1 × 300 mL) and evaporated *in vacuo* to afford the product (**193**) as a yellow foam (21.35 g, 97%); LC/MS 5.82 min (ES+) m/z (relative intensity) 689 ($[M + H]^+$, 100) 711 ($[M + Na]^+$, 35); $[\alpha]_D^{20}$ = -83° (*c* = 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.71 (s, 2 H, H3 aromatic), 6.84 (s, 2 H, H6 aromatic), 4.72 (dd, 2 H, *J* = 8.63, 4.41 Hz, H2), 4.34-4.29 (m, 4 H, Linker-O<u>CH$_2$</u>), 3.96 (s, 6

H, CH_3O x 2), 3.79 (s, 6 H, CO_2Me x 2), 3.35-3.28 (m, 2 H, H5), 3.21-3.13 (m, 2 H, H5), 2.46-2.38 (m, 2 H, Linker-$\underline{CH_2}$), 2.33 (dd, 2 H, J = 12.58, 7.74 Hz, H3), 2.11-2.03 (m, 2 H H3), 2.01-1.87 (m, 4 H H4); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 109.6 (C6 aromatic), 108.5 (C3 aromatic), 65.7 (Linker-O$\underline{CH_2}$), 60.6 (C2), 56.7 (CH_3O x 2), 52.3 (CO_2Me x 2), 48.3 (C5), 29.2 (C3), 28.6 (Linker-$\underline{CH_2}$), 23.0 (C4); IR (Golden gate): 2953, 2359, 1739 (C=O), 1640 (C=O), 1519, 1420, 1332, 1274, 1174, 1062, 870, 731 cm^{-1}; MS (ESI) *m/z* (relative intensity): 689 ([M + H]$^{+}$, 100%), 711 ([M + 23]$^{+}$, 40%), 785 (25), 372 (45), 364 (28); HRMS: [M + H]$^{+}$ Theoretical, $C_{31}H_{36}O_{14}N_4$ m/z 689.2301, found (ES^+) m/z 689.2325.

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (192).



193

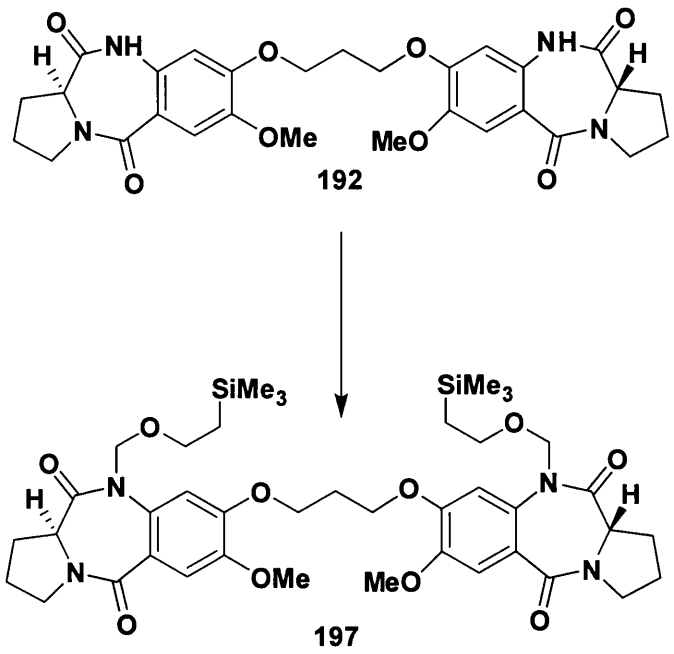


192

Palladium on charcoal catalyst (50 mg, 10% w/w) was added as a slurry in EtOAc (10 mL) (**CAUTION: pyrophoric**) to a solution of bis-nitro benzamide **193** (0.5 g, 0.73 mmol; 1.0 equiv.) in ethanol/EtOAc (1:1, 60 mL). The reaction mixture was agitated under an atmosphere of hydrogen (50 psi) in a Parr apparatus overnight after which time LC/MS showed the complete consumption of starting material. The mixture was filtered through celite, and the filter pad was washed with ethanol (3 x 10 mL). The solvent was removed under reduced pressure and the resulting oil was diluted with THF (0.8 mL) and water (5.6 mL) containing 0.02 mL of concentrated HCl. The solution was allowed to stir for 2.5 h at room temperature after which point LC/MS revealed the reaction had gone to completion. The product precipitated out of solution and was collected by vacuum filtration and dried to furnish **192** as a brown solid (0.36 g, 90%); LC/MS 4.88 min (ES+) m/z (relative intensity) 565 ($[M + H]^{+\cdot}$, 100) 588 ($[M + Na]^{+\cdot}$, 20); $[\alpha]_D^{23}$ = +397° (*c* = 0.5, DMSO); ^{1}H-NMR (d_6-DMSO, 400 MHz): δ 10.19 (s, 2 H, NH10), 7.26 (s, 2 H, H6), 6.74 (s, 2 H, H9), 4.34-4.29 (m, 4 H, Linker-O$\underline{CH_2}$), 4.06 (dd, 2 H, *J* = 7.94, 1.84 Hz, H11a), 3.79 (s, 6 H, CH_3O x 2), 3.60-3.52 (m, 2 H, H3), 3.51-3.44 (m, 2 H, H3), 2.50-2.47 (m, 2 H H1α), 2.27 (t, 2 H, *J* = 6.04 Hz, Linker-$\underline{CH_2}$), 1.99-1.86 (m, 4 H, H1β and H2), 1.85-1.75 (m, 2 H, H2); ^{13}C-NMR (d_6-DMSO, 100 MHz): δ 112.0 (C6 aromatic), 105.3 (C3 aromatic), 64.9 (Linker-O$\underline{CH_2}$), 56.3 (C11a), 55.6 (CH_3O x 2), 46.8 (C3), 28.6 (Linker-$\underline{CH_2}$), 25.6 (C1), 23.0 (C2); IR (Golden gate): 2953, 1684 (C=O), 1599 (C=O),

1493, 1447, 1369, 1263, 1227, 1119, 1026, 876, 757, 636 cm^{-1}; MS (ESI) *m/z* (relative intensity): 565 ($[M + H]^{+}$, 10%), 785 (100), 665 (8), 537 (20), 463 (12); HRMS: $[M + H]^{+}$ Theoretical, $C_{29}H_{32}O_8N_4$ m/z 565.2293, found (ES^{+}) m/z 565.2323.

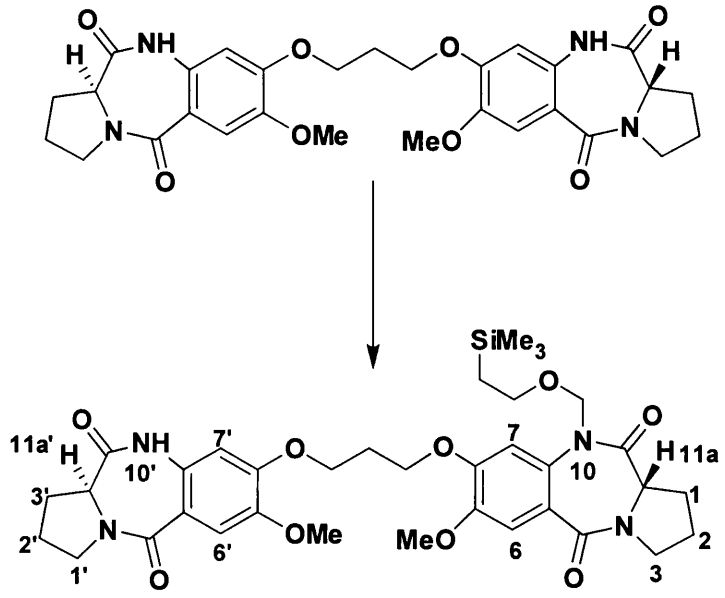
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-10-((2-(trimethylsilyl)ethoxy)methyl)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (197).



A solution of *n*-BuLi (10.3 mL of a 1.6 M solution in hexane, 16.55 mmol; 2.6 equiv.) was added dropwise to a stirred suspension of the bis-tetralactam (**192**) (3.59 g, 6.37 mmol; 1.0 equiv.) in anhydrous THF (50 mL) at – 30 °C under nitrogen atmosphere. The reaction mixture was allowed to stir for 2 h at which point a solution of the SEM-Cl (3.0 mL, 16.55 mmol; 2.6 equiv.) in dry THF (10 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature and left stirring for 20 h under nitrogen atmosphere after which point LC/MS revealed incomplete consumption of starting material (with traces amount of mono-N10-SEM protected bis-tetralactam). The reaction mixture was allowed to stir for a further 20 h where upon LC/MS still revealed the presence of bis-tetralactam (**192**) which was collected by vacuum filtration (1.7 g). The filtrate was removed by evaporation *in vacou* and the resulting residue was dissolved in EtOAc (100 mL) and washed with water (30 mL), brine (30 mL) dried

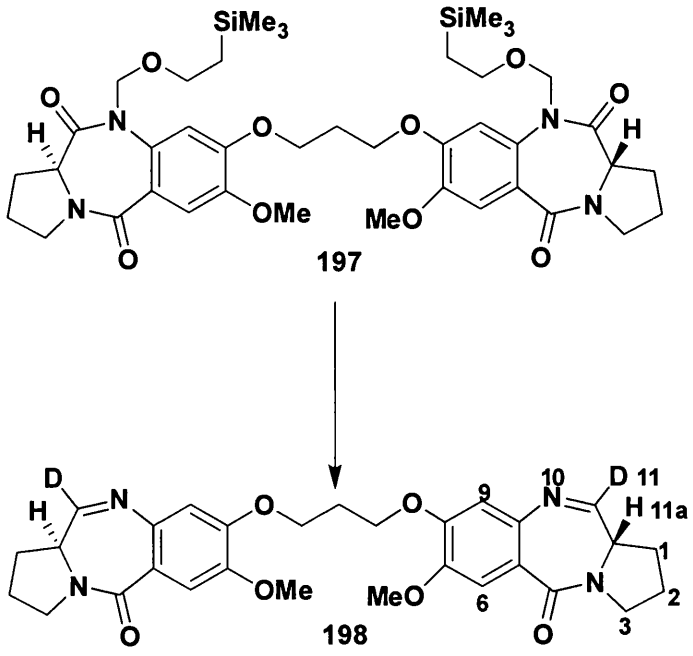
($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude product. Purification by flash chromatography (gradient elution: $CHCl_3$ to 99:1 v/v $CHCl_3$/MeOH) gave the pure bis-N10-SEM tetralactam (trace amounts of mono-N10-SEM protected bis-tetralactam was also isolated for NMR analysis) (**197**) as a yellow foam (2.5 g, 50%, (95% yield based upon recovered starting material 192)); LC/MS 7.85 min (ES+) m/z (relative intensity) 825 ($[M + H]^{+\cdot}$, 80), 848 ($[M + Na]^{+\cdot}$, 15); $[\alpha]_D^{24}$ = +218° (*c* = 0.5, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.34 (s, 2 H, H6), 7.21 (s, 2 H, H9), 5.46 (d, 2 H, *J* = 10.0 Hz, SEM-N$\underline{CH_2}$O), 4.69 (d, 2 H, *J* = 10.0 Hz, SEM-N$\underline{CH_2}$O), 4.27-4.21 (m, 4 H Linker-O$\underline{CH_2}$), 4.19 (dd, 2 H, *J* = 7.95, 2.25 Hz, H11a), 3.87 (s, 6 H, CH_3O x 2), 3.79-3.61 (m, 6 H, SEM-O$\underline{CH_2}$ and H3β), 3.59-3.50 (m, 2 H, H3α), 2.74-2.67 (m, 2 H, Hz, H1α), 2.39 (t, 2 H, *J* = 5.96 Hz Linker-$\underline{CH_2}$), 2.12-2.03 (m, 2 H, H2), 2.03-1.93 (m, 4 H, H1β and H2), 0.98-0.93 (m, 4 H, SEM-$\underline{CH_2}$Si$(CH_3)_3$), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 112.9 (C6), 108.3 (C9), 79.2 (SEM-N$\underline{CH_2}$O), 68.3 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 58.9 (C11a), 57.4 (CH_3O x 2), 48.0 (C3), 30.2 (Linker-$\underline{CH_2}$), 27.9 (C1), 25.1 (C2), 19.6 (SEM-$\underline{CH_2}$Si$(CH_3)_3$), 0.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2949, 1684 (C=O), 1635 (C=O), 1515, 1453, 1433, 1359, 1240, 1227, 1196, 1060, 1025, 856, 833, 750, 698 cm^{-1}; MS (ESI) *m/z* (relative intensity): 825 ($[M + H]^{+}$, 10%), 852 (20), 785 (10), 679 (15), 471 (8); HRMS: $[M + H]^{+}$ Theoretical, $C_{41}H_{60}O_{10}N_4Si_2$ m/z 825.3921, found (ES^+) m/z 825.3930

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]-(11a*S*/11'a*S*)-7,7'-methoxy-10-((2-(trimethylsilyl)ethoxy)methyl)bis[1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (Mono-Substituted N10-SEM tetralactam).



Trace amounts of mono-N10-SEM protected tetralactam was Isolated for NMR analysis; LC/MS 3.12 min (ES+) m/z (relative intensity) 695 ($[M + H]^{+\cdot}$, 60), 718 ($[M + Na]^{+\cdot}$, 10); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.66 (s, 1 H, NH10), 7.39 (s, 1 H, H6), 7.32 (s, 1 H, H6'), 7.19 (s, 1 H, H9), 6.59 (s, 1 H, H9'), 5.45 (d, 1 H, J = 10.0 Hz, SEM-N$\underline{CH_2}$O), 4.66 (d, 1 H, J = 10.0 Hz, SEM-N$\underline{CH_2}$O), 4.24-4.16 (m, 4 H Linker-O$\underline{CH_2}$), 4.15 (dd, 1 H, J = 7.71, 2.13 Hz, H11a), 3.98 (m, 1 H, H11'a), 3.84 (s, 6 H, CH_3O x 2), 3.77-3.59 (m, 4 H, SEM-O$\underline{CH_2}$ and H3β and H3'β), 3.58-3.47 (m, 2 H, H3α and H3'α), 2.72-2.63 (m, 2 H, Hz, H1α and H1'α), 2.35 (t, 2 H, J = 5.89 Hz Linker-$\underline{CH_2}$), 2.10-2.01 (m, 2 H, H2), 2.02-1.90(m, 4 H, H1β and H2), 0.97-0.89 (m, 2 H, SEM-$\underline{CH_2}$Si(CH_3)$_3$), -0.006 (s, 9 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 113.8 (C6'), 112.9 (C6), 108.4 (C9), 106.7 (C9'), 79.2 (SEM-N$\underline{CH_2}$O), 68.3 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 58.9 (C11a), 58.2 (C11a'), 57.5 (CH_3O x 2), 48.6 (C3'), 48.0 (C3), 30.2 (Linker-$\underline{CH_2}$), 27.9 (C1), 27.5 (C1'), 25.1 (C2), 24.9 (C2'), 19.6 (SEM-$\underline{CH_2}$Si(CH_3)$_3$), 0.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2950, 1688 (C=O), 1605 (C=O), 1515, 1454, 1433, 1360, 1245, 1186, 1196, 1058, 1024, 919, 857, 834, 726, 663 cm^{-1}; MS (ESI) *m/z* (relative intensity): 695 ($[M + H]^+$, 100%), 785 (20), 722 (25), 577 (12), 480 (8); HRMS: $[M + H]^+$ Theoretical, $C_{35}H_{46}O_9N_4Si_2$ m/z 695.3107, found (ES^+) m/z 695.3132.

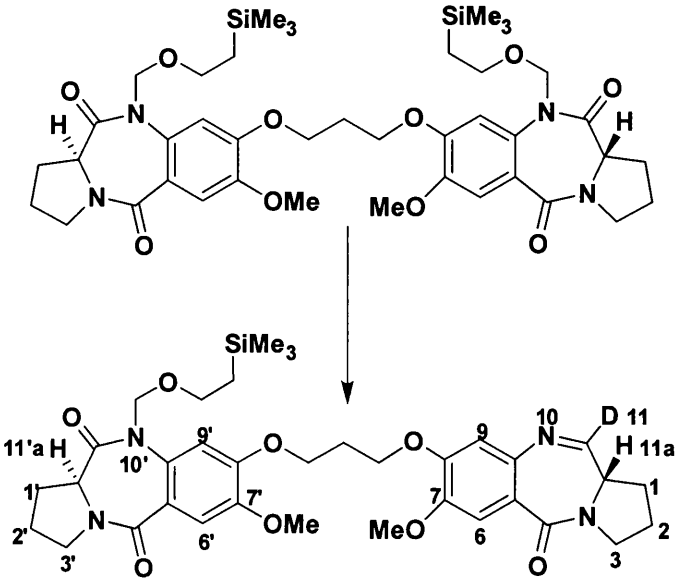
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-dione]] (198).



$NaBD_4$ (0.25 g, 6.07 mmol; 20.0 equiv.) was added to a stirred solution of the bis-N10-SEM tetralactam **197** (0.25 g, 0.303 mmol; 1.0 equiv.) in THF (12 mL) and ethanol (6 mL) and was allowed to stir for 8 h at room temperature under nitrogen atmosphere. The solvent was removed by evaporation in *vacuo* and the resulting residue was treated with water (70 mL) and was extracted with DCM (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL) and evaporated *in vacuo* to afford the SEM-carbinolamine intermediate. This was immediately dissolved in ethanol (25 mL) and water (12 mL) and treated with flash silica gel (~ 3 g). The reaction was allowed to stir at room temperature for 24 h after which time the formation of a large quantity of product was observed by TLC (10% MeOH/$CHCl_3$). The reaction mixture was filtered through a sinter funnel and rinsed a couple of times with EtOAc (2 x 40 mL). The organic filtrate layer was separated and the aqueous phase diluted with water (70 mL) and washed with EtOAc (3 x 35 mL). The combined organic layers were washed with water (25 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by flash chromatography (gradient elution: 100% $CHCl_3$ to 96:4 v/v $CHCl_3$/MeOH) afforded the pure bis-H-11 deuteriated imine (**198**) as an yellow foam (35 mg, 20%) as well as small amounts of mono-N10-SEM protected H-11

deuteriated imine; LC/MS 4.70 min (ES+) m/z (relative intensity) 535 ($[M + H]^{+\cdot}$, 100), (ES-) m/z (relative intensity) 533 ($[M - H]^{+\cdot}$, 90); $[\alpha]_D^{22} = +830°$ (*c* = 0.25, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.49 (s, 2 H, H6), 6.83 (s, 2 H, H9), 4.22-4.31 (m, 4 H Linker-O$\underline{CH_2}$), 3.90 (s, 6 H, CH_3O x 2), 3.79 (ddd, 2 H, *J* = 11.94, 7.06, 4.35 Hz, H3β), 3.68 (dd, 2 H, *J* = 6.98, 4.20 Hz, H11a), 3.56 (ddd, 2 H, *J* = 11.92, 7.10, 4.30 Hz, H3α), 2.39 (t, 2 H, *J* = 6.11 Hz, Linker-$\underline{CH_2}$), 2.32-2.26 (m, 4 H, H1α/β), 2.07-1.99 (m, 4 H, H2α/β); ^{13}C-NMR ($CDCl_3$, 100 MHz); δ 111.7 (C6), 110.8 (C9), 65.4 (Linker-O$\underline{CH_2}$), 56.1 (CH_3O x 2), 53.7 (C11a), 46.7 (C3), 29.6 (C1), 28.8 (Linker-$\underline{CH_2}$), 24.2 (C2); IR (Golden gate): 2946, 2198, 1597 (C=O), 1503 (C=O), 1428, 1259, 1217, 1026, 873, 755, 663 cm^{-1}; MS (ESI) *m/z* (relative intensity): 535 ($[M + H]^{+\cdot}$, 100%), 785 (50), 701 (8), 571 (12), 445 (10), 273 (7); HRMS: $[M + H]^{+\cdot}$ Theoretical, $C_{29}H_{30}D_2O_6N_4$ m/z 535.2520, found (ES^+) m/z 535.2500.

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]-(11a*S*/11'a*S*)-7,7'-methoxy-10'-((2-(trimethylsilyl)ethoxy)methyl)-(1',2',3',10',11',11'a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5',11'-dione)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-one]] (Mono-Substituted N10-SEM H-11 deuteriated imine).

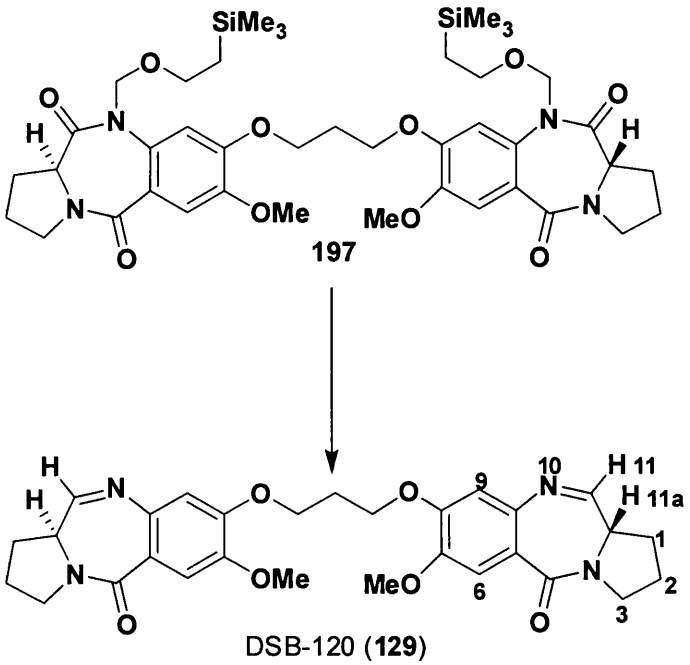


D = ^{2}H Deuteriated

Trace amounts of Mono-N10-SEM H-11 deuteriated imine was isolated for ^{1}H NMR analysis; LC/MS 6.17 min (ES+) m/z (relative intensity) 680 ($[M + H]^{+\cdot}$, 45), 702 ($[M + Na]^{+\cdot}$, 25); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.49 (s, 1 H, H6), 7.32 (s, 1 H, H6'), 7.21 (s, 1 H, H9'), 6.83 (s, 1 H, H9), 5.46 (d, 1 H, J = 9.98 Hz, SEM-N$\underline{CH_2}$O), 4.67 (d, 1 H, J = 9.99 Hz, SEM-N$\underline{CH_2}$O), 4.22-4.31 (m, 4 H Linker-O$\underline{CH_2}$), 4.10 (dd, 1 H, J = 7.88, 2.19 Hz, H11'a), 3.90 (s, 3 H, 7-CH_3O), 3.86 (s, 3 H, 7'-CH_3O), 3.84-3.75 (m, 2 H, H3β and H3'β), 3.74-3.58 (m, 3 H, SEM-O$\underline{CH_2}$ and H11a), 3.57-3.49 (m, 2 H, H3α and H3'α), 2.73-2.66 (m, 1 H, Hz, H1'α), 2.39 (t, 2 H, J = 6.08 Hz, Linker-$\underline{CH_2}$), 2.32-2.25 (m, 2 H, H1α/β), 2.12-1.92 (m, 5 H, H2, H2'α/β and H1'β), 0.95-0.91 (m, 2 H, SEM-$\underline{CH_2}$Si$(CH_3)_3$), 0.00 (s, 9 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz); δ 113.1 (C6'), 112.9 (C6), 112.2 (C9), 108.4 (C9'), 79.3 (SEM-N$\underline{CH_2}$O), 68.4 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 58.9 (C11'a), 57.5 (7-CH_3O/7'- CH_3O), 55.0 (C11a), 48.6 (C3/C3'), 31.0 (Linker-$\underline{CH_2}$), 30.0 (C1), 28.1 (C1'), 25.1 (C2'), 23.6 (C2), 19.7 (SEM-$\underline{CH_2}$Si$(CH_3)_3$), 0.0 (SEM-Si($\underline{CH_3}$)$_3$); MS (ESI) *m/z* (relative intensity): 680 ([*M* +

$H]^+$, 100%), 785 (80), 716 (12), 562 (25), 463 (10), 241 (8); HRMS: $[M + H]^+$ Theoretical, $C_{35}H_{45}DO_8N_4Si$ m/z 680.3220, found (ES^+) m/z 680.3232.

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-dione]] (129).

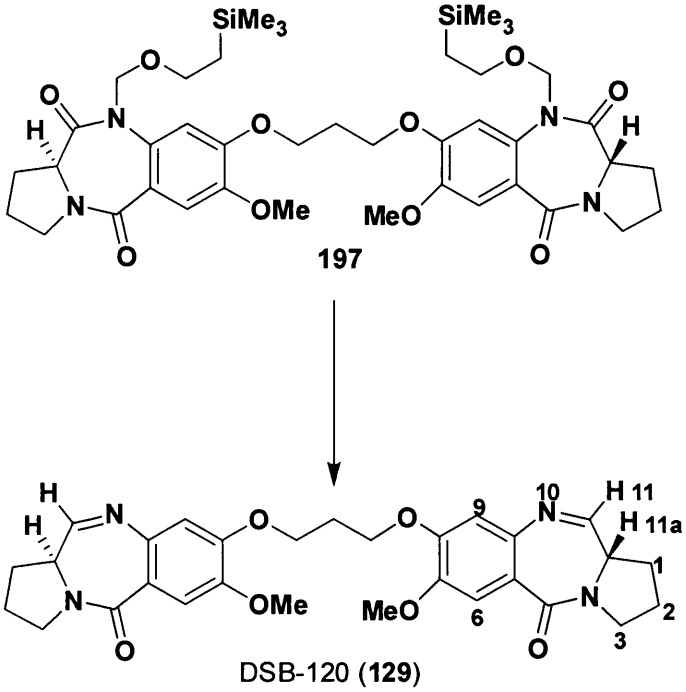


$LiBH_4$ (0.13 g, 6.07 mmol; 20.0 equiv.) was added to a stirred solution of the bis-N10-SEM tetralactam **197** (0.25 g, 0.303 mmol; 1.0 equiv.) in THF (12 mL) and ethanol (6 mL) and was allowed to stir for 5 h at room temperature under nitrogen atmosphere. The solvent was removed by evaporation in *vacuo* and the resulting residue was treated with water (70 mL) and was extracted with DCM (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL) and evaporated *in vacuo* to afford the SEM-carbinolamine intermediate. This was immediately dissolved in ethanol (25 mL) and water (12 mL) and treated with flash silica gel (~ 3 g). The reaction was allowed to stir at room temperature for 24 h after which time the formation of a large quantity of product was observed by TLC (10% $MeOH/CHCl_3$). The reaction mixture was filtered through a sinter funnel and rinsed a couple of times with EtOAc (2 x 40 mL). The organic filtrate layer was separated and the aqueous phase diluted with water (70 mL) and washed with EtOAc (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in*

vacuo to provide the crude product. Purification by flash chromatography (gradient elution: 100% $CHCl_3$ to 96:4 v/v $CHCl_3$/MeOH) afforded the pure DSB-120 (**129**) as an yellow foam (66 mg, 45%); LC/MS 2.33 min (ES+) m/z (relative intensity) 533 ($[M + H]^{+\cdot}$, 100), (ES) m/z (relative intensity) 531 ($[M - H]^{+\cdot}$, 95); $[\alpha]_D^{23}$ = +835° (*c* = 0.22, $CHCl_3$); δ 7.65 (d, 2 H, *J* = 4.35 Hz, H11), 7.50 (s, 2 H, H6), 6.85 (s, 2 H, H9), 4.35-4.20 (m, 4 H Linker-O$\underline{CH_2}$), 3.91 (s, 6 H, CH_3O x 2), 3.80 (ddd, 2 H, *J* = 11.43, 7.00, 4.50 Hz, H3β), 3.72-3.67 (m, 2 H, H11a), 3.55 (ddd, 2 H, *J* = 12.00, 8.01, 3.49 Hz, H3α), 2.40 (t, 2 H, *J* = 6.03 Hz, Linker-$\underline{CH_2}$), 2.33-2.26 (m, 4 H, H1α/β), 2.09-1.98 (m, 4 H, H2α/β); ^{13}C-NMR ($CDCl_3$, 100 MHz); δ 162.3 (C11), 111.7 (C6), 110.8 (C9), 65.4 (Linker-O$\underline{CH_2}$), 56.1 (CH_3O x 2), 53.7 (C11a), 46.6 (C3), 29.6 (C1), 28.8 (Linker-$\underline{CH_2}$), 24.1 (C2); MS (ESI) *m/z* (relative intensity): 533 ($[M + H]^{+\cdot}$, 100%), 785 (70), 567 (10), 416 (8), 288 (7); HRMS: $[M + H]^{+\cdot}$ Theoretical, $C_{29}H_{32}O_6N_4$ m/z 533.2394, found (ES^+) m/z 533.2415; (Thurston *et al.*, *J.Org.Chem.* Vol. 61, No. 23, 1996); [Lit ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.66 (d, 2 H, *J* = 4.4 Hz, H11), 7.51 (s, 2 H, H6), 6.85 (s, 2 H, H9), 4.22-4.33 (m, 4 H Linker-O$\underline{CH_2}$), 3.92 (s, 6 H, CH_3O x 2), 3.50-3.87 (m, 6 H, H3 and H11a), 2.28-2.45 (m, 8 H, H1), 2.01-2.17 (m, 2 H, H2); [Lit ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 164.6, 162.4 (C11), 150.6, 147.8, 140.6, 120.3 111.6 (C6), 110.7 (C9), 65.4 (Linker-O$\underline{CH_2}$), 56.1 (C11a), 53.7 (CH_3O x 2), 46.7 (C3), 29.6 (Linker-$\underline{CH_2}$), 28.8 (C1), 24.2 (C2)];

5.2.2.1 Optimization of the hydride reduction step (Including seconday Amine by-product Formation).

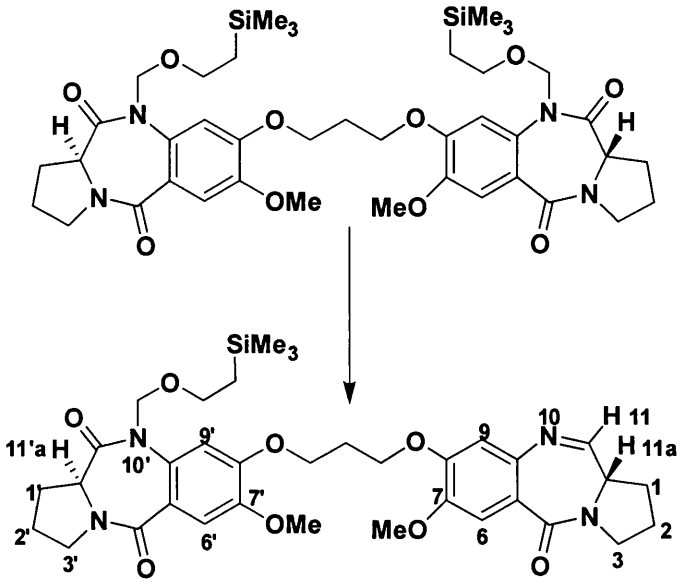
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-dione]] (129).



$NaBH_4$ (0.23 g, 6.07 mmol; 20.0 equiv.) was added to a stirred solution of the bis-N10-SEM tetralactam **197** (0.25 g, 0.303 mmol; 1.0 equiv.) in THF (12 mL) and ethanol (6 mL) and was allowed to stir for 8 h at room temperature under nitrogen atmosphere. The solvent was removed by evaporation in *vacuo* and the resulting residue was treated with water (70 mL) and was extracted with DCM (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL) and evaporated *in vacuo* to afford the SEM-carbinolamine intermediate. This was immediately dissolved in ethanol (25 mL) and water (12 mL) and treated with flash silica gel (~ 3 g). The reaction was allowed to stir at room temperature for 24 h after which time the formation of a large quantity of product was observed by TLC (10% $MeOH/CHCl_3$). The reaction mixture was filtered through a sinter funnel and rinsed a couple of times with EtOAc (2 x 40 mL). The organic filtrate layer was separated and the aqueous phase diluted with water (70 mL) and washed with EtOAc (3 x 35 mL). The combined organic layers

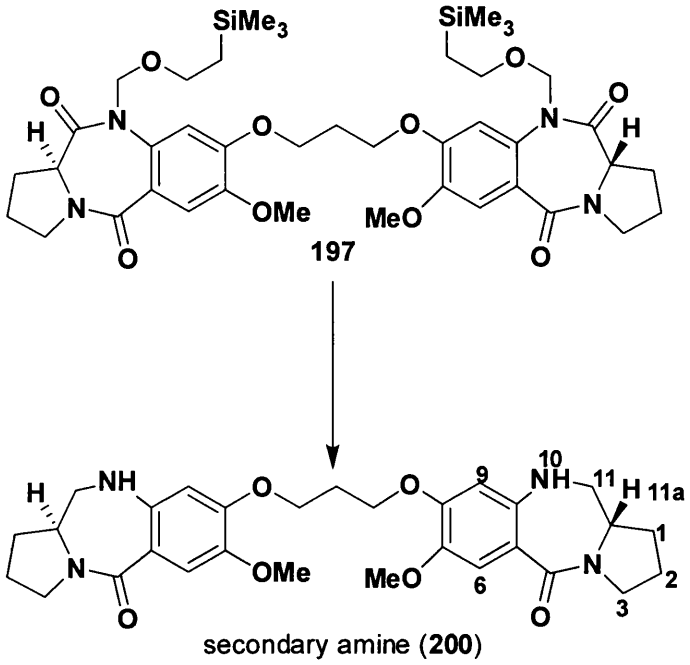
were washed with water (20 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by flash chromatography (gradient elution: 100% $CHCl_3$ to 96:4 v/v $CHCl_3$/MeOH) afforded the pure DSB-120 (**129**) as an yellow foam (40 mg, 25%) as well as small amounts of mono-N10-SEM protected imine; LC/MS 4.72 min (ES+) m/z (relative intensity) 533 ($[M + H]^{+\cdot}$, 90), (ES-) m/z (relative intensity) 531 ($[M - H]^{+\cdot}$, 40); $[\alpha]_D^{22}$ = +828° (*c* = 0.30, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.65 (d, 2 H, *J* = 4.38 Hz, H11), 7.50 (s, 2 H, H6), 6.85 (s, 2 H, H9), 4.34-4.19 (m, 4 H Linker-O$\underline{CH_2}$), 3.91 (s, 6 H, CH_3O x 2), 3.80 (ddd, 2 H, *J* = 11.98, 7.05, 4.31 Hz, H3β), 3.72-3.67 (m, 2 H, H11a), 3.56 (ddd, 2 H, *J* = 12.03, 8.01, 3.56 Hz, H3α), 2.41 (t, 2 H, *J* = 6.12 Hz, Linker-$\underline{CH_2}$), 2.33-2.27 (m, 4 H, H1α/β), 2.09-2.01 (m, 4 H, H2α/β); ^{13}C-NMR ($CDCl_3$, 100 MHz); δ 162.3 (C11), 111.7 (C6), 110.8 (C9), 65.4 (Linker-O$\underline{CH_2}$), 56.1 (CH_3O x 2), 53.7 (C11a), 46.6 (C3), 29.6 (C1), 28.8 (Linker-$\underline{CH_2}$), 24.2 (C2); HRMS: $[M + H]^{+\cdot}$ Theoretical, $C_{29}H_{32}O_6N_4$ m/z 533.2394, found (ES^+) m/z 533.2415.

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]-(11a*S*/11'a*S*)-7,7'-methoxy-10'-((2-(trimethylsilyl)ethoxy)methyl)-(1',2',3',10',11',11'a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5',11'-dione)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-one]] (Mono-Substituted N10-SEM imine).



Trace amounts of Mono-N10-SEM-imine was isolated for ^{1}H NMR analysis; LC/MS 6.22 min (ES+) m/z (relative intensity) 679 ($[M + H]^{+\cdot}$, 45), 702 ($[M + Na]^{+\cdot}$, 25); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.64 (s, 1 H, J = 4.34 Hz, H11), 7.51 (s, 1 H, H6), 7.33 (s, 1 H, H6'), 7.21 (s, 1 H, H9'), 6.83 (s, 1 H, H9), 5.45 (d, 1 H, J = 9.97 Hz, SEM-N$\underline{CH_2}$O), 4.68 (d, 1 H, J = 9.96 Hz, SEM-N$\underline{CH_2}$O), 4.21-4.33 (m, 4 H Linker-O$\underline{CH_2}$ and H11'a), 3.91 (s, 3 H, 7-CH_3O), 3.88 (s, 3 H, 7'-CH_3O), 3.85-3.76 (m, 2 H, H3β and H3'β), 3.75-3.60 (m, 3 H, SEM-O$\underline{CH_2}$ and H11a), 3.59-3.50 (m, 2 H, H3α and H3'α), 2.74-2.67 (m, 1 H, Hz, H1'α), 2.39 (t, 2 H, J = 6.07 Hz, Linker-$\underline{CH_2}$), 2.33-2.25 (m, 2 H, H1α/β), 2.10-1.94 (m, 5 H, H2, H2'α/β and H1'β), 0.93-0.90 (m, 2 H, SEM-$\underline{CH_2}$Si(CH_3)$_3$), 0.00 (s, 9 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz); δ 114.5 (C6'), 112.9 (C6), 108.3 (C9), 103.7 (C9'), 79.2 (SEM-N$\underline{CH_2}$O), 68.4 (SEM-O$\underline{CH_2}$), 67.8 (C11), 66.9 (Linker-O$\underline{CH_2}$), 62.5 (C11'a), 58.9 (C11a), 58.5 (7-CH_3O), 57.5 (7'-CH_3O), 48.2 (C3/C3'), 31.0 (Linker-$\underline{CH_2}$), 30.3 (C1), 27.9 (C1'), 25.1 (C2'), 25.0 (C2), 19.6 (SEM-$\underline{CH_2}$Si(CH_3)$_3$), 0.0 (SEM-Si($\underline{CH_3}$)$_3$); MS (ESI) *m/z* (relative intensity): 679 ($[M + H]^{+}$, 100%), 785 (45), 713 (12), 663 (10), 561 (29), 491 (38) 324 (20); HRMS: $[M + H]^{+}$ Theoretical, $C_{35}H_{46}O_8N_4Si$ m/z 679.3158, found (ES^+) m/z 679.3181.

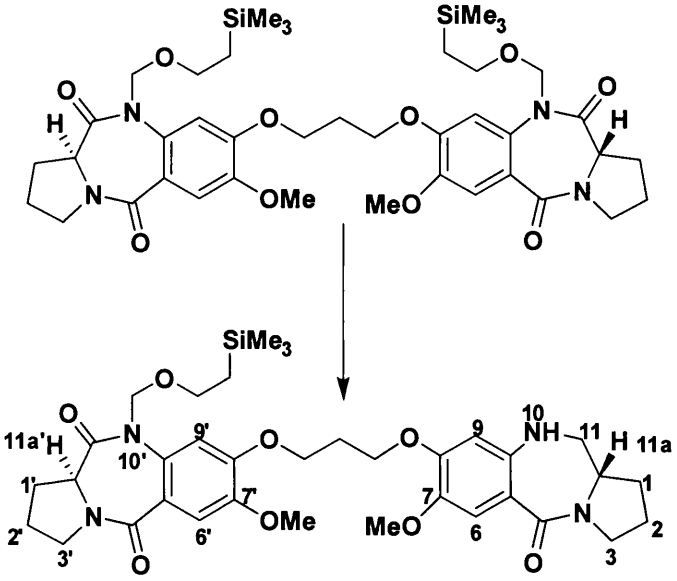
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-one]] (Secondary Amine, 200).



$NaBH_4$ (0.23 g, 0.95 mmol; 20.0 equiv.) was added to a stirred solution of the bis-methylbenzene **197** (0.25 g, 0.43 mmol; 1.0 equiv.) in THF (12 mL) and ethanol (6 mL) and was allowed to stir for 24 h at room temperature under nitrogen atmosphere. The solvent was removed by evaporation in *vacuo* and the resulting residue was treated with water (30 mL) and was extracted with DCM (3 x 15 mL). The combined organic layers were washed with water (10 mL), brine (20 mL) and evaporated *in vacuo* to afford the SEM-carbinolamine intermediate. This was immediately dissolved in ethanol (10 mL) and water (5 mL) and treated with flash silica gel (~ 3 g). The reaction was allowed to stir at room temperature for 24 h after which time the formation of a large quantity of product was observed by TLC (5% MeOH/$CHCl_3$). The reaction mixture was filtered through a sinter funnel and rinsed a couple of times with EtOAc (2 x 40 mL). The organic filtrate layer was separated and the aqueous phase diluted with water (40 mL) and washed with EtOAc (3 x 15 mL). The combined organic layers were washed with water (20 mL), brine (30 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by flash chromatography (gradient elution: 100% $CHCl_3$ to 96:4 v/v $CHCl_3$/MeOH) afforded the pure bis-secondary amine (**200**) as an white foam (95mg, 60%) as well as small amounts of mono-N10-SEM protected secondary amine; $[\alpha]_D^{22}$ = +90° (*c* = 0.8, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 6.73 (s, 2 H, H6), 6.21

(s, 2 H, H9), 4.35-4.27 (m, 2 H, H11a), 4.22 (t, 4 H, J = 6.12 Hz, Linker-O$\underline{CH_2}$), 3.69 (s, 6 H, CH_3O x 2), 3.65-3.51 (m, 6 H, NH10-$CH_2$11 and H3α), 2.31 (t, 4 H, J = 6.11 Hz, Linker-$\underline{CH_2}$), 2.12-2.03 (m, 2 H, H1) 1.84-1.75(m, 2 H, H2), 1.71-1.50 (m, 4 H, H2 and H1); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 114.7 (C6), 97.4 (C9), 67.2 (C11), 65.5 (Linker-O$\underline{CH_2}$), 61.1 (C11a), 57.7 (CH_3O x 2), 51.3 (C3), 29.3 (Linker-$\underline{CH_2}$), 28.6 (C1), 25.0 (C2); IR (Golden gate) 3329. 2944, 1620, 1483, 1238, 1068, 940, 678 cm^{-1}.

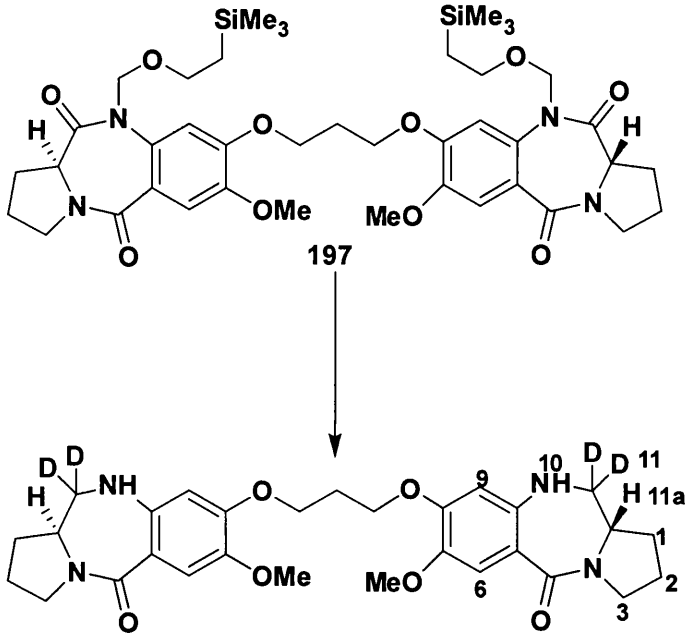
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]-(11a*S*/11'a*S*)-7,7'-methoxy-10'-((2-(trimethylsilyl)ethoxy)methyl)bis[1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5',11'-dione-(5-one)]] (Mono-Substituted N10-SEM secondary amine).



(A small amount of mono-N10-SEM protected secondary amine was isolated for ^{1}H NMR analysis); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.34 (s, 1 H, H6'), 7.21 (s, 1 H, H9'), 6.73 (s, 1 H, H6), 6.21 (s, 1 H, H9), 5.46 (d, 1 H, J = 10.0 Hz, SEM-N$\underline{CH_2}$O), 4.69 (d, 1 H, J = 10.0 Hz, SEM-N$\underline{CH_2}$O), 4.35-4.27 (m, 1 H, H11a'), 4.27-4.21 (m, 4 H Linker-O$\underline{CH_2}$), 4.13-4.08 (m, 1 H, H11a), 3.87 (s, 3 H, 7'-CH_3O), 3.69 (s, 3 H, 7-CH_3O), 3.71-3.64 (m, 4 H, SEM-O$\underline{CH_2}$ and NH10-$CH_2$11), 3.64-3.58 (m, 2 H, H3'α and H3α), 3.56-3.44 (m, 2 H, H3'β and H3β), 2.74-2.67 (m, 2 H, Hz, H1'α), 2.34 (t, 4 H, J = 5.95 Hz, Linker-$\underline{CH_2}$), 2.12-2.03 (m, 2 H, H1 and H2), 2.03-1.93 (m, 2 H, H1'β and H2'), 1.83-1.74 (m, 1 H, H2), 1.72-1.51 (m, 4 H, H2 and H1), 0.97-0.92 (m, 4 H, SEM-$\underline{CH_2}$Si(CH_3)$_3$), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 115.7 (C6), 112.9 (C6'), 108.3 (C9'), 97.4 (C9), 79.2 (SEM-N$\underline{CH_2}$O), 68.3 (SEM-

O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 61.1 (C11a), 58.9 (C11'a), 57.7 (7-CH_3O), 57.4 (7'-CH_3O), 51.3 (C3), 48.0 (C3'), 30.2 (Linker-$\underline{CH_2}$), 28.6 (C1), 27.9 (C1'), 25.1 (C2'), 25.0 (C2), 19.6 (SEM-$\underline{CH_2}Si(CH_3)_3$), 0.0 (SEM-Si($\underline{CH_3}$).

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-one]] (200, deuteriated).



D = ^2H deuteriated Secondary amine (**200**)

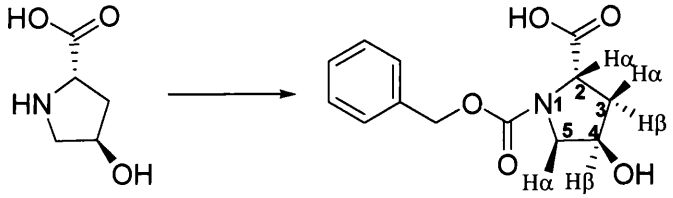
$NaBD_4$ (0.25 g, 0.95 mmol; 20.0 equiv.) was added to a stirred solution of the bis-tetralactam **197** (0.25 g, 0.43 mmol; 1.0 equiv.) in THF (12 mL) and ethanol (6 mL) and was allowed to stir for 24 h at room temperature under nitrogen atmosphere. The solvent was removed by evaporation in *vacuo* and the resulting residue was treated with water (30 mL) and was extracted with DCM (3 x 15 mL). The combined organic layers were washed with water (10 mL), brine (20 mL) and evaporated *in vacuo* to afford the SEM-carbinolamine intermediate. This was immediately dissolved in ethanol (10 mL) and water (5 mL) and treated with flash silica gel (~ 3 g). The reaction was allowed to stir at room temperature for 24 h after which time the formation of a large quantity of product was observed by TLC (5% MeOH/$CHCl_3$). The reaction mixture was filtered through a sinter funnel and rinsed a couple of times with EtOAc (2 x 40 mL). The organic filtrate layer was separated and the aqueous phase diluted with water

(40 mL) and washed with EtOAc (3 x 15 mL). The combined organic layers were washed with water (20 mL), brine (30 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by flash chromatography (gradient elution: 100% $CHCl_3$ to 96:4 v/v $CHCl_3$/MeOH) afforded the pure bis-deuteriated secondary amine analogue (**200**) as an yellow foam (90mg, 55%) as well as small amounts of mono-N10-SEM protected deuteriated secondary amine; LC/MS 6.56 min (ES+) m/z (relative intensity) 541 ($[M + H]^{+\cdot}$, 70); $[\alpha]_D^{22}$ = +75° (*c* = 0.5, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 6.78 (s, 2 H, H6), 6.26 (s, 2 H, H9), 4.39-4.32 (m, 2 H, H11a), 4.27 (t, 4 H, *J* = 6.12 Hz, Linker-O$\underline{CH_2}$), 3.74 (s, 6 H, CH_3O x 2), 3.65-3.56 (m, 2 H, H3α), 3.53-3.44 (m, 2 H, H3α), 2.37 (t, 4 H, *J* = 6.10 Hz, Linker-$\underline{CH_2}$), 2.17-2.09 (m, 2 H, H1), 1.90-1.81 (m, 2 H, H2), 1.78-1.56 (m, 4 H, H2 and H1); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 114.7 (C6), 97.3 (C9), 65.4 (Linker-O$\underline{CH_2}$), 61.1 (C11a), 57.6 (CH_3O x 2), 51.2 (C3), 29.2 (Linker-$\underline{CH_2}$), 28.5 (C1), 25.0 (C2);); IR (Golden gate) 3330, 2945, 1622, 1485, 1240, 1071, 945, 679 cm^{-1}.

5.3 Synthesis of Protected PBD C-Ring Precursor.

5.3.1 Synthesis of *O*-TBDMS Protected C-Ring

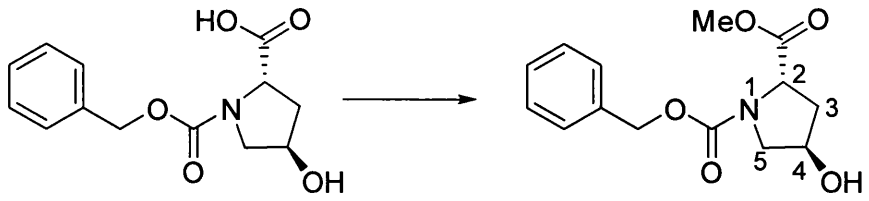
Synthesis of (2*S*, 4*R*)-*N*-benzyloxycarbonyl-2-carboxylic acid-4-hydroxypyrrolidine (204).



203 **204**

Trans-4-hydroxy-L-proline **203** (101.16 g, 0.77 mmol; 1.0 equiv.) and $NaHCO_3$ (162.0 g, 1.93 mmol; 2.5 equiv.) were dissolved in water (1500 mL) and a solution of benzylchloroformate (151.35 g or 127 mL, 0.89 mmol; 1.15 equiv.) in toluene (350 mL) was added dropwise. The reaction mixture was allowed to stir at room temperature for 20 h. The aqueous layer was separated and washed with diethyl ether (4 x 250 mL) then acidified to pH 2 with Conc.HCl. This was extracted with ethyl acetate (5 × 400 mL) and the combined extracts were dried ($MgSO_4$), evaporated *in vacuo* to afford the product (**204**) as a viscous white oil (200 g, 97%); LC/MS 5.82 min (ES+) m/z (relative intensity) 689 ($[M + H]^{+\cdot}$, 100) 711 ($[M + Na]^{+\cdot}$, 35); $[\alpha]_D^{20}$ = -83° (*c* = 1, $CHCl_3$);); LC/MS 6.10 min (ES+) m/z (relative intensity) 266 ($[M + H]^{+\cdot}$, 100); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.31-7.10 (m, 5 H, aromatic), 6.31-5.72 (broad s, 1 H, OH), rotamers: 5.10-4.91 (m, 2 H, $ArCH_2$ benzyl), 4.52-4.29 (m, 2 H, H2α and H4β), 3.63-3.41 (m, 2 H, H5α/β), 2.22-1.94 (m, 2 H, H3α/β); IR (golden gate): 3425 (OH), 3072, 2955, 1744 (C=O), 1632 (C=O), 1534, 1487, 1432, 1206, 1085, 856 cm^{-1}.

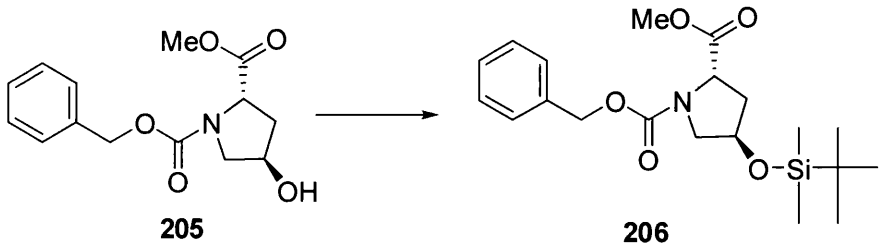
Synthesis of (2*S*, 4*R*)-*N*-benzyloxycarbonyl-2-methylester-4-hydroxypyrrolidine (205).



204 **205**

The acid C-ring **204** (200 g, 87.90 mmol; 1.0 equiv.) was dissolved in methanol (1200 mL) and Conc.H_2SO_4 (20 drops) was added. The solution was heated at reflux for 3 h after which time TLC (EtOAc) indicted the reaction had gone to completion. The reaction mixture was cooled and triethylamine was added and allowed to stir for a further 0.25 h. The solvent was evaporated *in vacuo* and the residue dissolved in EtOAc which was washed with brine, dried ($MgSO_4$) and evaporated *in vacuo* to afford the product (**205**) as a viscous oil (200 g, 99%); LC/MS 6.71 min (ES+) m/z (relative intensity) 280 ($[M + H]^{+\cdot}$, 95); ^{1}H-NMR (d_6-DMSO, 400 MHz): δ 7.42-7.31 (m, 5 H, aromatic), rotamers: 5.21-5.10 (m, 2 H, $ArCH_2$ benzyl), 4.48-4.32 (m, 2 H, H2α and H4β), 3.68 (s, 3 H, CH_3O), 3.56-3.49 (m, 2 H, H5α/β), 2.30-2.19 (m, 1 H H3α), 2.06-1.94 (m, 1 H, H3β); IR (golden gate): 2953, 2931, 2855, 1743, 1472, 1374, 1258, 1080, 1015, 835, 773 cm^{-1}; MS (ESI) *m/z* (relative intensity): 280 ($[M + H]^{+}$, 10%), 785 (100), 542 (15), 479 (45), 380 (35), 350 (70); HRMS: $[M + H]^{+}$ Theoretical, $C_{14}H_{17}O_5N$ m/z 280.1180, found (ES^+) m/z 280.1170.

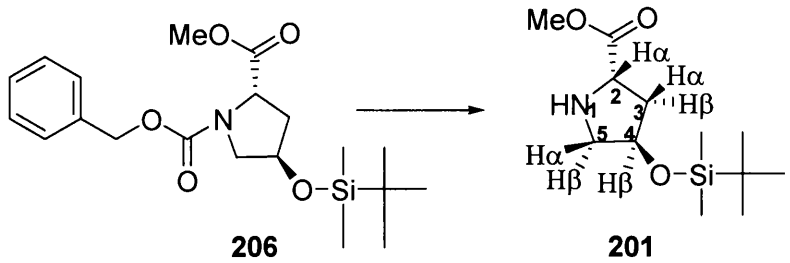
Synthesis of (2*S*,4*R*)-*N*-benzyloxycarbonyl-2-methylester-4-*t*-Butyldimethylsilyloxmethylpyrrolidine (206).



205 **206**

The C-ring **205** (5.58 g, 15.75 mmol; 1.0 equiv.) was dissolved in DMF (50 mL) to which was added TBDMS-Cl (3.10 g, 20.47 mmol; 1.3 equiv.), imidazole (2.68g, 39.40 mmol; 2.2 equiv.) and allowed to stir at room temperature for 3 h after which time TLC revealed the reaction had gone to completion. The reaction mixture was diluted with water (150 mL) and extracted with EtOAc (4 x 100 mL). The combined extracts were washed with water (2 x 150 mL), brine (300 mL) and dried ($MgSO_4$) evaporated in vacuo to afford the product (**206**) as a yellow foam (6.41g, 87%); LC/MS 7.55 min (ES+) m/z (relative intensity) 394 ($[M + H]^{+\cdot}$, 95) 417 ($[M + Na]^{+\cdot}$, 80); $[\alpha]_D^{25}$ = -52.45° (c = 0.3, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.36-7.27 (m, 5 H, aromatic), rotamers: 5.21-5.01 (m, 2 H, $ArCH_2$ benzyl), 4.51-4.40 (m, 2 H, H2α and H4β), 3.74 (s, 3 H, CH_3O), 3.65 (ddd, 1 H, J = 11.23, 6.26, 1.51 Hz, H5α), 3.10 (dd, 1 H, J = 11.48, 4.38 Hz, H5β), 2.25-2.14 (m, 1 H H3α), 2.08-1.99 (m, 1 H, H3β), 0.86 (s, 9 H, TBS CH_3 x 3), rotamers: 0.07-0.03 (m, 6 H, TBS CH_3 x 2); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 127.9 (aromatic), 70.4 (C4), 57.8 (C2), 55.2 (C5), 52.2 (CH_3O x 2), 38.9 (C3), 25.7 (TBS CH_3 x 3), -4.8 (TBS $\underline{CH_3}$ x 2); IR (ATR, ν_{max}/cm^{-1}): 2955, 2934, 2852, 1740, 1471, 1377, 1257, 1082, 1016, 834, 778; HRMS: $[M + H]^{+\cdot}$ Theoretical, $C_{20}H_{31}O_5NSi$ m/z 394.2044, found (ES^+) m/z 394.2036.

Synthesis of (2*S*,4*R*)-2-methylester-4-t- Butyldimethylsilyloxmethylpyrrolidine (201).

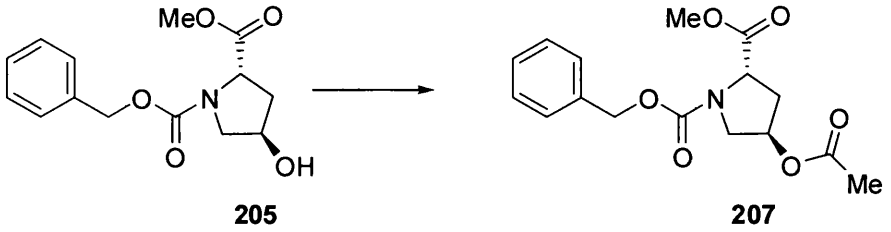


206 **201**

Palladium on charcoal catalyst (0.31 g, 10% w/w) was added as a slurry in EtOAc (**CAUTION! pyrophoric**) to a solution of **207** (3.31 g, 10.30 mmol; 1.0 Equiv.) in ethanol (20 mL). The reaction mixture was agitated in a Parr apparatus under H_2 (50 psi) for 2 h after which time LC/MS revealed complete consumption of starting material. The mixture was filtered through celite and the solvent was removed under reduced pressure to afford **202** as orange oil (1.45 g, 75%); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 4.33-4.27 (m, 1 H H4β), 3.97-3.88 (m, 1 H, H2α), 3.66 (s, 3 H, CH_3O), 3.10 (dd, 1 H, J = 11.48, 4.38 Hz, H5α), 2.79 (d, 1 H, J = 11.46 Hz, H5α), 2.02-1.94 (m, 1 H H3α), 1.91-1.83 (m, 1 H, H3β), 0.81 (s, 9 H, TBS CH_3 x 3), 0.00 (s, 6 H, TBS CH_3 x 2); IR (golden gate): 2955, 2934, 2852, 1740, 1471, 1377, 1257, 1082, 1016, 834, 778 cm^{-1}.

5.3.2 Synthesis of *O*-Acetoxy Protected C-Ring.

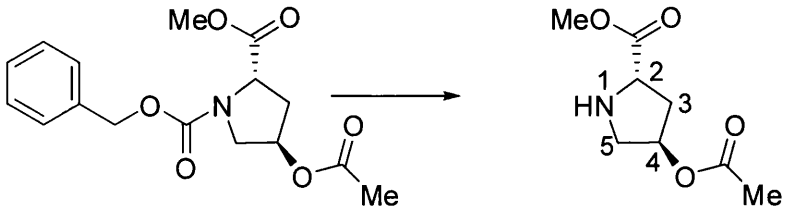
Synthesis of (2*S*, 4*R*)-*N*-benzyloxycarbonyl-2-methylester-4-hydroxyacetylpyrrolidine (207).



205 **207**

Acetyl chloride (1.20 mL, 16.11 mmol, 1.1 Equiv.) was added dropwise to a stirred solution of **205** (3.0 g, 10.74 mmol; 1.0 Equiv.) and Et_3N (1.5 mL, 14.0 mmol, 1.3 Equiv.) in DCM (50 mL) at room temperature and allowed to stir for 1 h after which time LC/MS indicated consumption of starting material. The reaction mixture was washed brine (3 x 100 mL), dried with $MgSO_4$ and concentrated *in vacuo* to afford **207** as yellow oil (3.31 g, 96%); LC/MS 7.82 min (ES+) m/z (relative intensity) 321 ($[M + H]^{+\cdot}$, 90) 344 ($[M + Na]^{+\cdot}$, 65); $[\alpha]_D^{22}$= –45.43° (*c* = 0.1, $CHCl_3$); $[\alpha]_D^{23}$ = –45° (*c* = 0.5, DMSO); ^{1}H-NMR (d_6-DMSO, 400 MHz): 7.25-7.21 (m, 5 H, Ar), 5.21-5.19 (m, 2 H, CH_2-Benzyl), 5.15–5.10 (m, 1 H, H4), 4.38 (dd, 1 H, *J* = 17.99, 8.03 Hz, H2), 3.26 (s, 3 H, CH_3O), 3.50-3.47 (m, 2 H, H5), 2.35-2.29 (m, 1 H, H3), 2.01 (s, 3 H, CH_3CO_2); IR (golden gate): 2955, 2857, 1743 (C=O), 1707, 1474, 1415, 1363, 1251, 1206, 1175, 1120, 1023, 837, 775, 695 cm^{-1}.

Synthesis of (2*S*, 4*R*)-*N*-benzyloxycarbonyl-2-methylester-4-hydroxyacetylpyrrolidine (202).



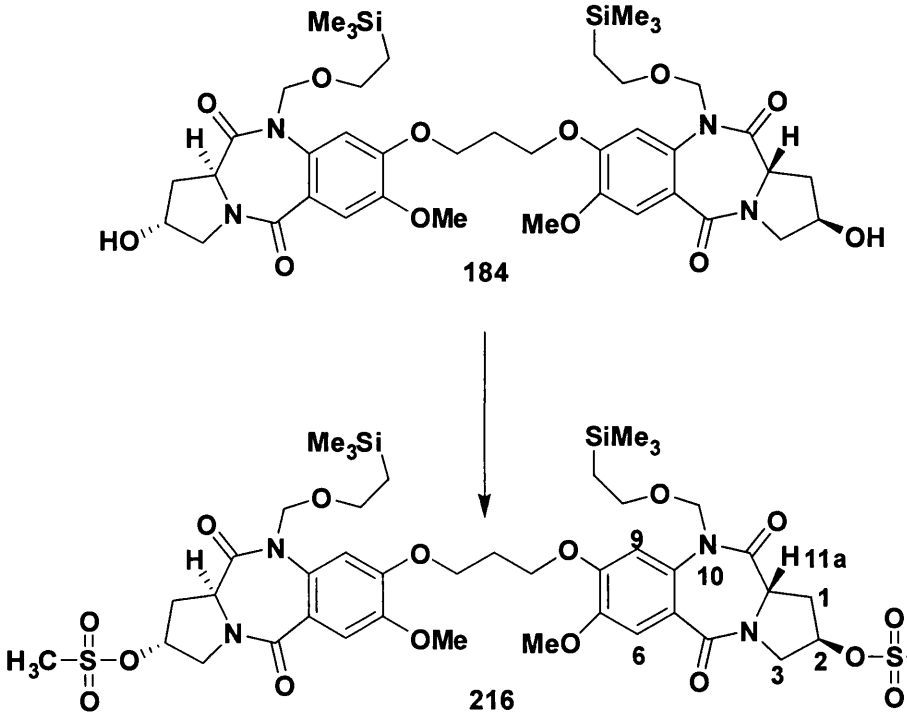
207 **202**

Palladium on charcoal catalyst (0.31 g, 10% w/w) was added as a slurry in EtOAc (**CAUTION! pyrophoric**) to a solution of **207** (3.31 g, 10.30 mmol; 1.0 Equiv.) in ethanol (20 mL). The reaction mixture was agitated in a Parr apparatus under H_2 (50 psi) for 2 h after which time LC/MS revealed complete consumption of starting material. The mixture was filtered through celite and the solvent was removed under reduced pressure to afford **202** as orange oil (1.45 g, 75%); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.13 – 5.17 (m, 1 H, H4), 3.30 – 3.35 (m, 1 H, H2), 3.23 (dd, 1 H, J = 12.43, 5.36 Hz, H5β), 2.86 (dd, 1 H, J = 12.42, 1.71 Hz, H5α), 1.98 (s, 3 H, CH_3CO_2), 1.76 – 1.82 (m, 2 H, H3α/β); IR (golden gate): 2954, 2933, 2858, 1743, 1470, 1374, 1252, 1085, 1015, 833, 778 cm^{-1}.

5.4 Exploiting the key Tetralactam Intermediate 184.

5.4.1 Successful Synthesis of C2-Vinyl Intermediate 219

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*,2*R*)-7-methoxy-2-(methanesulphonyl-oxy)-10-((2-(trimethylsilyl)ethoxy)methyl)-1,2,3,10,11, 11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (216).

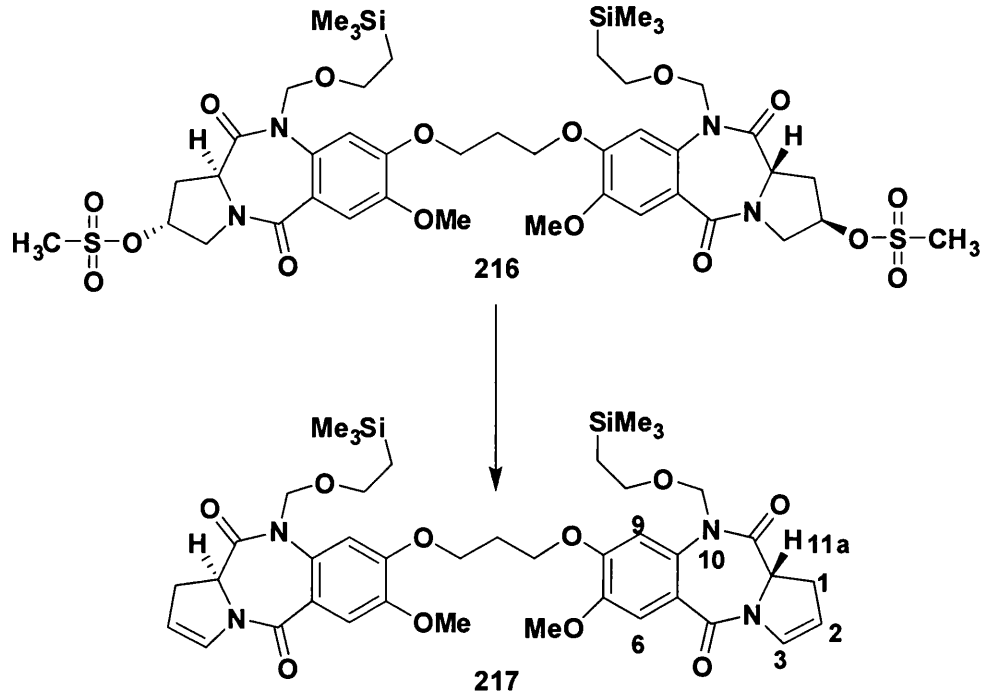


(**N: B:** The synthesis of pre-formed key Tetralactam intermediate **184** is described in section **5.5.1, successful synthesis of novel C2-aryl dimers** of the experimental section).

To a stirred solution of the bis-diol 184 (1.0 g, 1.168 mmol; 1.0 equiv.) in dry DCM (30 mL) at - 10 °C (ice/acetone) was added triethylamine (0.5 mL, 3.50 mmol; 3.0 equiv.) and then a solution of the methanesulfonyl chloride (0.2 mL, 2.57 mmol; 2.2 equiv.) in dry DCM (10 mL) dropwise. The reaction mixture was allowed to stir for 1 h at - 10 °C after which point TLC (100% EtOAc) revealed reaction had gone to completion. The reaction mixture was diluted with DCM (50 mL) and washed with water (50 mL), 10% HCl (2 x 50 mL), saturated $NaHCO_3$

(100 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the bis-methanesulfonyl (**216**) as a yellow foam (1.17 g, 99%): LC/MS 7.65 min (ES+) m/z (relative intensity) 1014 ([M + H]$^{+\cdot}$, 25); $[\alpha]_D^{24}$ = + 191° (*c* = 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.33 (s, 2 H, H9), 7.24 (s, 2 H, H6), 5.46 (d, 2 H, *J* = 10.0 Hz, SEM-N$\underline{CH_2}$O), 5.37-5.32 (m, 2 H, H2), 4.76 (d, 2 H, *J* = 10.0 Hz, SEM-N$\underline{CH_2}$O), 4.33 (t, 2 H, *J* = 7.0 Hz, H11a), 4.31-4.23 (m, 4 H, Linker-O$\underline{CH_2}$), 4.19 (d, 2 H, *J* = 13.71 Hz, H3β), 3.90 (s, 6 H, CH_3O x 2), 3.78-3.59 (m, 6 H, SEM-O$\underline{CH_2}$ and H3α), 3.16 (dt, 2 H, *J* = 14.56, 5.66 Hz, H1α), 3.06 (s, 6 H, CH_3SO_2R x 2), 2.59-2.35 (m, 2 H, Linker-$\underline{CH_2}$ and H1β), 0.95 (t, 4 H, *J* = 8.30 Hz, SEM-$\underline{CH_2}$Si(CH_3)$_3$), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 113.1 (C9), 108.5 (C6), 79.2 (SEM-N$\underline{CH_2}$O), 68.3 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 57.5 (CH_3O x 2), 57.1 (C11a), 53.9 (C2), 52.9 (C3), 39.8 (CH_3SO_2 x 2), 34.7 (C1), 30.2 (Linker-$\underline{CH_2}$), 19.5 (SEM-$\underline{CH_2}$Si(CH_3)$_3$), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2949, 1682 (C=O), 1638 (C=O), 1516, 1458, 1356, 1239, 1169, 1022, 956, 892, cm^{-1}; MS (ESI) *m/z* (relative intensity): 1014 ([*M* + H]$^{+\cdot}$, 100%), 849 (100), 675 (28), 491 (45); HRMS: [*M* + H]$^{+\cdot}$ Theoretical, $C_{43}H_{64}O_{16}N_4Si_2S_2$ m/z 1013.3370, found (ES^+) m/z 1013.3369.

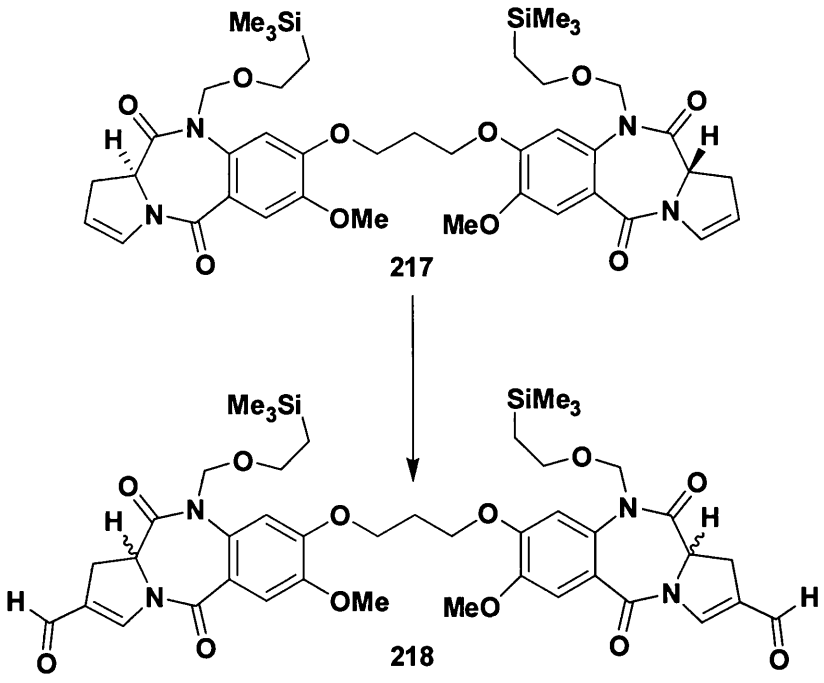
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (217).



DBU (0.51 mL, 3.44 mmol; 6.0 equiv.) was added to a solution of the bis-methanesulfonyl **216** (0.58 g, 0.57 mmol; 1.0 equiv.) in dry acentonitrile (15 mL). The reaction mixture was then heated in a microwave (190 °C, 195 psi) for 1 h at which point TLC (80:20 Et_2O/ACN) and LC-MS revealed reaction had gone to completion. The reaction mixture was diluted with DCM (15 mL), poured into 0.1 M citric acid solution (20 mL) and extracted with DCM (3 × 15 mL). The combined organic layers were washed with saturated $NaHCO_3$ (20 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude product. Purification by flash chromatography (gradient elution: 100% Et_2O to 80:20 v/v Et_2O/ACN) gave the pure bis-enamide (**217**) as a yellow foam (0.35 g, 65%): LC/MS 7.92 min (ES+) m/z (relative intensity) 822 ($[M + H]^+$, 20), 844 ($[M + Na]^+$, 25); $[\alpha]_D^{27}$ = + 129° (*c* = 0.85, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.35 (s, 2 H, H6), 7.26 (s, 2 H, H9), 6.90 (s, 2 H, H3), 5.48 (d, 2 H, *J* = 10.1 Hz, SEM-N$\underline{CH_2}$O), 5.39 (s, 2 H, H2), 4.82 (d, 2 H, *J* = 10.1 Hz, SEM-N$\underline{CH_2}$O), 4.50 (dd, 2 H, *J* = 10.40, 2.95 Hz, H11a), 4.33-4.24 (m, 4 H, Linker-O$\underline{CH_2}$), 3.89 (s, 6 H, CH_3O x 2), 3.80-3.62 (m, 6 H, SEM-O$\underline{CH_2}$), 3.57 (d, 2 H, *J* = 15.95, Hz, H1α), 2.89-2.78 (m, 2 H, H1β),

2.45-2.37 (m, 2 H, Linker-$\underline{CH_2}$), 0.96 (t, 4 H, J = 8.31 Hz, SEM-$\underline{CH_2}Si(CH_3)_3$), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 128.4 (C3), 113.9 (C2), 113.1 (C9), 108.6 (C6), 79.5 (SEM-N$\underline{CH_2}$O), 68.4 (SEM-O$\underline{CH_2}$), 65.8 (Linker-O$\underline{CH_2}$), 58.3 (C11a), 57.5 (CH_3O x 2), 32.3 (C1), 29.8 (Linker-$\underline{CH_2}$), 19.7 (SEM-$\underline{CH_2}Si(CH_3)_3$), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2951, 1687 (C=O), 1639 (C=O), 1516, 1454, 1356, 1238, 1065, 857, 750, cm^{-1}; MS (ESI) *m/z* (relative intensity):850 ([M + 28]$^{+}$, 100%), 822 ([M + H]$^{+}$, 35%), 713 (15), 544 (20), 460 (12); HRMS: [M + H]$^{+}$ Theoretical, $C_{41}H_{56}O_{10}N_4Si_2S_2$ m/z 821.3608, found (ES^{+}) m/z 821.3629.

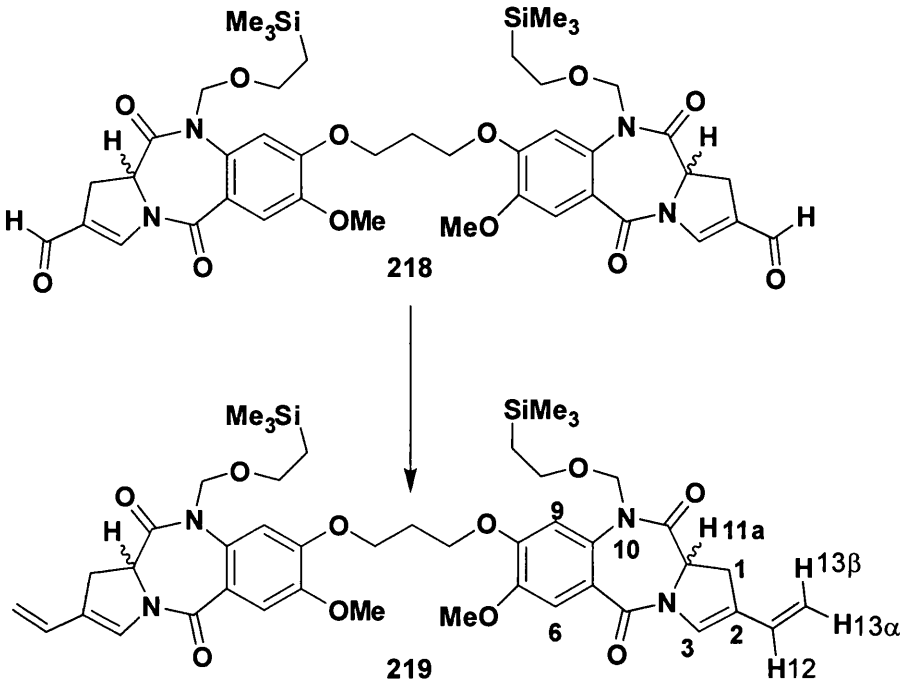
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*/11a*R*)-7-methoxy-2-(Formyl)-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (218).



To a stirred solution of the Vilsmeier reagent (1.0 g, 7.60 mmol; 26.0 equiv.) in dry DCM (12mL) at 0 °C under nitrogen atmosphere was added the bis-enamide **217** (0.24 g, 0.29 mmol; 1.0 equiv.) in dry DCM (12 mL) dropwise. After being stirred for 3.5 h at room temperature, the reaction mixture was added to a saturated solution (40%) of sodium carbonate at 0 °C and allowed to stir for 0.3 h at this temperature. The reaction mixture was then extracted with $CHCl_3$ (3 × 30 mL) and evaporated *in vacuo* to afford the crude product. Purification by flash

chromatography (gradient elution: 100% Et_2O to 80:20 v/v Et_2O/ACN) gave the pure bis-formyl (**218**) as a yellow foam (75 mg, 30%): LC/MS 7.75 min (ES+) m/z (relative intensity) 900 ([M + Na]$^{+\cdot}$, 20), (ES-) m/z (relative intensity) 876 ([M + H]$^{+\cdot}$, 15); $[\alpha]_D^{25}$ = 0° (*c* = 0.11, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 9.73 (s, 2 H, CHO-formyl), 7.76 (s, 2 H, H3), 7.31 (s, 2 H, H9), 7.27 (s, 2 H, H6), 5.49 (d, 2 H, *J* = 10.0 Hz, SEM-N$\underline{CH_2}$O), 4.77 (d, 2 H, *J* = 10.0 Hz, SEM-N$\underline{CH_2}$O), 4.76 (dd, 2 H, *J* = 10.30, 2.90 Hz, H11a), 4.34-4.23 (m, 4 H, Linker-O$\underline{CH_2}$), 3.89 (s, 6 H, CH_3O x 2), 3.90 (d, 2 H, *J* = 16.71 Hz, H1β), 3.80-3.62 (m, 4 H, SEM-O$\underline{CH_2}$), 2.97 (dd, 2 H, *J* = 16.72, 11.06 Hz, H1α), 2.47-2.43 (m, 2 H, Linker-$\underline{CH_2}$), 0.94 (t, 4 H, *J* = 9.56 Hz, SEM-$\underline{CH_2}$Si(CH_3)$_3$), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 142.8 (C3), 126.8 (CHO-formyl) 113.3 (C9), 108.8 (C6), 79.6 (SEM-N$\underline{CH_2}$O), 68.6 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 60.1 (C11a), 57.6 (CH_3O x 2), 30.2 (Linker-$\underline{CH_2}$), 28.8 (C1), 19.6 (SEM-$\underline{CH_2}$Si(CH_3)$_3$), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2919, 1689, 1649, 1604, 1515, 1452, 1430, 1403, 1341, 1236, 1173, 1129, 1063, 937, 857, 833, 762, 739 cm^{-1}; MS (ESI) *m/z* (relative intensity): 849 ([*M* + 28]$^{+\cdot}$, 100%), 877 ([*M* + H]$^{+\cdot}$, 60%), 821 (65), 801 (12); HRMS: [*M* + H]$^{+\cdot}$ Theoretical, $C_{43}H_{56}O_{12}N_4Si_2$ m/z 877.3506, found (ES$^+$) m/z 877.3207.

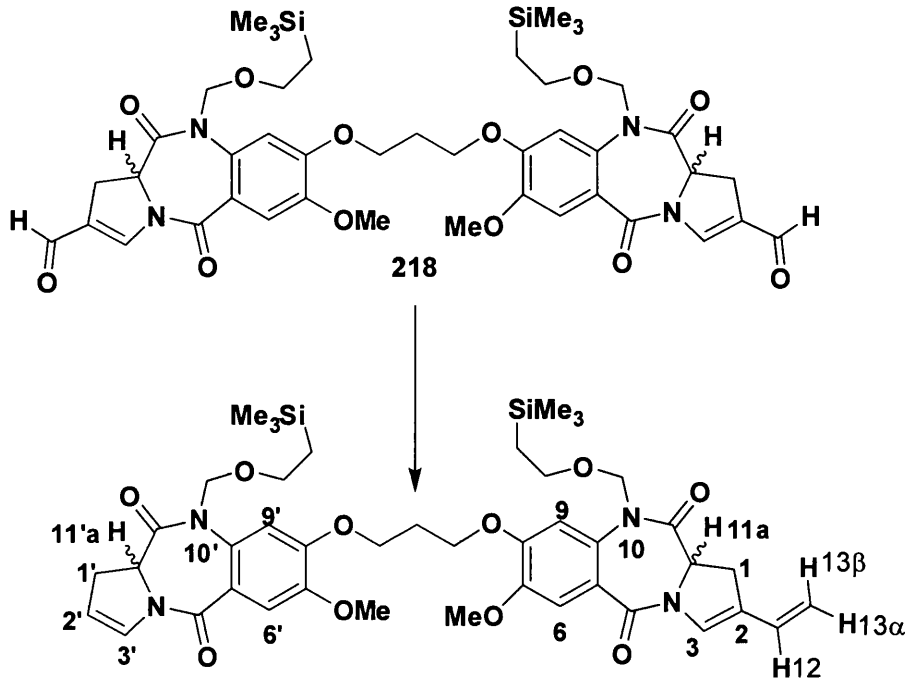
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*/11a*R*)-7-methoxy-2-(vinyl)-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (219).



To a stirred solution (Gregson *et al.*, 2004) of the methyltriphenylphosphonium bromide (0.39 g, 1.09 mmol; 4.0 equiv.) in dry THF (5 mL) at 0 °C under nitrogen atmosphere was added a solution of potassium-*t*-butoxide in dry THF (1.0 mL, 1.09 mmol; 4.0 equiv.). The resulting yellow ylid suspension was allowed to stir for 2 h at 0 °C at which point a solution of the bis-formyl **218** (0.24 g, 0.27 mmol; 1.0 equiv.) in dry THF (4 mL) was added to the reaction mixture and allowed to stir for 1 h at room temperature. The reaction mixture was partitioned between EtOAc (10 mL) and water (10 mL). The separated organic layers was washed with brine (15 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude product as a brown oil. Purification by flash chromatography (gradient elution: 50:50 v/v hexane/EtOAc to 80:20 v/v hexane/EtOAc) gave the pure bis-vinyl (**219**) as a yellow foam (0.5 g, 52%) as well as mono-substituted C2-vinyl compound (0.4 g, 41%): LC/MS 8.08 min (ES+) m/z (relative intensity) 896 ($[M + Na]^{+\cdot}$, 18), 874 ($[M + H]^{+\cdot}$, 15), 845 ($[M - CH{=}CH_2 + H]^{+\cdot}$, 10); $[\alpha]_D^{26} = 0°$ (*c* = 0.52, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.32 (s, 2 H, H9), 7.22 (s, 2 H, H6), 6.93 (s, 2 H, H3), 6.51 (dd, 2 H, *J* = 17.30, 10.60 Hz, H12 vinylic), 5.47 (d, 2 H, *J* = 10.10 Hz, SEM-N$\underline{CH_2}$O), 5.15 (dd, 2 H, *J* = 17.30, 10.80 Hz, H13α/β vinylic), 4.72 (d, 2 H, *J* = 10.10 Hz,

SEM-N$\underline{CH_2}$O), 4.52 (dt, 2 H, J = 10.80, 3.60 Hz, H11a), 4.30-4.19 (m, 4 H, Linker-O$\underline{CH_2}$), 3.86 (s, 6 H, CH_3O x 2), 3.78-3.60 (m, 6 H, SEM-O$\underline{CH_2}$ and H1β), 2.86 (dd, 2 H, J = 5.40, 2.69 Hz, H1α), (t, 2 H, J = 5.80 Hz, Linker-$\underline{CH_2}$), 0.93 (t, 4 H, J = 7.61 Hz, SEM-$\underline{CH_2}$Si(CH_3)$_3$), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 131.1 (C12 vinylic), 126.3 (C3), 116.4 (C13α/β vinylic), 113.1 (C9), 108.6 (C6), 79.6 (SEM-N$\underline{CH_2}$O), 68.5 (SEM-O$\underline{CH_2}$), 66.7 (Linker-O$\underline{CH_2}$), 58.9 (C11a), 57.5 (CH_3O x 2), 30.9 (C1), 30.2 (Linker-$\underline{CH_2}$), 19.6 (SEM-$\underline{CH_2}$Si(CH_3)$_3$), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2950, 1686 (C=O), 1636 (C=O), 1515, 1435, 1356, 1239, 1062, 834, 759 cm^{-1}; MS (ESI) *m/z* (relative intensity): 874 ([M + H]$^{\cdot+}$, 40%), 740 (100), 785 (58), 491 (48); HRMS: [M + H]$^{+}$ Theoretical, $C_{45}H_{60}O_{10}N_4Si_2$ m/z 873.3921, found (ES$^+$) m/z 873.3945.

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy][(11a*S*/*R*, 11'a*S*/*R*)-7,7'-methoxy-2-(vinyl)bis-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (Mono-substituted C2-vinyl tetralactam).



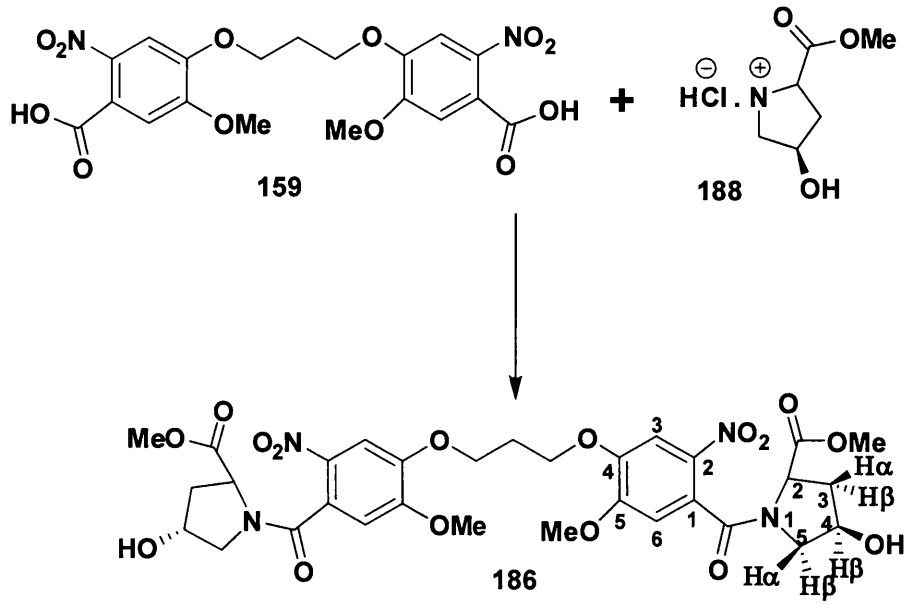
Recovered as side product from wittig procedure (see above, pg 162): LC/MS 8.00 min (ES+) m/z (relative intensity) 869 ($[M + Na]^{+\cdot}$, 15), 819 ($[M - CH{=}CH_2 + H]^{+\cdot}$, 10), 789 ($[M - OCH_3 + H]^{+\cdot}$, 20); $[\alpha]_D^{26} = 0°$ (*c* = 0.42, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.34 (s, 2 H, H9), 7.23 (s, 2 H, H6), 6.96 (s, 1 H, H3-Vinyl), 6.97-6.92 (m, 1 H, H3'-Enamide), 6.54 (dd, 2 H, *J* = 17.30, 10.60 Hz, H12 vinylic), 5.49 (d, 2 H, *J* = 10.00 Hz, SEM-N$\underline{CH_2}$O), 5.42-5.38 (m, 1 H, H2'-Enamide), 5.16 (dd, 2 H, *J* = 17.50, 10.30 Hz, H13α/β vinylic), 4.74 (d, 2 H, *J* = 10.10 Hz, SEM-N$\underline{CH_2}$O), 4.54 (dt, 2 H, *J* = 10.87, 3.55 Hz, H11a-vinyl), 4.45 (dt, 2 H, *J* = 10.49, 3.17 Hz, H11'a-enamide), 4.33-4.22 (m, 4 H, Linker-O$\underline{CH_2}$), 3.88 (s, 6 H, CH_3O x 2), 3.81-3.64 (m, 4 H, SEM-O$\underline{CH_2}$), 3.63 (d, 2 H, *J* = 9.56 Hz, H1β/H1'β), 2.96-2.79 (m, 2 H, H1α/H1'α), 2.42 (t, 2 H, *J* = 5.76 Hz, Linker-$\underline{CH_2}$), 0.95 (t, 4 H, *J* = 7.69 Hz, SEM-$\underline{CH_2}$Si(CH_3)$_3$), 0.04 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 130.0 (C12 vinylic), 128.3 (C13-vinylic), 126.3 (C3'-enamide), 116.4 (C13α/β vinylic), 113.9 (C2'-enamide), 113.0 (C9), 108.6 (C6), 79.5 (SEM-N$\underline{CH_2}$O), 68.3 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 58.9 (C11a-vinyl), 58.3 (C11a-enamide), 57.5 (CH_3O x 2), 30.9 (C1/C1'), 30.2 (Linker-$\underline{CH_2}$), 19.6 (SEM-$\underline{CH_2}$Si(CH_3)$_3$), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2957, 1686

(C=O), 1640 (C=O), 1516, 1436, 1355, 1241, 1188, 1025, 834, 719, 694 cm^{-1}; MS (ESI) *m/z* (relative intensity): 847 ($[M + H]^{+}$, 45%), 445 (100), 729 (55), 584 (50); HRMS: $[M + H]^{+}$ Theoretical, $C_{43}H_{58}O_{10}N_4Si_2$ m/z 847.3764, found (ES^{+}) m/z 847.3755.

5.5 Synthesis of Novel C2-Aryl PBD Dimers

5.5.1 Successful Synthesis of Novel C2-Aryl PBD imine Dimers.

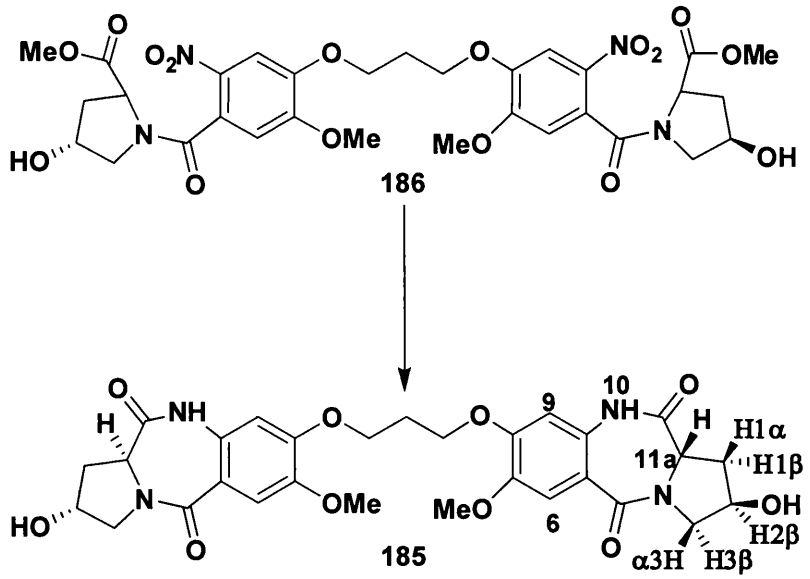
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(2*S*,4*R*)-(5-methoxy-2-nitro-1,4-phenylene)carbonyl]]bis[2-(carboxylic acid methyl ester)-4-hydroxypyrrolidine]] (186, Optimised Method)



Oxalyl chloride (23.06 g or 16.0 mL, 181.6 mmol; 3.0 equiv.) and DMF (0.2 mL) were added slowly to a suspension of bis-nitro acid **159** (28.20 g, 60.5 mmol; 1.0 equiv.) in dry DCM (285 mL). The mixture was allowed to stir at room temperature for 20 h. DCM and excess oxalyl chloride was removed under vacuum until viscous yellow oil was obtained. The oil was triturated with diethyl ether resulting in a yellow precipitate which was then filtered and dried in vacuum oven for 1 h. The yellow solid was then added portion wise over period of 0.25 h to a solution of 2*S*,4*R*-4-hydroxy-2-proline methyl ester **188** (24.73 g, 136.2 mmol; 2.25 equiv.) and Et_3N (42.0 mL, 302.6 mmol; 5.0 equiv.) in dry DCM (285 mL) at – 40 °C. LC/MS confirmed the reaction had gone to completion and the mixture was diluted with DCM (150 mL) and washed with saturated $NaHCO_3$ (1 × 300 mL), 1M HCl (1 × 300 mL), brine (1 × 300 mL) and evaporated *in vacuo* to afford the product (**186**) as a yellow foam (39.9 g, 92%); LC/MS 2.47 min (ES+) m/z (relative intensity) 743 ($[M + Na]^+$, 85); $[\alpha]_D^{24}$ = -279° (*c* = 0.42, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.63 (s, 2 H, H3 aromatic), 6.81 (s, 2 H, H6 aromatic),

4.75 (t, 2 H, J = 8.03 Hz, H2), 4.05 (s, 2 H, H4), 4.38-4.28 (m, 4 H, Linker-O$\underline{CH_2}$), 3.95 (s, 6 H, CH_3O x 2), 3.79 (s, 6 H, CO_2Me x 2), 3.43 (dd, 2 H, J = 11.10, 4.21 Hz, H5α), 3.03 (d, 2 H, J = 11.17 Hz, H5β), 2.46-2.33 (m, 4 H, Linker-$\underline{CH_2}$ and H3β), 2.12 (ddd, 2 H, J = 13.49, 7.89, 4.84 Hz, H3α); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 109.6 (C3 aromatic), 108.1 (C6 aromatic), 69.7 (C4), 68.9 (OH), 65.1 (Linker-O$\underline{CH_2}$), 57.4 (C2), 56.6 (CH_3O x 2), 56.4 (C5), 52.4 (CO_2Me x 2), 37.8 (C3), 29.0 (Linker-$\underline{CH_2}$); IR (Golden gate): 3427 (OH), 2954, 1745 (C=O), 1633 (C=O), 1532, 1488, 1435, 1349 (NO_2), 1205, 1084, 854 cm^{-1}; MS (ESI) *m/z* (relative intensity): 721 ([M + H]$^{+}$, 95%), 449 (100), 576 (25), 450 (30); HRMS: [M + H]$^{+}$ Theoretical, $C_{31}H_{36}O_{16}N_4$ m/z 721.2199, found (ES^{+}) m/z 721.2225.

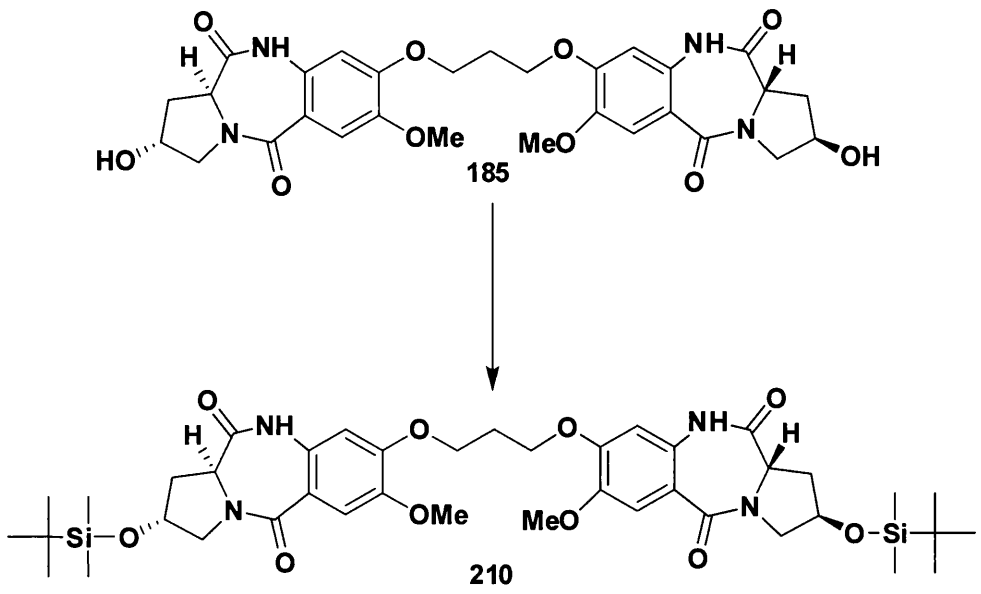
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*,2*R*)-7-methoxy-2-(hydroxy)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (185).



A solution of the bis-nitrobenzamide **186** (14.95 g, 20.5 mmol; 1.0 equiv.) in MeOH (600 mL) was added to a sample of Raney nickel (4 spatula end) and was heated at reflux. To this was added dropwise a solution of hydrazine hydrate (12.80 mL, 409.7 mmol; 20.0 equiv.) in MeOH (100 mL) and once effervescence ceased TLC (10% MeOH/$CHCl_3$) and LC/MS revealed reaction had gone to completion (mixture turn from yellow to a dark silver solution). The reaction mixture was immediately filtered to remove the Raney nickel without vacuum (**caution! Pyrophoric Nickel**) and the filtrate was reduced to volume by evaporation *in vacou*

at which point white solid precipitate formed. The mixture was left overnight to crystallise out and the resulting white solid was collected and dried to afford the pure bis-tetralactam (**185**) as a white solid (7.01 g, 57%): LC/MS 2.13 min (ES+) m/z (relative intensity) 597 ($[M + H]^{+\cdot}$, 50), 619 ($[M + Na]^{+\cdot}$, 20); $[\alpha]_D^{24} = +225°$ (c = 1, DMSO); ^{1}H-NMR (d_6-DMSO, 400 MHz): δ 10.24 (s, 2 H, NH10), 7.22 (s, 2 H, H6), 6.76 (s, 2 H, H9), 5.15 (s, 2 H, OH x 2), 4.26 (s, 2 H, H2), 4.17-4.04 (m, 6 H Linker-O$\underline{CH_2}$ and H11a), 3.75 (s, 6 H, CH_3O x 2), 3.56 (d, 2 H, J = 10.85 Hz, H3β), 3.45-3.27 (m, 2 H, H3α), 2.62-2.53 (m, 2 H, H1α), 2.27-2.18 (m, 2 H, Linker-$\underline{CH_2}$), 1.85-1.95 (m, 2 H, H1β); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 112.1 (C9), 105.3 (C6), 67.3 (C2), 64.9 (Linker-O$\underline{CH_2}$), 55.6 (CH_3O x 2), 55.3 (C11a), 53.9 (C3), 34.3 (C1), 28.3 (Linker-$\underline{CH_2}$); IR (Golden gate): 3509, 3460, 2955, 1682 (C=O), 1630 (C=O), 1573, 1481, 1351, 1252, 1059, 965 cm^{-1}; MS (ESI) *m/z* (relative intensity): 597 ($[M + H]^{+\cdot}$, 70%), 619 ($[M + Na]^{+\cdot}$, 30%), 785 (100), 469 (38); HRMS: $[M + H]^{+\cdot}$ Theoretical, $C_{29}H_{32}O_{10}N_4$ m/z 597.2191, found (ES^+) m/z 597.2208.

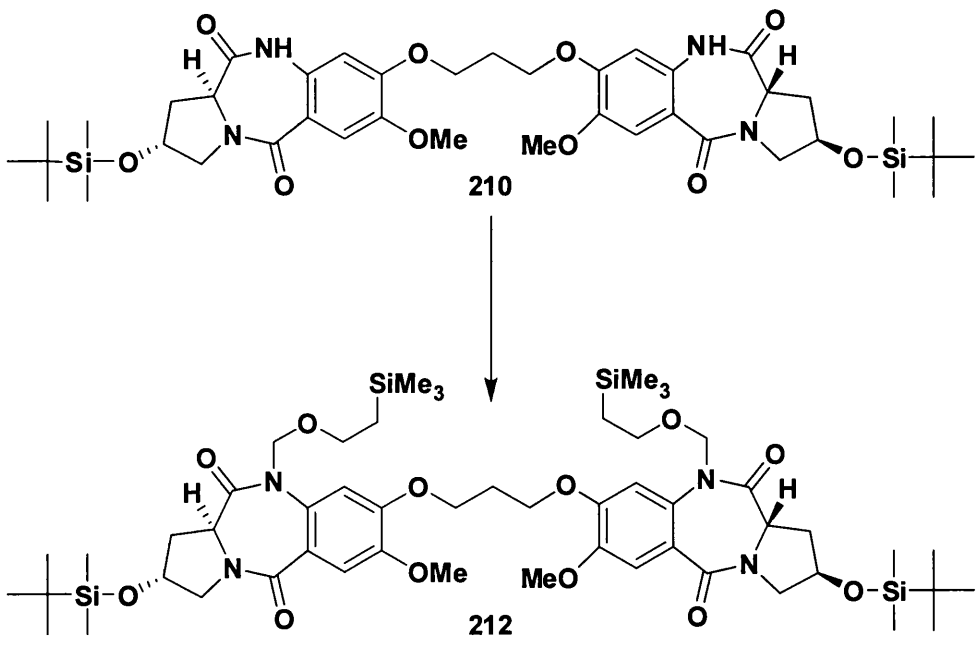
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*,2*R*)-7-methoxy-2-(*tert*-butyldimethylsilyloxy)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (210).



TBDMS-Cl (3.21 g, 21.3 mmol; 5.5 equiv.) and imidazole (3.29 g, 48.4 mmol; 12.5 equiv.) was added to a stirred cloudy solution of the bis-tetralactam **185** (2.31 g, 3.8 mmol; 1.0 equiv.) in anhydrous DMF (55 mL). The reaction mixture was allowed to stir for 3 h at room temperature under nitrogen atmosphere at which point LC/MS revealed reaction had gone to completion. The reaction mixture was poured onto ice/water (550 mL) and was allowed to stir to room temperature. The resulting precipitate was collected by vacuum filtration, washed with water, diethyl ether and then dried to afford the pure bis-TBDMS ether (**210**) as a white solid (2.90 g, 90%): LC/MS 7.98 min (ES+) m/z (relative intensity) 825 ($[M + H]^{+\cdot}$, 100); (ES-) m/z (relative intensity) 823 ($[M - H]^{+\cdot}$, 90);); $[\alpha]_D^{24}$ = + 325° (*c* = 1, MeOH); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.33 (s, 2 H, NH10), 7.44 (s, 2 H, H6), 7.26 (s, 2 H, H9), 4.51 (t, 2 H, *J* = 5.48 Hz, H2), 4.20-4.15 (m, 6 H Linker-O$\underline{CH_2}$ and H11a), 3.88 (s, 6 H, CH_3O x 2), 3.68 (t, 4 H, *J* = 5.94 Hz, H3α/β), 2.81 (dt, 4 H, *J* = 12.84, 4.98 Hz, H1α), 2.33 (t, 2 H, *J* = 5.94 Hz Linker-$\underline{CH_2}$), 2.03 (ddd, 2 H, *J* = 12.79, 8.18, 1.90, Hz, H1β), 0.86 (s, 18 H, TBS CH_3 x 6), 0.08 (s, 12 H, TBS CH_3 x 4); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 112.8 (C9), 105.2 (C6), 69.2 (C2), 65.4 (Linker-O$\underline{CH_2}$), 56.3 (CH_3O x 2), 55.7 (C11a), 54.2 (C3), 35.2 (C1), 28.6 (Linker-$\underline{CH_2}$), 25.7 (TBS CH_3 x 6), -4.8 (TBS $\underline{CH_3}$ x 4); IR (Golden gate): 2953, 1687 (C=O), 1642 (C=O), 1515, 1461, 1433, 1359, 1246, 1127, 1066, 832, 775 cm^{-1}; MS (ESI) *m/z* (relative intensity): 825 ($[M + H]^+$,

55%), 853 ([*M* + 28]$^{+}$, 10%), 785 (100), 479 (50), 467 (15); Elem. Anal. calculated for $C_{41}H_{60}N_4O_{10}Si_2$: C, 59.68; H, 7.33; N, 6.79%. Found: C, 59.54; H, 7.31; N, 6.73%. HRMS: [*M* + H]$^{+}$ Theoretical, $C_{41}H_{60}O_{12}N_4Si_2$ m/z 825.3926, found (ES^{+}) m/z 825.3956.

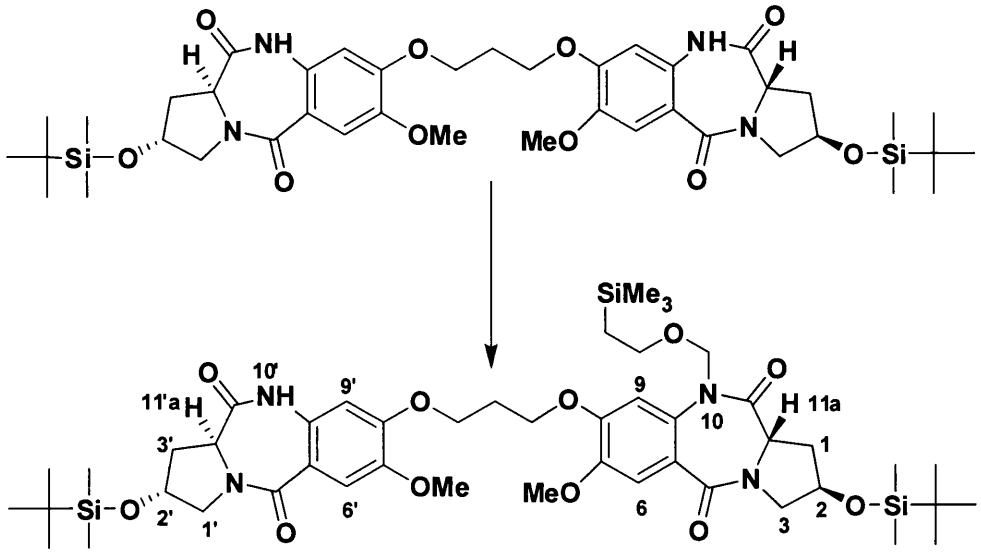
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*,2*R*)-7-methoxy-2-(*tert*-butyldimethylsilyloxy)-10-((2-(trimethylsilyl)ethoxy)methyl)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (212).



A solution of *n*-BuLi (1.60 mL of a 1.6 M solution in hexane, 2.55 mmol; 2.5 equiv.) was added dropwise to a stirred suspension of the bis-TBS ether **210** (0.84 g, 1.02 mmol; 1.0 equiv.) in anhydrous THF (15 mL) at – 30 °C under nitrogen atmosphere. The reaction mixture was allowed to stir for 1 h at which point a solution of the SEM-Cl (0.45 mL, 2.55 mmol; 2.5 equiv.) in dry THF (10 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature and left stirring overnight under nitrogen atmosphere after which point LC/MS and TLC (EtOAc) revealed reaction completion (with traces amount of mono-substituted-N10-SEM protected bis-TBS ether). The THF was removed by evaporation *in vacou* and the resulting residue was dissolved in EtOAc (100 mL) and washed with water (30 mL), brine (30 mL) dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude

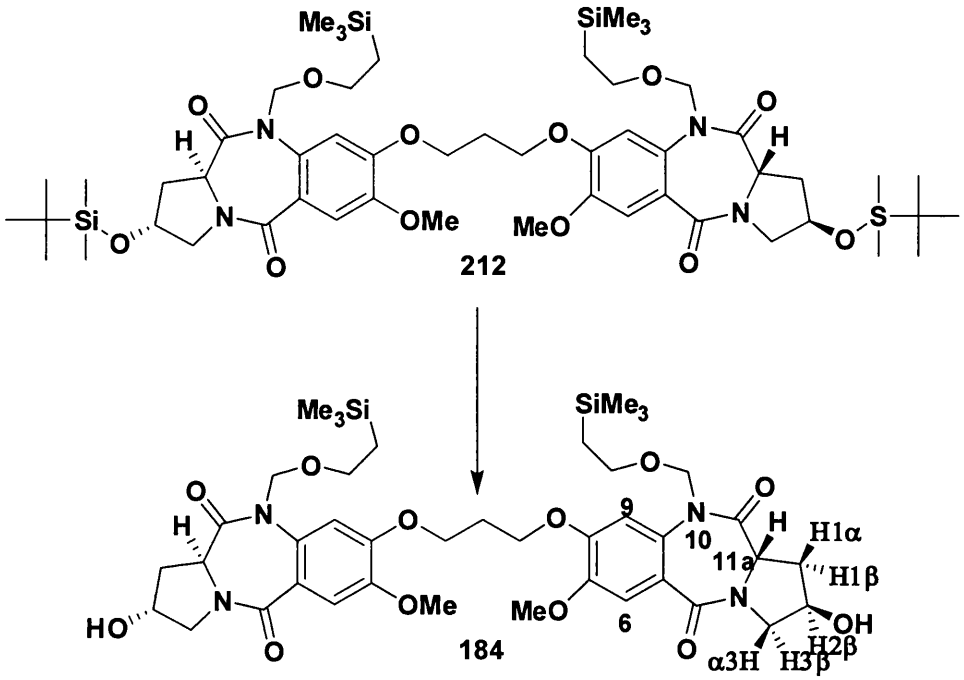
product. Purification by flash chromatography (gradient elution: 80:20 v/v Hexane/EtOAc to 50:50 v/v Hexane/EtOAc) gave the pure bis-diol (trace amounts of mono-N10-SEM protected bis-TBS ether was isolated for NMR analysis) (**212**) as a yellow foam (0.52 g, 50%): LC/MS 4.75 min (ES+) m/z (relative intensity) 1085 ($[M + H]^{+\cdot}$, 85); $[\alpha]_D^{25}$ = + 199° (*c* = 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.32 (s, 2 H, H6), 7.21 (s, 2 H, H9), 5.46 (d, 2 H, *J* = 10.0 Hz, SEM-N$\underline{CH_2}$O), 4.67 (d, 2 H, *J* = 10.0 Hz, SEM-N$\underline{CH_2}$O), 4.56 (t, 2 H, *J* = 5.80 Hz, H2), 4.28-4.22 (m, 4 H Linker-O$\underline{CH_2}$), 4.19 (dd, 2 H, *J* = 8.24, 3.92 Hz, H11a), 3.88 (s, 6 H, CH_3O x 2), 3.79-3.60 (m, 6 H, SEM-O$\underline{CH_2}$ and H3β), 3.53 (dd, 2 H, *J* = 11.9, 5.51 Hz, H3α), 2.83 (dt, 2 H, *J* = 12.72, 4.27 Hz, H1α), 2.41 (t, 2 H, *J* = 5.85 Hz Linker-$\underline{CH_2}$), 2.01 (dd, 2 H, *J* = 14.86, 6.43, Hz, H1β), 0.95 (t, 4 H, *J* = 8.27 Hz, SEM-$\underline{CH_2}Si(CH_3)_3$), 0.85 (s, 18 H, TBS CH_3 x 6), 0.08 (s, 12 H, TBS CH_3 x 4), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 112.8 (C6), 108.2 (C9), 79.2 (SEM-N$\underline{CH_2}$O), 70.8 (C2), 68.3 (SEM-O$\underline{CH_2}$), 66.7 (Linker-O$\underline{CH_2}$), 57.4 (CH_3O x 2), 57.3 (C11a), 54.9 (C3), 36.8 (C1), 30.1 (Linker-$\underline{CH_2}$), 27.0 (TBS CH_3 x 6), 19.6 (SEM-$\underline{CH_2}Si(CH_3)_3$), 0.0 (TBS $\underline{CH_3}$ x 4), -3.5 (SEM-Si($\underline{CH_3}$)$_3$); MS (ESI) *m/z* (relative intensity): 1085 ($[M + H]^{+}$, 75%), 1113 ($[M + 28]^{+}$, 48%), 812 (20), 609 (45), 449 (15); HRMS: $[M + H]^{+}$ Theoretical, $C_{53}H_{88}O_{12}N_4Si_4$ m/z 1085.5548, found (ES^+) m/z 1085.5543.

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]-(11a*S*/2*R*, 11'a*S*/2'*R*)-7,7'-methoxy-10-((2-(trimethylsilyl)ethoxy)methyl)bis[-2-(*tert*-butyldimethylsilyloxy))-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (Mono-substituted N10-SEM tetralactam).



Trace amounts of mono-N10-SEM protected Bis-TBS ether was Isolated for NMR analysis; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.47 (s, 1 H, NH10), 7.41 (s, 2 H, H6), 7.32 (s, 2 H, H6'), 7.19 (s, 2 H, H9), 6.55 (s, 2 H, H9'), 5.45 (d, 1 H, J = 10.0 Hz, SEM-NCH_2O), 4.65 (d, 1 H, J = 10.0 Hz, SEM-NCH_2O), 4.53 (t, 1 H, J = 11.43 Hz, H2), 4.47 (t, 1 H, J = 10.68 Hz, H2'), 4.22-4.12 (m, 4 H, Linker-OCH_2 and H11a), 3.86 (s, 6 H, CH_3O x 2), 3.74-3.63 (m, 4 H, SEM-OCH_2 and H3'/H3β), 3.53 (dd, 2 H, J = 11.9, 5.30 Hz, H3/H3'α), 2.84-2.76 (m, 2 H, H1/H1'α), 2.35 (t, 2 H, J = 5.46 Hz Linker-CH_2), 2.04-1.95 (m, 2 H, J = 14.86, 6.43, Hz, H1/H1'β), 0.93 (t, 2 H, J = 8.17 Hz, SEM-$CH_2Si(CH_3)_3$), 0.83 (s, 18 H, TBS CH_3 x 6), -0.06 (s, 12 H, TBS CH_3 x 4), -0.14 (s, 9 H, SEM-$Si(CH_3)_3$).

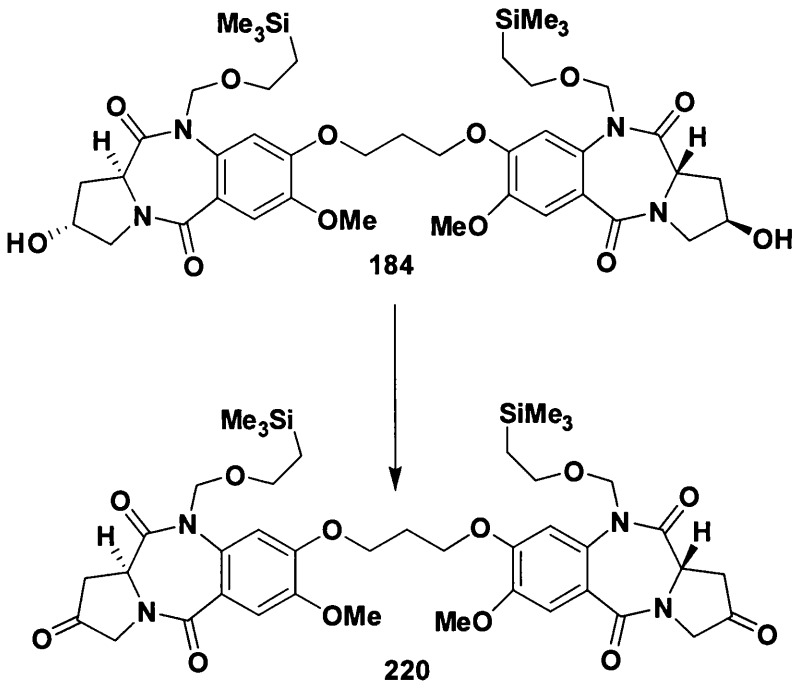
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*,2*R*)-7-methoxy-2-(hydroxy)-10-((2-(trimethylsilyl)ethoxy)methyl)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (184).



A 1.0 M solution of TBAF (0.95 mL, 0.95 mmol; 2.2 equiv.) was added to a stirred solution of the bis-TBDMS ether **212** (0.47 g, 0.43 mmol; 1.0 equiv.) in dry THF (10 mL). The reaction mixture was allowed to stir for 3.5 h at room temperature at which point TLC (5% MeOH/$CHCl_3$) revealed reaction completion. The reaction mixture was poured into a cooled (ice) solution of NH_4Cl (100 mL) and extracted with $CHCl_3$ (3 × 30 mL). The organic layer was washed with brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude product. Purification by flash chromatography (gradient elution: 100%$CHCl_3$ to 97:3 v/v $CHCl_3$/MeOH) gave the pure bis-diol (**184**) as a yellow foam (0.37 g, 100%): LC/MS 6.72 min (ES+) m/z (relative intensity) 879 ($[M + Na]^+$, 20); $[\alpha]_D^{24}$ = + 215° (*c* = 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.28 (s, 2 H, H9), 7.20 (s, 2 H, H6), 5.44 (d, 2 H, *J* = 10.0 Hz, SEM-N$\underline{CH_2}$O), 4.72 (d, 2 H, *J* = 10.0 Hz, SEM-N$\underline{CH_2}$O), 4.63-4.58 (m, 2 H, H2), 4.25 (t, 4 H, *J* = 5.80 Hz, Linker-O$\underline{CH_2}$), 4.18 (t, 2 H, *J* = 7.04 Hz, H11a), 3.90 (d, 2 H, *J* = 12.78 Hz, H3β), 3.85 (s, 6 H, CH_3O x 2), 3.78-3.59 (m, 4 H, SEM-O$\underline{CH_2}$), 3.56 (dd, 2 H, *J* = 12.64, 4.53 Hz, H3α), 2.97-2.89 (m, 2 H, H1α), 2.38 (t, 2 H, *J* = 5.6 Hz, Linker-$\underline{CH_2}$), 2.12-2.04 (m, 2 H, H1β), 0.95 (t, 4 H, *J* = 8.25 Hz, SEM-$\underline{CH_2}$Si$(CH_3)_3$), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR

($CDCl_3$, 100 MHz): δ 112.9 (C9), 108.3 (C6), 79.4 (SEM-N$\underline{CH_2}$O), 70.5 (C2), 68.4 (SEM-O$\underline{CH_2}$), 66.5 (Linker-O$\underline{CH_2}$), 57.6 (C11a), 57.5 (CH_3O x 2), 55.3 (C3), 36.4 (C1), 30.4 (Linker-$\underline{CH_2}$), 19.6 (SEM-$\underline{CH_2}$Si(CH_3)$_3$), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 3377 (OH), 2953, 1685 (C=O), 1624 (C=O), 1516, 1434, 1359, 1237, 1178, 1057, 833, 752 cm^{-1}; MS (ESI) *m/z* (relative intensity): 857 ($[M+H]^+$, 100%), 885 ($[M+28]^+$, 48%), 785 (58), 711 (10), 466 (25); HRMS: $[M+H]^+$ Theoretical, $C_{41}H_{60}O_{12}N_4Si_2$ m/z 857.3819, found (ES^+) m/z 857.3798.

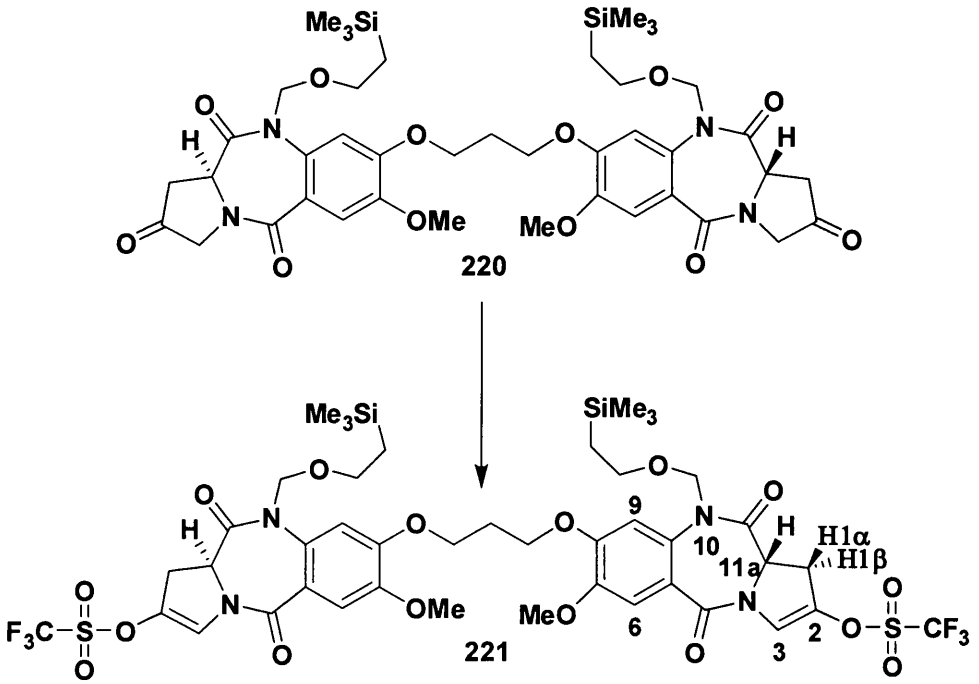
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-10-((2-(trimethylsilyl)ethoxy)methyl)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (220).



A solution of DMSO (3.50 mL, 49.44 mmol; 6.0 equiv.) in anhydrous DCM (90 mL) was added dropwise to a stirred solution of oxalyl chloride (12.36 mL of a 2.0 M solution in DCM, 24.72 mmol; 3.0 equiv.) at – 60 °C under nitrogen atmosphere. After stirring at – 60 °C for 10 min, a solution of the bis-Diol **184** (7.05 g, 8.24 mmol; 1.0 equiv.) in dry DCM (170 mL) was added dropwise to the mixture, which was then stirred for a further 20 min at – 60 °C. A solution of TEA (16.0 mL, 115.36 mmol; 14.0 equiv.) in dry DCM (90 mL) was added dropwise to the mixture at – 60 °C under nitrogen atmosphere. The reaction mixture was allowed to warm to 0 °C and was diluted with DCM (200 mL). The mixture was washed with

1N HCl (2 x 150 mL), Water (150 mL), brine (150 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude product. Purification by flash chromatography (gradient elution: 60:40 v/v hexane/EtOAc to 80:20 v/v EtOAc/Hexane) gave the pure bis-ketone (**220**) as a yellow foam (3.5 g, 50%): LC/MS 7.53 min (ES+) m/z (relative intensity) 875 ($[M + Na]^{+\cdot}$, 35); $[\alpha]_D^{22} = +290°$ (*c* = 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.31 (s, 2 H, H6), 7.24 (s, 2 H, H9), 5.49 (d, 2 H, *J* = 10.06 Hz, SEM-N$\underline{CH_2}$O), 4.74 (d, 2 H, *J* = 10.06 Hz, SEM-N$\underline{CH_2}$O), 4.59 (dd, 2 H, *J* = 9.83, 3.06 Hz, H11a), 4.30-4.24 (m, 4 H, Linker-O$\underline{CH_2}$), 4.20 (d, 2 H, *J* = 20.0 Hz, H3β), 3.88 (s, 6 H, CH_3O x 2), 3.83 (s, 2 H, H3α), 3.78-3.61 (m, 4 H, SEM-O$\underline{CH_2}$), 3.54 (d, 2 H, *J* = 19.25, Hz, H1α), 2.76 (dd, 2 H, *J* = 18.90, 10.26 Hz, H1β), 2.41 (t, 2 H, *J* = 5.90 Hz, Linker-$\underline{CH_2}$),), 0.97-0.91 (m, 4 H, SEM-$\underline{CH_2}$Si$(CH_3)_3$), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 112.8 (C6), 108.3 (C9), 79.4 (SEM-N$\underline{CH_2}$O), 68.5 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 57.5 (CH_3O x 2), 56.1 (C11a), 53.6 (C3), 38.6 (C1), 30.2 (Linker-$\underline{CH_2}$), 19.6 (SEM-$\underline{CH_2}$Si$(CH_3)_3$), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2951, 1763 (ketone), 1685 (C=O), 1640 (C=O), 1515, 1458, 1358, 1205, 1064, 1021, 834, 765, 693 cm^{-1}; MS (ESI) *m/z* (relative intensity): 853 ($[M + H]^{+}$, 100%), 855 ($[M + 2H]^{+}$, 52%), 864 (12), 846 (10); HRMS: $[M + H]^{+}$ Theoretical, $C_{41}H_{56}O_{12}N_4Si_2$ m/z 853.3506, found (ES^+) m/z 853.3506.

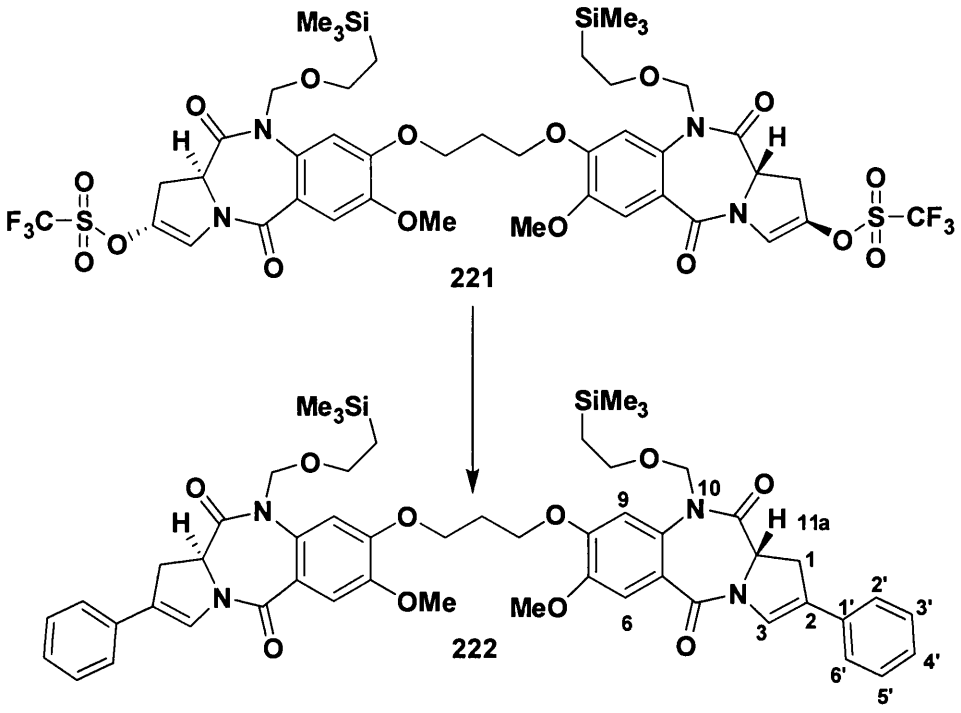
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-[[(trifluromethyl)sulfonyl]oxy]-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5*H*-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione]] (221).



Anhydrous triflic anhydride, taken from a freshly opened ampoule (0.78 mL, 4.65 mmol; 2.2 equiv.) was added rapidly in one portion to a vigorously stirred solution of the bis-ketone **220** (1.8 g, 2.11 mmol; 1.0 equiv.), and anhydrous pyridine (0.41 mL, 5.06 mmol; 2.4 equiv.) in dry DCM (60 mL) at room temperature under a nitrogen atmosphere. The reaction mixture was allowed to stir for 3 h under nitrogen atmosphere at which point (colour change from orange to dark red was observed) TLC (EtOAc) revealed complete consumption of starting material. The reaction mixture was poured into cold saturated $NaHCO_3$ (150 mL) and extracted with DCM (3 x 60 mL). The combined organic layers were then washed with saturated $CuSO_4$ (90 mL), brine (90 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude product. Purification by flash chromatography (gradient elution: 90:10 v/v hexane/EtOAc to 60:40 v/v Hexane/EtOAc) gave the pure bis-enol triflate (**221**) as a yellow foam (1.2 g, 50%): LC/MS 8.18 min (ES+) m/z (relative intensity) 1117 ($[M + H]^+$, 20); $[\alpha]_D^{21}$ = +281° (*c* = 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.33 (s, 2 H, H6), 7.26 (s, 2 H, H9), 7.13 (t, 2 H, *J* = 1.98 Hz, H3), 5.51 (d, 2 H, *J* = 10.05 Hz, SEM-N$\underline{CH_2}$O), 4.76 (d, 2 H, *J* = 10.05 Hz, SEM-N$\underline{CH_2}$O), 4.62 (dd, 2 H, *J* = 10.98 3.65 Hz, H11a), 4.32-4.23 (m, 4 H, Linker-O$\underline{CH_2}$), 3.94-3.92 (m, 2 H,

H1α), 3.88 (s, 6 H, CH_3O x 2), 3.81-3.64 (m, 4 H, SEM-O$\underline{CH_2}$), 2.76 (ddd, 2 H, J = 16.38, 11.04, 2.31 Hz, H1β), 2.43 (t, 2 H, J = 5.89 Hz, Linker-$\underline{CH_2}$), 0.92 (t, 4 H, J = 8.31 Hz, SEM-$\underline{CH_2}Si(CH_3)_3$), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 120.1 (C3), 113.1 (C6), 108.6 (C9), 79.8 (SEM-N$\underline{CH_2}$O), 68.7 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 57.9 (CH_3O x 2), 57.5 (C11a), 32.0 (C1), 30.2 (Linker-$\underline{CH_2}$), 19.7 (SEM-$\underline{CH_2}Si(CH_3)_3$), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2982, 1691 (C=O), 1642 (C=O), 1513, 1452, 1361, 1206, 1063, 1020, 832, 932, 755 cm^{-1}; MS (ESI) *m/z* (relative intensity): 1117 ([M + H]$^{+}$, 75%), 1089 ([M - 28]$^{+}$, 40%), 656 (100), 519 (50); HRMS: [M + H]$^{+}$ Theoretical, $C_{43}H_{54}O_{16}N_4Si_2S_2F_6$ m/z 1117.2491, found (ES^{+}) m/z 1117.2535.

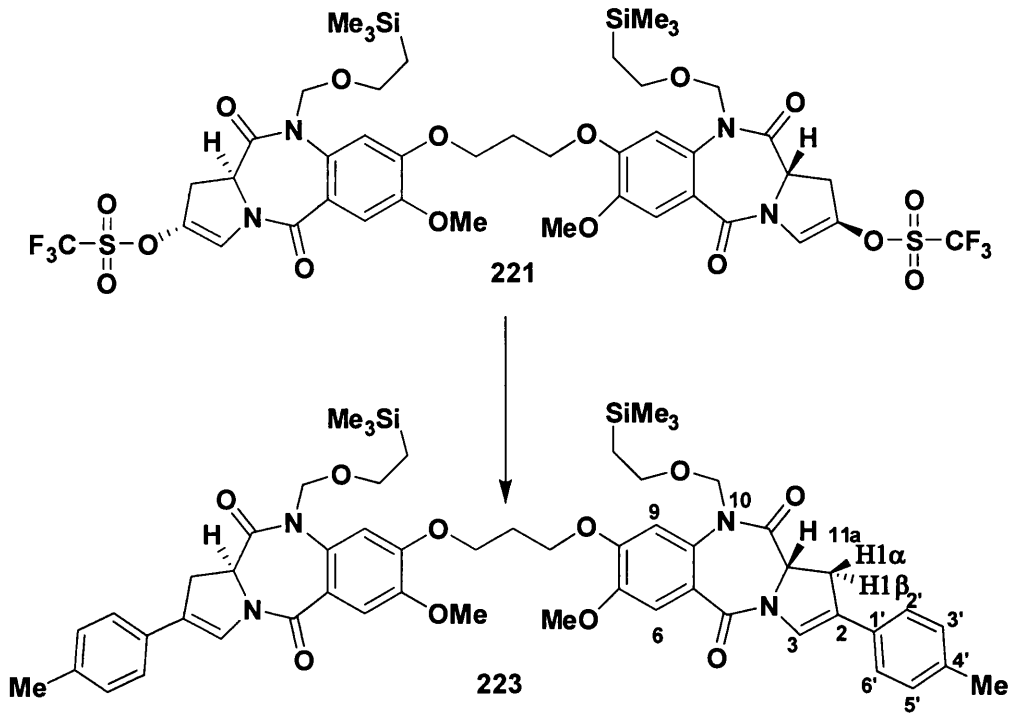
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(Phenyl)-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (222).



$Pd(PPh_3)_4$ (0.02 g, 0.018 mmol; 0.04 equiv.) was added to a stirred solution of the bis-enol triflate **221** (0.5 g, 0.45 mmol; 1.0 equiv.), phenylboronic acid (0.13 g, 1.035 mmol; 2.3 equiv.), Na_2CO_3 (0.27 g, 2.52 mmol; 5.6 equiv.), toluene (4.3 mL), ethanol (4.3 mL), water (2.3 mL). The reaction mixture was allowed to stir for 24 h at room temperature under nitrogen atmosphere after which point TLC (100% EtOAc) and LC/MS revealed complete consumption of starting material. The solvent was removed by evaporation in *vacuo* and the resulting residue redissolved in EtOAc (150 mL) and was washed with water (40 mL), brine (60 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude product. Purification by flash chromatography (gradient elution: 80:20 v/v hexane/EtOAc to 50:50 v/v hexane/EtOAc) afforded the pure bis-C2-phenyl (**222**) as a yellow foam (0.41 g, 93%): LC/MS 8.22 min (ES+) m/z (relative intensity) 973 ($[M + H]^{+\cdot}$, 80); $[\alpha]_D^{20}$ = +341° (*c* = 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.40 (d, 6 H, *J* = 7.96 Hz, H2'/H6' and H3), 7.37 (s, 2 H, H6), 7.31 (t, 2 H, *J* = 7.37 Hz, H4'), 7.23 (t, 2 H, *J* = 7.22 Hz, H3'/H5' and H9), 5.49 (d, 2 H, *J* = 10.05 Hz, SEM-N$\underline{CH_2}$O), 4.75 (d, 2 H, *J* = 10.05 Hz, SEM-N$\underline{CH_2}$O), 4.60 (dd, 2 H, *J* = 10.57, 3.35 Hz, H11a), 4.27 (t, 4 H, *J* = 5.86 Hz, Linker-O$\underline{CH_2}$), 3.90 (s, 6 H, CH_3O x 2), 3.80 (d, 2 H, *J* = 16.09 Hz,

H1α), 3.81-3.62 (m, 4 H, SEM-O$\underline{CH_2}$), 3.14 (ddd, 2 H, J = 16.20, 10.61, 1.98 Hz, H1β), 2.42 (t, 2 H, J = 5.84 Hz, Linker-$\underline{CH_2}$), 0.95 (t, 4 H, J = 7.35 Hz, SEM-$\underline{CH_2}Si(CH_3)_3$), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 129.9 (C2'/C6'), 128.9 (C4'), 126.5 (C3'/C5'), 123.1 (C3), 113.1 (C9), 108.6 (C6), 79.6 (SEM-N$\underline{CH_2}$O), 68.5 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 58.9 (C11a), 57.5 (CH_3O x 2), 32.6 (C1), 30.2 (Linker-$\underline{CH_2}$), 19.6 (SEM-$\underline{CH_2}Si(CH_3)_3$), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2949, 1686 (C=O), 1638 (C=O), 1514, 1451, 1357, 1268, 1064, 833, 754, 690 cm^{-1}; MS (ESI) *m/z* (relative intensity): 973 ($[M + H]^{+}$, 75%), 785 (100), 1000 (25), 693 (20), 490 (18); HRMS: $[M + H]^{+}$ Theoretical, $C_{53}H_{64}O_{10}N_4Si_2$ m/z 973.4234, found (ES^{+}) m/z 973.4280.

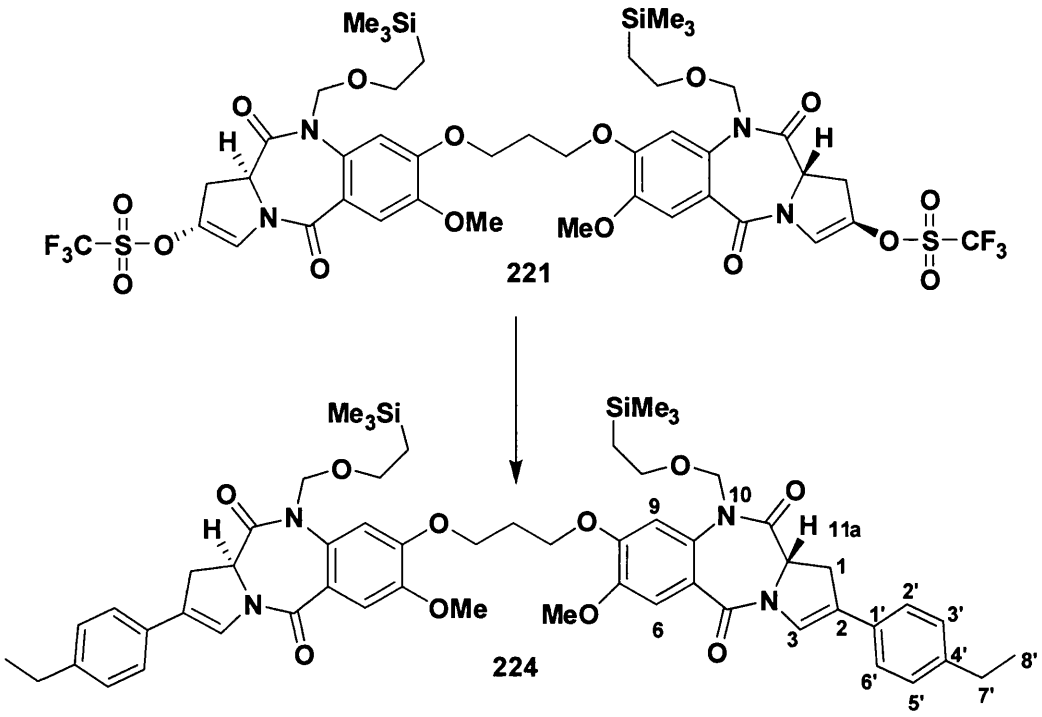
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(*p*-Methylbenzene)-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (223).



$Pd(PPh_3)_4$ (83 mg, 0.007 mmol; 0.04 equiv.) was added to a stirred solution of the bis-enoltriflate **221** (0.2 g, 0.179 mmol; 1.0 equiv.), *para*-methylbenzeneboronic acid (0.06 g, 0.412 mmol; 2.3 equiv.), Na_2CO_3 (0.11 g, 1.003 mmol; 5.6 equiv.), toluene (5 mL), ethanol (5 mL), water (2 mL). The reaction mixture was allowed to stir for 24 h at room temperature under nitrogen atmosphere after which point TLC (100% EtOAc) and LC/MS revealed complete consumption of starting material. The solvent was removed by evaporation in *vacuo* and the resulting residue redissolved in EtOAc (50 mL) and was washed with water (30 mL), brine (30 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude product. Purification by flash chromatography (gradient elution: 90:10 v/v hexane/EtOAc to 50:50 v/v hexane/EtOAc) afforded the pure bis-C2-methylbenzene (**223**) as a yellow foam (85 mg, 48%): LC/MS 8.75 min (ES+) m/z (relative intensity) 1001 ($[M + H]^+$, 60), 1023 ($[M + Na]^+$, 15); $[\alpha]_D^{24} = +352°$ (*c* = 0.85, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.37 (broad s, 4 H, H6 and H3), 7.30 (d, 4 H, *J* = 7.94 Hz, H2'/H6'), 7.25 (s, 2 H, H9), 7.13 (d, 4 H, *J* = 7.94 Hz, H3'/H5'), 5.49 (d, 2 H, *J* = 10.04 Hz, SEM-N$\underline{CH_2}$O), 4.75 (d, 2 H, *J* = 10.04 Hz, SEM-N$\underline{CH_2}$O), 4.59 (dd, 2 H, *J* = 10.47, 3.25 Hz, H11a), 4.27 (t, 4 H, *J* = 5.78 Hz, Linker-O$\underline{CH_2}$),

3.97-3.93 (m, 2 H, H1α), 3.89 (s, 6 H, CH_3O x 2), 3.81-3.62 (m, 4 H, SEM-O$\underline{CH_2}$), 3.11 (ddd, 2 H, J = 17.78, 10.65, 1.60 Hz, H1β), 2.42 (t, 2 H, J = 5.61 Hz, Linker-$\underline{CH_2}$), 2.32 (s, 6 H, 4'-CH_3 x 2), 0.95 (t, 4 H, J = 7.99 Hz, SEM-$\underline{CH_2}Si(CH_3)_3$), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 130.6 (C2'/C6'), 126.4 (C3'/C5'), 122.4 (C3), 113.1 (C6), 108.6 (C9), 79.6 (SEM-N$\underline{CH_2}$O), 68.4 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 58.8 (C11a), 57.5 (CH_3O x 2), 32.7 (C1), 30.2 (Linker-$\underline{CH_2}$), 22.5 (4'-CH_3 x 2), 19.6 (SEM-$\underline{CH_2}Si(CH_3)_3$), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2952, 1689 (C=O), 1643 (C=O), 1515, 1458, 1358, 1272, 1053, 1022, 829, 930, 755, 698 cm^{-1}; MS (ESI) *m/z* (relative intensity): 1117 ([M + H]$^{+}$, 75%), 1089 ([M - 28]$^{+}$, 40%), 656 (100), 519 (50); HRMS: [M + H]$^{+}$ Theoretical, $C_{55}H_{68}O_{10}N_4Si_2$ m/z 1001.4547, found (ES^{+}) m/z 1001.4537.

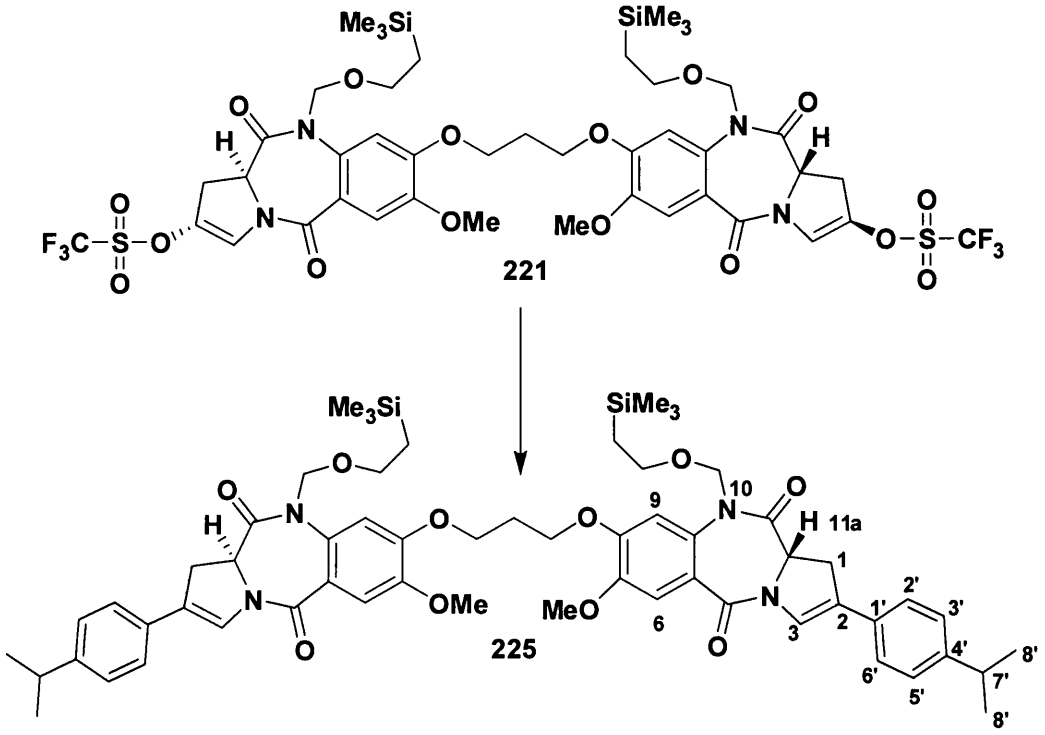
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(4-ethylphenyl)-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (224).



$Pd(PPh_3)_4$ (0.008 mg g, 0.007 mmol; 0.04 equiv.) was added to a stirred solution of the bis-enol triflate **221** (0.2 g, 0.179 mmol; 1.0 equiv.), 4-ethylphenylboronic acid (0.062 g, 0.4118 mmol; 2.3 equiv.), Na_2CO_3 (0.12 g, 1.003 mmol; 5.6 equiv.), toluene (2.0 mL), ethanol (2.0 mL), water (1 mL). The reaction mixture was allowed to stir for 24 h at room temperature under nitrogen atmosphere after which point TLC (100% EtOAc) and LC/MS revealed complete consumption of starting material. The solvent was removed by evaporation in *vacuo* and the resulting residue redissolved in EtOAc (50 mL) and was washed with water (15 mL), brine (20 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude product. Purification by flash chromatography (gradient elution: 90:10 v/v hexane/EtOAc to 50:50 v/v hexane/EtOAc) afforded the pure bis-C2-ethylphenyl (**224**) as a yellow foam (0.14 g, 72%): LC/MS 4.58 min (ES+) m/z (relative intensity) 1029 ($[M + H]^+$, 30); $[\alpha]_D^{23}$ = +314° (*c* = 1.3, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.38-7.37 (m, 4 H, H6 and H3), 7.33 (d, 4 H, *J* = 8.17 Hz, H2'/H6'), 7.25 (s, 2 H, H9), 7.14 (d, 4 H, *J* = 8.21 Hz, H3'/H5'), 5.49 (d, 2 H, *J* = 10.06 Hz, SEM-N$\underline{CH_2}$O), 4.76 (d, 2 H, *J* = 10.06 Hz, SEM-N$\underline{CH_2}$O), 4.58 (dd, 2 H, *J* = 10.56, 3.35 Hz, H11a), 4.28 (t, 4 H, *J* = 5.91 Hz, Linker-O$\underline{CH_2}$), 3.96-3.94 (m, 2 H, H1α), 3.89 (s, 6 H, CH_3O x 2), 3.81-3.62 (m,

4 H, SEM-O$\underline{CH_2}$), 3.11 (ddd, 2 H, J = 16.26, 10.62, 1.99 Hz, H1β), 2.61 (q, 4 H, J = 7.56 Hz, 2 x 7'-CH_2), 2.42 (t, 2 H, J = 5.84 Hz, Linker-$\underline{CH_2}$), 1.21 (t, 6 H, J = 7.52 Hz, 2 x 8'-CH_3), 0.95 (t, 4 H, J = 7.35 Hz, SEM-$\underline{CH_2}Si(CH_3)_3$), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 129.4 (C3'/C5'), 126.5 (C2'/C6'), 122.4 (C6), 113.1 (C3), 108.6 (C9), 79.6 (SEM-N$\underline{CH_2}$O), 68.4 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 58.8 (C11a), 57.5 (CH_3O x 2), 32.7 (C1), 30.2 (Linker-$\underline{CH_2}$), 29.9 (7'-CH_2 x 2), 19.6 (SEM-$\underline{CH_2}Si(CH_3)_3$), 16.8 (8'-$CH_3$ x 2), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2959 , 1689 (C=O), 1638 (C=O), 1604, 1514, 1453, 1431, 1357, 1269, 1208, 1140, 1100, 1062, 1021, 832, 751, 688 cm^{-1}; MS (ESI) *m/z* (relative intensity): 1029 ([M + H]$^+$, 27%), 785 (40), 743 (30), 491 (100), 417 (18); HRMS: [M + H]$^+$ Theoretical, $C_{57}H_{72}O_{10}N_4Si_2$ m/z 1029.4860, found (ES$^+$) m/z 1029.4891.

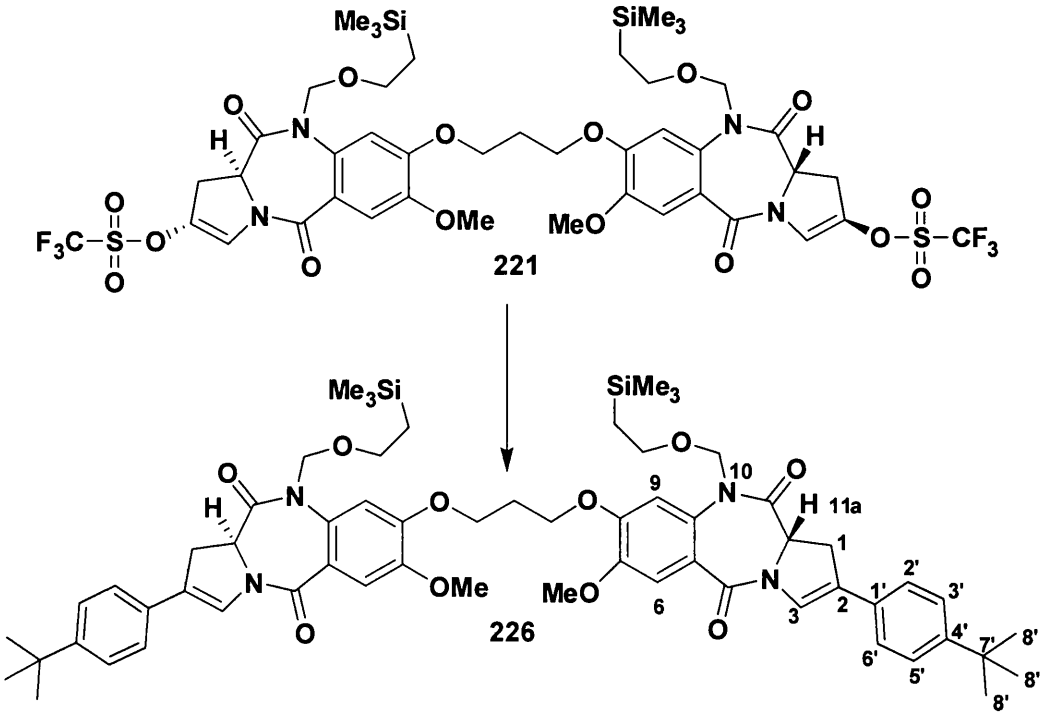
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(4-*iso*-propylphenyl)-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (225).



$Pd(PPh_3)_4$ (83 mg g, 0.007 mmol; 0.04 equiv.) was added to a stirred solution of the bis-enoltriflate **221** (0.2 g, 0.179 mmol; 1.0 equiv.), 4-*iso*-propylphenylboronic acid (0.07 g, 0.412 mmol; 2.3 equiv.), Na_2CO_3 (0.12 g, 1.003 mmol; 5.6 equiv.), toluene (2.0 mL), ethanol (2.0 mL), water (1 mL). The reaction mixture was allowed to stir for 24 h at room temperature under nitrogen atmosphere after which point TLC (100% EtOAc) and LC/MS revealed complete consumption of starting material. The solvent was removed by evaporation in *vacuo* and the resulting residue redissolved in EtOAc (50 mL) and was washed with water (15 mL), brine (20 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude product. Purification by flash chromatography (gradient elution: 90:10 v/v hexane/EtOAc to 40:60 v/v hexane/EtOAc) afforded the pure bis-C2-*iso*-propylphenyl (**225**) as a yellow foam (0.151 g, 80%): LC/MS 3.60 min (ES-) m/z (relative intensity) 1055 ($[M - H]^+$, 45); $[\alpha]_D^{25}$ = +293° (*c* = 1.47, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.39-7.37 (m, 4 H, H6 and H3), 7.34 (d, 4 H, *J* = 8.24 Hz, H2'/H6'), 7.26 (s, 2 H, H9), 7.17 (d, 4 H, *J* = 8.29 Hz, H3'/H5'), 5.50 (d, 2 H, *J* = 10.067 Hz, SEM-N$\underline{CH_2}$O), 4.77 (d, 2 H, *J* = 10.07 Hz, SEM-N$\underline{CH_2}$O), 4.59 (dd, 2 H, *J* = 10.54,

3.33 Hz, H11a), 4.29 (t, 4 H, J = 5.88 Hz, Linker-O$\underline{CH_2}$), 3.97-3.94 (m, 2 H, H1α), 3.89 (s, 6 H, CH_3O x 2), 3.82-3.62 (m, 4 H, SEM-O$\underline{CH_2}$), 3.12 (ddd, 2 H, J = 16.26, 10.60, 1.94 Hz, H1β), 2.92-2.83 (m, 2 H, 2 x 7'-CH), 2.43 (t, 2 H, J = 5.81 Hz, Linker-$\underline{CH_2}$), 1.23 (d, 12 H, J = 6.92 Hz, 2 x 8'-$(CH_3)_2$), 0.96 (t, 4 H, J = 8.31 Hz, SEM-$\underline{CH_2}Si(CH_3)_3$), 0.01 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 128.0 (C3'/C5'), 126.5 (C2'/C6'), 122.4 (C6), 113.1 (C3), 108.6 (C9), 79.5 (SEM-N$\underline{CH_2}$O), 68.3 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 58.8 (C11a), 57.5 (CH_3O x 2), 35.3 (2 x 7'-CH), 32.7 (C1), 30.2 (Linker-$\underline{CH_2}$), 25.2 (2 x 8'-$(CH_3)_2$), 19.6 (SEM-$\underline{CH_2}Si(CH_3)_3$), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2959, 1687 (C=O), 1638 (C=O), 1604, 1514, 1453, 1431, 1357, 12698, 1243, 1207, 1141, 1100, 1054, 1018, 832, 752, 680 cm^{-1}; MS (ESI) *m/z* (relative intensity): 1057 ([M + H]$^{+}$, 28%), 813 (20), 785 (70), 665(40), 371 (52); HRMS: [M + H]$^{+}$ Theoretical, $C_{59}H_{76}O_{10}N_4Si_2$ m/z 1057.5173, found (ES^{+}) m/z 1057.5200.

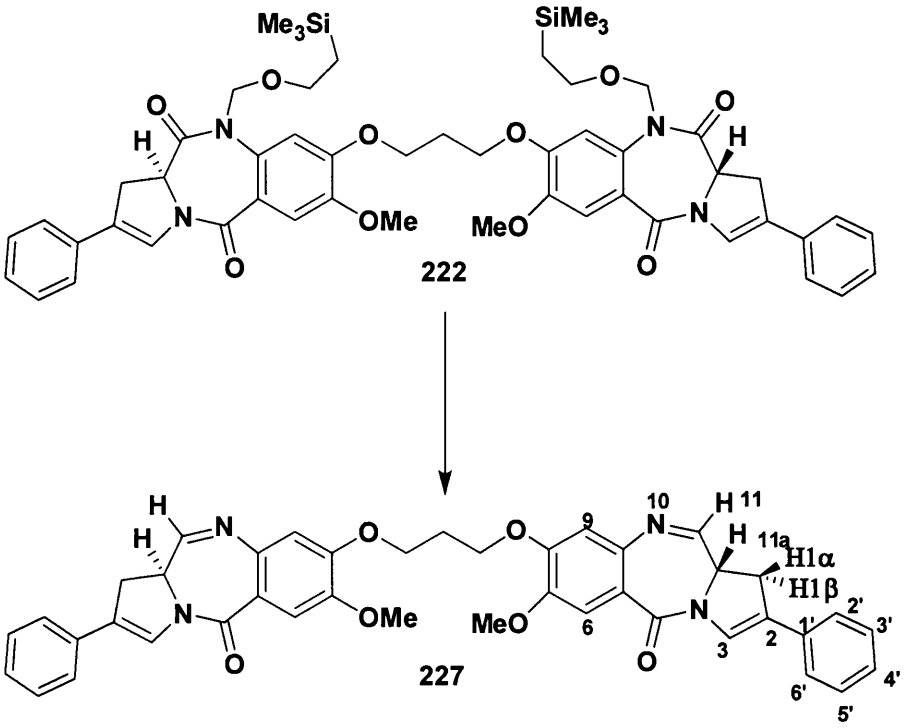
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(4-*tert*-ButylPhenyl)-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (226).



$Pd(PPh_3)_4$ (80 mg, 0.007 mmol; 0.04 equiv.) was added to a stirred solution of the bis-enoltriflate **221** (0.183 g, 0.164 mmol; 1.0 equiv.), 4-*tert*-butylphenylboronic acid (0.07 g, 0.38 mmol; 2.3 equiv.), Na_2CO_3 (0.11 g, 0.92 mmol; 5.6 equiv.), toluene (2.0 mL), ethanol (2.0 mL), water (1 mL). The reaction mixture was allowed to stir for 24 h at room temperature under nitrogen atmosphere after which point TLC (100% EtOAc) and LC/MS revealed complete consumption of starting material. The solvent was removed by evaporation in *vacuo* and the resulting residue redissolved in EtOAc (50 mL) and was washed with water (15 mL), brine (20 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude product. Purification by flash chromatography (gradient elution: 90:10 v/v hexane/EtOAc to 40:60 v/v hexane/EtOAc) afforded the pure bis-C2-*tert*-butylphenyl (**226**) as a yellow foam (0.149 g, 83%): LC/MS 3.60 min (ES+) m/z (relative intensity) 1085 ($[M + H]^{+\cdot}$, 80); $[\alpha]_D^{24}$ = +285° (*c* = 1.47, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.39-7.37 (m, 4 H, H6 and H3), 7.35-7.29 (m, 4 H, H2'/H6' and H3'/H5'), 7.26 (s, 2 H, H9), 5.50 (d, 2 H, *J* = 10.067 Hz, SEM-N$\underline{CH_2}$O), 4.77 (d, 2 H, *J* = 10.07 Hz, SEM-N$\underline{CH_2}$O), 4.59 (dd, 2 H, *J* = 10.54, 3.33 Hz, H11a), 4.29 (t, 4 H, *J* = 5.89 Hz, Linker-O$\underline{CH_2}$), 3.97-3.94 (m, 2 H, H1α), 3.89 (s, 6 H, CH_3O x 2), 3.83-3.62 (m, 4

H, SEM-O$\underline{CH_2}$), 3.12 (ddd, 2 H, J = 16.29, 10.62, 1.94 Hz, H1β), 2.43 (t, 2 H, J = 5.82 Hz, Linker-$\underline{CH_2}$), 1.22 (s, 18 H, 2 x 8'-$(CH_3)_3$), 0.96 (t, 4 H, J = 8.28 Hz, SEM-$\underline{CH_2}Si(CH_3)_3$), 0.02 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 126.8 (C3'/C5'), 126.3 (C2'/C6'), 122.5 (C6), 113.1 (C3), 108.6 (C9), 79.5 (SEM-N$\underline{CH_2}$O), 68.3 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 58.8 (C11a), 57.5 (CH_3O x 2), 32.6 (C1), 32.5 (2 x 8'-$(CH_3)_3$), 30.2 (Linker-$\underline{CH_2}$), 19.6 (SEM-$\underline{CH_2}Si(CH_3)_3$), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2925, 1686 (C=O), 1637 (C=O), 1599, 1452, 1431, 1405, 1357, 1269, 1243, 1204, 1139, 1098, 1063, 1018, 930, 855, 833, 809, 752, 664 cm^{-1}; MS (ESI) *m/z* (relative intensity): 1085 ([M + H]$^+$, 60%), 785 (40), 929 (35), 785 (75), 491 (100); HRMS: [M + H]$^+$ Theoretical, $C_{61}H_{80}O_{10}N_4Si_2$ m/z 1085.5486, found (ES$^+$) m/z 1085.5446.

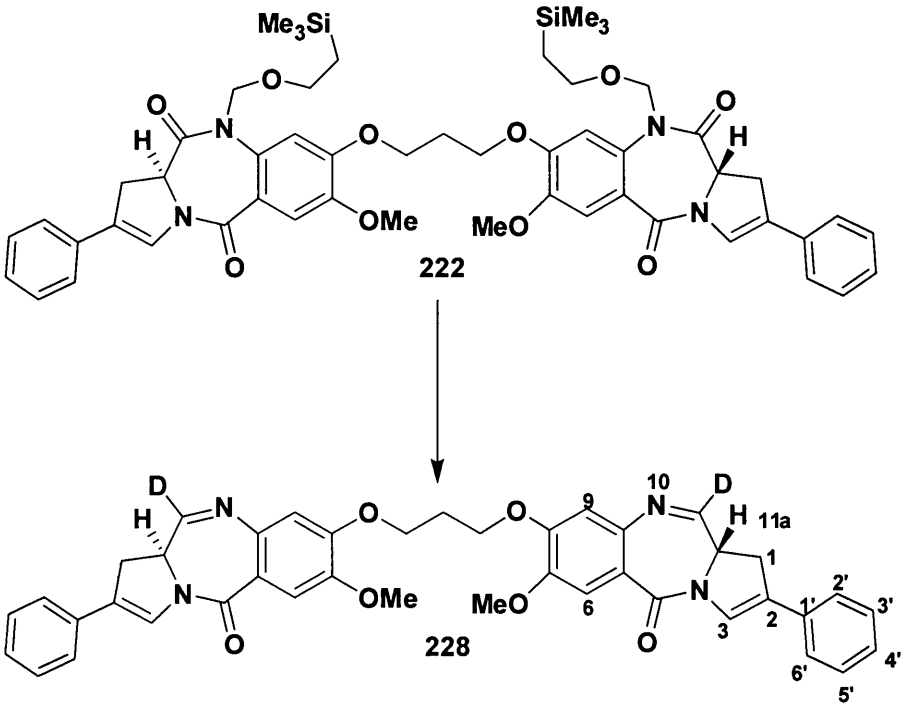
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(phenyl)-1,11a-dihydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-one]] (227).



$LiBH_4$ (0.12 g, 4.09 mmol; 20.0 equiv.) was added to a stirred solution of the bis-N10-SEM tetralactam **222** (0.20 g, 0.20 mmol; 1.0 equiv.) in THF (12 mL) and ethanol (6 mL) and was allowed to stir for 3 h at room temperature under nitrogen atmosphere. The solvent was removed by evaporation in *vacuo* and the resulting residue was treated with water (70 mL) and was extracted with DCM (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL) and evaporated *in vacuo* to afford the SEM-carbinolamine intermediate. This was immediately dissolved in ethanol (25 mL) and water (12 mL) and treated with flash silica gel (~ 5 g). The reaction was allowed to stir at room temperature for 3 h after which time the formation of a large quantity of product was confirmed by TLC (5% MeOH/$CHCl_3$). The reaction mixture was filtered through a sinter funnel and rinsed a couple of times with EtOAc (2 x 40 mL). The organic filtrate layer was separated and the aqueous phase diluted with water (70 mL) and washed with EtOAc (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by flash chromatography (gradient elution: 100% $CHCl_3$ to 98:2 v/v $CHCl_3$/MeOH) afforded the pure bis-C2-phenyl imine (**226**) as an yellow foam (81 mg, 58%): LC/MS 8.22 min (ES+) m/z (relative intensity) 681 ($[M + H]^{+\cdot}$,

60); $[\alpha]_D^{24}$ = +740° (*c* = 0.81, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.86 (d, 2 H, *J* = 3.95 Hz, H11), 7.51 (s, 2 H, H6), 7.48 (s, 2 H, H3), 7.39-7.31 (m, 10 H, C2-Phenyl aromatic), 6.86 (s, 2 H, H9), 4.36 (ddd, 2 H, *J* = 11.34, 5.15, 2.28 Hz, H11a), 4.32-4.26 (m, 4 H, Linker-O$\underline{CH_2}$), 3.92 (s, 6 H, CH_3O x 2), 3.56 (ddd, 2 H, *J* = 16.31, 11.58, 1.92 Hz, H1β), 3.37 (ddd, 2 H, *J* = 16.39, 11.71, 1.65 Hz, H1α), 2.41 (t, 2 H, *J* = 6.05 Hz, Linker-$\underline{CH_2}$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 162.5 (C11), 128.9 (C2'/C6'), 127.5 (C4'), 124.8 (C3'/C5'), 123.6 (C3), 111.9 (C6), 111.2 (C9), 65.4 (Linker-O$\underline{CH_2}$), 56.2 (CH_3O x 2), 53.9 (C11a), 35.4 (C1), 28.9 (Linker-$\underline{CH_2}$); IR (Golden gate): 2873, 1626 (C=O), 1599 (C=O), 1501, 1426, 1263, 1204, 1054, 829, 754, 685 cm^{-1}; MS (ESI) *m/z* (relative intensity): 681 ($[M + H]^+$, 20%), 553 (10), 401 (25), 323 (100), 274 (15); HRMS: $[M + H]^+$ Theoretical, $C_{41}H_{36}O_6N_4$ m/z 681.2711, found (ES^+) m/z 681.2708.

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(phenyl)-1,11a-dihydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-one]] (228, H11-deuteriated).

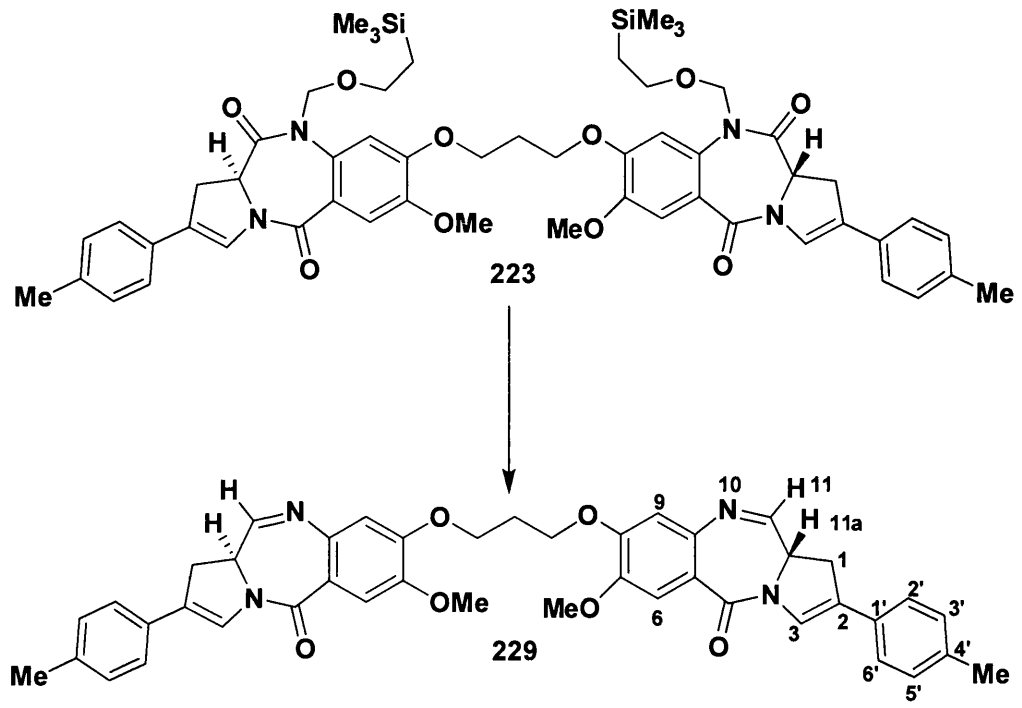


D = Deuteriated

$NaBD_4$ (0.1 g, 4.12 mmol; 20.0 equiv.) was added to a stirred solution of the bis-N10-SEM tetralactam **222** (0.20 g, 0.21 mmol; 1.0 equiv.) in THF (12 mL) and ethanol (6 mL) and was allowed to stir for 8 h at room temperature under nitrogen atmosphere. The solvent was removed by evaporation in *vacuo* and the resulting residue was treated with water (70 mL) and was extracted with DCM (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL) and evaporated *in vacuo* to afford the SEM-carbinolamine intermediate. This was immediately dissolved in ethanol (25 mL) and water (12 mL) and treated with flash silica gel (~ 4 g). The reaction was allowed to stir at room temperature for 24 h after which time the formation of a large quantity of product was observed by TLC (5% $MeOH/CHCl_3$). The reaction mixture was filtered through a sinter funnel and rinsed a couple of times with EtOAc (2 x 40 mL). The organic filtrate layer was separated and the aqueous phase diluted with water (70 mL) and washed with EtOAc (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by flash chromatography (gradient elution: 100% $CHCl_3$ to 98:2 v/v $CHCl_3$/MeOH) afforded the pure bis-C2-phenyl deuteriated imine

(**228**) as an yellow foam (41 mg, 38%): LC/MS 3.10 min (ES+) m/z (relative intensity) 683 ([M + H]$^{+\cdot}$, 80), 705 ([M + Na]$^{+\cdot}$, 38); $[\alpha]_D^{25}$ = +700° (*c* = 0.47, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.52 (s, 2 H, H6), 7.50 (s, 2 H, H3), 7.41-7.32 (m, 10 H, C2-Phenyl aromatic), 6.87 (s, 2 H, H9), 4.38 (dd, 2 H, *J* = 11.52, 5.25 Hz, H11a), 4.33-4.26 (m, 4 H, Linker-O$\underline{CH_2}$), 3.92 (s, 6 H, CH_3O x 2), 3.58 (ddd, 2 H, *J* = 16.35, 11.62, 1.97 Hz, H1β), 3.37 (ddd, 2 H, *J* = 16.36, 11.99, 1.66 Hz, H1α), 2.41 (t, 2 H, *J* = 6.06 Hz, Linker-$\underline{CH_2}$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 128.7 (C2'/C6'), 127.5 (C4'), 124.8 (C3'/C5'), 123.6 (C3), 111.9 (C6), 111.2 (C9), 65.4 (Linker-O$\underline{CH_2}$), 56.2 (CH_3O x 2), 53.9 (C11a), 35.4 (C1), 28.9 (Linker-$\underline{CH_2}$); IR (Golden gate): 2919, 1624 (C=O), 1595 (C=O), 1503, 1423, 1263, 1341, 1262, 1210, 1183, 1054, 834, 791, 665 cm^{-1}; MS (ESI) *m/z* (relative intensity): 683 ([*M* + H]$^{+\cdot}$, 100%), 785 (30), 548 (10), 408 (50), 324 (46), 256 (17); HRMS: [*M* + H]$^{+\cdot}$ Theoretical, $C_{41}H_{34}D_2O_6N_4$ m/z 683.2833, found (ES$^+$) m/z 683.2820.

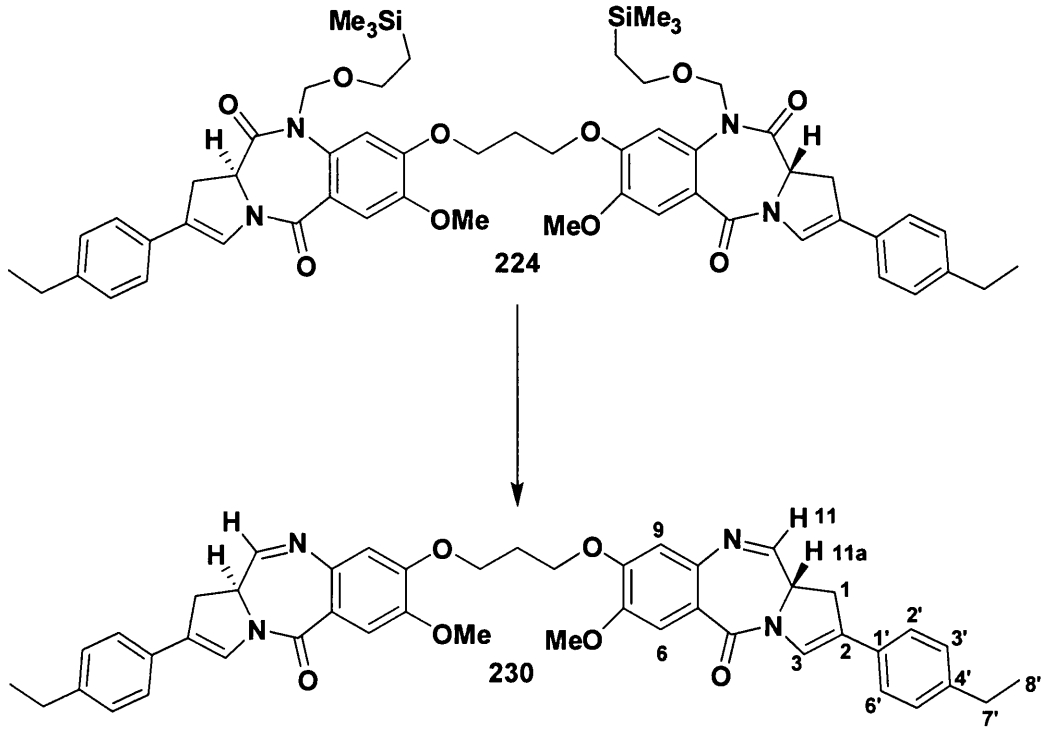
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(*p*-methylbenzene)-1,11a-dihydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-one]] (229).



$LiBH_4$ (0.04 g, 1.66 mmol; 20.0 equiv.) was added to a stirred solution of the bis-methylbenzene **223** (83 mg, 0.083 mmol; 1.0 equiv.) in THF (5 mL) and ethanol (2.5 mL). The reaction mixture was allowed to stir for 3 h at room temperature under nitrogen atmosphere after which point LC-MS revealed complete conversion of starting material to the electrophillic N10/C11 imine (LC/MS 6.68 min (ES+) m/z (relative intensity) 709 ($[M + H]^{+\cdot}$, 45), 731 ($[M + Na]^{+\cdot}$, 75). The solvent was removed by evaporation in *vacuo* and the resulting residue was treated with water (30 mL) and was extracted with DCM (3 x 15 mL). The combined organic layers were washed with water (10 mL), brine (20 mL) and evaporated *in vacuo* to afford the SEM-carbinolamine intermediate. This was immediately dissolved in ethanol (10 mL) and water (5 mL) and treated with flash silica gel (~ 4 g). The reaction was allowed to stir at room temperature for 24 h at after which time formation of product was observed by TLC (5% $MeOH/CHCl_3$). The reaction mixture was filtered through a sinter funnel and rinsed a couple of times with EtOAc (2 x 40 mL). The organic filtrate layer was separated and the aqueous phase diluted with water (40 mL) and washed with EtOAc (3 x 15 mL). The combined organic layers were washed with water (20 mL), brine (30 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by flash chromatography (gradient elution:

100% $CHCl_3$ to 98:2 v/v MeOH/$CHCl_3$) afforded the pure bis-C2-methylphenyl imine (**229**), after repeated cycles of $CHCl_3$ evaporation, as an yellow foam (30mg, 52%): LC/MS 6.70 min (ES+) m/z (relative intensity) 709 ($[M + H]^{+\cdot}$, 45), 731 ($[M + Na]^{+\cdot}$, 75); $[\alpha]_D^{16}$ = +644° (*c* = 0.32, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.87 (d, 2 H, *J* = 3.94 Hz, H11) 7.52 (s, 2 H, H6), 7.45 (s, 2 H, H3), 7.28 (d, 4 H, *J* = 8.05 Hz, H3'/H5'), 7.15 (d, 4 H, *J* = 8.04 Hz, H2'/H6'), 6.86 (s, 2 H, H9), 4.42-4.18 (m, 6 H, Linker-O$\underline{CH_2}$ and H11a), 3.93 (s, 6 H, CH_3O x 2), 3.11 (ddd, 2 H, *J* = 16.33, 11.50, 1.91 Hz, H1β), 3.93 (dd, 2 H, *J* = 15.63, 4.46, Hz, H1α), 2.43 (t, 2 H, *J* = 5.92 Hz, Linker-$\underline{CH_2}$), 2.34 (s, 6 H, CH_3 x 2); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 162.6 (C11), 124.8 (C2'/C6'), 124.4 (C3'/C5'), 122.8 (C3), 113.3 (C6), 112.8 (C9), 65.4 (Linker-O$\underline{CH_2}$), 56.2 (CH_3O x 2), 53.8 (C11a), 35.5 (C1), 28.9 (Linker-$\underline{CH_2}$), 21.2 (CH_3 x 2); IR (Golden gate): 2919, 1625 (C=O), 1599 (C=O), 1506, 1454, 1427, 1245, 1207, 1173, 1093, 1050, 810, 935, 832, 810, 752, 690 cm^{-1}; MS (ESI) *m/z* (relative intensity): 709 ($[M + H]^{+}$, 95%), 789 (80), 743 (45), 560 (38), 324 (100) HRMS: $[M + H]^{+}$ Theoretical, $C_{43}H_{40}O_6N_4$ m/z 709.3049, found (ES^+) m/z 709.3021.

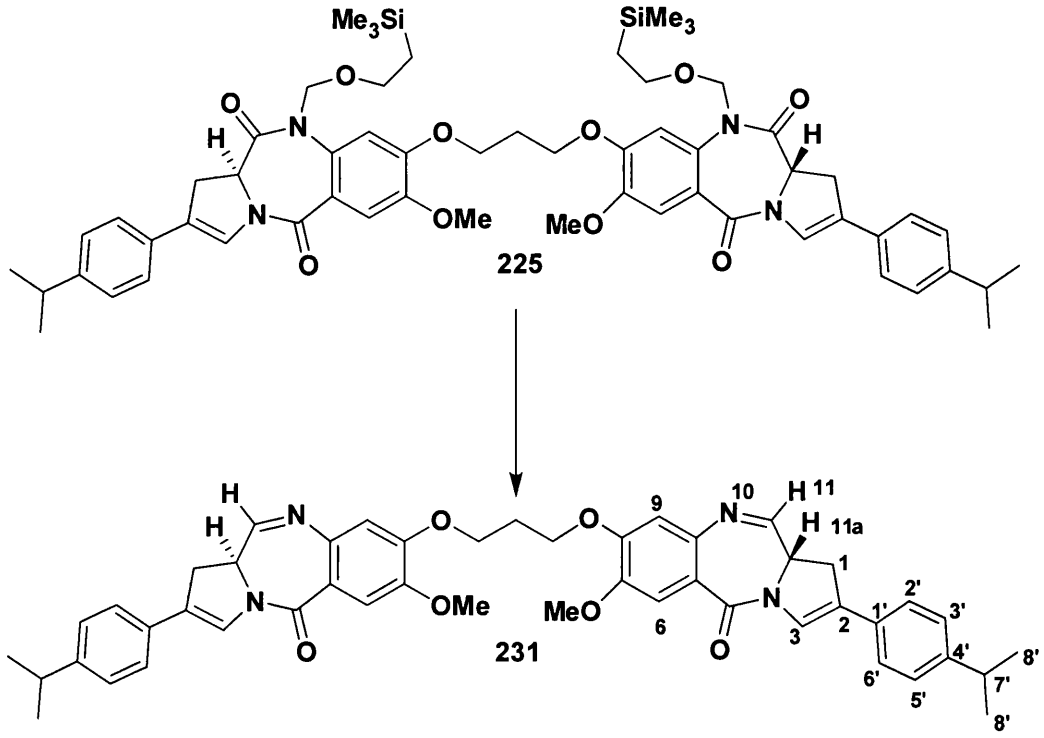
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(4-ethylphenyl)-1,11a-dihydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-one]] (230).



$LiBH_4$ (0.06 g, 2.53 mmol; 20.0 equiv.) was added to a stirred solution of the bis-N10-SEM tetralactam **224** (0.13 g, 0.13 mmol; 1.0 equiv.) in THF (6 mL) and ethanol (3 mL) and was allowed to stir for 3 h at room temperature under nitrogen atmosphere. The solvent was removed by evaporation in *vacuo* and the resulting residue was treated with water (70 mL) and was extracted with DCM (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL) and evaporated *in vacuo* to afford the SEM-carbinolamine intermediate. This was immediately dissolved in ethanol (13 mL) and water (6 mL) and treated with flash silica gel (~ 5 g). The reaction was allowed to stir at room temperature for 24 h after which time the formation of a large quantity of product was confirmed by TLC (5% MeOH/$CHCl_3$). The reaction mixture was filtered through a sinter funnel and rinsed a couple of times with EtOAc (2 x 40 mL). The organic filtrate layer was separated and the aqueous phase diluted with water (70 mL) and washed with EtOAc (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by flash chromatography (gradient elution: 100% $CHCl_3$ to 98:2 v/v $CHCl_3$/MeOH) afforded the pure bis-C2-ethylphenyl imine (**230**) as a

yellow foam (74 mg, 77%): LC/MS 7.47 min (ES+) m/z (relative intensity) 736 ($[M + H]^+$, 100); $[\alpha]_D^{22}$ = +620° (*c* = 0.74, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.85 (d, 2 H, *J* = 3.95 Hz, H11), 7.52 (s, 2 H, H6), 7.45 (s, 2 H, H3), 7.31 (d, 4 H, *J* = 8.09 Hz, H2'/H6'), 7.17 (d, 4 H, *J* = 8.05 Hz, H3'/H5'), 6.86 (s, 2 H, H9), 4.42-4.20 (m, 6 H, H11a and Linker-O$\underline{CH_2}$), 3.92 (s, 6 H, CH_3O x 2), 3.55 (ddd, 2 H, *J* = 16.34, 11.57, 1.55 Hz, H1β), 3.41-3.33 (m, 2 H, H1α), 2.63 (q, 4 H, *J* = 7.54 Hz, 2 x 7'-CH_2), 2.42 (t, 2 H, *J* = 5.95 Hz, Linker-$\underline{CH_2}$), 1.23 (t, 6 H, *J* = 7.62 Hz, 2 x 8'-CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 162.5 (C11), 128.2 (C3'/C5'), 124.9 (C2'/C6'), 123.4 (C3), 111.9 (C6), 111.2 (C9), 65.4 (Linker-O$\underline{CH_2}$), 56.1 (CH_3O x 2), 53.8 (C11a), 35.4 (C1), 28.8 (Linker-$\underline{CH_2}$), 28.6 (7'-CH_2 x 2), 15.4 (8'-CH_3 x 2); IR (Golden gate): 2959 , 1625 (C=O), 1596 (C=O), 1506, 1454, 1425, 1382, 1262, 1209, 1100, 1049, 1000, 821, 780, 680 cm^{-1}; MS (ESI) *m/z* (relative intensity): 737 ($[M + H]^+$, 100%), 785 (30), 516 (15), 6435 (28), 340 (8); HRMS: $[M + H]^+$ Theoretical, $C_{45}H_{44}O_6N_4$ m/z 737.3334, found (ES^+) m/z 737.3348.

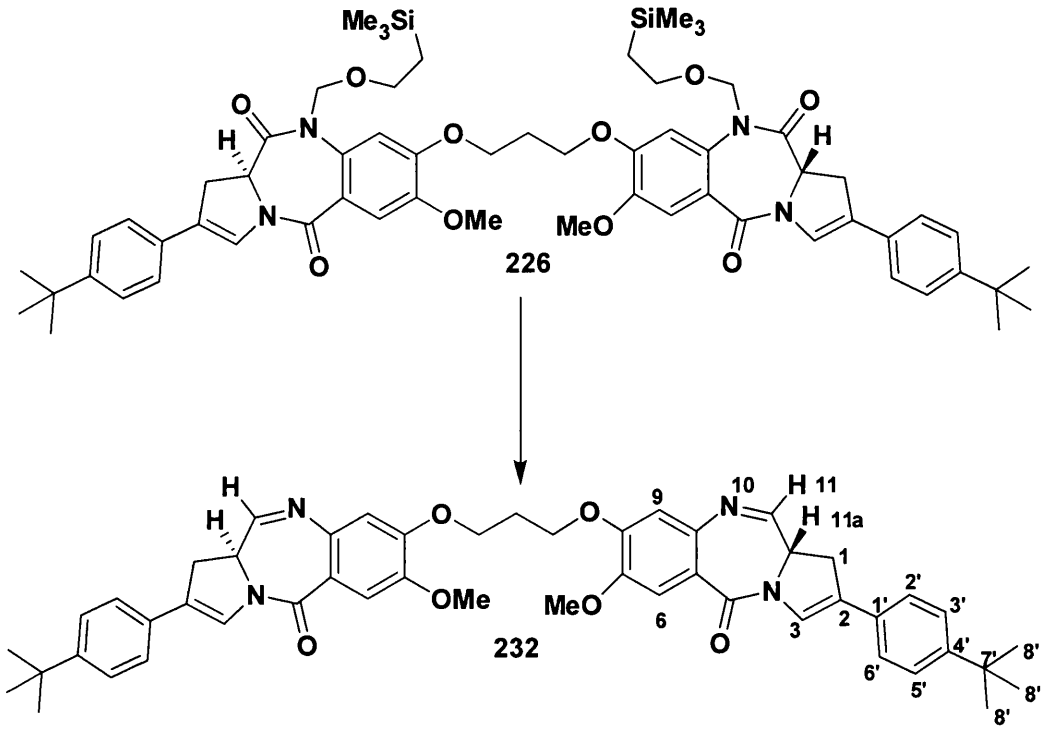
Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(4-*iso*-propylphenyl)-1,11a-dihydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-one]] (231).



$LiBH_4$ (0.11 g, 2.65 mmol; 20.0 equiv.) was added to a stirred solution of the bis-N10-SEM tetralactam **225** (140 mg, 0.13 mmol; 1.0 equiv.) in THF (6 mL) and ethanol (3 mL) and was allowed to stir for 3 h at room temperature under nitrogen atmosphere. The solvent was removed by evaporation in *vacuo* and the resulting residue was treated with water (70 mL) and was extracted with DCM (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL) and evaporated *in vacuo* to afford the SEM-carbinolamine intermediate. This was immediately dissolved in ethanol (13 mL) and water (6 mL) and treated with flash silica gel (~ 5 g). The reaction was allowed to stir at room temperature for 24 h after which time the formation of a large quantity of product was confirmed by TLC (5% $MeOH/CHCl_3$). The reaction mixture was filtered through a sinter funnel and rinsed a couple of times with EtOAc (2 x 40 mL). The organic filtrate layer was separated and the aqueous phase diluted with water (70 mL) and washed with EtOAc (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by flash chromatography (gradient elution: 100% $CHCl_3$ to 98:2 v/v $CHCl_3$/MeOH) afforded the pure bis-C2-*iso*-propylphenyl imine (**231**)

as a yellow foam (55 mg, 54%): LC/MS 7.72 min (ES+) m/z (relative intensity) 765 ($[M + H]^{+\cdot}$, 100); $[\alpha]_D^{23}$ = +618° (*c* = 0.55, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.86 (d, 2 H, *J* = 3.92 Hz, H11), 7.52 (s, 2 H, H6), 7.45 (s, 2 H, H3), 7.32 (d, 4 H, *J* = 8.17 Hz, H2'/H6'), 7.21 (d, 4 H, *J* = 8.14 Hz, H3'/H5'), 6.87 (s, 2 H, H9), 4.41-4.25 (m, 6 H, H11a and Linker-O$\underline{CH_2}$), 3.93 (s, 6 H, CH_3O x 2), 3.57 (ddd, 2 H, *J* = 16.32, 11.64, 1.64 Hz, H1β), 3.42-3.34 (m, 2 H, H1α), 2.94-2.84 (m, 2 H, 2 x 7'-CH), 2.42 (t, 2 H, *J* = 5.85 Hz, Linker-$\underline{CH_2}$), 1.25 (d, 12 H, *J* = 6.86 Hz, 2 x 8'-$(CH_3)_2$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 163.9 (C11), 128.2 (C3'/C5'), 126.3 (C2'/C6'), 124.2 (C3), 113.3 (C6), 112.6 (C9), 66.9 (Linker-O$\underline{CH_2}$), 57.5 (CH_3O x 2), 55.2 (C11a), 36.8 (C1), 35.2 (2 x 7'-CH), 30.2 (Linker-$\underline{CH_2}$), 25.2 (2 x 8'-$(CH_3)_2$); IR (Golden gate): 2897, 1687 (C=O), 1641 (C=O), 1597, 1516, 1452, 1431, 1356, 1263, 1243, 1201, 1067, 1100, 1023, 825, 762, 644 cm^{-1}; MS (ESI) *m/z* (relative intensity): 765 ($[M + H]^{+\cdot}$, 37%), 785 (100), 734 (8), 576 (7), 449 (6); HRMS: $[M + H]^{+\cdot}$ Theoretical, $C_{47}H_{48}O_6N_4$ m/z 765.3647, found (ES^+) m/z 765.3679.

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(4-*tert*-butylphenyl)-1,11a-dihydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-one]] (232).

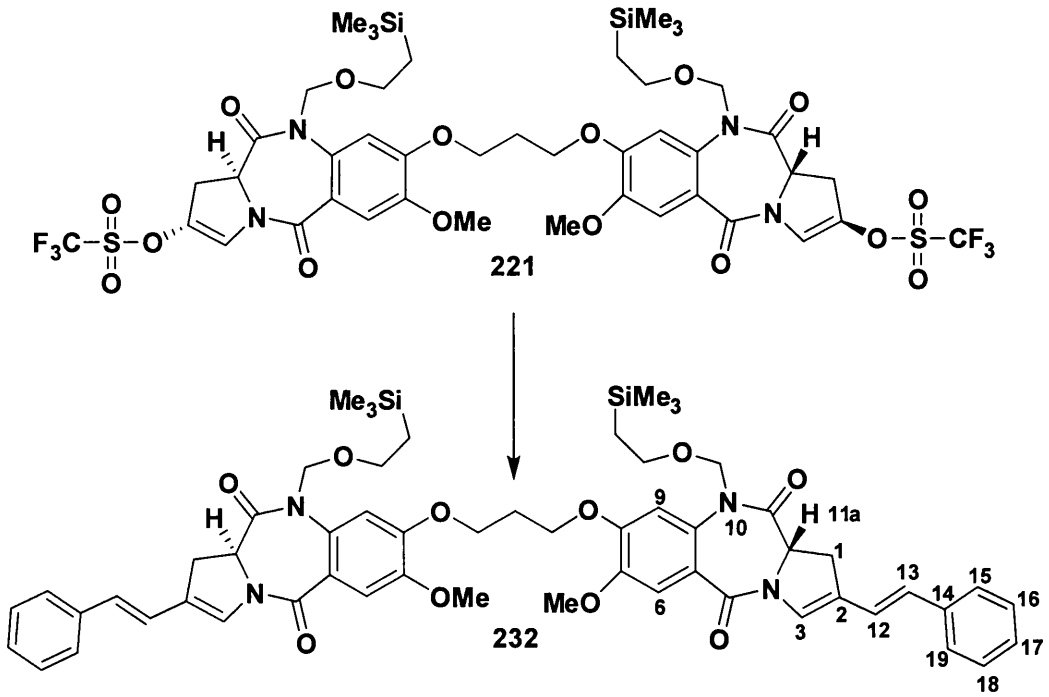


$LiBH_4$ (0.06 g, 2.69 mmol; 20.0 equiv.) was added to a stirred solution of the bis-N10-SEM tetralactam **226** (146 mg, 0.14 mmol; 1.0 equiv.) in THF (6 mL) and ethanol (3 mL) and was allowed to stir for 3 h at room temperature under nitrogen atmosphere. The solvent was removed by evaporation in *vacuo* and the resulting residue was treated with water (70 mL) and was extracted with DCM (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL) and evaporated *in vacuo* to afford the SEM-carbinolamine intermediate. This was immediately dissolved in ethanol (13 mL) and water (6 mL) and treated with flash silica gel (~ 5 g). The reaction was allowed to stir at room temperature for 24 h after which time the formation of a large quantity of product was confirmed by TLC (5% MeOH/$CHCl_3$). The reaction mixture was filtered through a sinter funnel and rinsed a couple of times with EtOAc (2 x 40 mL). The organic filtrate layer was separated and the aqueous phase diluted with water (70 mL) and washed with EtOAc (3 x 35 mL). The combined organic layers were washed with water (20 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by flash chromatography (gradient elution: 100% $CHCl_3$ to 98:2 v/v $CHCl_3$/MeOH) afforded the pure bis-C2-*tert*-butylphenyl (**232**) as a yellow foam (60 mg, 55%): LC/MS 7.87 min (ES+) m/z (relative intensity) 793 ($[M + H]^+$,

95); $[\alpha]_D^{24}$ = +625° (*c* = 0.6, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.87 (d, 2 H, *J* = 3.95 Hz, H11), 7.52 (s, 2 H, H6), 7.47 (s, 2 H, H3), 7.38 (d, 4 H, *J* = 8.17 Hz, H2'/H6'), 7.36 (d, 4 H, *J* = 8.14 Hz, H3'/H5'), 6.87 (s, 2 H, H9), 4.42-4.25 (m, 6 H, H11a and Linker-O$\underline{CH_2}$), 3.94 (s, 6 H, CH_3O x 2), 3.59 (ddd, 2 H, *J* = 16.32, 11.55, 1.77 Hz, H1β), 3.43-3.36 (m, 2 H, H1α), 2.44 (t, 2 H, *J* = 6.03 Hz, Linker-$\underline{CH_2}$), 1.33 (s, 18 H, 2 x 8'-$(CH_3)_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 162.5 (C11), 125.7 (C3'/C5'), 124.6 (C2'/C6'), 122.9 (C3), 111.9 (C6), 111.2 (C9), 65.4 (Linker-O$\underline{CH_2}$), 56.2 (CH_3O x 2), 53.9 (C11a), 35.5 (C1), 31.2 (2 x 8'-$(CH_3)_3$), 28.8 (Linker-$\underline{CH_2}$); IR (Golden gate): 2940, 2335, 1620 (C=O), 1592 (C=O), 1506, 1447, 1427, 1354, 1261, 1204, 1181, 1096, 1018, 873, 825, 754, 654 cm^{-1}; MS (ESI) *m/z* (relative intensity): 793 ([*M* + H]$^+$, 47%), 785 (100), 734 (31), 627 (10), 485 (20), 240 (41); HRMS: [*M* + H]$^+$ Theoretical, $C_{49}H_{52}O_6N_4$ m/z 793.3959, found (ES^+) m/z 793.3950.

5.5.2 Synthesis of C2-Styryl Intermediate 232

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(styryl)-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (232).

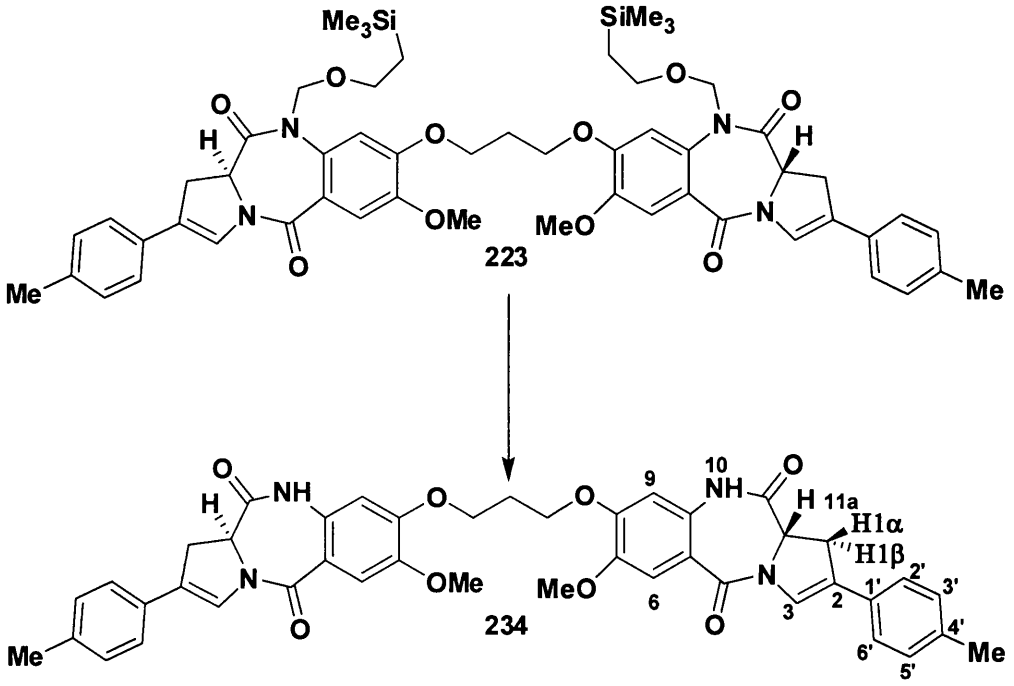


$Pd(PPh_3)_4$ (12 mg, 0.179 mmol; 0.04 equiv.) was added to a stirred solution of the bis-enol triflate **221** (0.5 g, 0.45 mmol; 1.0 equiv.), *trans*-styrylboronic acid (0.45 g, 1.03 mmol; 2.3 equiv.), $NaCO_3$ (0.27 g, 2.51 mmol; 5.6 equiv.), toluene (5 mL), ethanol (5 mL), water (3 mL). The reaction mixture was allowed to stir for 24 h at room temperature under nitrogen atmosphere after which point TLC (100% EtOAc) and LC/MS revealed complete consumption of starting material. The solvent was removed by evaporation in *vacuo* and the resulting residue redissolved in EtOAc (10 mL) and was washed with water (50 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude product. Purification by flash chromatography (gradient elution: 80:20 v/v hexane/EtOAc to 60:40 v/v hexane/EtOAc) afforded the pure bis-styryl (**232**) as a yellow foam (100 mg, 20%): LC/MS 8.40 min (ES+) m/z (relative intensity) 1025 ($[M + H]^+$, 65), 1048 ($[M + Na]^+$, 10); $[\alpha]_D^{24}$ = +298° (*c* = 0.42, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.38 (d, 4 H, *J* = 7.56 Hz, H15/H19), 7.35 (s, 2 H, H6), 7.29 (t, 4 H, *J* = 7.40 Hz, H16/H18), 7.25 (s, 2 H, H9), 7.20 (d, 2 H, *J* = 7.40 Hz, H17), 7.06 (s,

2 H, H3), 6.93 (d, 2 H, J = 16.03 Hz, H12), 6.48 (d, 2 H, J = 16.01 Hz, H13), 5.49 (d, 2 H, J = 10.08 Hz, SEM-N$\underline{CH_2}$O), 4.74 (d, 2 H, J = 10.08 Hz, SEM-N$\underline{CH_2}$O), 4.56 (dd, 2 H, J = 10.50, 3.30 Hz, H11a), 4,27 (t, 4 H, J = 8.73 Hz, Linker-O$\underline{CH_2}$), 3.88 (s, 6 H, CH_3O x 2), 3.80 (d, 2 H, J = 17.57 Hz, H1β), 3.71-3.62 (m, 6 H, SEM-O$\underline{CH_2}$), 3.01 (dd, 2 H, J = 15.30, 10.81 Hz, H1α), 2.42 (t, 2 H, J = 5.84 Hz, Linker-$\underline{CH_2}$), 0.95 (t, 4 H, J = 8.36 Hz, SEM-$\underline{CH_2}$Si$(CH_3)_3$), 0.00 (s, 18 H, SEM-Si($\underline{CH_3}$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 131.3 (C12), 130.0 (C15/C19), 128.9 (C17), 127.6 (C16/18), 126.7 (C3), 123.4 (C13), 113.1 (C9), 108.6 (C6), 79.6 (SEM-N$\underline{CH_2}$O), 68.5 (SEM-O$\underline{CH_2}$), 66.8 (Linker-O$\underline{CH_2}$), 59.0 (C11a), 57.5 (CH_3O x 2), 30.2 (C1), 31.3 (Linker-$\underline{CH_2}$), 19.6 (SEM-$\underline{CH_2}$Si$(CH_3)_3$), 00.0 (SEM-Si($\underline{CH_3}$)$_3$); IR (Golden gate): 2955, 1682 (C=O), 1630 (C=O), 1512, 1449, 1351, 1232, 1065, 832, 752 cm^{-1}; MS (ESI) *m/z* (relative intensity): 1025 ([M + H]$^{+}$, 35%), 785 (90), 610 (75), 457 (100); HRMS: [M + H]$^{+}$ Theoretical, $C_{57}H_{68}O_{10}N_4Si_2$ m/z 1025.4547, found (ES^{+}) m/z 1025.4514.

5.5.3 Synthesis of Tetralactam C2-Methylbenzene Intermediate 234

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(methylbenzene)-1,10,11,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5,11-dione]] (234).

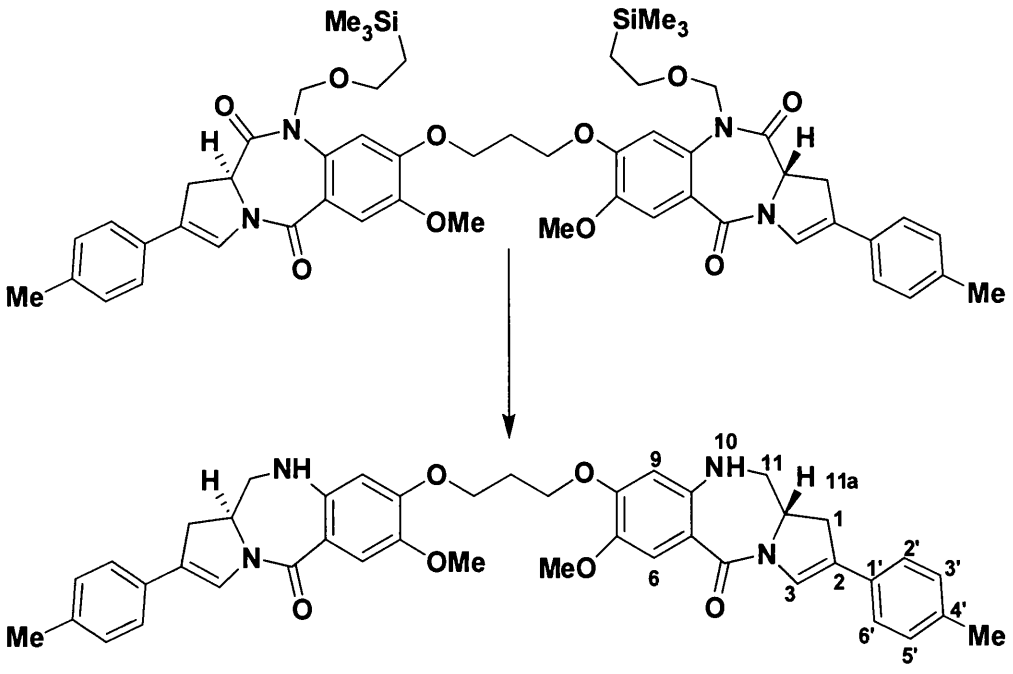


A 1.0 M solution of TBAF (0.30 mL, 0.30 mmol; 10.0 equiv.) was added to a stirred solution of the bis-methylbenzene **223** (30 mg, 0.030 mmol; 1.0 equiv.) in dry THF (5 mL). The reaction mixture was allowed to stir for 1 h at reflux under nitrogen atmosphere at which point TLC (5% MeOH/$CHCl_3$) revealed complete consumption of starting material. The reaction mixture was poured into a cooled (ice) solution of NH_4Cl (100 mL) and extracted with $CHCl_3$ (3 × 30 mL). The organic layer was washed with brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to afford the crude product. Purification by flash chromatography (gradient elution: 100%$CHCl_3$ to 97:3 v/v $CHCl_3$/MeOH) gave the pure bis-methylbenzene tetralactam (**234**) as a yellow foam (4 mg, 7%); LC/MS 7.25 min (ES+) m/z (relative intensity) 741 ($[M + H]^+$, 100), 764 ($[M + Na]^+$, 10); $[\alpha]_D^{23}$ = +365° (*c* = 0.02, DMSO); ^{1}H-NMR (d_6-DMSO, 400 MHz): δ 10.45 (s, 2 H, NH10), 7.51 (broad s, 2 H, H3), 7.45 (d, 4 H, *J* = 8.09 Hz, H3'/H5'), 7.32 (s, 2 H, H6), 7.19 (d, 4 H, *J* = 8.09 Hz, H2'/H6'), 6.81 (s, 2 H, H9), 4.74 (dd, 2 H, *J* = 10.85, 3.69 Hz, H11a), 4.27-4.13 (m, 4 H, Linker-O$\underline{CH_2}$), 3.83 (s, 6 H, CH_3O x 2), 3.67 (d, 2 H, *J* = 16.00 Hz, H1α), 3.32 (s, 6 H, CH_3 x 2), 3.15 (ddd, 2 H, *J* = 16.33, 11.03, 2.14 Hz, H1β), 2.39-2.25

(m, 2 H, Linker-$\underline{CH_2}$); IR (Golden gate): 3214 (NH), 2952, 1681 (C=O), 1642 (C=O), 1512, 1483, 1350, 1272, 1174, 1047, 1025, 894, 922, 755, 697 cm^{-1}; MS (ESI) *m/z* (relative intensity): 741 ($[M + H]^+$, 100%), 764 ($[M + Na]^+$, 38%), 683 (20), 595 (28), 491 (35); HRMS: $[M + H]^+$ Theoretical, $C_{43}H_{40}O_8N_4$ m/z 741.2919, found (ES^+) m/z 741.2914.

5.5.3.1 ^{1}H NMR analysis of bis-seconday amine (By-product from hydride reduction step).

Synthesis of 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11a*S*)-7-methoxy-2-(*p*-methylbenzene)-1,10,11,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-one]] (Bis-C2-methylbenzene secondary amine).



Rf value = 0.42 cm (5% MeOH/$CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.15 (d, 4 H, J = 8.15 Hz, H3'/H5'), 7.10 (d, 4 H, J = 8.14 Hz, H2'/H6'), 6.94 (s, 2 H, H3), 6.87 (s, 2 H, H6), 6.51 (s, 2 H, H9), 5.01 (broad s, 2 H, NH10), 4.90-4.78 (m, 2 H, H11a), 4.34 (t, 2 H, J = 6.0 Hz Linker-O$\underline{CH_2}$), 3.93 (dd, 2 H, J = 15.54, 5.48, Hz, H11β), 3.82-3.70 (m, 8 H, CH_3O x 2 and H11α), 3.28 (ddd, 2 H, J = 16.54, 11.01, 1.98 Hz, H1β), 2.58 (dd, 2 H, J = 16.21, 3.57, Hz, H1α), 2.42 (t, 2 H, J = 6.04 Hz, Linker-$\underline{CH_2}$), 2.32 (s, 6 H, CH_3 x 2); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 129.3 (C2'/C6'), 124.9 (C3), 124.8 (C3'/C5'), 115.1 (C6), 96.9 (C9), 67.5 (C11), 65.3 (Linker-

O$\underline{CH_2}$), 62.3 (C11a), 57.5 (CH_3O x 2), 33.6 (C1), 29.3 (Linker-$\underline{CH_2}$), 21.1 (CH_3 x 2); IR (Golden gate): 3216 (NH), 2920, 1680 (C=O), 1645 (C=O), 1481, 1446, 1421, 1304, 1275, 1212, 1170, 895, 870, 838, 814, 756, 698 cm^{-1}; D_2O Shake= δ 5.01 (broad s, 2 H, NH10) disappeared after D_2O shake.

5.6 Methodology Employed in the K_{562} Leukeamia Single Cell Line Cytotoxicity Assays.

5.6.1 Alamar Blue K_{562} Leukeamia Single Cell Line Cytotoxicity Assay

K562 human chronic myeloid leukaemia cells were maintained in RPMI medium supplemented with 10% Fetal Calf Serum and 2 mM glutamine. 190 µl K562 suspensions was added (1.10^4 cells/ml) to each well of columns 2 to 11 of a 96 well plate, and 190 µl medium was added to each well of columns 1 and 12. The compound solution (in 100% DMSO) was serially diluted across a 96 well plate. Each resulting point was then further diluted 1/10 into aqueous, and each point was added in triplicate to the cell plate (5% v/v). To cell –ve blanks and compound –ve control wells, 10% DMSO was added at 5% v/v. Assay plates were incubated for 96 hours at 37°C in a humidified atmosphere containing 5% CO_2. Following incubation, 20 µl Alamar Blue (1 µM, final concentration) was added to each well. The plates were then kept for a further 4 h at 37 °C in a humidified atmosphere containing 5% CO_2, and were read at 580-620 nm fluorescence emission using an Envision™ plate reader. The data was analysed using GraphPad Prism and the IC_{50} value was read as the dose required reducing the final optical density to 50% of the control value.

5.6.2 The MTT K_{562} Leukeamia Single Cell Line Cytotoxicity Assay

Cell culture conditions:

A2780 parental cell line, A2780 2000r (SG2000-) cell line and A2780 CisR cell line were grown in Dulbecco's Modified Eagles' Media (DMEM) containing ~10% Foetal Calf Serum (FCS) and ~1% 200 mM L-Glutamine solution and grown in Corning Cellbind 75 cm^2 flasks. A2780 2000r (SG2000+) cell line was grown as above but with the addition of 150pM SG2000 in every passage.

Assay plate conditions:

190µl cell suspension was added (at 1 x 10^4) to each well of columns 2 to 11 of a 96 well plate (Nunc 96F flat bottom TC plate). 190 µl of media was added to each well of columns 1 and 12. The media was Dulbecco's Modified Eagles' Media (DMEM) (which included~10% Foetal Calf Serum (FCS) and ~1 % 200mM L-Glutamine solution).

Plates were incubated overnight at 37 °C before addition of drug if cells were adherent. 200 µM SG compound solutions (in 100% DMSO) were serially diluted across a 96 well plate. Each resulting point was then further diluted 1/10 into sterile distilled water (SDW). Cisplatin was then added to the SDW plate as described in section 2.1. Each point was then added in triplicate to the cell plates, 5% v/v.

To the cell negative blanks and compound negative control wells, 10 % DMSO was added at 5% v/v. Assay plates were incubated for the following durations at 37 °C in 5 % CO_2 in a humidified incubator for 72 hours. Following incubation, MTT solution to a final concentration of 1.5µM was added to each well. Plates were then incubated for a further 4 hours at 37 °C in 5 % CO_2 in a humidified incubator. The media was then removed, and the dye was solubilised in 200µl DMSO (99.99%).

Data analysis:

Plates were read at 540 nm absorbance using an Envision plate reader. Data was analysed using Microsoft Excel and GraphPad Prism and IC_{50} values obtained.

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