DOCTOR OF PHILOSOPHY

Approaches to the synthesis of guanidine containing natural products

Buck, Matthew

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Approaches to the synthesis of guanidine containing natural products

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A thesis presented in partial fulfilment of
the requirements for the degree of

Doctor of Philosophy

in the

School of Chemistry

by

Matthew Buck

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Contents

Abbreviations

CDI	Carbonyldiimidazole
CSA	Camphor sulfonic acid
CYN	Cylindrospermopsin
DCC	N,N'-Dicyclohexylcarbodiimide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DE	Diethyl ether
DEAD	Diethyl azodicarboxylate
DIAD	Diisopropyl azodicarboxylate
DIBAL-H	Diisobutylaluminium hydride
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	N,N-Dimethyl formamide
DMS	Dimethyl sulfide
DMSO	Dimethyl sulfoxide
EA	Ethyl acetate
EPSRC	Engineering and Physical Sciences Research Council
EU	European Union
HPLC	High performance/pressure liquid chromatography
Imid	Imidazole
LHS	Left hand side
m-CPBA	*meta*-Chloroperoxybenzoic acid
ME	Methanol
MOM	Methoxymethyl acetal
MS	Mass spectrometry
NMO	N-Methylmorpholine N-oxide
NMR	Nuclear magnetic resonance
PCC	Pyridinium chlorochromate
PE	Petroleum ether (40-60)
PHB	Polyhydroxybutyrate
PMB	*para*-Methoxybenzyl
*p*MBCl	4-*para*-Methoxybenzyl chloride

Pyr	Pyridine
Rf	Retention factor
RHS	Right hand side
TBA	*tert*-butyl alcohol
TBAF	Tetrabutylammonium fluoride
TBSCl	*Tert*-butyldimethylsilyl chloride
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMSCl	Trimethylsilyl chloride
TPAP	Tetrapropylammonium perruthenate
TROCCl	2,2,2-Trichlorethoxycarbonyl chloride
UV	Ultraviolet

Abstract

The thesis is divided into three parts:

A: *synthetic approach to the LHS of cylindrospermopsin*: An intermediate (3R,4S)-6-(benzyloxy)-4-((tert-butyl dimethyl silyl)oxy)-3-methyl hexane-1,2-diol (I) (Figure I) for the synthesis of the alkaloid cylindrospermopsin was prepared in 6 steps from propane-1,3-diol. However, the key step in the synthesis the Sharpless asymmetric dihydroxylation of an alkene using AD-mix ß gave very poor diastereoselectivity. An intermediate Brown crotylation step gave a 93:7 selectivity as shown by the formation of mandelate esters and their NMR analysis.

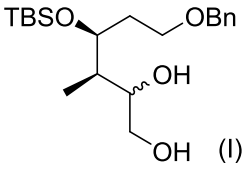


Figure I: Intermediate structure (I).

B: *The total synthesis of Tiruchanduramine*: Tiruchanduramine (II) was prepared in a convergent manner over 8 steps in 4.5% overall yield from the known ß-carboline carboxylic acid (III) and the cyclic guanidine (IV), which was prepared in 7 steps from 3-amino-1-propanol. The two fragments were coupled using CDI in THF/DMF.

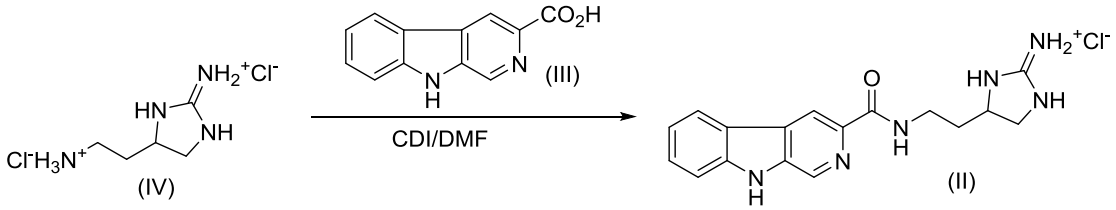


Figure II: Coupling of (III) and (IV) to yield Tiruchanduramine (II).

C: *Approaches to the synthesis of nitensidine E:* The intermediate thiourea (V) was prepared in 5-steps from *cis*-1,4-butene diol in an attempt to investigate the Pd^0 mediated cyclisation of N-Methoxyguanidines.

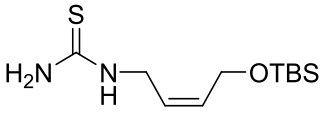


Figure III: Thiourea (V)

Section A:

A Synthetic approach to the LHS of cylindrospermopsin:

Introduction

Cylindrospermopsin was first isolated by Moore *et al* [1] from cyanobacteria, *cylindrospermopsis raciborskii (woloszynska) seenaya* and *subba raju* which were thought to be non-toxic. Upon the outbreak of hepatoenteritis on palm island (Palm island mystery disease), the disease was traced back to the cyanobacteria *cylindrospermopsis raciborskii (woloszynska) seenaya* which 148 people had been exposed to through drinking water.[2] The cylindrospermopsin alkaloids (Figure 1) are a group of five water soluble alkaloids that are found to be produced by numerous cyanobacteria.[3],[4] The toxicological properties of these alkaloids are of great concern and have been studied greatly.[5],[6] The three compounds of interest are cylindrospermopsin (1), 7-*epi*-cylindrospermopsin (2) and 7-deoxy-cylindrospermopsin (3). In addition it has been discovered that a strain of cyanobacteria found in Thailand[7] that is closely related to the cyanobacteria that produces (1), (2) and (3), produced 7-*deoxy*-desulfo-cylindrospermopsin (4) and 7-deoxy-desulfo-12-acetylcylindrospermopsin (5).

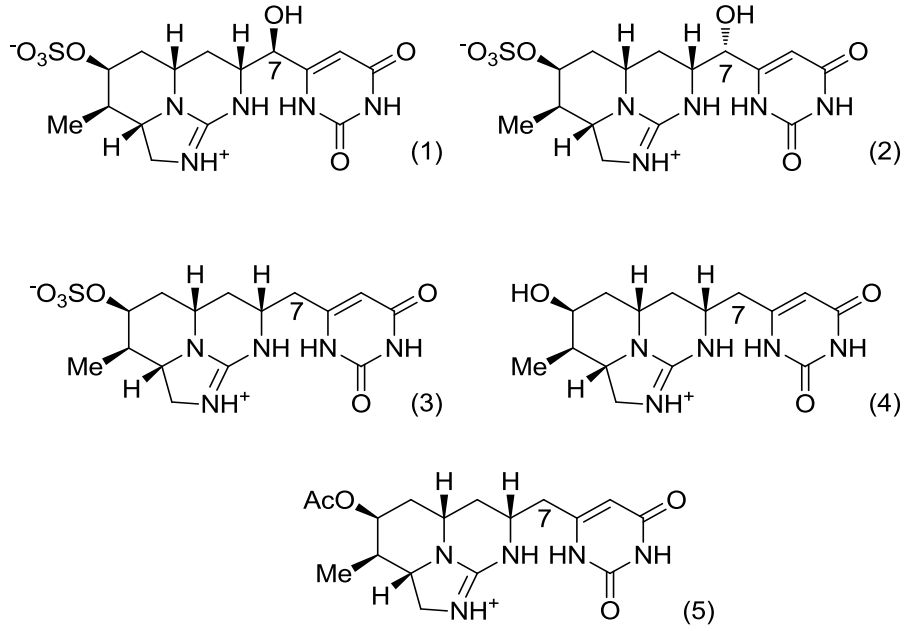


Figure 1: The Cylindrospermopsin Alkaloids.

It is now known that the cylindrospermopsin alkaloids are produced by many different cyanobacteria on almost every continent. The cyanobacteria that produce these toxins are not limited by climate as they are prevalent in a variety of climates

such as Australia,[8] Egypt[9] and Mexico[10] but also countries with a cooler climate such as Germany[11] and France.[12]

Considerable research has been undertaken into the detection of the cylindrospermopsin alkaloids in the environment, as they are considered to be toxic and have a considerable effect human and animal health.

Detection of cylindrospermopsin

Chromatographic techniques are fast and usually reliable, and as a result much of the work in the area of the detection of these alkaloids has focused on these types of techniques. The techniques that have shown the most promise are HPLC-PDA (Photodiode Array Detector)[13] and HPLC-MS.[14] Although HPLC-MS^2 (Tandem mass spectrometry) has shown a particular promise of being able to detect concentrations as low as 0.1 ng mL^{-1} in a sample of water and 1 ng g^{-1} in samples of fish.[15] This means that HPLC-MS^2 could be used as a method of early detection of the cylindrospermopsin alkaloids in reservoirs and other important sources of water used by humans and animals.

An alternative to chromatographic techniques is the use of biological assays. These have the distinct disadvantage of the amount of time it takes to produce an answer and some biological assays would involve the use of living creatures which means these methods also come with ethical questions and considerations.

Many biological assays have been tested to find a method of detection for the cylindrospermopsin alkaloids such as mice,[16] speckled cockroach,[17] tobacco pollen germination[18] and mustard plant seedling growth inhibition.[19] Some of these methods come with one or both of the main concerns of biological assays i.e. time and ethical considerations.

A promising biological assay for the detection of the cylindrospermopsin alkaloids is the use of commercially available kits that utilise the rabbit reticulocyte translation system. It has been demonstrated that these kits can detect the alkaloids at a similar sensitivity to the HPLC methods.[20] There is also a great deal of research focused on the development of a PCR (Polymerase Chain Reaction) assay, whilst initial efforts could successfully detect the cylindrospermopsin alkaloids[21] they could not differentiate the strain producing the toxins. Fergusson *et. al*. produced a PCR test that could not only detect the alkaloids it could differentiate between the strains

producing them in less than one day in the lab or in the field.[22]

Treatmeant of water contaminated with CYN

Whilst the strains of cyanobacteria that produce the cylindrospermopsin alkaloids are varied and are able to thrive in many climates, the effect of rapid climate change is likely to magnify the problem of the toxins being produced by these cyanobacteria.[23] Water is a natural resource which is becoming increasingly put under pressure due to increases of human population and increasing pollution of water sources. It is also one of the main ways that humans are exposed to these toxins produced by cyanobacteria, for example, through drinking water or water pumped to homes.

Methods that are traditionally used to treat water have been successful in the removal of the cyanobacteria but not the toxins dissolved in the water.[24] These traditional methods of water treatment may even in some cases lead to an increase in concentrations of these toxins in the water being treated.[25] More toxicological studies are needed to find an acceptable safe limit of exposure through drinking water, although some countries have already imposed a guideline limit in drinking water, for example, the EU[26] has set it at 0.1 μg L^{-1}.

Methods of water treatment can be by chemical or non-chemical methods, with each providing a solution to a different problem to contaminated water treatment. Chemical methods are used extensively in water treatment and as such been the subject of study of water contaminated with cyanobacteria and there toxins, such as the cylindrospermopsin alkaloids. Ozonolysis[27] has shown to be one of the most useful methods of deactivating and removal of various cyanobacteria that produce the cylindrospermopsin alkaloids and the toxins dissolved in the water. Permanganate[28] on the other hand was shown to be ineffective in deactivating the cyanobacteria and the toxins.

Non-chemical methods of water treatment commonly include the use of photo-degradation using UV radiation, however, it is known that this method is not completely effective at purifying water at the currently utilised radiation levels. However, if titanium dioxide is added as a photocatalyst at a concentration of 0.1 g L^{-1} the half-life of both the toxin and cyanobacteria has been shown to be 42 seconds.[29]

The most promising method of non-chemical water treatment seems to be the

use of nanofiltration[30] which uses molecular weight cut off (M.W.C.O.) membranes. Various water samples contaminated with cylindrospermopsin were passed through varying porosities and it was found that membranes with a molecular cut off weight of 100 Da were able to effectively remove between 90-100% of the toxins at concentrations of 16 µg L^{-1}.

In a follow up study[31] contaminated samples were treated with conventional water treatment methods such as flocculation, dissolved air floatation and sand filtration. They found that the use of membranes with a porosity size of 300Da or less were able to remove between 90-100% of the toxins in the water.

The least effective method of water purification seems to be bioremediation using microbes, animals and/or plants to clean the environment. Several microbes have been investigated in order to test their ability to remove cylindrospermopsin from the environment. The most effective microbe found to date is *Bifidobacterium longum* 46[32] which was able to remove 31.6% of the cylindrospermopsin in the sample within 24 h at a constant incubation temperature of 37^{o}C which is not a realistic representation of the environments the cyanobacteria that produces cylindrospermopsin are found.

Finding the right microbe that is able to replicate the success of *Bifidobacterium longum* 46 or better under more realistic conditions could nevertheless be the major breakthrough this field needs.

Toxicology

In recent years many different toxins produced by cyanobacteria have been found. Saxitoxin[33] is a potent neurotoxin found in shellfish, it is responsible for (PSP) Paralytic Shellfish Poisoning and has an LD_{50} of 5.7 µg/Kg.

Whilst not as toxic, cylindrospermopsin shows some evidence of cytotoxicity,[34],[35],[36] genotoxicity[37],[38] and carcinogenicity.[39],[40] It is a potent hepatotoxin as seen from the outbreak of hepatoenteritis on Palm Island, Australia. Cylindrospermopsin has also been attributed to the deaths of livestock in two cases.[41],[42] The mode of action of cyclindrospermopsin within several organ systems such as kidneys,[16] thymus,[43] heart and spleen[44] is not fully understood.

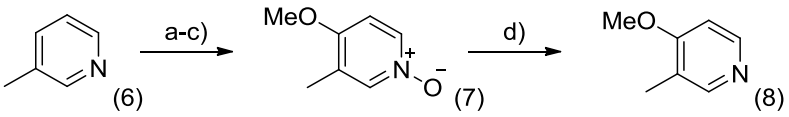
Synthesis of Cylindrospermopsin

The task of synthesising cylindrospermopsin is not easy as the molecule contains 6 stereogenic centres, a sulfonated tricyclic guanidine and an attached uracil ring. Three of five molecules that comprise the cylindrospermopsin alkaloids differ from each other at the C-7 position. Only recently two new alkaloids belonging to the cylindrospermopsin family of alkaloids were isolated from a strain of *cylindrospermopsis raciborskii* found off the coast of New Zealand. To date (2017) four total syntheses of member of the cylindrospermopsin family have been reported.

Snider was the first to report the synthesis of (1) in racemic form over 21 steps in a 1.5-2% overall yield.[45],[46],[47] Following this, the Weinreb research group reported the total synthesis of both cylindrospermopsin and 7-*epi*-cylindrospermopsin and were able to correct an original miss-assignment of the stereochemistry at C-7 by preparing firstly 7-*epi*-cylindrospermopsin (2) in 33 steps and 0.26% overall yield. This was followed by their synthesis of cylindrospermopsin (1) in 36 steps and in 0.20-0.25% overall yield.[48],[49],[50] Following this, White detailed the asymmetric synthesis of 7-*epi*-cylindrospermopsin (2) in 25 steps with an overall yield of 0.39%.[51],[52] The Williams research group reported the preparation of all three metabolites in the most efficient manner to date. They prepared cylindrospermopsin in 19 steps in a 0.34-0.57% yield, 7-*epi*-cylindrospermopsin (2) in 19 steps with a 0.47-0.82% yield and racemic 7-*deoxy*-cylindrospermopsin (3) in 20 steps with a 0.62-1.05% yield.[53],[54]

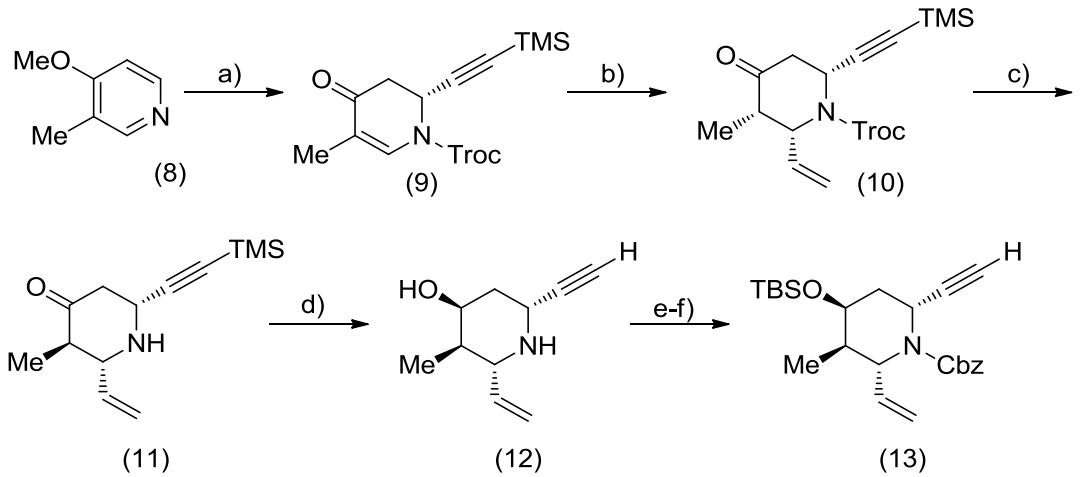
Snider group [45],[46],[47]

The Snider research group first performed various model studies towards the synthesis of both the left and right hand sides of cylindrospermopsin.[45],[46] They then went on to successfully prepare cylindrospermopsin in a racemic manner.[47] They began with 4-methoxy-3-methylpyridine (8) which was prepared from 3-picoline (6) in 4 steps (Scheme 1).



Scheme 1: a) H_2O_2, HOAc, reflux, b) HNO_3, H_2SO_4, 95°C, 24 h, c) K_2CO_3, MeOH, reflux, 4 h, d) 10% Pd/C, H_2 (45 atm.), EtOH.

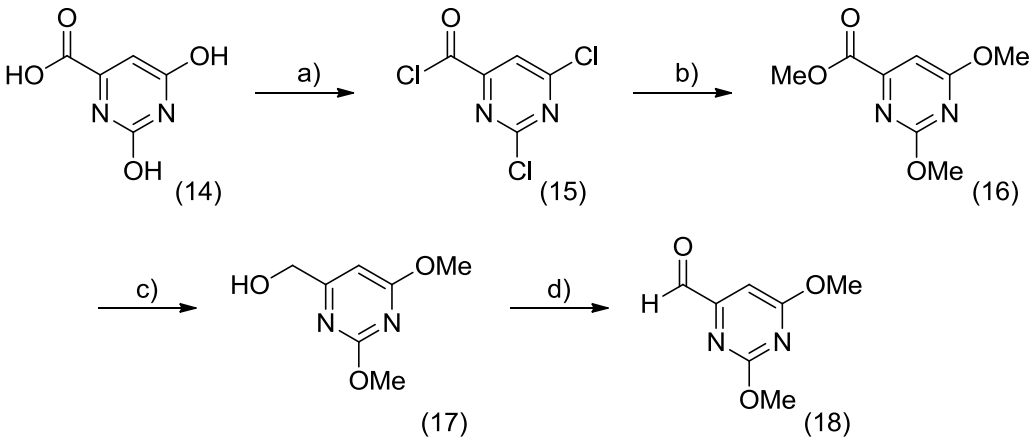
Once they obtained 4-methoxy-3-methylpyridine (8) it was treated with TrocCl (to activate the pyridine to nucleophilic attack) and trimethylsilylethynylmagnesium bromide at -30°C with an acidic work up gave (9) in an 80% yield based on recovered 4-methoxy-3-methylpyridine (8) (Scheme 2). They then utilized the copper catalized addition of vinyl magnesium bromide in the presence of TMSCl at -78°C to give (10) in a 92% yield. The Troc group was then cleaved using zinc dust in acetic acid to give the piperidone (11) which was then reduced to (12) using *L*-Selectride in a 90% yield over 2 steps. This gave the correct relative stereochemistry for ring A. They then protected the amine and alcohol functionalities with benzyl chloroformate (Cbz) and *t*-butyldimethylsilyl chloride (TBS) in 96% and 89% yields respectively.



Scheme 2: a) TrocCl, THF, -30°C, TMSC≡CMgBr, b) $CuBr{\cdot}SMe_2$, -78°C, vinyl magnesium bromide, TMSCl, THF, c) Zn, HOAc, d) *L*-selectride, e) CbzCl, Na_2CO_3, THF, f) TBSCl, imidazole, DMAP, dichloromethane.

With the protected fragment (13) in hand (Scheme 2) the next step was to couple this with the pyrimidine aldehyde (18). This was prepared from orotic acid (14) which was treated with phosphorus oxychloride and phosphorus pentachloride to

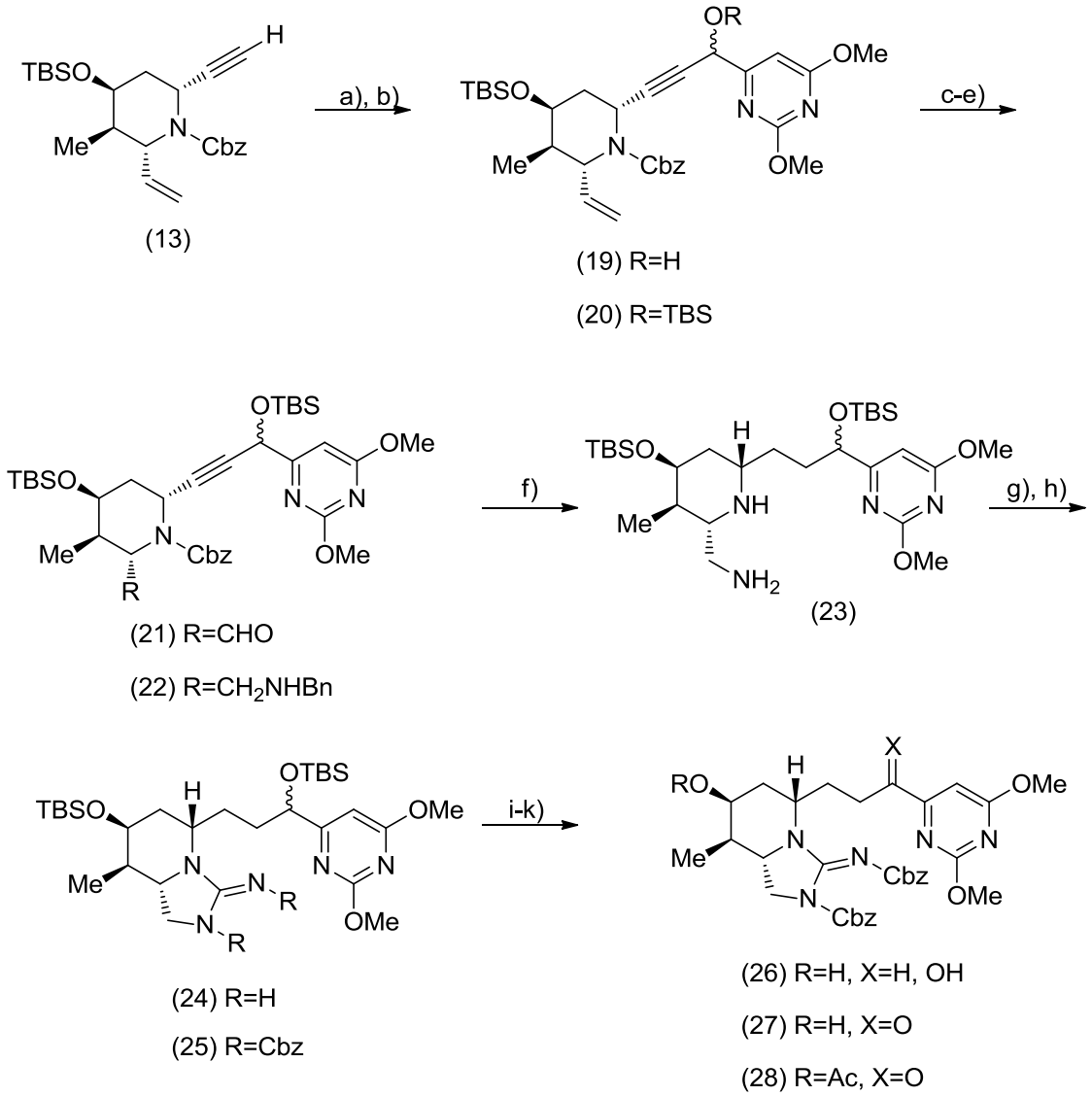
produce 2,4-dichloro-4-pyrimidine carbonylchloride (15) in a 64% yield. (15) was then refluxed with sodium methoxide to give methyl-2,6-dimethoxy-4-pyrimidinecarboxylate (16) in a 65% yield. Snider *et al*. then continued on from this to reduce the ester to an alcohol (17) using lithium borohydride in a 93% yield which was then oxidised to an aldehyde by Dess-Martin oxidation in a 90% yield to give the desired pyrimidine fragment (18) (Scheme 3).



Scheme 3: a) $POCl_3$, PCl_5, b) NaOMe, CH_3OH, reflux, c) $LiBH_4$, THF, d) DMP, dichloromethane, rt, 1 h.

To couple the two fragments together, (13) was treated with the Grignard reagent ethylmagnesium bromide to give the corresponding alkynylmagnesium bromide which was then coupled with (18) to give the alcohol (19) in a 83% yield (Scheme 4). The alcohol (19) was then protected as the silyl ether (20) again using TBSCl in a 88% yield. Compound (20) was treated with ozone to give aldehyde (21), after a reductive workup, in a 72% yield. The aldehyde (21) was then condensed with benzylamine to give the imine which was then reduced to the benzylamine (22) in 68% yield using sodium cyanoborohydride. The benzylamine (22) then underwent catalytic hydrogenation using 5% Pd/C in methanol which cleaved the Cbz and benzyl protecting groups and also reduced the triple bond to give the diamine (23). Conversion of the diamine to the guanidine was performed by the slow addition of cyanogen bromide and then cyclization of the primary cyanamide to give the guanidine (24). This was then protected to give the corresponding Cbz protected guanidine (25) in a 45% yield from (22). The silyl ethers were then removed using TBAF to give the corresponding alcohols (26) in an 83% yield and the alcohol at the

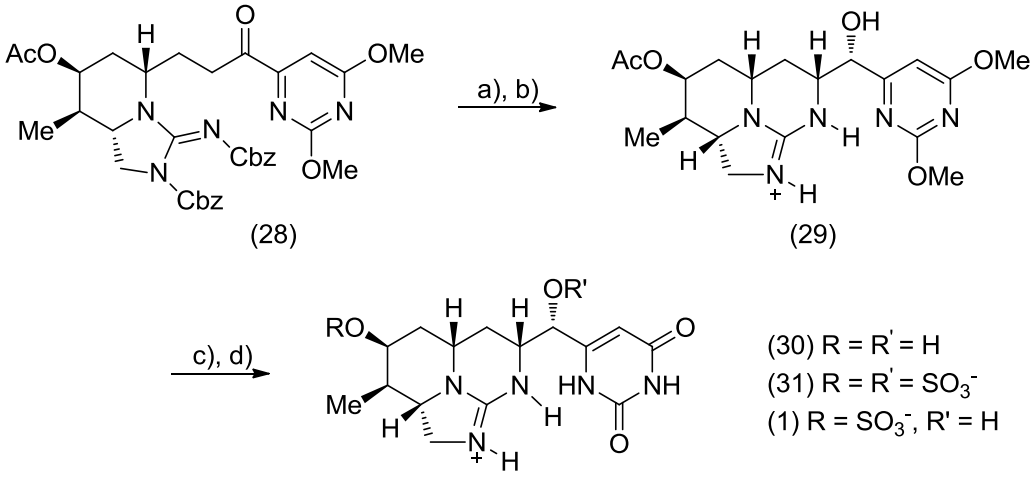
C-7 position was oxidized using manganese dioxide to give the ketone (27) in an 87% yield. The alcohol in the ring system was protected using acetic anhydride in pyridine to give (28) in an 87% yield.



Scheme 4: a) EtMgBr, THF, 0°C, (18), b) TBSCl, Imidazole, DMAP, dicloromethane, c) O_3, DMS, dichloromethane, -78°C, d) NH_2Bn, HOAc, PhH, e) $NaCNBH_3$, MeOH, f) 5% Pd/C, H_2, MeOH, g) CNBr, PhH, h) NaH, CbzCl, THF, i) TBAF, j) MnO_2, dichloromethane, k) Ac_2O, Pyr, rt.

The ketone (28) was then brominated (Scheme 5) to give the tricyclic ring system as a mixture of bromo ketones, in which one of the Cbz protecting groups was deprotected. Hydrogenation of the mixture gave the guanidine (29) in a 42% yield,

which was then hydrolysed using concentrated hydrochloric acid to give the diol (30) in a 95% yield. The final step of the synthesis involved the sulfonation of the ring-hydroxyl group using SO_3 and anhydrous pyridine to give cylindrospermopsin (1) in a 50-70% yield. The reaction also gave the bisulfate (31).



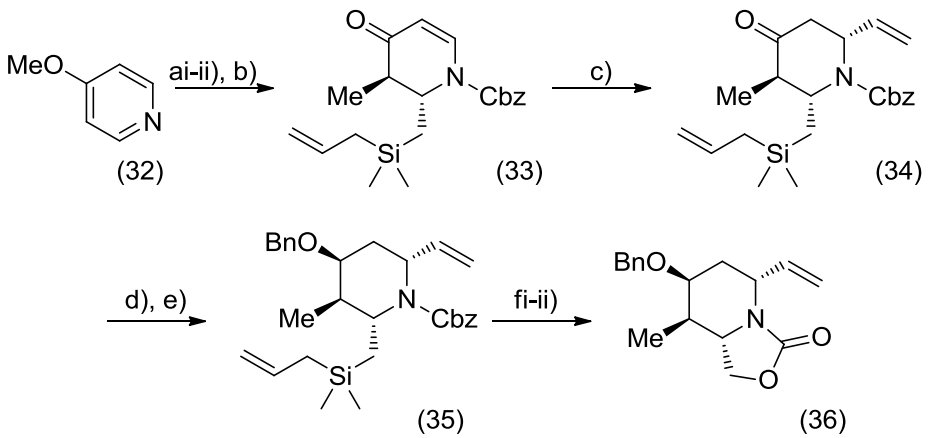
Scheme 5: a) $CuBr_2$, EtOAc, rt, 30 min, b) H_2, $Pd(OH)_2/C$, MeOH, c) conc HCl, reflux, d) $SO_3 \cdot DMF$, Pyr, DMF.

The Snider group compared the toxicity of the racemic synthetic cylindrospermopsin and the diol (30) to that of natural cylindrospermopsin in an *in vitro* hepatocyte assay. They confirmed the activity of cylindrospermopsin and also found the diol (30) depletes the cellular antioxidant glutathione more than racemic cylindrospermopsin and is at least as active as naturally occurring cylindrospermopsin (1). The GSH test is an indicator for the forecast of cell death as depletion in GSH in hepatocytes always precedes cell death. This observation suggests that the sulfate group probably plays no role in the biological activity of cylindrospermopsin.

Weinreb group [48],[49],[50]

Following Snider *et al.* Weinreb *et al*. published the total stereoselective synthesis of 7-epi-cylindrospermopsin (2). Originally their target was cylindrospermopsin (1) but their synthesis led to the reassignment of the stereochemistry at the C-7 position of cylindrospermopsin.

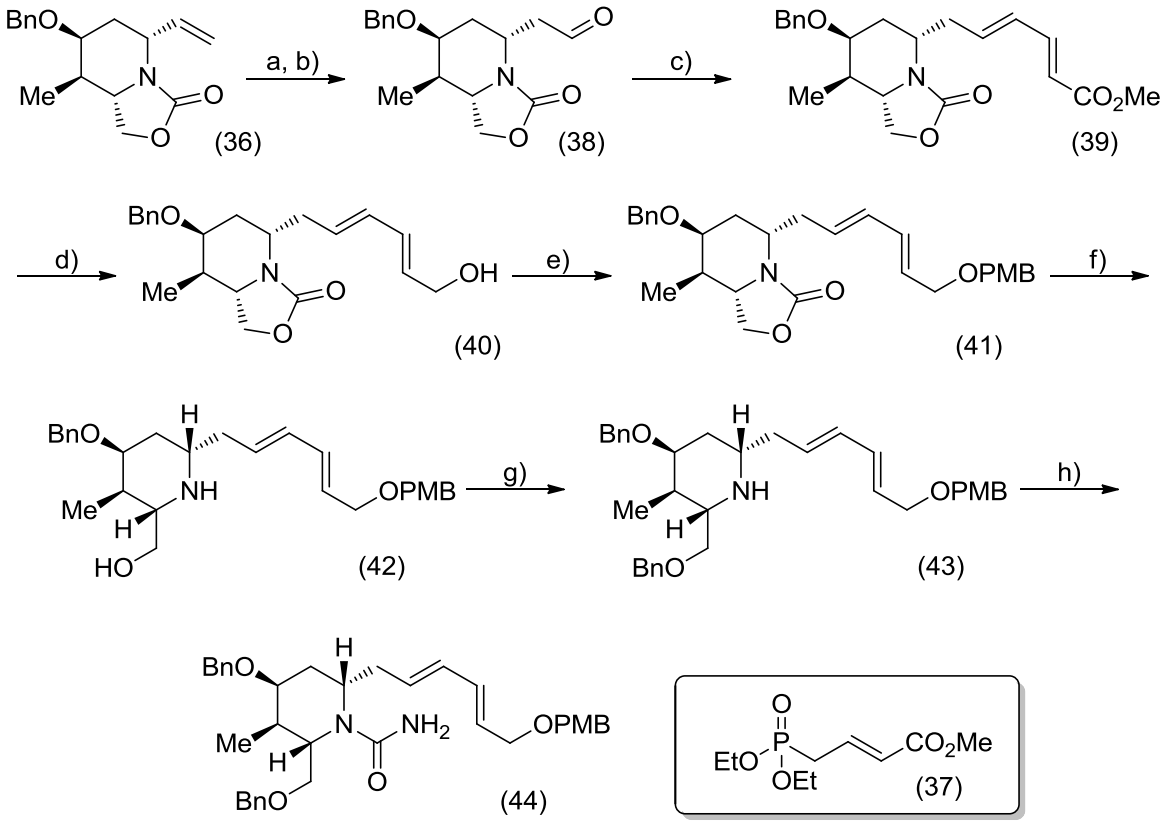
Their synthesis began with the reaction of 4-methoxypyridine (32) with benzyl chloroformate followed by treatment (allyldimethylsilyl)methylmagnesium bromide, to give an intermiediate enone was then deprotonated and then selectively alkylated to give the enone (33) in an 88% yield. Using the work of Comins *et al*.,[55] Weinreb then proceeded with the conjugate addition of vinyl cuprate to the enone (33) to give the ketone (34) in a 98% yield. The ketone (34) was subsequently reduced using *L*-selectride stereoselectively to give an alcohol, which was protected using benzyl bromide to give the benzyl ether (35) in an 81% yield. The following step used the Tamao oxidation procedure, which converted the silane directly to the cyclic carbamate (36) in an 88% yield.



Scheme 6: a) i) CbzCl, THF, -20°C, ii) Et_2O, (allyldimethylsilyl)methylmagnesium bromide, b) NaHMDS, THF, MeI, c) CH_2=CHMgBr, CuI, THF, d) *L*-selectride, THF, e) NaH, THF, TBA, BnBr, f) i) KHF_2, $CHCl_3$, TFA, ii) MeOH, $NaHCO_3$, THF, H_2O_2.

The hydroboration of the cyclic carbamate (36) (Scheme 7) with disiamylborane (Sia_2BH) followed by treatment with hydrogen peroxide/NaOH gave the corresponding alcohol in a 97% yield. This compound was then oxidised to the aldehyde (38) under Swern conditions in a 84% yield, which was then coupled with the phosphonate (37) which gave the (*E*,*E*)-diene ester (39). This ester (39) was then reduced using DIBAl-H which produced the allylic alcohol (40) which was then protected as the *p*-methoxybenzyl ether (PMB) (41) in a 96% yield. Hydrolysis under basic conditions of the cyclic carbamate gave the amino alcohol (42) in a quantitative yield the alcohol functionality of which was protected as its benzyl ether (43) in a

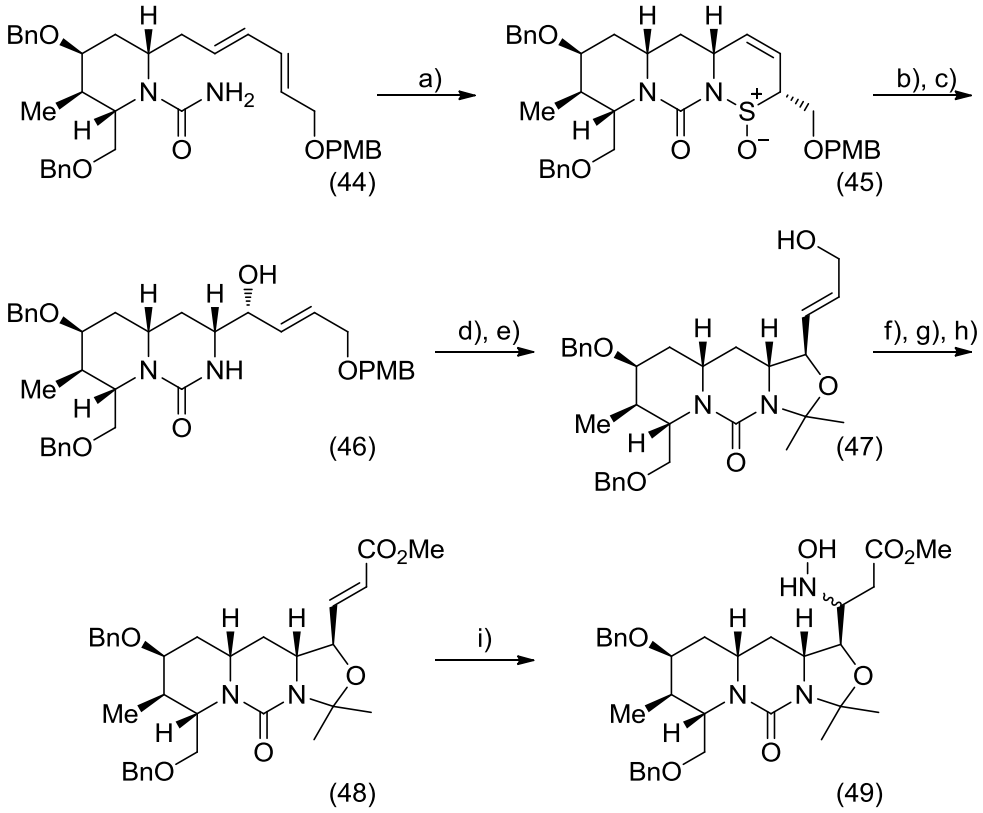
65% yield. Conversion of the amino functionality to the urea (44) was achieved by modification of a method developed by Magnus *et al*.[56] in an 85% yield.



Scheme 7: a) i) Sia_2BH, THF, ii) H_2O_2, NaOH, b) Swern oxidation, c) $LiOH{\cdot}H_2O$, THF, (39), d) DIBAl-H, BF_3Et_2O, dichloromethane, e) NaH, THF, TBA, PMBCl, f) NaOH, H_2O, EtOH, g) NaH, THF, TBA, BnBr, h) KOCN, HOAc, pyr, NEt_3.

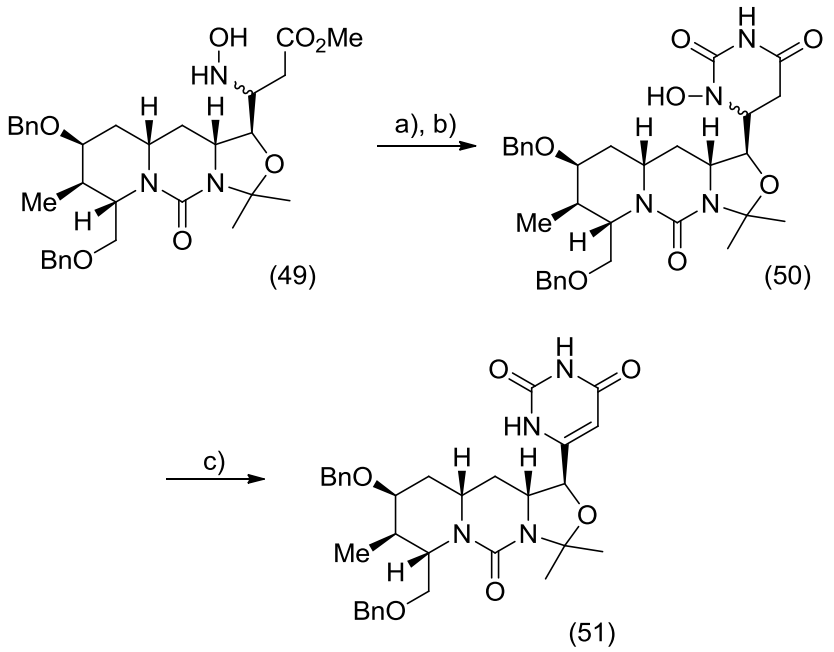
Urea derivative (44) (Scheme 8) was then treated with thionyl chloride and imidazole at -78 °C gave the tricyclic adduct (45) in an 81% yield. Treatment of the tricyclic adduct (45) with phenylmagnesium bromide followed by trimethyl phosphate in methanol gave the single stereoisomer of the allylic alcohol (46) in an 84% yield. Weinreb *et al*. next constructed the D-ring using a methodology developed previously in a model study.[57] Their plan was to first protect the alcohol in the c-7 position as the MOM ether and use the conjugate addition of a nitrogen nucleophile but this failed, the reasoning behind this was that the bulky MOM group was preventing the addition step. Weinreb *et al.* then decided that they would use a methodology that had been used to protect and introduce a rigid system by producing the acetonide from the

amino alcohol (46) a method which had previously been utilized by Hart *et al.*[58] in their model study. Then the PMB protecting group was removed using DDQ in a 78% yield to give the primary alcohol (47). The α,ß-unsaturated ester (48) was then produced in an 81% yield by oxidation of the alcohol and then esterification. The ester (48) was then treated with N,O-*bis*-trimethylsilylhydroxylamine which gave the conjugate addition product (49) as a single stereoisomer in an 82% yield.



Scheme 8: a) $SOCl_2$, imidazole, dichloromethane, -78°C-rt, b) PhMgBr, THF/dichloromethane, c) $(MeO)_3P$, MeOH, d) $Me_2C(OMe)_2$, Me_2CO, CSA, e) DDQ, H_2O, dichloromethane, f) Dess-Martin, g) $NaClO_2$, *t*-BuOH, H_2O, h) *i*-Pr_2NEt, MeI, DMF, i) TMSONHTMS, THF, EtOH.

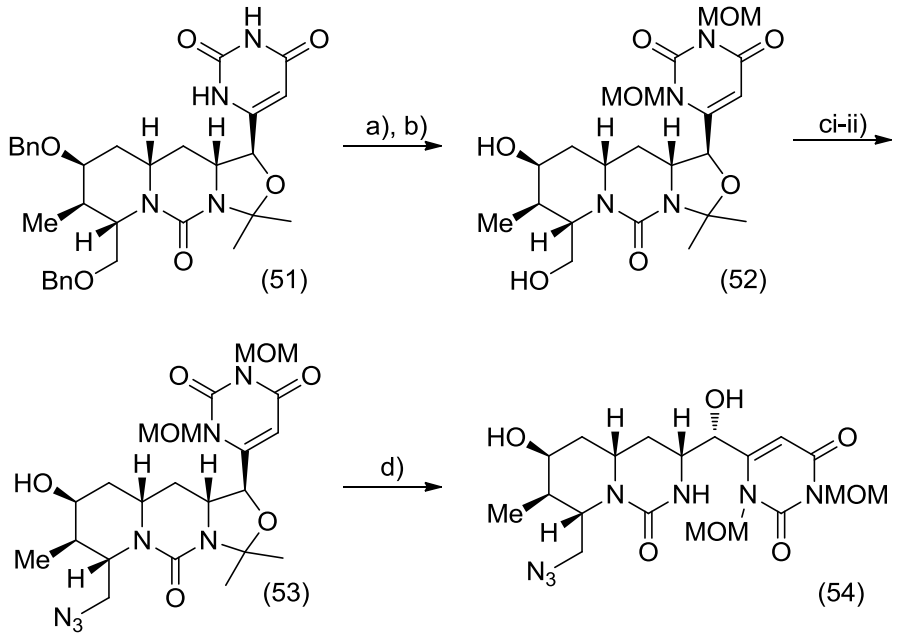
The hydroxylamine (49) (Scheme 9) was then treated with phenyl chloroformate followed by ammonium hydroxide to give the *N*-hydroxydihydrouracil (50) in a 65% yield, from which water was eliminated using triflic anhydride to give (51) in a 73% yield which contains the completed uracil D-ring.



Scheme 9: a) PhOCOCl, NEt_3, THF, b) NH_4OH, *i*-PrOH, c) Tf_2O, pyr, dichloromethane.

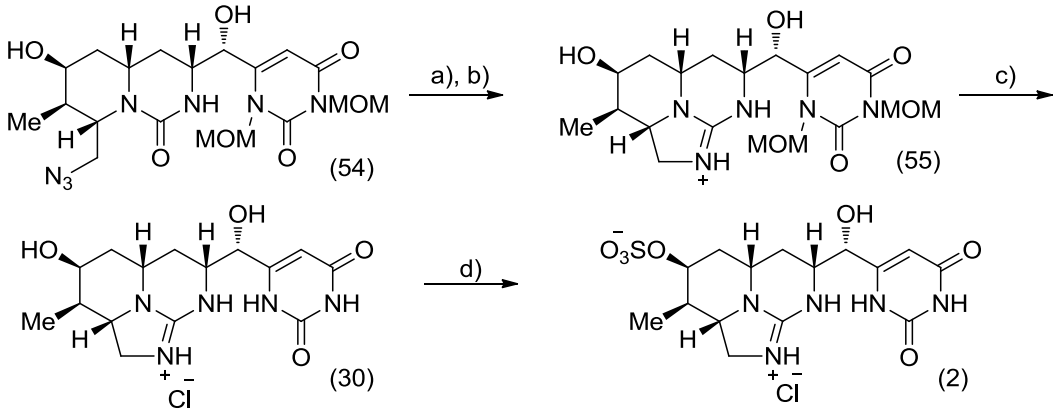
At this point two distinct synthetic pathways were followed. Firstly, Weinreb *et al*. attempted to prepare cylindrospermopsin and based on the original assignment and in fact succeeded in the total synthesis of 7-*epi*-cylindrospermopsin (2). Attempts were made to convert (51) into cylindrospermopsin by formation of the C-ring, however this proved difficult and it was eventually found that the uracil ring was interfering with the activation of the urea. After a number of attempts to construct the guanidine containing C-ring of cylindrospermopsin it was eventually solved by *N*-protecting the uracil functionality.

Thus the uracil was *N*-protected using the MOM protecting group in an 80% yield (Scheme 10). This was followed by the de-protection of the alcohol groups by catalytic hydrogenolysis in a 71% yield to give (52). The next step was to produce the azide by treating the primary alcohol of (52) with triphosgene followed by sodium azide to give the azide (53) in a 86% yield. At this point the C-7 alcohol was de-protected by use of dilute hydrochloric acid to give the diol (54) in a 72% yield.



Scheme 10: a) Me_3SiCl, MOMCl, *i*-Pr_2NEt, dichloromethane, 80%, b) $Pd(OH)_2$, EtOH, H_2, 71%, C) i) triphosgene, THF, rt, ii) NaN_3, DMF, 65°C, 86%, d) HCl, THF, H_2O, 85°C, 72%.

Activation of the urea with MeOTf (Scheme 11) was followed by the catalytic hydrogenation of the azide which led to the cyclization of the C-ring to give the guanidine (55). This was then followed by the removal of the MOM protecting groups by hydrolysis under acidic conditions gave the diol precursor (30) in a 43% yield over the 3 steps.



Scheme 11: a) MeOTf, 2,6-di-*tert*-butyl pyridine, dichloromethane, 78°C- rt, b) 10% Pd/C, EtOH, H_2, c) 12N HCl, 95°C, 43% over 3 steps, d) SO_3.DMF, Pyr, DMF, Na_2SO_4, rt, 70%.

It was at this point a discrepancy in the literature was noticed due to the differences in the NMR data between their diol (30) and the diol (30) reported by Snider *et al*. (Table 1).

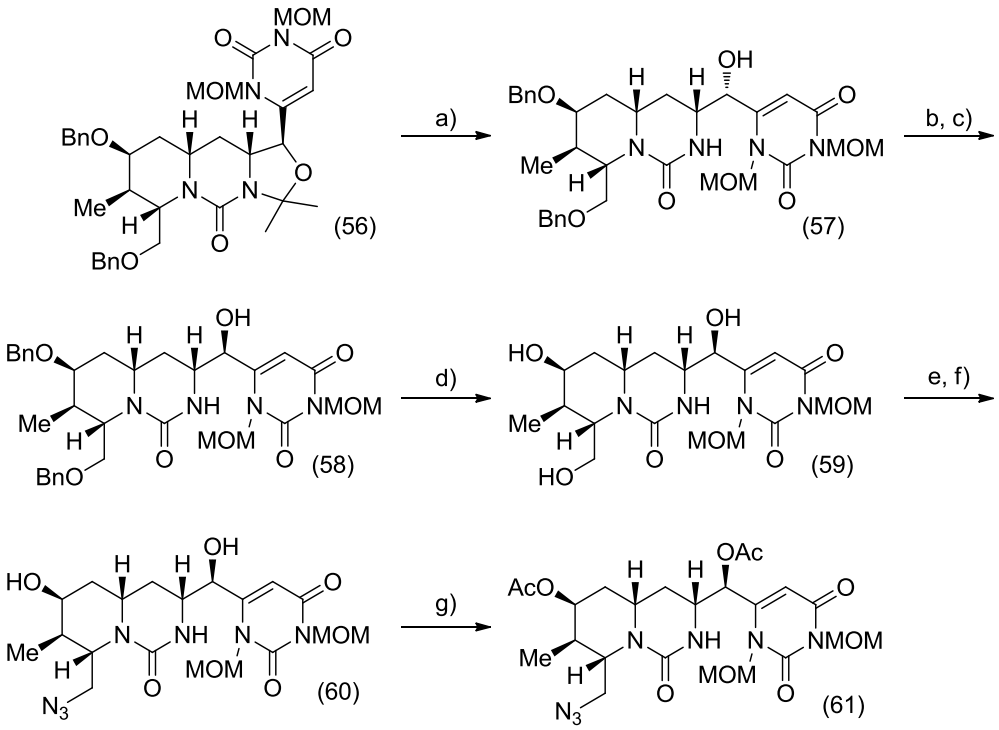
compound	$\delta_{c\text{-}7}$ppm	*j* Hz
Weinreb's diol (30)	4.5	6.6
Snider's diol (30)	4.7	4

Table 1: comparison of NMR data of the C-7 position for the diol (30) and Snider's diol.

This NMR data led to the reassignment of the structures of cylindrospermosin (1) and 7-*epi*-cylindrospermopsin (2). The synthesis was completed by sulfonation with sulfur trioxide to give 7-*epi*-cylindrospermopsin (2) in a 70% yield.

Cylindrospermopsin

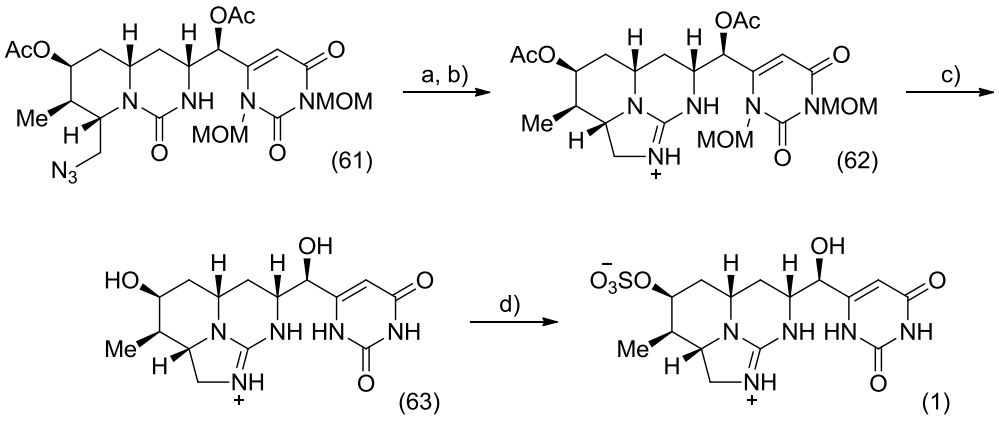
Weinreb *et al*. later re-visited the synthesis of cylindrospermopsin (Scheme 12), and to install the correct stereochemistry at the C-7 position they began with the *N*-protected uracil intermediate (56). The first step was to de-protect the C-7 alcohol by acidic hydrolysis of the acetonide to give the alcohol (57) in an 85% yield. The alcohol at the C-7 position was now inverted by using Mitsonobu chemistry which gave the inverted alcohol (58) in a 61% yield.



Scheme 12: a) HCl, H_2O, THF, 85%, b) PPh_3, *p*-NBA, DEAD, PhH, c) MeOH, K_2CO_3, 61%, d) $Pd(OH)_2$, EtOH, cyclohexene, e) Triphosgene, THF, f) NaN_3, DMF, Δ, 70%, g) Ac_2O, pyr, DMAP, 78%.

This was followed by the removal of the benzyl ether protecting groups by catalytic hydrogenolysis to give the triol (59) in a 95% yield. The azide was then installed, as before using triphosgene and sodium azide, to give (60) in a 70% yield. The resulting azide was not very soluble in dichloromethane so the diol was protected as the acetate (61) using acetic anhydride in pyridine in a 78% yield. Activation of the urea proceeded as before with the exposure of (61) to MeOTf followed by

hydrogenation of the azide which lead to the guanidine salt (62) (Scheme 13). The acetyl and MOM protecting groups were then removed under acidic hydrolysis conditions in a 61% yield over 3 steps to give the diol (63). The NMR data of this diol matched that of Snider's diol (30) and that of the literature which confirmed the reassignment of the stereochemistry of the C-7 position of cylindrospermopsin. Again, the synthesis was completed by utilizing the same method as Snider *et al*. for the installation of the sulfur trioxide functionality.



Scheme 13: a) MeOTf, 2,6-di-*tert*-butylpyridine, dichloromethane, b) 10% Pd/C, H_2, EtOH, c) 12N HCl, Δ, 61% over 2 steps, d) SO_3.DMF, Pyr, DMF, Na_2SO_4, rt.

Weinreb *et al*. thus prepared both cylindrospermopsin (1) In 36 steps and 7-*epi*-cylindrospermopsin (2) in 33 steps and also correctly assigned the stereochemistry at the C-7 position for each metabolite.

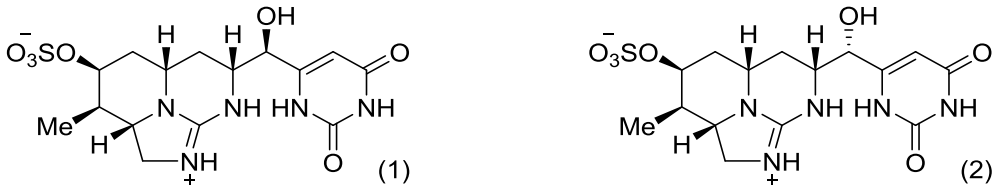


Figure 2: Confirmed stereochemistry of Cylindrospermopsin (1) and 7-*epi* cylindrospermopsin (2).

White's group[51],[52]

Initially, White *et al.* set out to prepare cylindrospermopsin (1) but during their work Weinreb *et al*. corrected the assignment of the C-7 stereochemistry of cylindrospermopsin which meant that their efforts were actually directed towards 7-*epi*-cylindrospermopsin (2). In their retrosynthesis they disconnected cylindrospermopsin into two fragments which they called, the "Western" (64) and "Eastern" (65) fragments respectively (Figure 3).

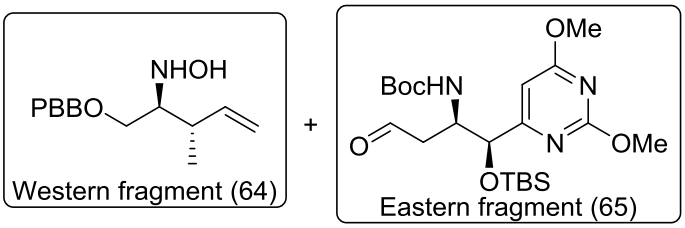
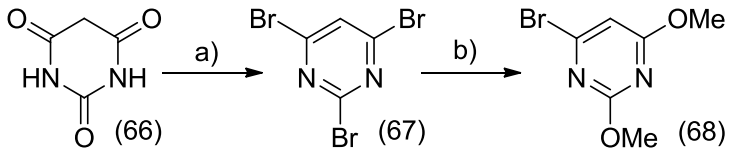


Figure 3: Western and Eastern fragment of the retrosynthesis of White *et. al.*

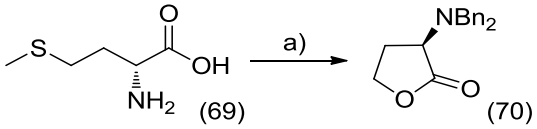
The initial stages of their synthesis focused on the Eastern fragment with the construction of the dimethoxypyrimidine unit. Starting with barbituric acid (66) this underwent treatment with phosphorus oxybromide and triethylamine in hot toluene to give tribromopyrimidine (67) in a quantitative yield. Tribromopyrimidine was reacted with sodium methoxide which displaced two of the three bromo groups to give the dimethoxypyrimidine (68) in an 84% yield (Scheme 14).



Scheme 14: a) $POBr_3$, Et_3N, toluene, Δ, 99%, b) NaOMe (2.0eqv.), MeOH, 84%.

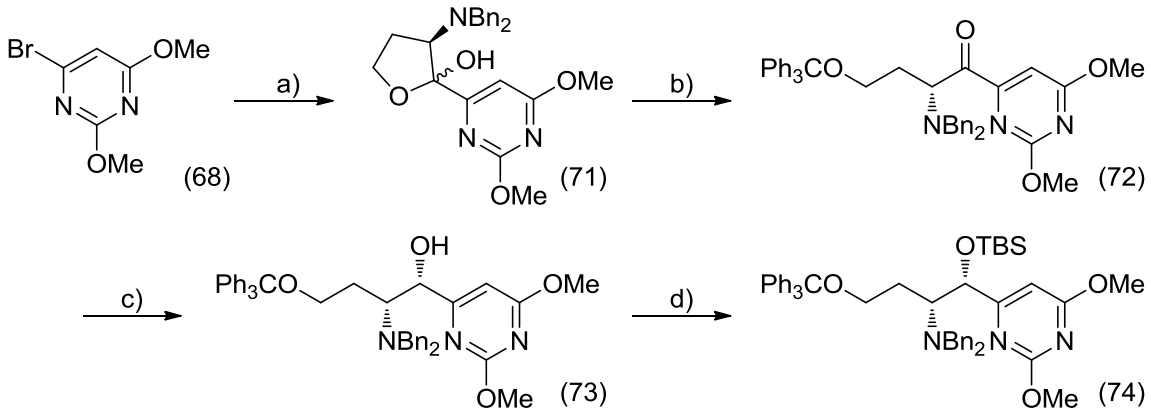
The next stage required the use of the chiral reagent (R)-2-(dibenzylamino)butyrolactone (70) which was prepared by adapting a method used to prepare the enantiomer of this from (S)-(+)-methionine by Reetz *et al.*[59] White et al simply switched to (R)-(-)-methionine (69) to prepare the required compound

(Scheme 15). They also improved the enantiomeric purity to >98% from 90% by recrystallizing the product.



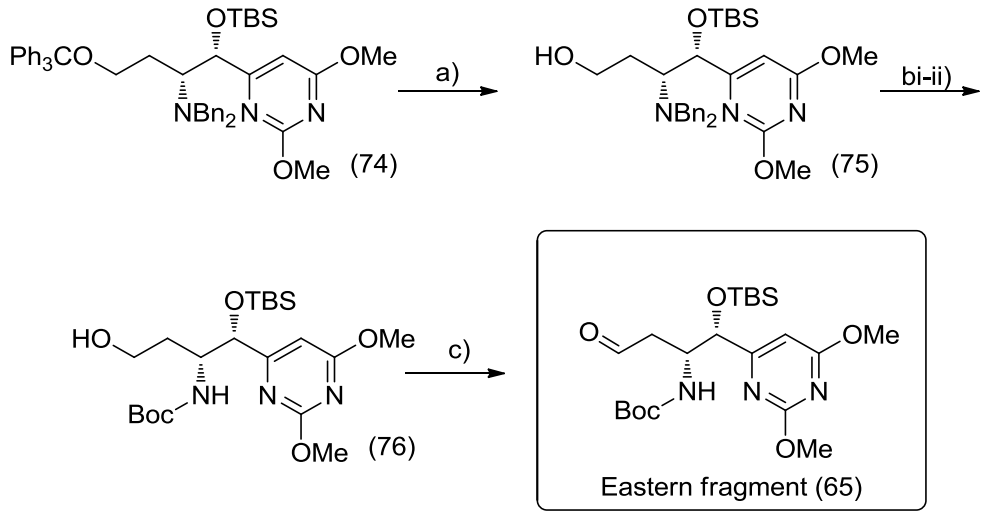
Scheme 15: Adapted synthesis using Reetz *et al*'s method, a) BnBr, K_2CO_3.

Dimethoxypyrimidine (68) was lithiated using n-BuLi to give the corresponding lithiated pyrimidine, which was then treated with the lactone (70) (Scheme 16). Interestingly, if the lactone is used alone this leads to approximately 25% racimisation of the product which was found by incorporating deuterium into the work up of the experiment. They completely inhibited this process by the addition of cerium chloride to the reaction mixture and eventually were able to prepare the product (71) in a 97% yield. This was then reacted with trityl chloride and triethylamine in the presence of a catalytic amount of DMAP to give the α-amino ketone (72) in a 93% yield. This was then reduced using *L*-selectride which gave a 12:1 ratio of the desired epimer of (73). The alcohol (73) was then converted to its TBS ether (74) in an 87% yield.



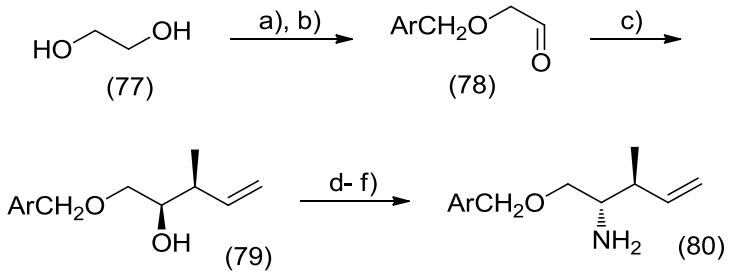
Scheme 16: a) n-BuLi, $CeCl_3$, -78°C-rt, (72), 97%, b) Ph_3CCl, Et_3N, DMAP, dichloromethane, 93%, c) *L*-selectride, THF, -78°C, 84%, d) TBSOTf, Et_3N, THF, 87%.

The next stages of the synthesis started with the removal of the trityl group to give the primary alcohol (75) in a quantitative yield. At this point, White *et al.* encountered some problems producing the aldehyde because the tertiary amine was too reactive and led to the formation of side products during the oxidation of the primary alcohol. They had to then suppress the activity of the amine by protecting it as a carbamate in two steps firstly hydrogenolysis to remove the benzyl groups using palladium hydroxide and hydrogen in an 81% yield, then using $(Boc)_2O$ to give the carbamate (76) in a 68% yield. The oxidation of the primary alcohol using TPAP and NMO gave the desired Eastern fragment (65) in a 91% yield. The Eastern fragment (65) was thus produced in 9 steps in an overall yield of 20% from barbituric acid (66) (Scheme 17).



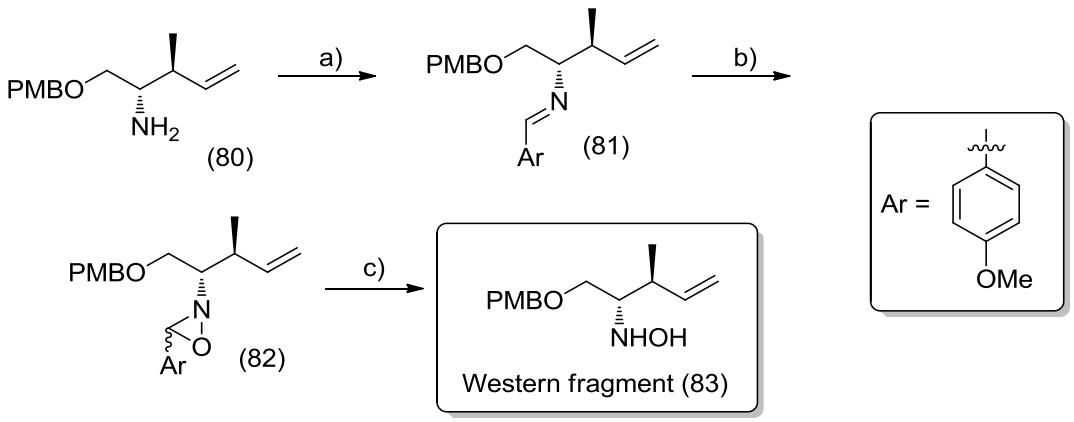
Scheme 17: a) HCO_2H, THF, quantitative yield, b) i) $Pd(OH)_2$, H_2, EtOH, 81%, ii) $(Boc)_2O$, Et_3N, dichloromethane, 68%, c) TPAP, NMO, dichloromethane, 91%.

The Western fragment (64) was then prepared from ethylene glycol (77) (Scheme 18) which was converted into then mono-*p*-bromobenzyl ether in a 65% yield which was then oxidised under Dess-Martin conditions to give the aldehyde (78) in a 90% yield. The aldehyde (78) then underwent asymmetric crotylation to give the *syn*-homoallylic alcohol (79) in a 45% yield and a 94% ee. This was them converted into amine (80) via the corresponding mesylate, which was then displaced using sodium azide, the product of which was reduced to the amine (80) using triphenylphosphine in a 56% overall yield.



Scheme 18: a) NaH, *p*-$BrC_6H_4CH_2Br$, THF, 65%, b) DMP, dichloromethane, 90%, c) Z-2-Butene, n-BuLi, *t*-BuOK, (+)-$(ipc)_2BOMe$, d) Ms_2O, pyr., e) NaN_3, DMF, f) PPh_3, THF-H_2O, 56%.

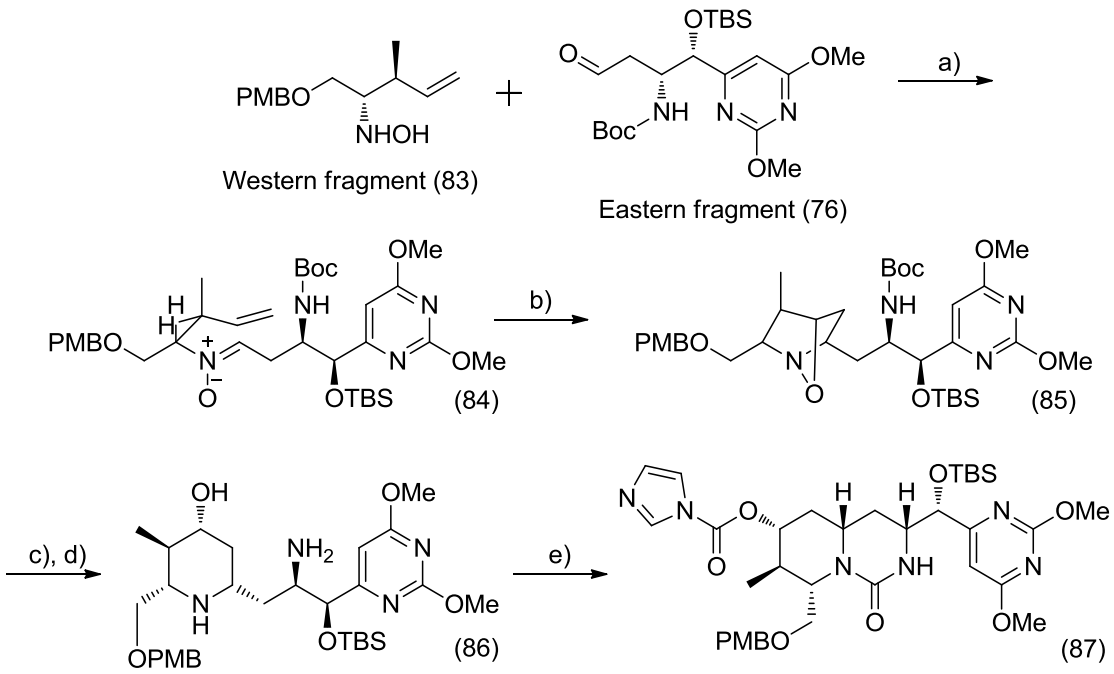
The next task was to oxidize the amine (80) to the hydroxylamine without oxidizing the vinyl group which was achieved using a method developed by Holmes *et al.*[60] Using this method, White *et al.* first condensed the amine (80) with *p*-anisaldehyde to give the imine (81), and then this was oxidised to the oxaziridine (82) as a 1:1 mixture of diastereomers. The mixture was then exposed to hydroxylamine hydrochloride which gave the Western fragment (83) in an overall yield of 33% from the amine (80).



Scheme 19: a) *p*-$MeOC_6H_4CHO$, Na_2CO_3, MeOH, 60°C, b) *m*-CPBA, dichloromethane, 0°C, c) $HONH_2 \cdot HCl$, MeOH, 0°C-rt, 40% over 3 steps.

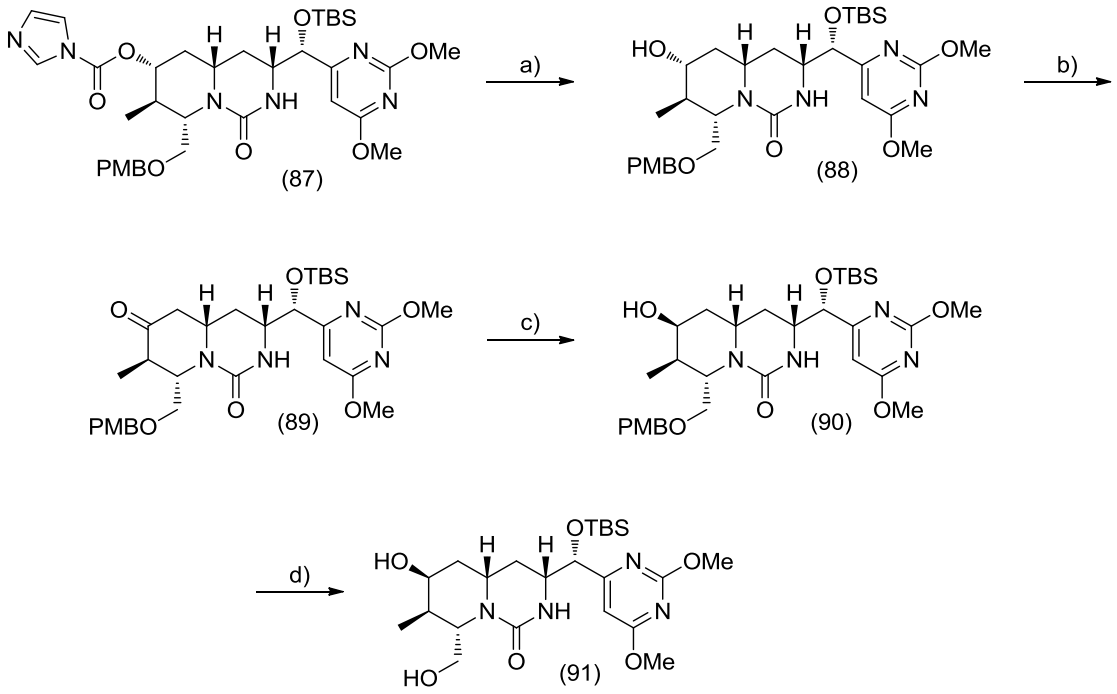
The two fragments were then coupled together to form the nitrone (84) in 60% yield which in turn was refluxed in toluene to give the bicyclic product (85). The bicyclic product was unstable to chromatography so the N-O bond was reduced using

Zn dust and ammonium chloride followed by the deprotection of carbamate protected amine using acidic methanol to give the free amine (86) in an overall yield of 68% from the nitrone (84). The amine was then bridged with the piperidine nitrogen to give the cyclic urea (87) using CDI in an 85% yield.



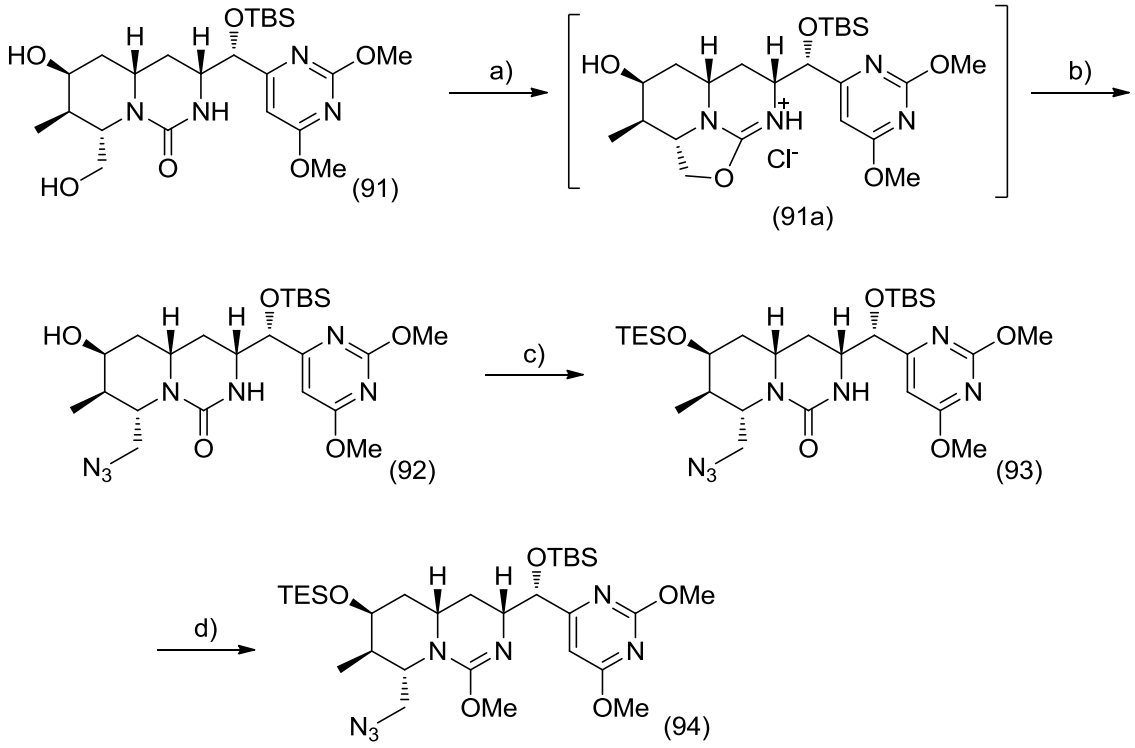
Scheme 20: a) MeOH, Δ, 3Å mol sieves, b) PhMe, Δ, c) Zn, NH_4Cl, THF-H_2O, Δ, d) HCl, MeOH, 98%, e) CDI, dichloromethane, 85%.

The cyclic urea (87) contains an imidazoyl carbamate (Scheme 21) which was cleaved from the cyclic urea using methanolic potassium carbonate in a quantitative yield to give the alcohol (88). The stereochemistry of this alcohol is the opposite of that required for 7-*epi*-cylindrospermopsin (2) and the next two steps in the synthesis were needed to invert this stereocenter. Alcohol (88) was thus oxidised under Dess-Martin conditions to give the ketone (89) in a 98% yield, which was then reduced using *L*-selectride to give the alcohol (90) with the correct configuration for 7-*epi*-cylindrospermopsin (2). The alcohol (90) then underwent hydrogenolysis to remove the PHB to give the primary alcohol (91) in 76% yield.



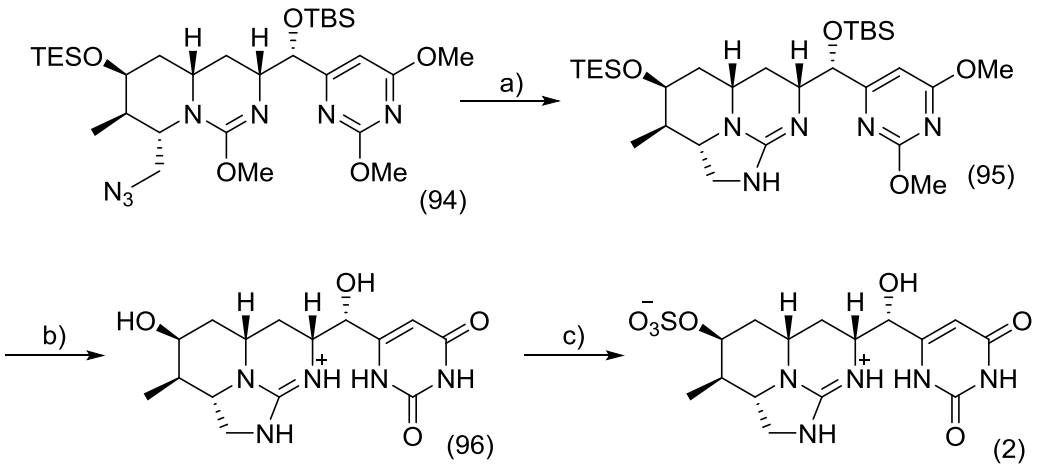
Scheme 21: a) K_2CO_3, MeOH, quantitative yield, b) Dess-Martin periodinane, dichloromethane, 98%, c) LiBH $(sec\text{-}Bu)_3$, THF, -78-0°C, 85%, d) H_2, $Pd(OH)_2$, EtOH, 76%.

The primary alcohol (91) was then treated with triphosgene which formed a tricyclic intermediate (91a) which was then reopened using sodium azide to give the azide (92) in a 49% yield over two steps. The alcohol in (92) was protected using trimethylsilyl triflate to give the ether (93) in a 99% yield. The urea in (93) was then exposed to potassium hexamethyldisilazide and then trimethyloxonium fluoroborate to give the methylisourea (94) (Scheme 22).



Scheme 22: a) $(Cl_3CO)_2CO$, THF, b) NaN_3, DMF, Δ, 49%, c) TESOTf, EtN_3, THF, 99%, d) KHMDS, $Me_3O^+BF_4^-$, dichloromethane, 0-25°C.

The methylisourea (94) was unstable to chromatography and was thus hydrogenated directly to the corresponding amine which spontaneously cyclised to form the cyclic guanidine (95). Deprotection of the silyl protection groups and the methyl ethers gave the diol (96) in a 21% yield from (93) (3 steps). Finally, diol (93) was selectively sulfonated with sulfur trioxide pyridine complex, as reported by Snider *et al*., to give 7-*epi*-cylindrospermopsin (2) in a 63% yield (Scheme 23).



Scheme 23: a) H_2, Pd/C, EtOH, b) HCl, Δ, 21% over 2 steps, c) SO_3.DMF, Pyr, DMF, Na_2SO_4, rt, 63%.

White *et al*. showed that their spectroscopic and optical rotation data matched that of the naturally isolated 7-*epi*-cylindrospermopsin (2), which further confirmed Weinreb's correction of the assignment of the C-7 position of cylindrospermopsin.

Williams group [53],[54]

Williams *et al*. set out to prepare the three main cylindrospermopsin alkaloids, cylindrospermopsin (1), 7-*epi*-cylindrospermopsin (2) and 7-deoxy-cylindrospermopsin (3) via a common strategy. They determined that a common starting point would be the carboxylic acid (97) (Figure 4).

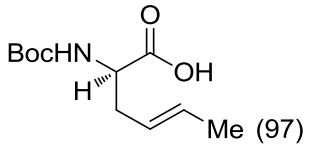
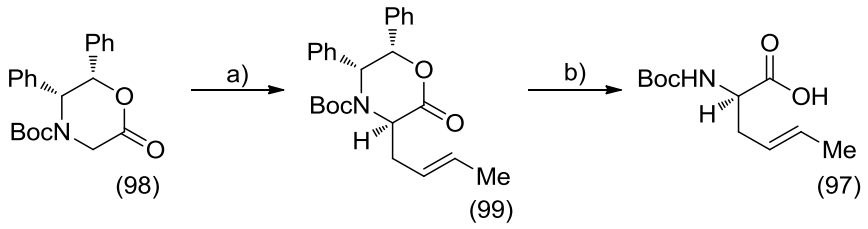


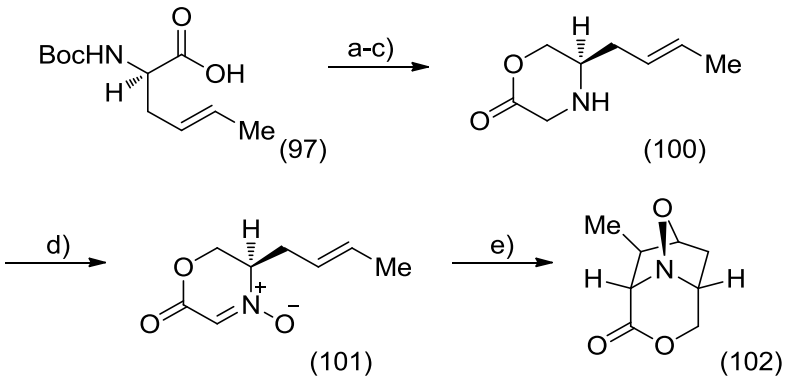
Figure 4: Williams's common intermediate (97).

Two routes were investigated to reach this intermediate but only one gave both enantiomers in a higher optical purity. This route started from oxazinone (98), which was alkylated with crotyl iodide to give (99). Removal of the auxiliary using lithium metal in ammonia gave the enantiomer (97) in a 68-87% yield (Scheme 24).



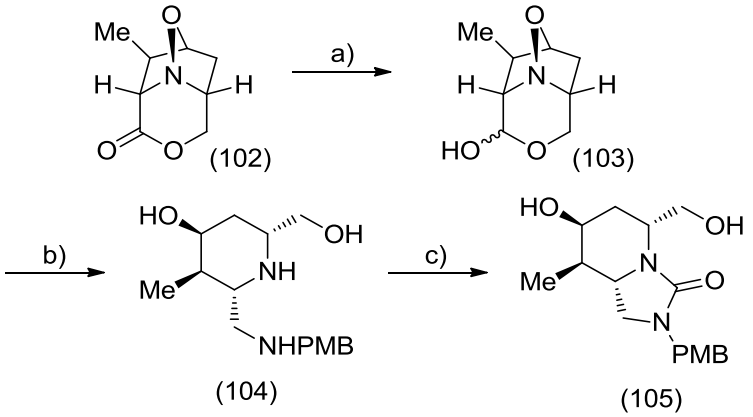
Scheme 24: a) KHMDS, THF, crotyl iodide, -78°C-rt, 92%, b) Li, NH_3, THF, EtOH, 68-87%.

Intermediate (97) was deprotected and esterified before being reduced to give crotylglycinol which was converted to morpholine (100) using α-bromophenyl acetate, in a 41-52% yield over three steps. Oxidation of the secondary amine in (100) using *m*-CPBA gave the oxazinone-*N*-oxide (101) in an 84% yield. Heating (101) at 200°C gave the tricyclic product (102) in a 78% yield (Scheme 25).



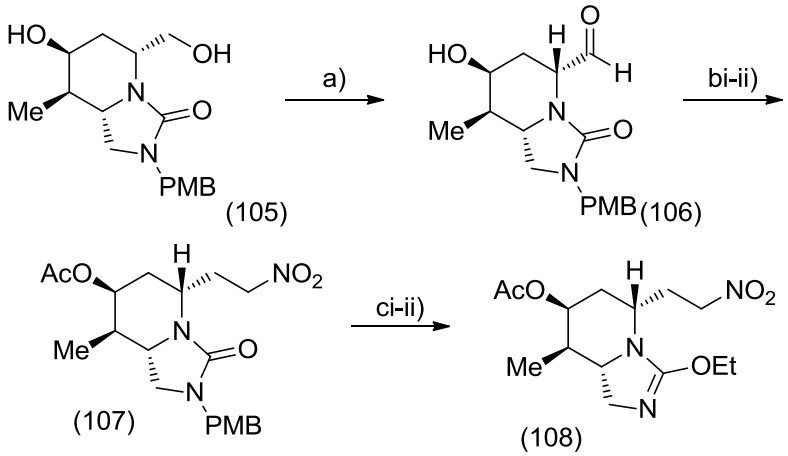
Scheme 25: a) AcCl, MeOH, b) $LiAlH_4$, THF, c) $PhOC(O)CH_2Br$, *i*-Pr_2NEt, MeCN, 41-52%, d) *m*CPBA, Na_2HPO_4, dichloromethane, -78°C, 84%, e) PhMe, 200°C, sealed tube, 78%.

The tricyclic intermediate (102) was reduced with DIBAl-H to give the lactol (103) in an 87% yield which in turn underwent reductive amination using $PMBNH_2$ to give (104). The formation of the cyclic urea (105) was obtained using bis-*p*-nitrophenylcarbonate in a 67% yield over 2 steps (Scheme 26).



Scheme 26: a) DIBAl-H, -78°C, 87%, b) 10% Pd/C, $PMBNH_2$, EA, H_2(1atm), c) (*p*-$O_2NC_6H_4O)_2CO$, MeCN.

The primary alcohol in the cyclic urea (105) was then oxidized using TEMPO, $PhI(OAc)_2$ and methanesulfonic acid to give the aldehyde (106) in a 75% yield. The next steps were the homologation of the aldehyde (106) using lithiated nitromethane to give a mixture of inseperable diastereomers of nitroaldol products. This mixture then underwent treatment with acetic anhydride which protected the secondary alcohol and dehydrated the aldol product, which on reduction with borohydride gave the nitroalkene (107) in a 56% yield over the two steps. The intermediate (107) was then deprotected using TFA which activated the urea which was then O-alkylated using Et_3OBF_4, to give (108) in a 62% yield over 2 steps (Scheme 27).

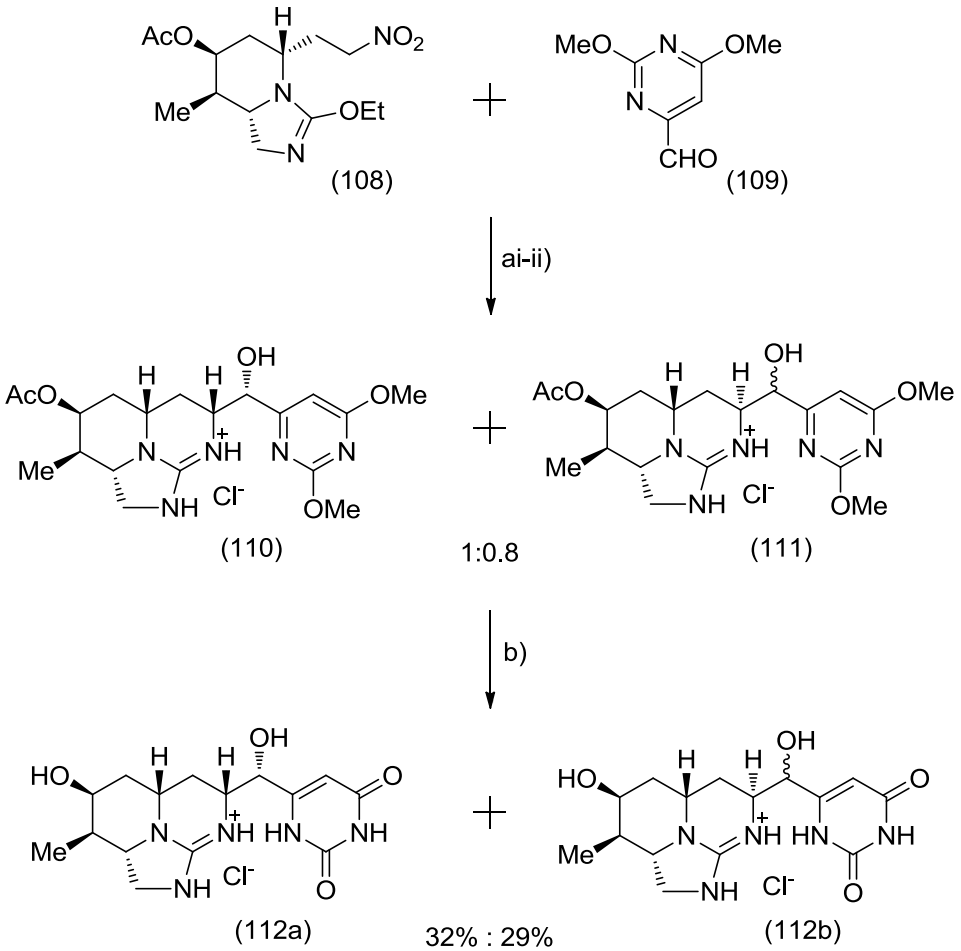


Scheme 27: a) TEMPO (0.4 eqv.), $PhI(OAc)_2$ (1.5 eqv.), $MeSO_3H$ (0.01 eqv.), 74%, b) i) $MeNO_2$, n-BuLi, ii) Ac_2O, DMAP then $NaBH_4$, EtOH, c) i) TFA, reflux, ii) Et_3OBF_4, Cs_2CO_3, 62% over 2 steps.

Compound (108) represents the advanced synthetic intermediate for the preparation of all three alkaloids and this intermediate can be converted into each of the three alkaloids.

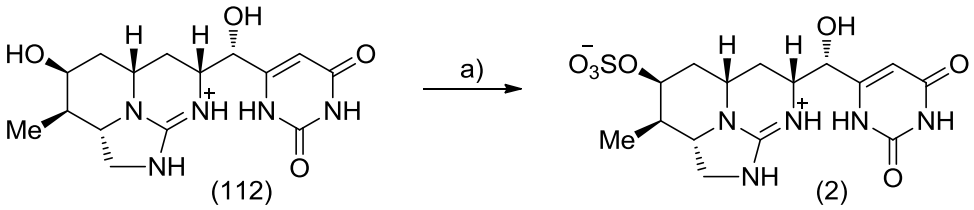
7-*epi*-Cylindrospermopsin (2)

Treatment of (108) with 2,6-dimethoxypyrimidine-4-carbaldehyde (109) gave the two diastereomers (110) and (111) in a ratio of 1:0.8 favouring the diastereomer (110) required for 7-*epi*-cylindrospermopsin (2). This inseparable mixture was then treated with conc. HCl which hydrolysed the acetyl- and methoxy-groups to give a separable mixture of (112a) and (112b) in a 32% and 29% yield respectively (Scheme 28).



Scheme 28: a) i) TBAF (2.0eqv.), THF, -15°C, 15 min, ii) H_2, $Pd(OH)_2$, MeOH, 5% HOAc, b) conc. HCl, reflux, 12 h.

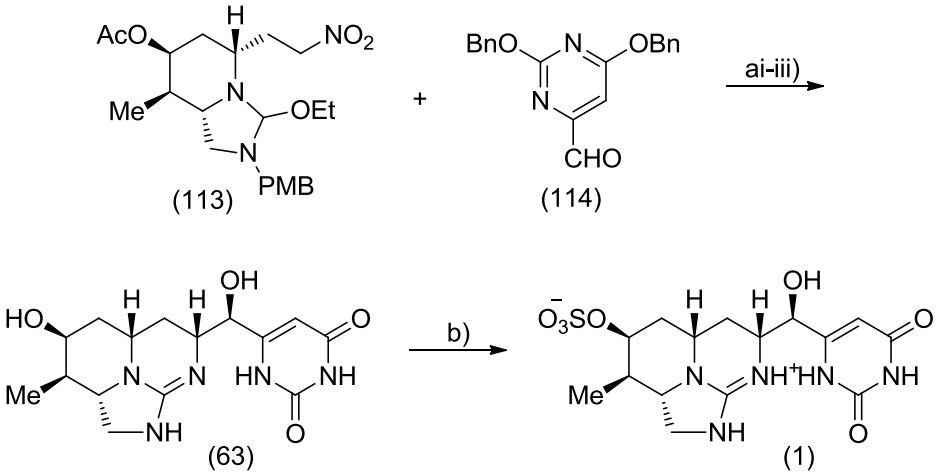
The diol (112a) was then reacted with sulfur trioxide pyridine complex to give 7-*epi*-cylindrospermopsin (2) in a 59% yield and the corresponding bi-sulfate in a 2:1 ratio. The overall yield for this synthesis of 7-*epi*-cylindrospermopsin (2) was 0.82% yield over 19 steps (Scheme 29).



Scheme 29: a) SO_3.DMF, Pyr, DMF, Na_2SO_4, rt, 3Å MS, 59%.

Synthesis of Cylindrospermopsin (1)

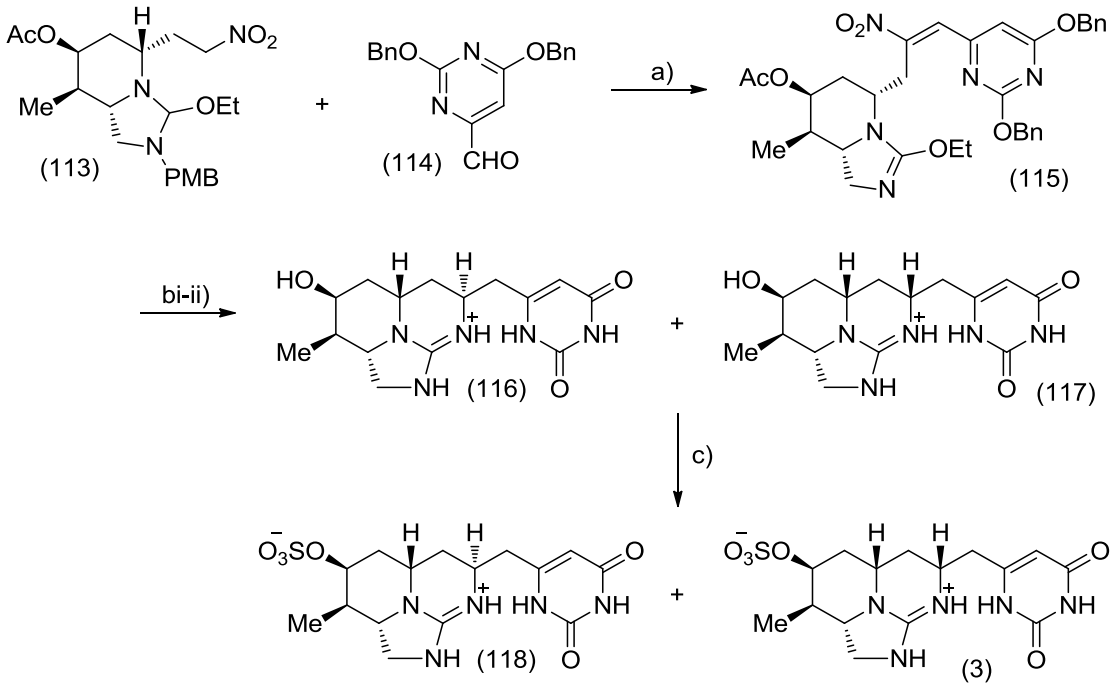
The synthesis of cylindrospermopsin (1) was achieved by firstly performing a TBAF mediated aldol reaction between nitroalkane (113) and di-benzyloxypyrimidine aldehyde (114). Reduction of the nitro group in the crude product of this reaction effected formation of the B-ring via an intramolecular guanidinylation. Deprotection of the acetate and pyrimidine protectiong groups in conc. HCl followed by HPLC purification gave diol (63) in a 20% yield. Diol (63) was then sulfonated using sulfur trioxide pyridine complex to give cylindrospermopsin (1) in a 60% yield. This preparation represents the first enantioselective synthesis of cylindrospermopsin (Scheme 30).



Scheme 30: a) i) 1.0 eqv. TBAF, THF, -15°C, 0.5 h, ii) H_2, $Pd(OH)_2$, MeOH, 5% HOAc, iii) conc HCl, reflux, 0.5 h, b) SO_3.DMF, Pyr, DMF, Na_2SO_4, rt, 3Å MS, 60%.

Synthesis of 7-deoxy-cylindrospermopsin (3)

The synthesis of 7-deoxy-cylindrospermopsin (3) was also achieved from the intermediate (113) and the aldehyde (114) by performing an aldol condensation reaction catalysed by acetic anhydride and caesium fluoride, which led to the nitroalkene (115) in a 67% yield. Treatment with sodium borohydride, followed by reduction with hydrogen gas and $Pd(OH)_2$, led to the formation of an inseparable mixture of guanidines. These were hydrolysed with concentrated HCl to give a 1:1 mixture of diastereomers (116) and (117). Sulfonation using sulfur trioxide pyridine complex gave 7-deoxy-cylindrospermopsin (3) and its diastereomer (118) in a yield of 33% (Scheme 31).



Scheme 31: a) CsF, Ac_2O, MeCN, 67%, b) i) $NaBH_4$, EtOH then H_2, $Pd(OH)_2$, MeOH, 5% AcOH, ii) HCl, reflux, 0.5 h, c) SO_3.DMF, Pyr, DMF, Na_2SO_4, rt, 3Å MS, 33%.

In conclusion, the Williams group developed two enantioselective syntheses of both cylindrospermopsin (1) in 19 steps in an overall yield of 0.57% and 7-*epi*-cylindrospermopsin (2) in 19 steps with an overall yield of 0.82%. They also achieved

the the first reported synthesis of the biosynthetic intermediate 7-deoxy-cylindrospermopsin (3) in 20 steps with an overall yield of 1.05%.

The biosynthesis of the cylindrospermospin alkaloids

Runnegar *et al.* investigated the biosynthesis of cylindrospermopsin within *C. raciborskii* via ^{13}C labelled feeding experiments using acetate and glycine as the biosynthetic precursor units.

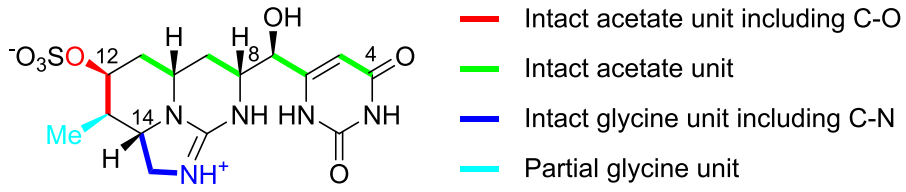
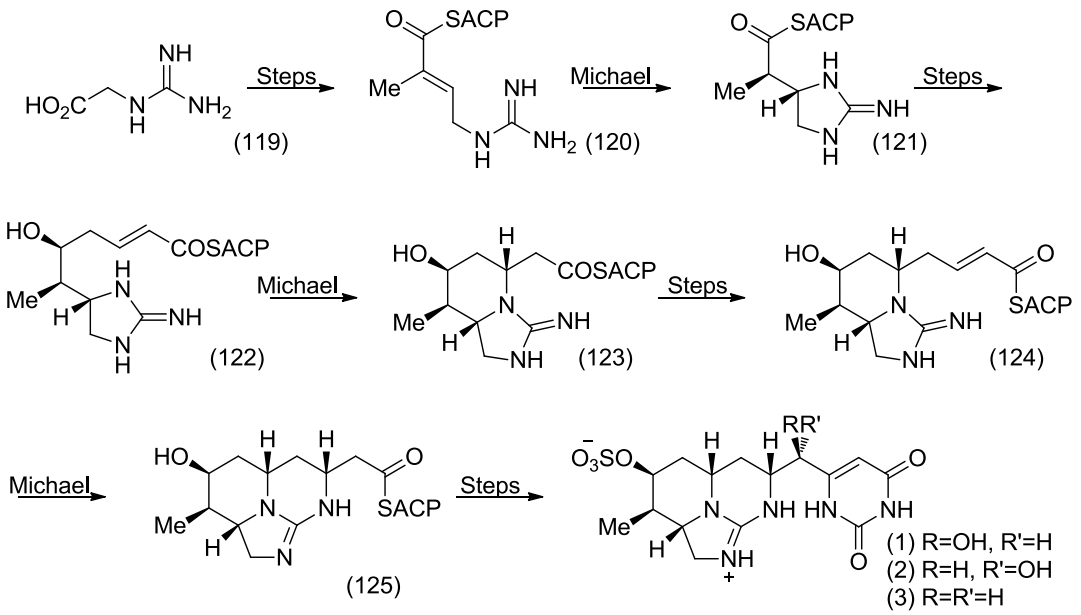


Figure 5: Acetate and glycine units found in cylindrospemopsin

This study concluded that cylindrospermopsin is formed via a series of polyketide elongations of guanidino acetic acid.[61] The feeding experiments also indicated that C-4 to C-13 are derived from five acetate units arranged head to tail as are the oxygen atoms attached to C-4 and C-12. The C-14, C-15 and N-16 are from a single glycine unit with the methyl group C-17 being from a different glycine unit (Figure 5).

The formation of cylindrospermopsin in nature has been proposed to start from a novel *L*-arginine glycine amidino transferase enzyme.[62],[63] The rings of the tricyclic core of cylindrospermopsin are constructed in the order C-A-B order starting from guanidine acetic acid (119) and are achieved via sequential polyketide extensions and Michael type ring closures. The uracil ring system is formed from a donor such as urea or arginine followed by dehydration and further addition reactions. The final steps of the biosynthesis are tailored to specific members of the cylindrospermopsin family.[64]

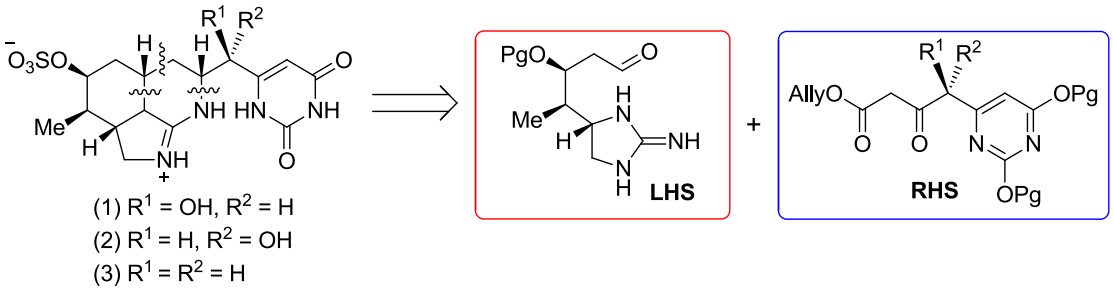


Scheme 32: Biosynthesis of cylindrospermopsin

Sulfonation of the C-12 hydroxyl group is thought to be achieved using a sulfotransferase enzyme which gives 7-deoxy-cylindrospermopsin (3). Hydroxylation of the C-7 bridging carbon atom to give either cylindrospermopsin (1) or 7-*epi*-cylindrospermopsin (2) (Scheme 32) it thought to proceed by a hydroxylation step likely being catalyzed by CyrI, a proline hydroxylase homologue.[65]

Previous work in the Murphy research group

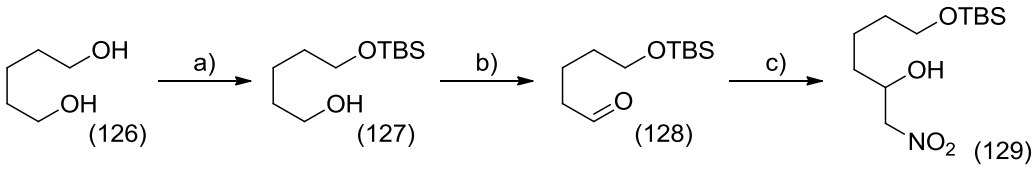
The synthetic strategy adopted in the Murphy research group[66] is based upon a biomimetic synthetic strategy in which the formation of the cyclic guanidine core is achieved early on in the synthesis. The retrosynthesis of cylindrospermopsin makes disconnections which separate the tricyclic guanidinium core from the substituted uracil system to give a RHS and LHS synthon. It is proposed synthesis to couple these fragments under tethered Biginelli conditions.[67] If successful, this synthetic route has the potential to be the shortest route to the cylindrospermopsin alkaloids.



Scheme 33: Retrosynthesis of cylindrospermopsin

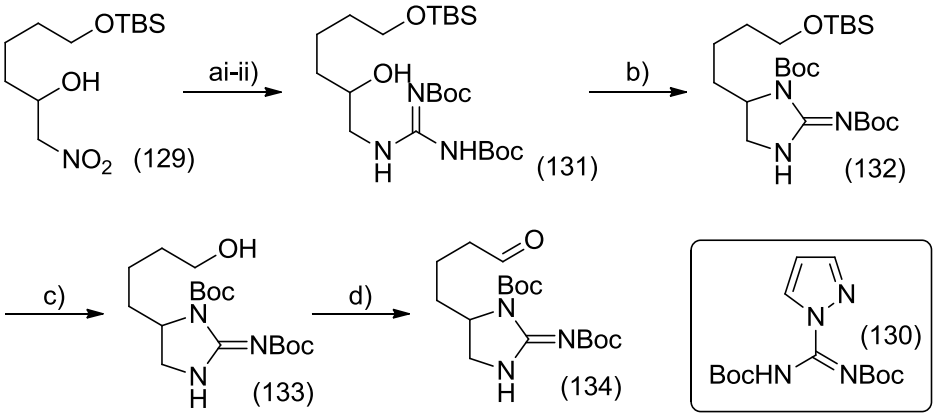
The model study of Evans and Murphy[67] focused on the construction of the LHS fragment which contains the tricyclic guanidinium core and three of the six stereogenic centres. This synthesis unlike previous methodology constructs the C-ring early in the synthesis and uses the inherent stereochemistry at the ring junction to establish the other two rings in one-step and in a stereospecific manner.

The model study approach started with 1,5-pentane diol (126) which was mono-silylated using TBSCl to give (127) in a 61% yield. The remaining alcohol was then oxidised under Swern conditions to give the aldehyde (128) in an 89% yield. The aldehyde (128) then underwent a nitro-aldol (Henry) reaction with nitromethane to give the nitro-alcohol (129) in an 81% yield (Scheme 34).



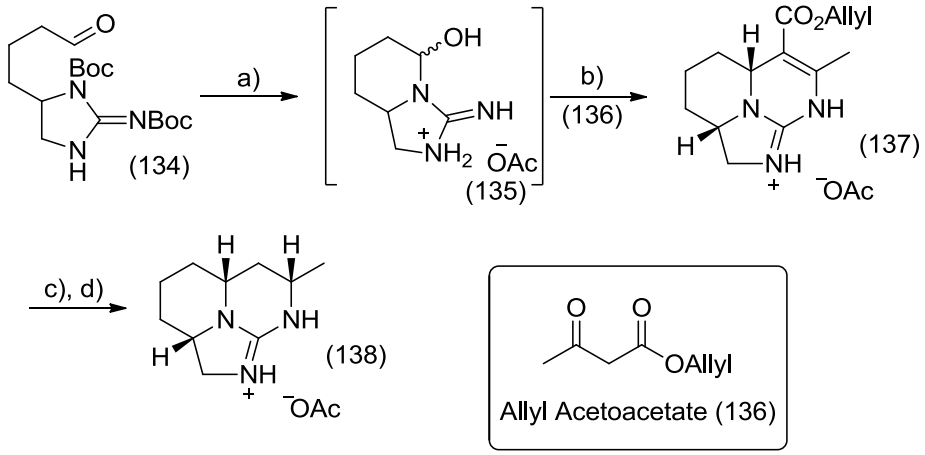
Scheme 34: a) TBSCl, NaH, THF, 2 h, 61%, b) Oxalyl chloride, DMSO, NEt_3, dichloromethane, -78°C, 3 h, 89%, c) $MeNO_2$, DIPEA, dichloromethane, 36 h, 81%.

The nitro alcohol (129) was the reduced to the corresponding amine which was then guanidinylated in situ with N,N′-di-Boc-1H-pyrazole-1-carboxamidine (130) to give (131) in a 78% yield over two steps. The guanidine (131) then underwent an Appel type cyclisation to give the cyclic guanidine (132) in a 96% yield. This intermediate contains the fully constructed C-ring of the tricyclic ring system. The silyl ether in (132) was then removed using TBAF to give the corresponding alcohol (133) in a quantitative yield. The alcohol was then oxidized under Dess-Martin conditions to give the aldehyde (134) in a 96% yield (Scheme 35).



Scheme 35: a) i) $NiCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH, NEt_3, 0°C, 3 h, ii) (130), 48 h, 78% over 2 steps, b) PPh_3, I_2, imidazole, dichloromethane, -20°C, 1 h, 96%, c) TBAF, THF, 0°C-rt, 24 h, 99%, d) DMP, pyr, dichloromethane, 24 h, 96%.

The Boc groups in the aldehyde intermediate (134) were removed by treatment with acetic acid to give the hemiaminal (135) which was then condensed with allyl acetoacetate (136) under Biginelli conditions to give the tricyclic system (137) in a 43% yield. The final step of the model study was to remove the allyl ester by treatment of (137) with $Pd(PPh_3)_4$ in THF/methanol, followed by reduction of the enamine intermediate using sodium cyanoborohydride under acidic conditions to give the saturated tricyclic guanidine (138) in a 57% yield (Scheme 36).

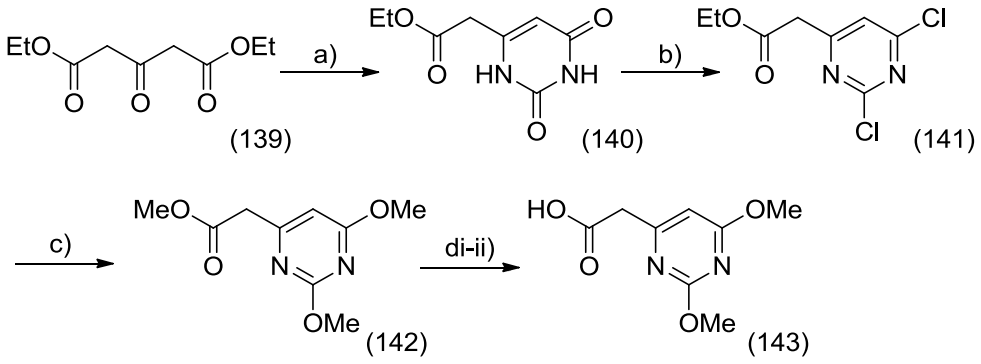


Scheme 36: a) HOAc, 24 h, b) Morpholine acetate, (136), Na_2SO_4, CF_3CH_2OH, 70°C, 12 d, 43%, c) $Pd(PPh_3)_4$, pyrrolidine, THF/MeOH, 1.5 h, d) $NaBH_3CN$, HOAc/MeOH, 16 h, 57% over 2 steps.

With only 12 steps, this synthesis of the tricyclic core of cylindrospermopsin represents the most efficient method. Other work in the Murphy research group has focused on the synthesis of the RHS fragment, which contains not only the uracil ring but also the C-7 stereocentre.

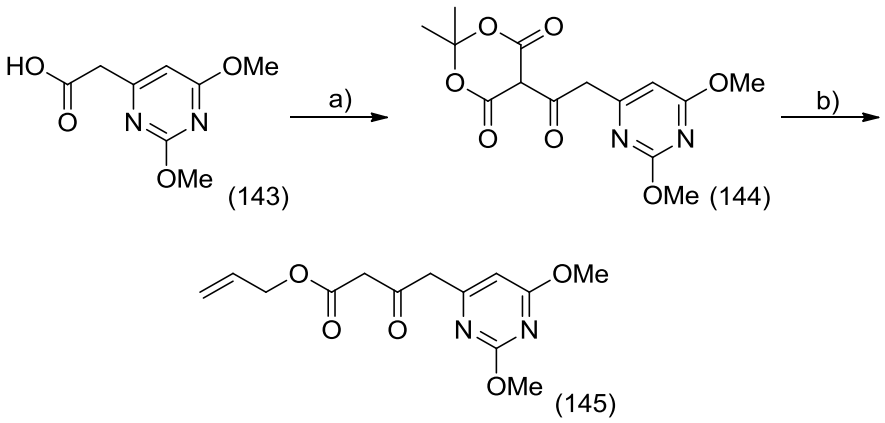
Preparation of a 7-deoxy-cylindrospermopsin RHS synthetic equivalent

The synthesis[68] of the D-ring of 7-deoxy-cylindrospermopsin (3) began with diethyl 1,3-acetonedicarboxylate (139) which on treatment with urea under acidic conditions at reflux in a benzene/ethanol mixture gave the pyrimidine (140) in a 80% yield. The pyrimidine (140) was then reacted with $POCl_3$ under basic conditions to give the dichloropyrimidine (141) in a 76% yield. This was then treated with sodium methoxide to give the pyrimidine intermediate (142) in a 40-57% yield. The methyl ester in (142), was then hydrolysed using sodium hydroxide followed by the addition of acid to give (143) in a 96% yield (Scheme 37).



Scheme 37: a) Urea, H_2SO_4, PhH, EtOH, 10 d, 80%, b) $POCl_3$, *i*-$PrNEt_2$, PhMe, Δ, 3 h, 76%, c) NaOMe, MeOH, 3 h, 40-57%, d) i) NaOH, MeOH, 16 h, ii) 1M HCl, 96%.

The acid (143) was activated with CDI and then exposed treated with Meldrums acid to give the intermediate (144), which on refluxing in allyl alcohol gave the ß-ketoester (145) in a 28% yield overall from (143) (Scheme 38).

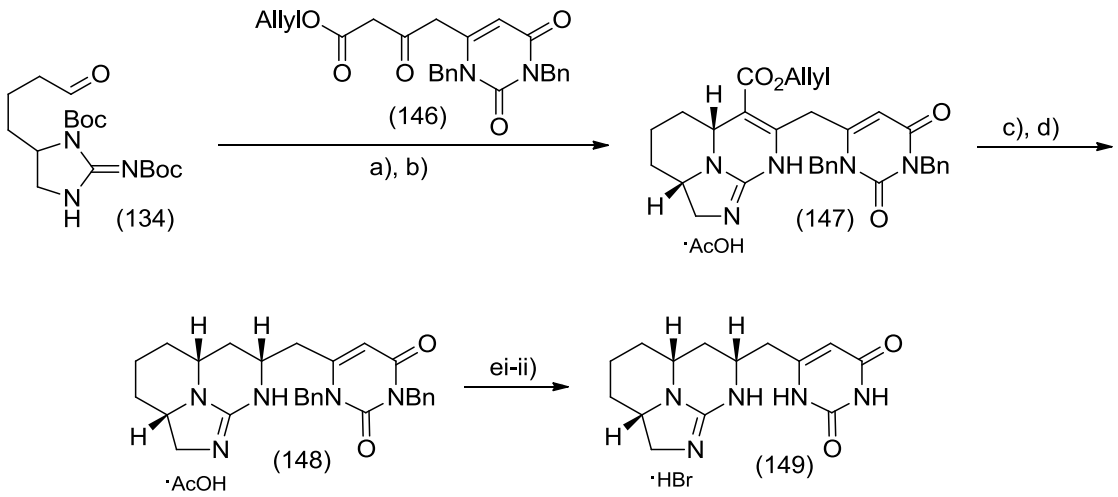


Scheme 38: a) CDI, DMAP, Meldrums acid, b) Allyl alcohol, PhH, Δ.

The intermediate (145) has been used (see below) in the synthesis of a tetracyclic analogue of cylindrospermopsin and with a suitable LHS synthetic equivalent would allow access to 7-deoxy-cylindrospermopsin (3). Work is still underway to prepare oxidised analogues of (145) to allow access to cylindrospermopsin (1) and 7-*epi*-cylindrospermopsin (2).

Synthesis of a tetracyclic analogue of cylindrospermopsin

Within the Murphy group a tetracyclic analogue (149) of 7-deoxy-cylindrospermopsin (3) has been prepared from the previously prepared aldehyde (134) which was subjected to a tethered Biginelli condensation with the β-keto ester (146) using the previously reported conditions[66],[67] Under these conditions it was possible to isolate the tetracyclic guanidine (147) in 19% yield, the relative stereochemistry of which was determined by 2D NOESY NMR. Deallylation/decarboxylation of (147) was achieved by treatment with $Pd(PPh_3)_4$, followed by reduction with $NaBH_3CN$ to give guanidine (148) in 75% yield. Again 2D NOESY NMR was used to determine the orientation of the newly introduced methine proton. Deprotection of the *N*-benzyl groups was achieved by treatment with BBr_3 which furnished the 7-deoxy-cylindrospermopsin analogue (149) in 93% yield (Scheme 39).



Scheme 39: Preparation of analogue (149), a) AcOH, 24 h, b) Morpholine acetate, (146), Na_2SO_4, CF_3CH_2OH, 100°C, 12 d, 19%, c) $Pd(PPh_3)_4$, pyrolidine, THF-MeOH (1:1), 4 h, d) $NaBH_3CN$, AcOH-MeOH (1:1), 0°C to rt, 16 h, 75% (2 steps), e) i) BBr_3, Xylene, 130°C, 16 h, ii) MeOH, rt, 16 h, 93%.

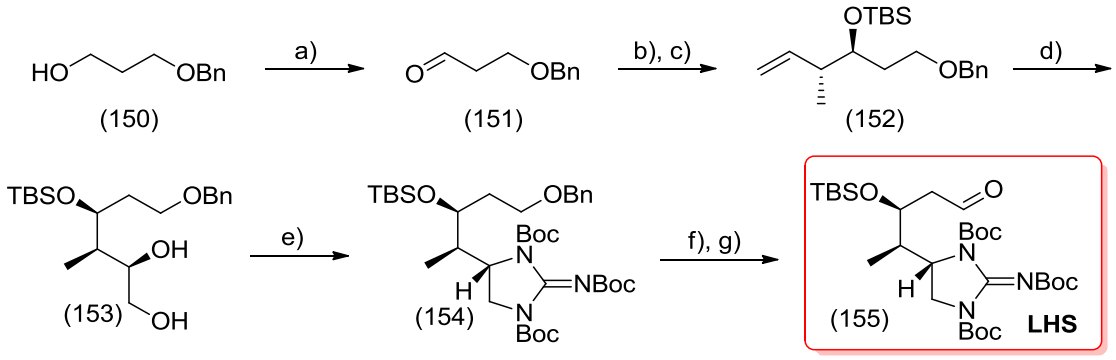
In conclusion, Murphy and co-workers have developed a model synthesis of a 7-deoxy-cylindrospermopsin analogue (149) in 13 steps and 0.7% overall yield. This synthesis might be open to modification to prepare 7-deoxy-cylindrospermopsin (3) in significantly fewer steps than the current known racemic synthesis of this metabolite (20 steps, 0.62-1.05%).

With a viable route for the preparation of 7-deoxy-cylindrospermopsin (3) in hand efforts were directed towards the preparation of and 7-*epi*-cylindrospermopsin (2) and cylindrospermopsin (1).

Aims

The aim of the first section of this thesis is to investigate the preparation of the LHS synthetic equivalent (155). The initially proposed synthesis of (155) begins with 3-benzyloxy-1-propanol (150).[69] Oxidation of the primary alcohol under Swern conditions should yield aldehyde (151), which on treatment with (+)-(*E*)-crotyl-Ipc-2-borane[70] followed by silyl protection of the alcohol should give alkene (152). This alkene, under Sharpless *bis*-hydroxylation conditions[71] should give the diol (153) whose conversion to the guanidine (154) can be achieved under Mitsunobu conditions. Removal of the benzyl group by hydrogenolysis followed by oxidation of

the resulting alcohol with Dess-Martin periodinane should yield the desired LHS aldehyde (155), which is analogous to (134) (Scheme 40).



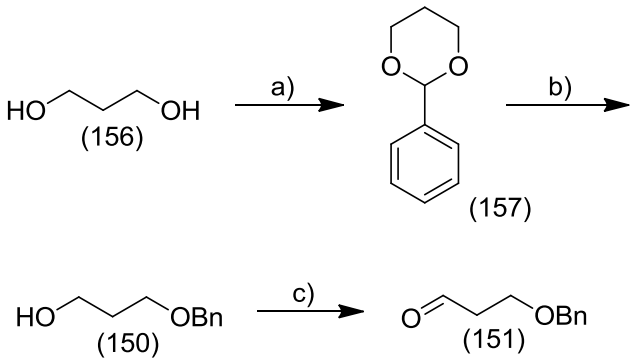
Scheme 40: a) oxalyl chloride, DMSO, NEt_3, dichloromethane, -78°C, b) (+)-Ipc_2BOMe, (*E*)-2-butene, *t*BuOK, *n*BuLi, $BF_3 \cdot Et_2O$, THF, -78°C, c) TBSCl, imid, DMF, d) AD-mix-β, K_2OsO_4, *t*BuOH/H_2O, e) PPh_3, DIAD, *N*,*N*′,*N*″-*Tris*-Boc-guanidine, f) H_2 Pd/C EtOAc, g) Dess-Martin Periodinane.

Following the successful preparation of (155) coupling with the RHS synthetic equivalent (146) should allow the access to 7-deoxy-cylindrospermopsin (3) using the methodology developed previously.[66],[67]

Results and discussion

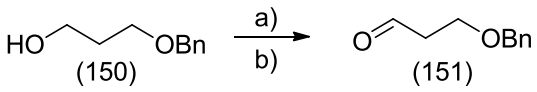
The preparation of the alcohol (150) began with the condensation of 1,3-propane-diol (156) and benzaldehyde under Dean-Stark conditions to form the acetal (157) required to access the benzyl protecting group. This occurred in a quantitative yield, after work up with sodium metabisulfite to remove excess benzaldehyde. The acetonide (157) was then reduced using DIBAL-H to give mono-protected diol (150) via a ring opening reaction. Initially the reaction yielded the product in yields varying from 69-71% (8 reactions), with the low yield being attributed to the workup procedure as the aluminium salts formed generated a viscous emulsion which did not separate well on extraction. Eventually it was found that if the reaction was quenched at 0°C instead of at room temperature the resulting viscous aqueous layer did not form, but instead seemed to form a clear or slightly cloudy aqueous layer which did not form an emulsion. Yields of 98% were routinely obtained. The reason behind this

the initial poor yields was not investigated, but it was concluded that it might be due to the rate at which the aluminium salt is formed during the quenching of the reaction.



Scheme 41: a) PhCHO, PhMe, CSA, Dean-Stark, Δ, 24 h, quantitative yield, b) DIBAl-H, -78°C, 98%, c) PCC, dichloromethane, 24 h 32-40%.

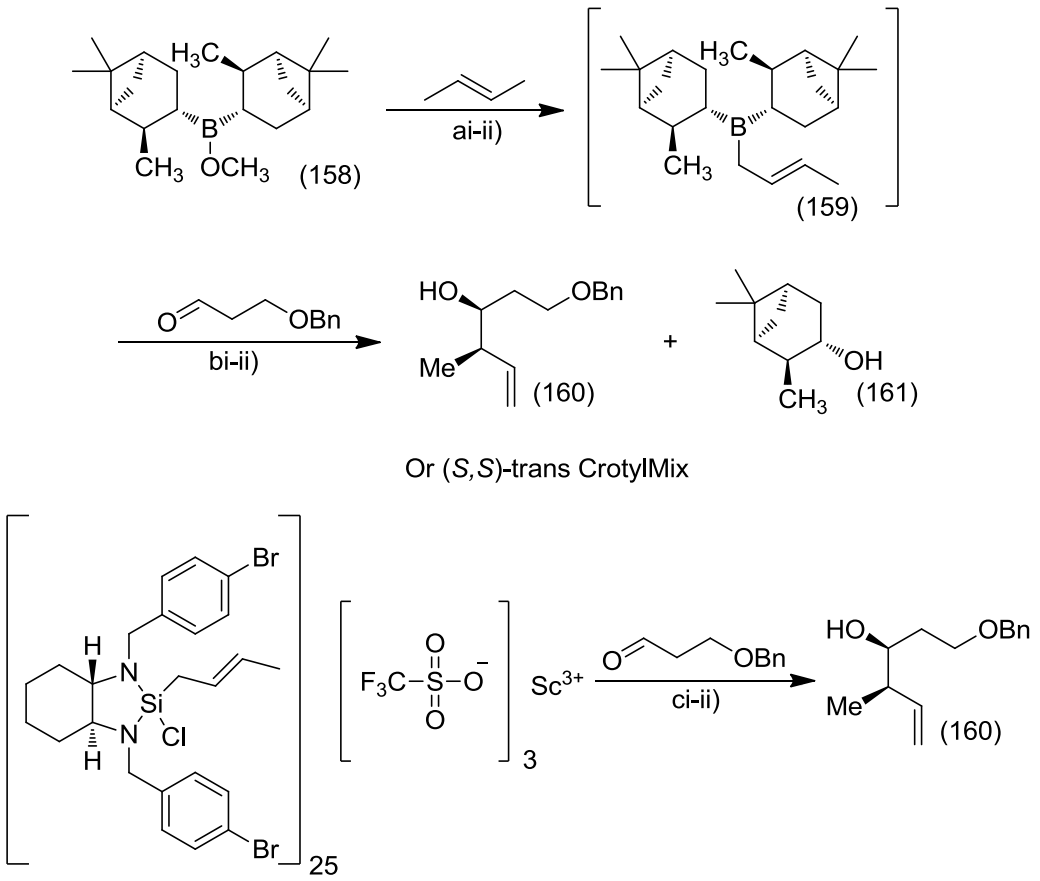
The resulting mono-protected diol (150) was oxidised using PCC to give moderate yields of 32-40% (6 attempts) (Scheme 41). This result was disappointing and the low yields were attributed to the difficulties in the work up procedure as the chromium by-products formed a sticky tar-like substance, which was difficult to extract. This was aided to some extent by the addition of Celite© but the yields only improved slightly (35-48%) (Scheme 42). Due to these problems and the low yields the PCC oxidation did not seem a good choice for the oxidation. The Swern oxidation, however, proceeded with a 95% yield, and the crude product showed no signs of major impurities on TLC or by proton NMR spectroscopy. After work up, the aldehyde (151) was purified by chromatography but had a noticeable smell of DMS (dimethyl sulfide). This was not perceived as a problem, however on proceeding to the Brown crotylation protocol it was found that using the aldehyde obtained by the Swern method gave very poor yields and a complex mixture of products. It was noted that no starting aldehyde was recovered from the reaction. The reason for this failure is unclear but possibly the residual DMS reacts preferentially with the boron complex. We thus reverted to the PCC oxidation method for the preparation of the aldehyde (151).



Scheme 42: a) PCC, dichloromethane, Celite, 12 h, 35-48%; or b) Oxalyl chloride, dichloromethane, -78°C, N_2 atmosphere, DMSO, NEt_3, 90-99% yield.

The next step in the synthesis is the Brown crotylation of the aldehyde (151) which will install the two stereogenic centres found in the A-ring of the alkaloids. This reaction involves the use of the chiral reagent (158) which is treated with metallated *trans*-2-butene to give (+)-(*E*)-crotyl-Ipc-2-borane (159).[70] Addition of aldehyde (151) and boron trifluoride yielded the crotyl alcohol (160) together with the alcohol by-product (161).

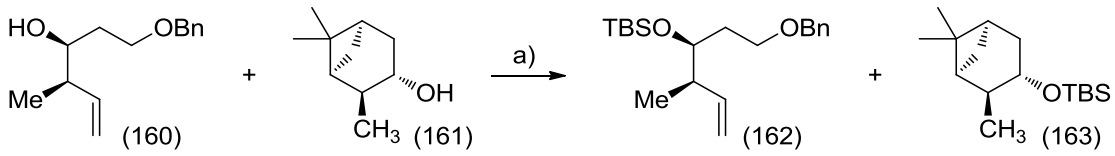
Attempts at the purification of (160**)** by column chromatography were problematic as the major by-product in this reaction, alcohol (161) eluted a similar Rf to the product and was inseparable even on repeated chromatography. The literature suggested that the alcohol by-product (161) could be removed by sublimation[72]. The method described heating the mixture to 65°C under water aspiration would give effective sublimation. However we attempted this method and in our hands it was unsuccessful as no sublimation was observed under these conditions. We tried the process at a lower pressure (1-2 mmHg) which was also unsuccessful. Even at an increased temperature, no evidence of sublimation was observed in the apparatus used. This method also appeared to cause decomposition of the crude product, and it was decided that this method and multiple chromatography was not a cost effective or efficient method for the purification of the crotyl alcohol (160).



Scheme 43: a) i) *t*-BuOK, THF, n-BuLi, 20 min, -78°C, *trans*-2-butene, -45°C, 30 min, ii) (+)-$(IPC)_2BOMe$, -78°C, 30 min, b) i) Aldehyde (150), BF_3OEt_2, 5 h, rt, ii) NaOH, H_2O_2 (30%), 1 h, rt, c) i) dichloromethane, 0°C, ii) Et_2O, HCl, 1 h.

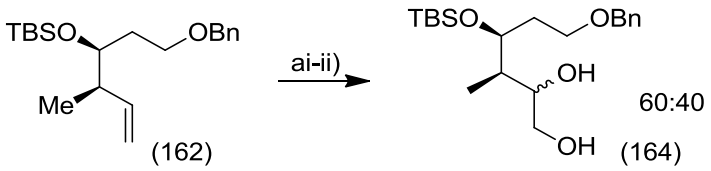
An alternative crotylation method was also utilized to produce (160) using (*S*, *S*)-trans crotyl mix. The reaction did produce (160) in a reasonable yield although it was not cost effective and did not warrant a switch to this reaction.

Instead, we decided to protect the crude mixture of alcohols as their TBS-ethers, to see if the mixture of silyl ethers (162) and (163) could be separated. Thus the mixture of alcohols was treated with an excess of TBSCl and imidazole with a catalytic amount of DMAP. After stirring for 3 d, work up and chromatography proved successful in separating the desired silyl ether (162) in a 43% yield over 2 steps.



Scheme 44: a) TBDMSCl, dichloromethane, DMAP, imidazole, 3 d, 43% (2 steps).

With the ether (162) in hand we proceeded to investigate the Sharpless asymmetric dihydroxylation using AD mix ß which should produce the diol (164) and installs the third stereogenic centre in the incorrect orientation (this will be inverted later). We initially tried the recommended conditions (1:1 water: tert-butyl alcohol, 0°C, 24 h) but on analysis of the proton and carbon NMR spectra of the crude product it was apparent that diasteromeric mixture of alcohols (164) was formed.



Scheme 45: a) i) *t*-BuOH/H_2O (1:1), AD-mix ß, see (table 2), ii) Na_2SO_3, 1 h, rt.

Entry	Conditions		Yield	diastereomeric ratio
	Temp	pH		
1	rt	7	63%	65:35
2	rt	14	67%	60:40
3	0°C	7	0%	NA
4	0°C	14	30%	55:45

Table 2: Effect of changes in reaction conditions

The ratio of alcohols could be determined as an approximate ratio of 65:35 (major product not determined) (Table 2, entry 1) from the integration of the methyl signals of the two diasteroisomeric products at δ_H 0.83 (3H, d, *J* 7.8 Hz, Me) ppm and δ_H 0.98 (3H, d, *J* 7.1 Hz, Me) ppm. This result seemed to indicate that the selectivity for this reaction is quite poor compared to the reported literature for this reaction. (Figure 6)

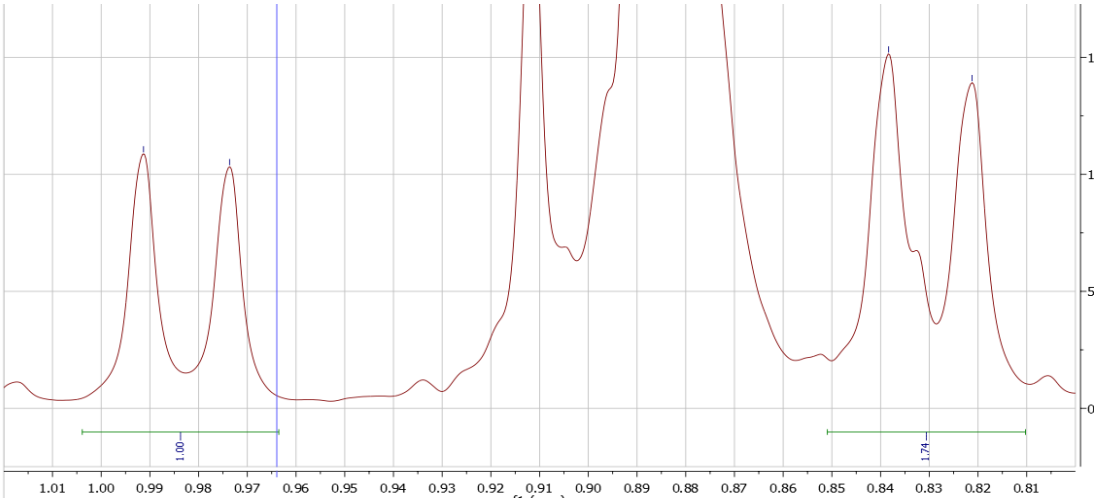


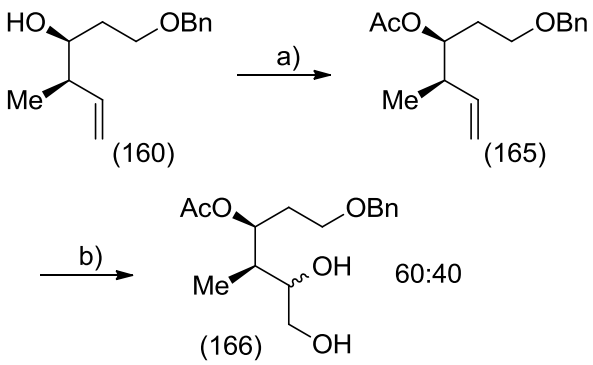
Figure 6: Diagnostic NMR spectrum of diol mixture (164).

This result was disappointing, but it was suggested in the literature[73] that performing the reaction at a higher pH was one possible way of improving selectivity. Repeating this reaction at pH 14 unfortunately gave a ratio of 60:40 (entry 2). Both of these reactions were repeated at a lower temperature, however this failed to improve selectivity and led to much lower yield in both cases (Table 2, entries 1-2).

These results were surprising as this reaction usually gives good selectivity and high yields. We had proposed that the bulky TBS-protecting group which partially eclipses the terminal alkene might lead to an advantageous selectivity in the dihydroxylation reaction. Indeed it is apparent that in a minimized energy conformation of (162) (chemdraw 3D) (Figure 7) the lowest energy configuration the TBS-protecting group does appear to eclipse one face of the alkene but the chain containing the benzyl protecting group appears to eclipse the other face. This observation might explain the poor facial selection of the Sharpless reagent and thus the low diastereoselectivity.

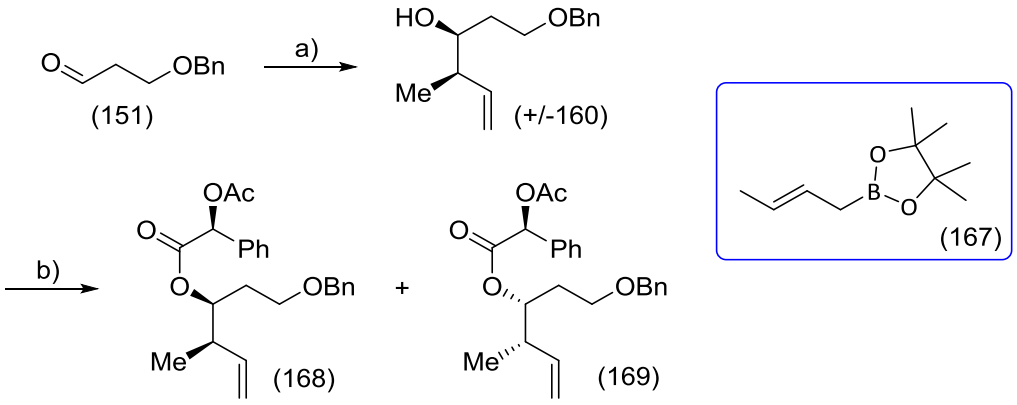
Figure 7: Minimized energy conformation of (162). (Chemdraw 3D)

It was felt that a smaller protecting group on the alcohol might improve selectivity. In order to test this hypothesis acetylation of crude alcohol mixture (160) was carried out using acetic anhydride/pyridine to give the acetate (165) in 19% over two steps. Unfortunately dihydroxylation under conditions previously employed again gave a mixture of diols (166) in a 53:47 ratio (as determined by integration of the acetyl methyl signals of the two diasteroeisomers). This was a lower selectivity than that observed in the dihydroxylation of the silyl protected alkene (162) thus the introduction of the OAc group seems to have had a detrimental effect.



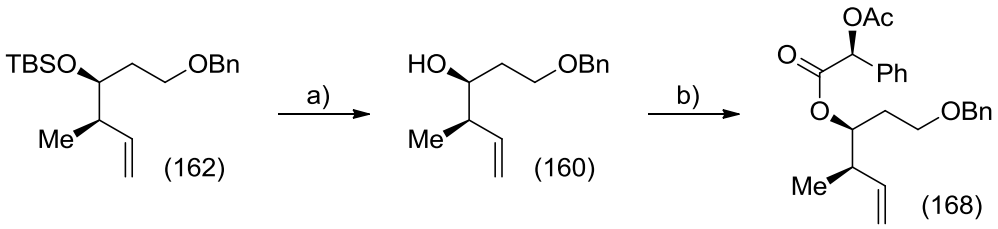
Scheme 46: a) Ac_2O, dichloromethane, pyr., 24 h, 20%, b) *t*-BuOH/H_2O (1:1), AD-mix ß, 0°C-rt, 1 d, 24%.

We next proposed that the initial crotylation step was possibly not as sterereoselective as we hoped and thus investigated the selectivity of this reaction. To determine the lack of selectivity in these processes we investigated the levels of asymmetric induction in the original Brown crotylation step. In order to do this a racemic sample of alcohol was prepared using the *trans*-crotylboronic acid pinacol ester (167) which gave (+/-160) in 23% yield. Reaction of this with an excess of (S)-(+)-O-acetylmandelic acid, in the presence of DCC and DMAP gave a 1:1 mixture of mandelate esters (168) and (169).



Scheme 47: a) (167), dichloromethane, -78°C-rt, 24 h, 23%, b) (S)-(+)-*O*-acetylmandelic acid, dichloromethane, DMAP, DCC, 3 d.

The silyl ether (162) that had been previously used in the dihydroxylation reactions was deprotected using TBAF in THF to give the crotyl alcohol (160) in quantitative yield. Reaction of (160) with an excess of *(S)*-(+)-*O*-acetylmandelic acid, in the presence of DCC and DMAP gave predominately the mandelate ester (168) as shown by comparison of its NMR data with the NMR of the racemate. (Scheme 47)



Scheme 48: a) TBAF 1 M, THF, rt, 24 h, quantitative yield, b) (S)-(+)-*O*-Acetylmandelic acid, dichloromethane, DMAP, DCC, 3 d.

Analysis of the two mandelate esters by 1H NMR spectroscopy gave two clear signals at δ_H 5.48 (1H, ddd, *J* 17.1, 10.3, 8.1 Hz, CH) ppm and δ_H 5.67-5.79 (1H, m, CH) ppm for the vinyl CH protons. Similarly diastereotopic signals were present for the mandelate CH-proton at δ_H 5.88 (1H, s, CH) ppm and δ_H 5.89 (1H, s, CH) as well as the methyl group at δ_H 0.69 (3H, d, *J* 6.9 Hz, Me) ppm and δ_H 1.03 (3H, d, J 6.9 Hz, Me) ppm. The vinyl protons were suitable for the determination of diasteremomeric excess as they were in a clear part of the spectrum and the ratio of these two signals from integration is a direct measure of the enantiomeric excess of the original alcohol. Integration of the signals of the mandelate from the racemic sample of (168) and (169) gave essentially a 50:50 ratio as was expected. Analysis of the mandelate ester (168) from the asymmetric crotylation gave a relative integration of the signals as 91:9 ratio indicating an 82% ee (Figure 8). It was thus apparent from these NMR experiments that a lack of selectivity in the crotylation step is not the source of the problem with the Sharpless dihydroxylation.

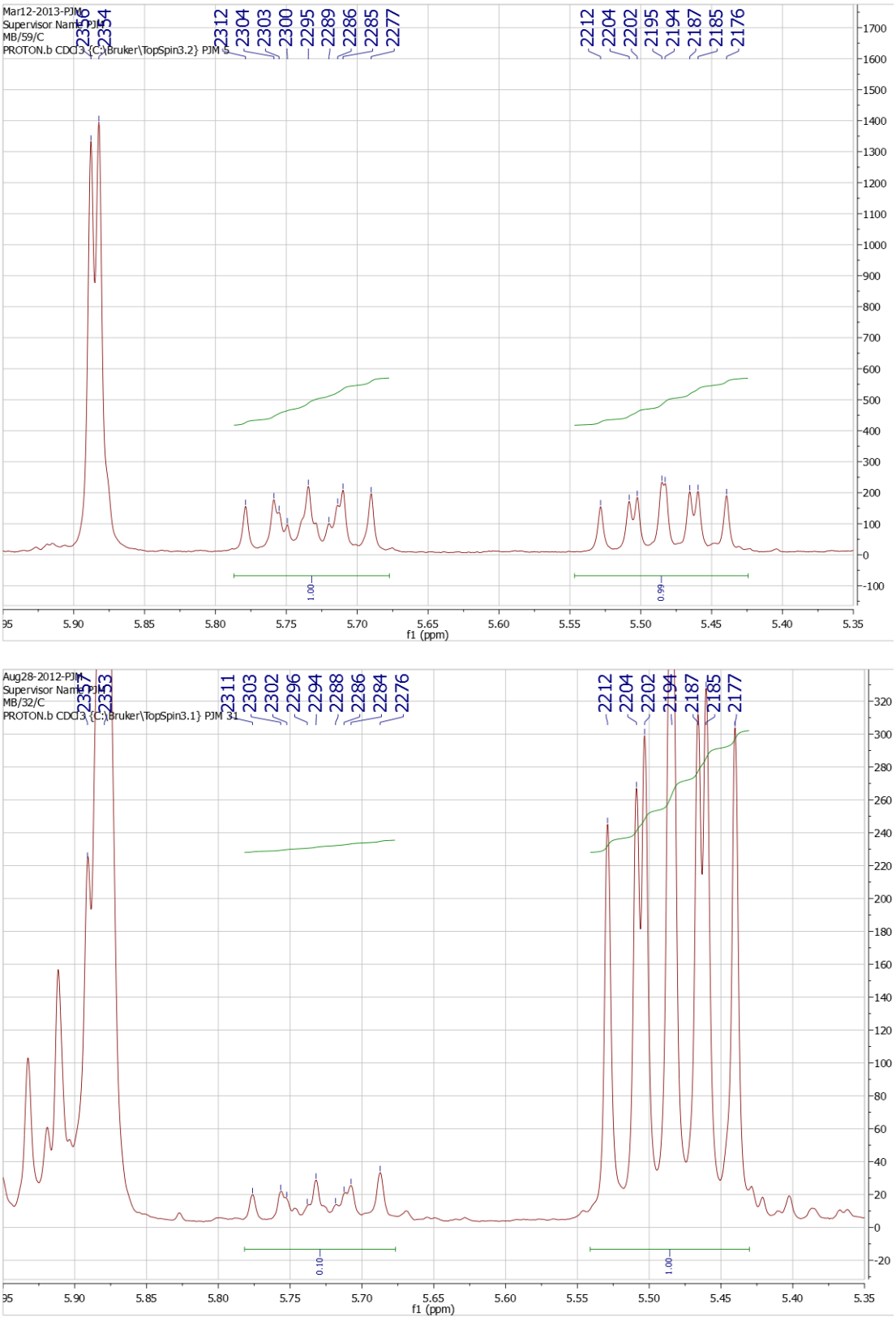


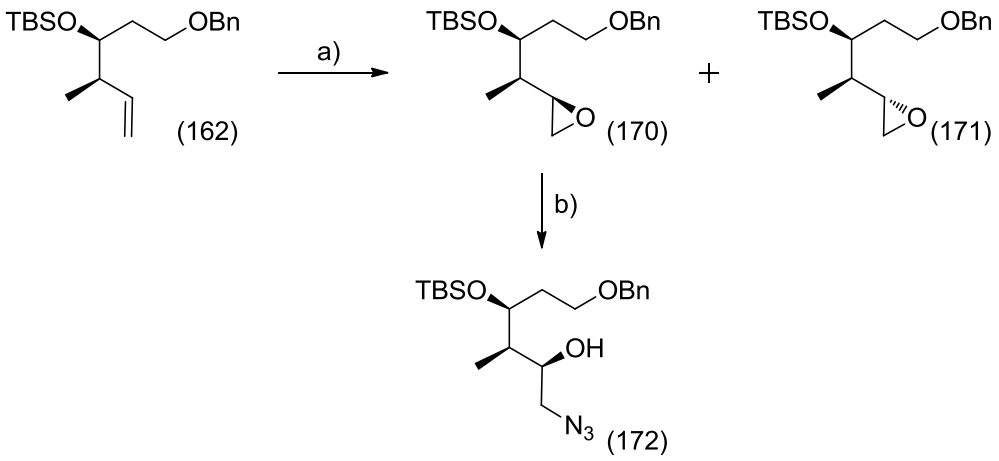
Figure 8: Comparison of the ^{1}H NMR data for the racemic mandelate (168)/(169) (top trace) and the mandelate (168) (lower trace).

Conclusion and Future work

At this point the focus of the project shifted to the synthesis of two related alkaloids as the work detailed in this chapter was funded by the EPSRC and a postdoctoral worker took over the project.

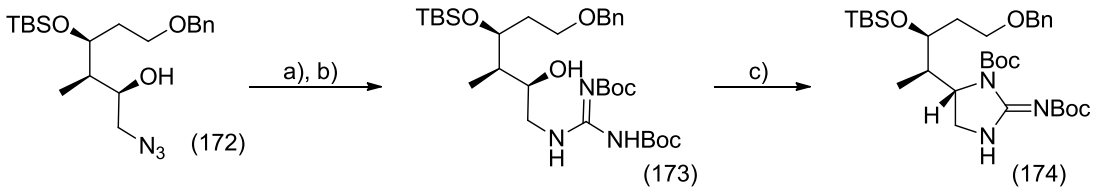
This preliminary work has contributed to the program directed towards the synthesis of the LHS of cylindrospermopsin with the key step being the Brown crotylation, which installs two of the stereogenic centres in the correct orientation. The reaction did give rise to problems as the product was heavily contaminated with alcohol (163) which co-eluted with the product on chromatography however when these were both converted to the silyl ethers (164) and (165) they were easily separated by chromatography. The oxidation of the terminal alkene of (164) by using a Sharpless di-hydroxylation using AD-mix ß to install the third stereogenic centre was successful, however it did not produce a single isomer and instead gave a 60:40 diastereomeric mixture of diols which could not be separated. The solution to this problem remains a sticking point in synthetic efforts towards cylindrospermopsin (1) as the work performed by the EPSRC PDRA indicated that the selective derivatisation of this alkene was not a trivial matter.

Work performed subsequently by Dr Dan Evans, to the work above focused on the epoxidation of silylether (162) which led to a 58-42 mixture of the epoxides (170) and (171) which were separable on careful chromatography. Reaction of (170) with sodium azide in an ethanol/water mix gave the alcohol (172) in 88% yield. (Scheme 49)



Scheme 49: formation of azide (172), a) *m*-CPBA, dichloromethane, 0 °C to rt, b) NaN_3, EtOH-H_2O, (4:1), 70°C, 48 h, 88%.

Reduction of the azide (172) under Staudinger conditions to give an intermediate amine which was guanylated using (130) to give the guanidine (173) in an almost quantitative yield over 2 steps. Cyclisation of (173) under Appel conditions gave the cyclic guanidine (174) in a 67% yield.



Scheme 50: New route to fragment (174), a) PPh_3, THF/H_2O (4:1), 48 h, b) (130), CH_3CN, 48 h, 99% (2 steps), c) PPh_3, I_2, imidazole, CH_3CN, 0°C to rt, 16 h, 67%.

This sequence of reactions demonstrates the potential for the method as the correct stereochemistry was established for the 5-membered ring system. Further work on this substrate proved problematic as the deprotection of the benzyl group was not easily achieved without the concomitant loss of one of the Boc-protecting groups. Further oxidation of the alcohol to the aldehyde was also not easily achieved in the presence of the mono-protected guanidine.[74]

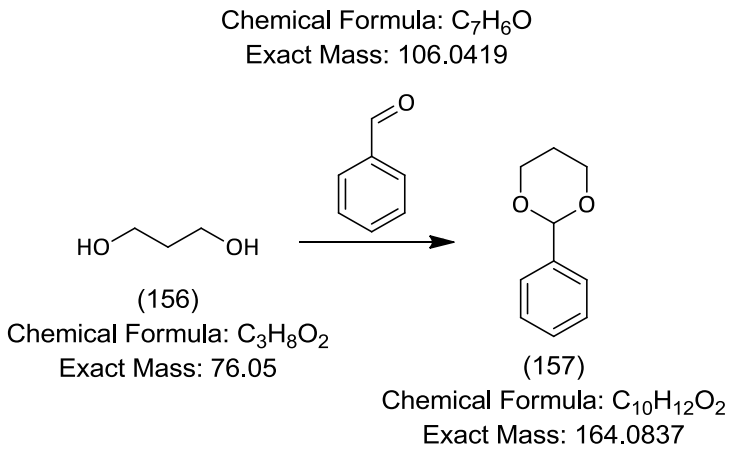
Experimental

Column chromatography was carried out on silica gel (60A) and TLCs were conducted on precoated Kieselgel 60 F254 (Art. 5554; Merck) with the eluent specified in each case. All non-aqueous reactions were conducted in oven-dried apparatus under a static atmosphere of argon. Diethyl ether, THF and dichloromethane were dried by a Pure Solv MD-3 solvent purification system. Dry methanol and DMF was purchased from Aldrich. Chemical shifts are reported as δ values relative to chloroform (7.26/77.16 ppm), methanol (3.31/49.0 ppm) and DMSO (2.50/39.52 ppm) as internal standards. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 or 500 spectrometer with an internal deuterium lock at ambient temperature at 400 or 500 MHz with internal references of δ_H 7.26 and δ_C 77.16 ppm for $CDCl_3$, δ_H 3.31 and δ_C 49.0 ppm for CD_3OD and δ_H 2.54 ppm and δ_C 39.52 ppm for DMSO. Mass spectra data were obtained at the EPSRC Mass Spectrometry Service Centre at the University of Wales, Swansea. Low resolution Chemical Ionisation (CI) and Electrospray Ionisation (ESI) mass spectra were recorded on a Micromass Quattro II spectrometer and high resolution mass spectra were recorded on either a Finnigan MAT 900 XLT or a Finnigan MAT 95 XP. Infrared samples were prepared as thin films or solutions using sodium chloride plates or as KBr discs; spectra were recorded on a Bruker Tensor 37 FT-IR. Melting points (Mp.) were determined using a Gallenkamp MF370 instrument and are uncorrected.

Materials

Reagents and starting materials were purchased from commercial suppliers and used without further purification unless otherwise noted. All anhydrous solvents used in reactions were distilled over either sodium wire and benzophenone (THF/DE) or calcium hydride (dichloromethane), and used either immediately or stored over molecular sieves prior to use. Flash column chromatography was performed on Davisil® silica gel (35-70 microns) with the eluent specified in each case, TLC was conducted on precoated E.Merck silica gel 60 F_{254} glass plates.

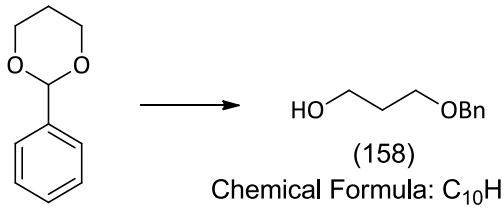
2-Phenyl-1,3-dioxane (157)[70]



A solution of benzaldehyde (47 g, 45 mL, 0.44 mol), 1,3-propanediol (156) (38 mL, 0.53 mol, 1.2 eqv.) and camphorsulfonic acid (0.5 g) in toluene (300 mL) was stirred and refluxed under Dean-Stark conditions for 4 h. The reaction mixture was then cooled and diluted with diethyl ether (100 mL) and stirred for 5 min and the solution washed with sodium hydroxide (2 M, 2 x 100 mL), sodium bisulfate solution (aq., sat., 100 mL), water (100 mL) and brine (100 mL) then dried ($MgSO_4$) and evaporated under vacuum to give crude (157) (73 g, 0.44 mol) as white crystals in quantitative yield.

Rf 0.11 (50% EA in PE, PMA, I_2); $\boldsymbol{\delta_H}$ δ 1.40-1.48 (1H, m, CH), 2.17-2.30 (1H, m, CH), 3.98 (2H, app dt, *J* 2.4, 12.3 Hz, 2 x CH), 4.27 (2H, dd, *J* 5.0, 12.3 Hz, CH), 5.52 (1H, s, CH), 7.33-7.42 (3H, m, 3 x CH), 7.49-7.53 (2H, m, 2 x CH). $\boldsymbol{\delta_C}$ 25.3, 67.0, 101.2, 125.6, 127.8, 128.4, 138.4; ν_{max} 3093, 3072, 3038, 2965, 2853, 1653, 1646, 1634, 1378, 1238, 1108, 1013.

3-(benzyloxy)propan-1-ol (158)[70]



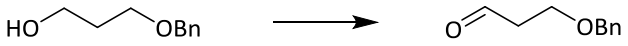
(157)
Chemical Formula: $C_{10}H_{12}O_2$
Exact Mass: 164.08

(158)
Chemical Formula: $C_{10}H_{14}O_2$
Exact Mass: 166.10

In a 1 L RBF, a solution of DIBAL-H (1 M. 54.0 mL, 54.0 mmol, 3.0 eqv.) in hexanes, was added in a dropwise manner to a cooled (0°C) stirred solution of 2-phenyl-1,2-dioxane (157) (3.0 g, 18.0 mmol) in dry dichloromethane (50 mL). On completion (TLC), the solution was diluted with dichloromethane (100 mL) following which, ethyl acetate (50 mL) was added slowly followed by the slow addition of methanol (15 mL) to destroy any remaining DIBAL-H. After 10 min Rochelle's salt (15 g of potassium sodium tartrate in 50 mL water) was added dropwise, which initially resulted in the formation of a gel, which cleared to give two distinct layers on stirring for ca. 30 min. The organic layer was separated, dried ($MgSO_4$) and evaporated under reduced pressure to give crude 3-(benzyloxy)propan-1-ol (150) (3.3 g). Purification by silica gel chromatography (25% EA in PE) gave (150) (2.10 g, 12.5 mmol) in 69% as a colourless oil. Repetition of this reaction at -78°C gave a 71% yield. Repetition of this reaction with cooling to 0°C during the work up and addition of the Rochelle's salt gave (150) in 98% yield.

Rf 0.18 (25% EA in PE, PMA, UV); $\boldsymbol{\delta_H}$ 1.83-1.89 (2H, p, *J* 5.8 Hz, CH_2), 2.70 (1H, br s, OH), 3.65 (2H, t, *J* 5.8 Hz, CH_2), 3.74-3.77 (2H, t, *J* 5.8 Hz, CH_2), 4.52 (2H, s, CH_2), 7.26-7.37 (5H, m, Ph); $\boldsymbol{\delta_C}$ 32.2, 61.6, 69.2, 73.2, 126.9, 127.7, 128.5, 138.1; ν_{max} 3385 , 2939, 1659, 1485, 1460, 1367, 1198, 1081, 917, 748, 702

3-(benzyloxy)propanal (151)[75],[76]



(150)
Chemical Formula: $C_{10}H_{14}O_2$
Exact Mass: 166.10

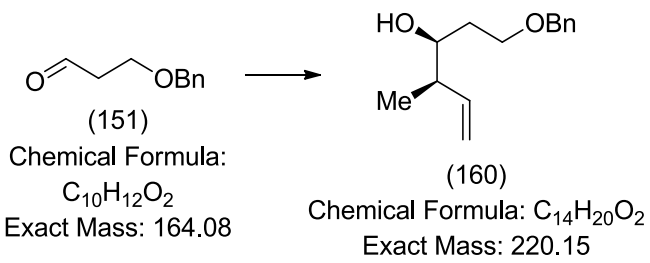
(151)
Chemical Formula: $C_{10}H_{12}O_2$
Exact Mass: 164.08

By PCC oxidation. Celite© (8.0 g) was added to a solution of 3-(benzyloxy)propan-1-ol (150) (2.08 g, 12.0 mmol) in dry dichloromethane (59 mL) and the mixture cooled (0°C) and stirred for 30 min. PCC (4.1 g, 18.8 mmol, 1.5 eqv.) dissolved in dichloromethane (50 mL) was added in a dropwise manner over 45 min and the mixture stirred overnight. The reaction was diluted with diethylether (100 mL) and filtered through a pad of layered Celite© (2 cm) and silica (2 cm). The residue in the flask was triturated with dichloromethane (50 mL) which was diluted with diethylether (100 mL) and passed through the filter pad. After repeating this twice more evaporation of the filtrate gave crude product (151) as a yellow/brown viscous oil (1.6 g). Purification by column chromatography (20% EA in PE) gave 3-(benzyloxy)propanal (151) (1.10 g, 6.90 mmol) in 48% yield as a colourless oil.

By Swern oxidation. DMSO (6.5 mL, 91.3 mmol, 2.2 eqv.) was added in a dropwise manner to a cooled (-78°C) and stirred solution of oxalyl chloride (7.1 mL, 83.0 mmol, 2.0 eqv.) in dichloromethane (300 mL). After 15 min alcohol (150) (6.9 g, 41.5 mmol) dissolved in dichloromethane (15 mL) was added in a dropwise manner and the mixture stirred for 45 min. Triethylamine (35.0 mL, 249.0 mmol, 6.0 eqv.) was then added in a dropwise manner and the mixture stirred for 15 min. After warming to rt over 30 min, water (500 mL) was added, the organic layers was separated and the aqueous layer further extracted with dichloromethane (3 x 100mL). The combined organic layers were washed with water (2 x 200 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give (151) (6.4 g, 39.0 mmol in 95% yield as a colourless oil. The crude product was then purified by column chromatography (10-50% EA in PE) to give (151) (5.99 g, 36.5 mmol, 89%).

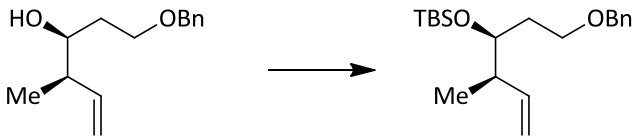
Rf 0.50 (30% EA in PE); $\boldsymbol{\delta_H}$ 2.68 (2H, dt, *J* 1.8, 6.1 Hz, CH_2), 3.81 (2H, t, *J* 6.1, CH_2), 4.54 (2H, s, CH_2), 7.28-7.39 (5H, m, Ph), 9.70 (1H, t, *J* 1.8 Hz, CHO); $\boldsymbol{\delta_C}$ 43.8, 63.8, 73.2, 127.7, 127.7, 128.4, 137.9, 201.1; $\boldsymbol{\nu_{max}}$ 3040, 2865, 1728, 1495, 1446, 1372, 1214, 1104, 1022, 909, 734, 690.

(*3S*,*4R*)-1-(benzyloxy)-4-methylhex-5-en-3-ol (160)[70]



Pottasium *t*-butoxide (3.5 g, 28.9 mmol, 1.7 eqv.) was suspensed in dry THF (50 mL) and cooled (-78°C) following which a hexane solution of nBuLi (2.5 M, 11.6 mL, 28.9 mmol, 1.7 eqv.) was added and the mixture stirred to form a yellow solution. Liquified *trans*-2-butene (6.0 mL, 43.7 mmol, 2.4 eqv.) was then added via cannula. The reaction was stirred at -45°C for 20 min then re-cooled (-78°C) and (+)-$(Ipc)_2$BOMe (168) (11.7 g, 37.0 mmol, 2.03 eqv.) was added as a solid in portions over 5 min and stirring continued for 30 min. Boron trifluoride diethyl etherate (5.3 g, 38.2 mmol, 4.7 mL, 2.1 eqv.) was then added dropwise over 5 mins, which was followed by the addition of aldehyde (151) (3.0 g, 18.2 mmol) as a solution in THF (10 mL) over 5 mins. The reaction was stirred to room temperature over 5 h whereupon sodium hydroxide solution (3 N, aq., 30 mL) was added in a dropwise manner, followed by a solution of hydrogen peroxide (30%, aq., 30 mL) and the mixture stirred for 1 h. The mixture was separated and the aqueous layer extracted with dichloromethane (50 mL) and the combined organic layers dryied ($MgSO_4$) and evaporated under reduced pressure gave crude (160) (15.3 g). Attempts at purification by column chromatography and sublimation were unsuccessful (see discussion) and this crude compound was carried forward to the next step.

(((3*S*,4*R*)-1-(benzyloxy)-4-methylhex-5-en-3-yl)oxy)(tert-butyl)dimethylsilane (162)



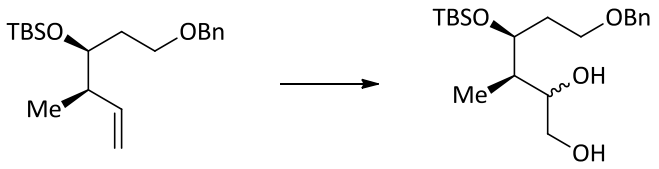
(160)
Chemical Formula: $C_{14}H_{20}O_2$
Exact Mass: 220.15

(162)
Chemical Formula: $C_{20}H_{34}O_2Si$
Exact Mass: 334.23

Crude alcohol (160) (15.3 g, from previous step) was dissolved in dichloromethane (100 mL) and imidazole (12.5 g, 184.0 mmol) was added. After stirring for 45 min, the reaction mixture was cooled (0°C) and TBDMSCl (16.6 g, 111.0 mmol) was added portion wise over 10 min. A catalytic quantity of DMAP (100.0 mg) was added and the mixture stirred for 6 d. Water (100 mL) was added and the solution was stirred for 10 min followed by a further 400 mL of water and the aqueous layer was then separated and extracted with ethyl acetate (3 x 150 mL). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure to give a crude product (27.3 g), which was purified by column chromatography (20% EA in PE) to give (162) (2.6 g, 7.8 mmol) in 43% yield (over 2 steps) as a clear oil.

Rf 0.78 (20% EA in PE); $\boldsymbol{\delta_H}$ 0.04 (3H, s, Me), 0.06 (3H, s, Me), 0.88 (9H, s, 3 x Me), 1.00 (3H, d, *J* 6.8 Hz, Me), 1.61-1.78 (2H, m, CH_2), 2.24-2.35 (1H, m, CH), 3.46-3.57 (2H, m, CH_2), 3.75-3.81 (1H, m, CH), 4.45 (1H, d, *J* 11.9, CH), 4.51 (1H, d, *J* 11.9, CH), 4.95-5.03 (2H, m, 2 x CH), 5.78 (1H, ddd, *J* 7.4, 10.2, 17.5 Hz, CH), 7.24-7.37 (5H, m, Ph); $\boldsymbol{\delta_C}$ -4.4, -4.3, 14.7, 18.3, 26.1, 33.4, 43.6, 67.5, 72.7, 73.0, 114.7, 127.6, 127.8, 128.5, 138.8, 140.8; $\boldsymbol{\nu_{max}}$ 2957, 2928, 2885, 2858, 1725, 1462, 1454, 1472, 1361, 1257, 1092, 1110, 1030, 836, 775; **HRMS** (CI) found 335.2399, $C_{20}H_{35}O_2Si$ ($[M+H]^+$) requires 335.2401.

(3*R*,4*S*)-6-(benzyloxy)-4-((tert-butyldimethylsilyl)oxy)-3-methylhexane-1,2-diol (164)[71]



(162)
Chemical Formula: $C_{20}H_{34}O_2Si$
Exact Mass: 334.23

(164)
Chemical Formula: $C_{20}H_{36}O_4Si$
Exact Mass: 368.24

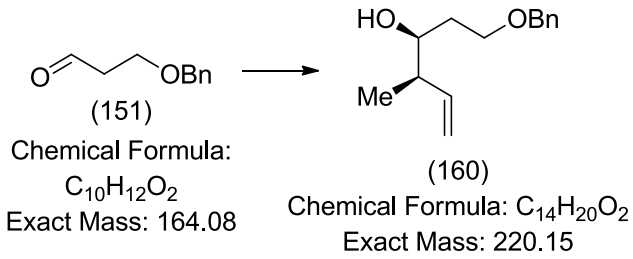
Example procedure: AD-mix β (4.2 g, 2.8 mmol) was added to a mixture of *t*-BuOH (15 mL) and water (15 mL) with vigorous stirring and cooling (0°C). A solution of alkene (162) (1.0 g, 2.9 mmol) dissolved in *t*-BuOH (5 mL) and water (5 mL) was added and the mixture stirred at this temperature for 24 h. Sodium sulfite (4.5 g, 35.0 mmol) was then added and the solution stirred for a further hour whilst warming to rt. Dichloromethane (30 mL) was added, the layers separated and the aqueous layer extracted with further dichloromethane (3 x 50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to give crude mixture (3.4 g). Purification by column chromatography (25% EA in PE) gave (164**)** (0.67 g, 1.8 mmol) in 63% yield as a clear oil as a mixture of diasteroisomers (See discussion). Details of the other experiments that were conducted to determine the effects of pH and temperature are shown below (table 3). Conditions were identical apart from the experiments which required the pH changing in which NaOH (aq. 2M) was added to give a stable pH of 14 and experiments which required cooling were kept in an ice bath during daylight hours and in a fridge overnight to prevent warming.

(Table 3)

(162)		AD-mix b		NaOH to	temp	Yield		%
G	mmol	g	mmol	pH 14	°C	g	mmol	yield
0.335	1	1.4	1	N	rt	0.23	0.63	63
0.1	0.29	0.46	0.29	Y	rt	0.07	0.19	67
1	2.9	4.2	2.9	N	0°C	0.67	1.83	63
0.1	0.29	0.46	0.29	Y	0°C	0.03	0.09	30

Rf 0.33 (25% EA in PE); δ_H 0.83 (3H, d, J 7.8 Hz, Me, major diasteroisomer) ppm, δ_H 0.98 (3H, d, J 7.1 Hz, Me, minor diasteroisomer); ν_{max} 3365, 2877, 1542, 1460, 1395, 1259, 1092, 1045, 1030, 760, 728, 707; **HRMS** (CI) found 368.2436 , $C_{20}H_{36}O_4Si$ ($[M+H]^+$) requires 368.2434.

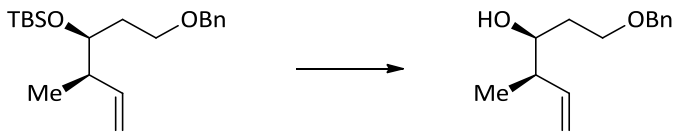
(3*S*,4*R*)-1-(benzyloxy)-4-methylhex-5-en-3-ol (160)[78]



From (S,S)-trans crotyl mix: (*S*,*S*)-trans crotyl mix (1.0 g, 0.9 mmol, 1.1 eqv.), was added to a cooled (0°C) solution of aldehyde (151) (140.0 mg, 0.9 mmol) dissolved in dry dichloromethane (15.0 mL) and the mixture stirred. After 30 min, the reaction mixture was concentrated and diethyl ether (8.0 mL) and HCl (1M. aq., 8.0 mL) were added and the mixture stirred for an hour. The mixture was then filtered and the filter pad washed with diethyl ether (2 x 10 mL) and the aqueous layer separated and extracted with diethyl ether (3 x 10 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated to give a crude product (0.20 g). Purification by column chromatography (20% EA in hexane) gave (160) (79.0 mg, 0.30 mmol) in 36% yield as a colourless viscous oil. Data was in agreement with the literature.

Rf 0.30 (20% EA in hexane); $\boldsymbol{\delta_H}$ 1.05 (3H, d, *J* 6.9 Hz, Me), 1.69-1.79 (2H, m, CH_2), 2.19-2.30 (1H, m, CH), 2.72 (1H. br s, OH), 3.60-3.77 (3H, m, CH/CH_2), 4.53 (2H, s, CH_2), 5.01-5.12 (2H, m, 2 x CH), 5.76-5.86 (1H, m, CH), 7.26-7.38 (5H, m, Ph); $\boldsymbol{\delta_C}$ 16.0, 33.8, 44.2, 69.4, 73.5, 74.4, 115.7, 127.8, 127.9, 128.6, 138.2, 140.6; $\boldsymbol{\nu_{max}}$ 3445, 3067, 3031, 2963, 2929, 2869, 1640, 1496, 1454, 1419, 1365, 1207, 1096, 1028, 1001, 960, 913, 736, 698. **HRMS** (CI) found 221.1546, $C_{14}H_{21}O_2$ ($[M+H]^+$) requires 221.1536

(*3S,4R*)-1-(benzyloxy)-4-methylhex-5-en-3-ol (160) [78]

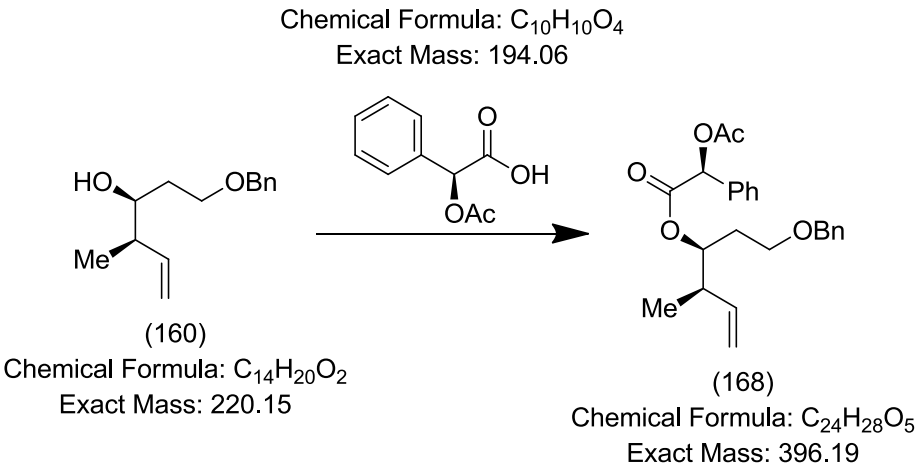


(162)
Chemical Formula: $C_{20}H_{34}O_2Si$
Exact Mass: 334.23

(160)
Chemical Formula: $C_{14}H_{20}O_2$
Exact Mass: 220.15

A THF solution of TBAF (1 M, 0.65 mL, 0.65 mmol 1.1 eqv.) was added to a cooled (0°C) solution of silyl ether (162) (200.0 mg, 0.598 mmol) dissolved in THF (1.0 mL). After warming to rt overnight the reaction was diluted with dichloromethane (100 mL) and washed with ammonium chloride solution (sat., 100 mL) and brine (100 mL). After drying ($MgSO_4$) and evaporation, the alcohol (160) (139.6 mg) was obtained as a clear colourless viscous oil which was used in the next step without further purification. Data was as previously reported.

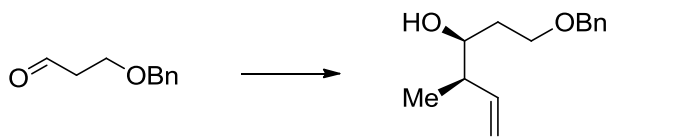
(3*S*,4*R*)-1-(benzyloxy)-4-methylhex-5-en 3-yl-(*S*)-2-acetoxy-2-phenylacetate (168)[79]



DCC (260.0 mg, 1.3 mmol) was added to a cooled (0°C) solution of (*S*)-(+)-*O*-acetylmandelic acid (250.0 mg, 1.3 mmol), alcohol (160) (139.6 mg, 0.6 mmol) and DMAP (~10 mg) in dichloromethane (10 mL). After stirring at rt for 3 d, the solution was filtered and the precipitate was washed with hexane (3 x 40 mL). The combined filtrates were washed with HCl solution (aq., 1M, 100 mL), sodium bicarbonate (aq., sat., 100 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give crude (168) (286.5 mg) which was analysed by ^{1}H NMR (see discussion).

Major diastereoisomer δ_H 0.69 (3H, d, *J* 6.9 Hz, Me), 1.82 (2H, app q, *J* 6.5 Hz, CH_2), 2.17 (3H, s, Me), 2.20-2.29 (1H, m, CH), 3.39-3.55 (2H, m, CH_2), 4.40 (1H, d *J* 11.6 Hz CH), 4.46 (1H, d *J* 11.6 Hz CH) 4.78-4.88 (2H, m, 2 x CH) 5.07-5.14 (1H, m, CH), 5.48 (1H, ddd, *J* 17.1, 10.3, 8.1 Hz, CH), 5.88 (1H, s, CH) 7.27-7.51 (10H, m, 2 x Ph).

+/-(*3S,4R*)-1-(benzyloxy)-4-methylhex-5-en-3-ol (160)[80]

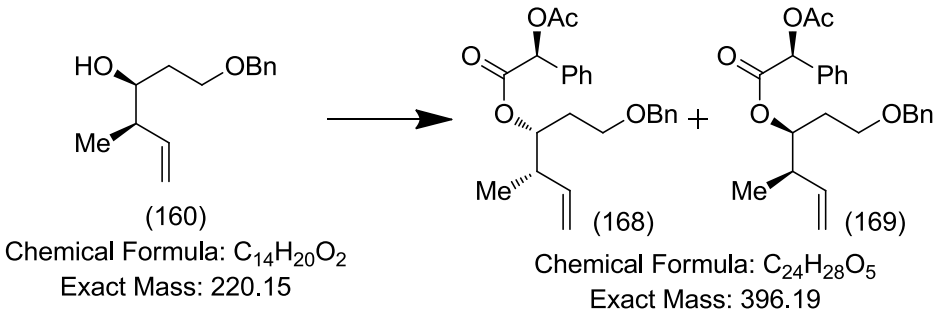


(151)
Chemical Formula: $C_{10}H_{12}O_2$
Exact Mass: 164.08

+/-(160)
Chemical Formula: $C_{14}H_{20}O_2$
Exact Mass: 220.15

E-Crotylboronic acid pinacol ester (167) (300.0 mg, 1.1 mmol) was added to a cooled (-78°C) and stirred solution of aldehyde (151) (165.0 mg, 1.0 mmol) in dichloromethane (10mL). After warming to rt over 24 h, water (10 mL) was added and the mixture stirred for 10 min. After separation, the aqueous layer was further extracted with dichloromethane (2 x 20 mL) and the combined organic layers dried ($MgSO_4$) and evaporated under reduced pressure to give crude (160) (364.7 mg). Purification by column chromatography (20% EA in hexane) gave (+/-160) (50.0 mg, 0.227 mmol) in 23% yield as a colourless oil. Data was identical to that reported for compound (160).

(3*R*,4*S*)-1-(benzyloxy)-4-methylhex-5-en-3-yl (*S*)-2-acetoxy-2-phenylacetate (168) and (3*S*,4*R*)-1-(benzyloxy)-4-methylhex-5-en-3-yl (*S*)-2-acetoxy-2-phenylacetate (169)[79]

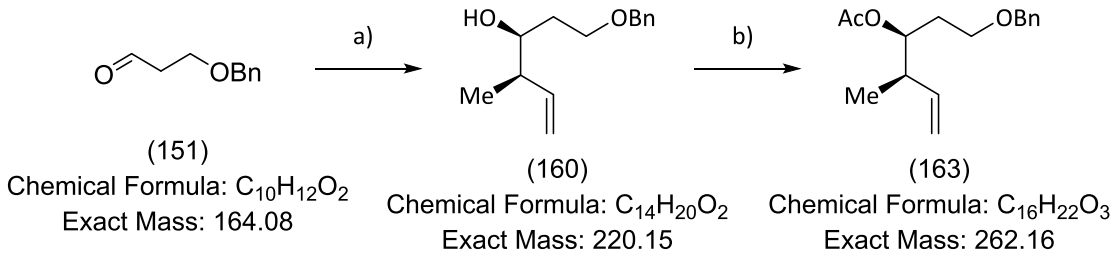


DCC (94.0 mg, 0.46 mmol) was added to a stirred and cooled (0°C) solution of (*S*)-(+)-O-acetylmandelic acid (90.0 mg, 0.46 mmol), alcohol +/-(153) (50.0 mg, 0.227 mmol) and DMAP (~10 mg) dissolved in dichloromethane (5 mL). After stirring at rt for 3 d, the solution was filtered and the precipitate was washed with hexane (3 x 40 mL). The combined filtrates were washed with HCl solution (1 M, aq., 100 mL), sodium bicarbonate solution (sat., 100 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give crude (168) and (169) (160.0 mg) which was analysed by ^{1}H NMR to determine its diastereomeric excess (see discussion)

Major diastereoisomer δ_H 0.69 (3H, d, *J* 6.9 Hz, Me), 1.82 (2H, app q, *J* 6.5 Hz, CH_2), 2.17 (3H, s, Me), 2.20-2.29 (1H, m, CH), 3.39-3.55 (2H, m, CH_2), 4.40 (1H, d *J* 11.6 Hz CH), 4.46 (1H, d *J* 11.6 Hz CH) 4.78-4.88 (2H, m, 2 x CH) 5.07-5.14 (1H, m, CH), 5.48 (1H, ddd, *J* 17.1, 10.3, 8.1 Hz, CH), 5.88 (1H, s, CH) 7.27-7.51 (10H, m, 2 x Ph)

Minor diastereoisomer δ_H (partial data) 1.03 (3H, d, *J* 6.9 Hz, Me), 2.20 (3H, s, Me), 5.67-5.79 (1H, m, CH), 5.89 (1H, s, CH).

(3*S*,4*R*)-1-(benzyloxy)-4-methylhex-5-en-3-yl acetate (163)[81]



(151)
Chemical Formula: $C_{10}H_{12}O_2$
Exact Mass: 164.08

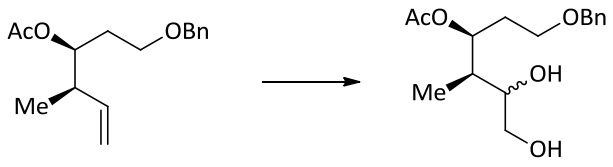
(160)
Chemical Formula: $C_{14}H_{20}O_2$
Exact Mass: 220.15

(163)
Chemical Formula: $C_{16}H_{22}O_3$
Exact Mass: 262.16

a) Aldehyde (151) (1.9 g, 11.3 mmol), was crotylated under the previously employed conditions to give crude alcohol (160) (2.5 g, 11.3 mmol). b) Acetic anhydride (7.1 mL, 20.2 mmol, 5.0 eqv.) was added to cooled (0°C) solution of the crude alcohol (160) (2.5 g, 11.3 mmol), pyridine (15.0 mL, 32.3 mmol, 8.0 eqv.) and DMAP (cat) in dichloromethane (20 mL). After 24 h aqueous hydrochloric acid (1 M. aq., 100 mL) was added, the organic layer separated and the aqueous layer extracted with further dichloromethane (3 x 50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to give the crude product (1.80 g). Purification by column chromatography (5-10% DE in PE) gave (163) (550.1 mg, 2.09 mmol) as a colourless oil in 19% yield over 2 steps. Data was in agreement with the literature.

Rf 0.35 (10% DE in PE); $\boldsymbol{\delta_H}$ 1.01 (3H, d, *J* 6.9 Hz, Me), 1.75-1.90 (2H, m, CH_2), 2.00 (3H, s, Me), 2.35-2.46 (1H, m, CH), 3.41-3.53 (2H, m, CH_2), 4.47 (2H, s, CH_2), 5.00-5.07 (3H, m 3 x CH) 5,73 (1H, ddd, 8.0, 9.8, 17.7 CH), 7.25-7.37 (5H, m, Ph); $\boldsymbol{\delta_C}$ 15.9, 21.2, 31.8, 42.0, 67.1, 73.2, 74.2, 115.8, 127.7, 127.9, 128.5, 138.5, 139.5, 170.9; $\boldsymbol{\nu_{max}}$ 3057, 2933, 2842, 1680, 1649, 1466, 1435, 1372, 1213, 1087, 1054, 959, 758, 663.

(*3S*,*4R*)-1-(benzyloxy)-5,6-dihydroxy-4-methylhexan-3-yl acetate (166)[71]



(163)
Chemical Formula: $C_{16}H_{22}O_3$
Exact Mass: 262.16

(166)
Chemical Formula: $C_{16}H_{24}O_5$
Exact Mass: 296.16

Example procedure: AD-mix β (0.62 g, 0.04 mmol) was added to a mixture of *t*-BuOH (15 mL) and water (15 mL) with vigorous stirring and cooling (0°C). A solution of alkene (163) (100.0 mg, 0.04 mmol) dissolved in *t*-BuOH (5 mL) and water (5 mL) was added and the mixture stirred at this temperature for 24 h. Sodium sulfite (0.58 g, 4.6 mmol) was then added and the solution stirred for a further hour whilst warming to rt. Dichloromethane (30 mL) was added, the organic layer separated and the aqueous layer extracted with further dichloromethane (3 x 50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to give crude (166) (87.3 mg). Purification by column chromatography (25% EA in PE) gave (166) (26.8 mg, 0.080 mmol) which was a 53:47 mixture of diasteroisomers as a gum in 24% yield.

(Table 4)

(163)		AD-mix b		NaOH to pH 14	temp °C	Yield		% yield
g	mmol	g	mmol			mg	mmol	
0.1	0.04	0.62	0.04	N	rt	2.85	9.60	24
0.1	0.04	0.62	0.04	Y	0°C	0.59	2.00	5

Rf 0.31 (25% EA in PE); δ_H (partial data) 2.02 (3H, s, Me, minor diasteroisomer) ppm, δ_H 2.03 (3H, s, Me, major diasteroisomer).

Section B:

B: The total synthesis of tiruchanduramine.

Introduction

The guanidine containing natural product tiruchanduramine (175) was isolated from the marine ascidian *synoicum macroglossum* collected at the Tiruchandur coast, India in 2002 by Ravinder *et al.*[82] The genus *synoicum* has yielded several other interesting compound including spiroketals such as the Prunolides A-C and the furan-2(5H)-one Rubrolide A[83] (Figure 9).

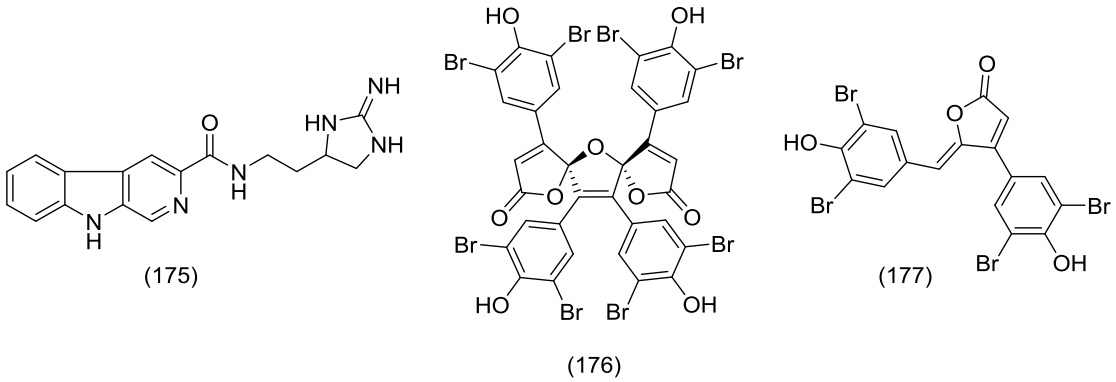


Figure 9: Tiruchanduramine (175), Prunolide A (176) and Rubrolide A (177).

Ravinder *et al.* extracted Tiruchanduramine from the sample by first extracting with dichloromethane and methanol which was then partitioned between water and EtOAc. The water extracts were then freeze dried to yield a residue which was then triturated with methanol. The soluble extracts were then subjected to gel filtration followed by column chromatography to give pure tiruchanduramine. After extensive NMR studies the structure was determined as (175) although the absolute stereochemistry could not be assigned. The structure of tiruchanduramine contains a ß-carboline-3-carboxylate, an amine side chain and a 5 membered guanidine ring system.

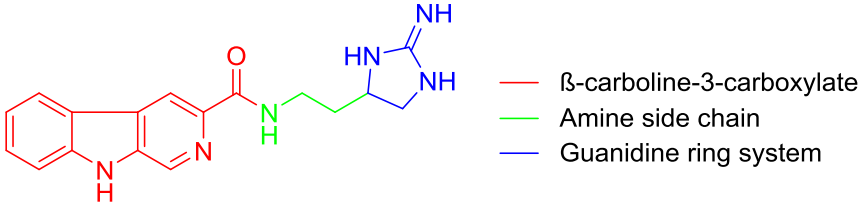


Figure 10: Structural elements in tiruchanduramine (175).

Tiruchanduramine (175) is unusual in that ß-carboline-3-carboxylates have not previously been isolated from ascidians. Tiruchanduramine (175) also contains an enduracididinamine moiety, which is the decarboxylation product of the unusual amino acid enduracididine (178). This has also not been reported in a secondary metabolite or marine natural product before.[84] This amino acid itself is found in the macrocyclic depsipeptide teixobactin (179) which was found to be a potent antibiotic which is interesting as it does not show signs of bacteria evolving detectable resistance against it[85] (Figure 11).

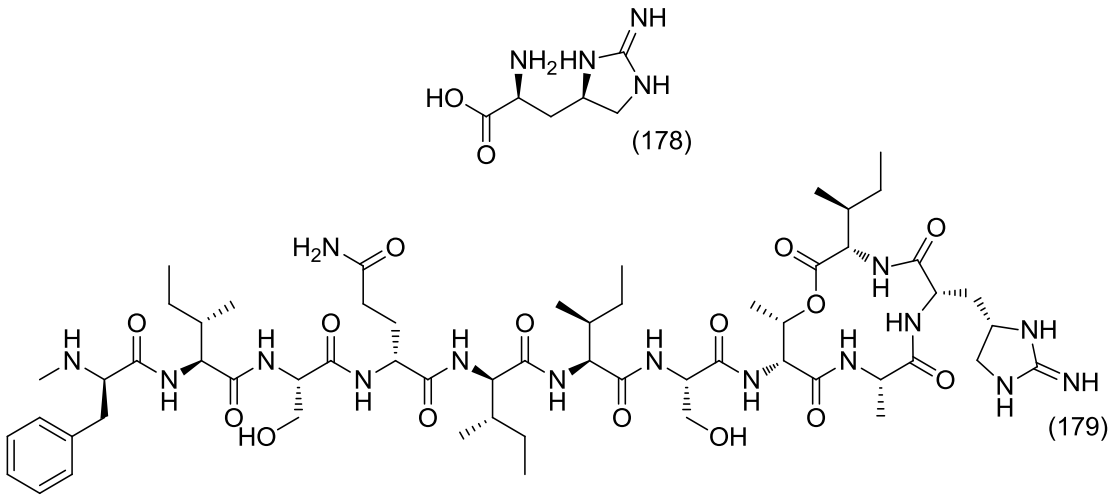
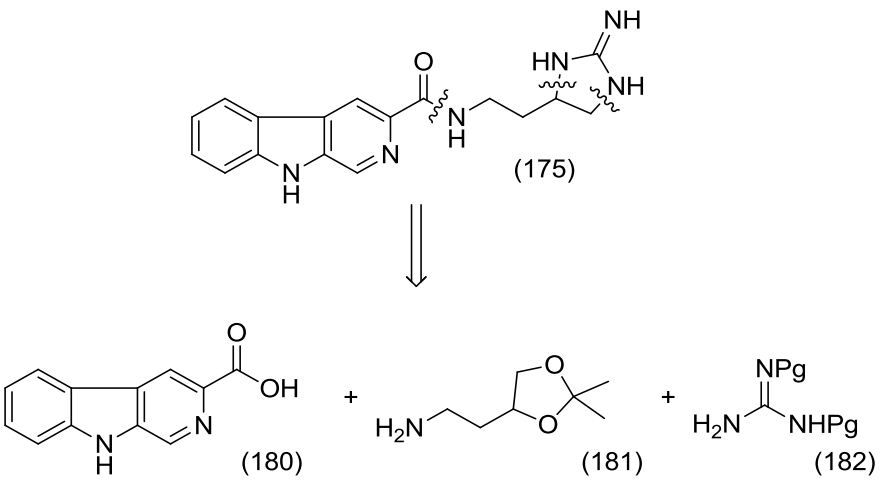


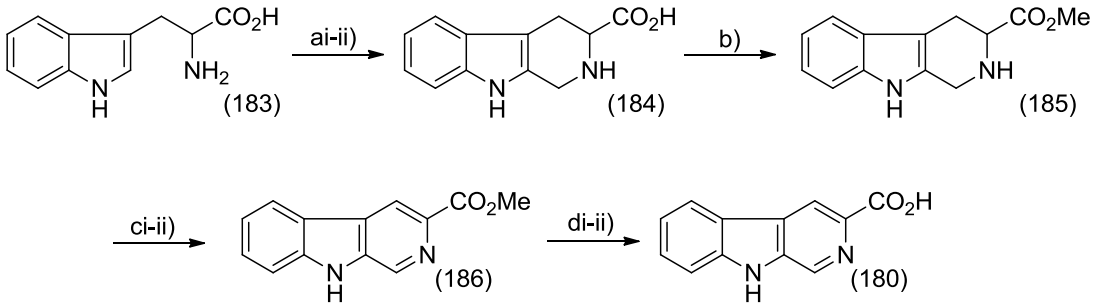
Figure 11: Enduracididinamine (178) and the macrocyclic depsipeptide teixobactin (179).

Ravinder also reported a racemic synthesis of tiruchanduramine and his retrosynthetic analysis made 3 disconnections to give 3 fragments, ß-carboline-3-carboxylic acid (180) an aliphatic side chain synthon (181) and finally a guanidine synthon (182) (Scheme 50).



Scheme 51: Ravinders retrosynthetic analysis.

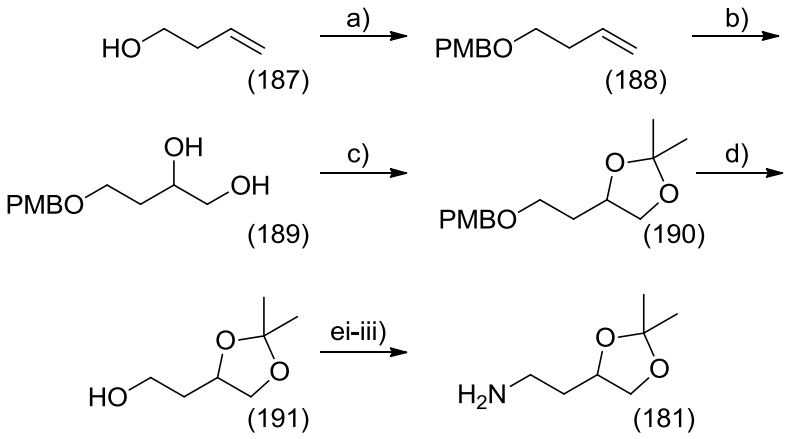
He prepared the ß-carboline-3-carboxylic acid (180) by a literature method[86, 87] starting from the amino acid *L*-tryptophan (183) which firstly underwent a Pictet-Spengler reaction using formaldehyde to give (184) in a 97% yield. The carboxylic acid functionality was then protected as the methyl ester using classic Fischer esterification conditions to give (185) in a 90% yield. The unsaturated ring in (185) was then oxidised using lead tetraacetate to give (186) in a 57% yield, this was followed by the hydrolysis of the ester in a 96% yield to give the completed ß-carboline-3-carboxylic acid fragment (180).



Scheme 52: Synthesis of ß-carboline-3-carboxylic acid (180). a) i) NaOH, H_2O, formalin, 3.5 h, Δ, ii) HCl., b) HCl (g), MeOH, 44 h, c) i) AcOH, $Pb(OAc)_4$, ii) oxalic acid, 40 h, rt, d) i) NaOH, EtOH, 45 min, Δ, ii) HCl.

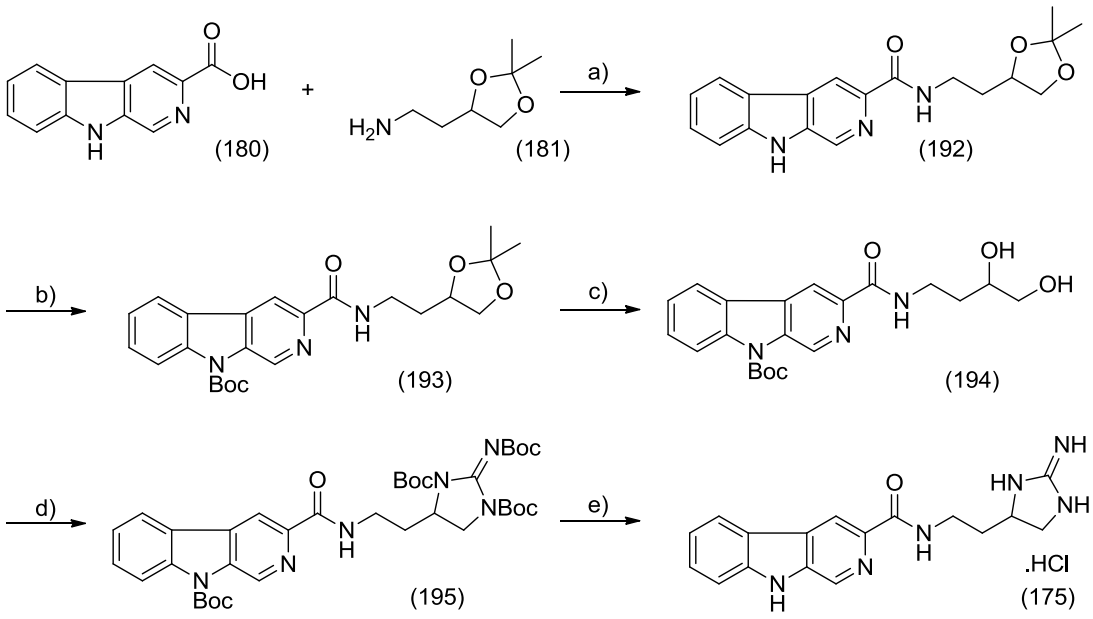
The synthesis of the aliphatic side chain (181) started with homoallyl alcohol (187) which was firstly protected as its PMB ether (188) in a 92% yield. The alkene

(188) then underwent di-hydroxylation using the Sharpless dihydroxyaltion to give (189) in a 70% yield. The diol (189) was then protected as the acetonide (190) in 82% yield. The primary alcohol in (190) was then converted to the corresponding amine (191) in 4 steps, firstly deprotection to give (192), followed by conversion to the tosyl ester in an 80% yield. This tosylate was then converted to the corresponding azide in an 86% yield which in turn was then reduced to give the amine (181) in a 92% yield.



Scheme 53: Synthesis of the aliphatic side chain (181), a) PMBBr, NaH, THF, 92%, b) OsO_4, NMO, acetone/water 7:3, 70%, c) 2,2-DMP, PTSA, 82%, d) DDQ, dichloromethane/water 9:1, 90%, e) i) *p*-TsCl, pyr, 0°C, 80%, ii) NaN_3, DMF, 86%, iii) 10% Pd/C, H_2, 92%.

The completion of the synthesis required the coupling of the ß-carboline-3-carboxylic acid (180) and the aliphatic side chain (181). This was achieved using EDCI and HOBT as the coupling agents to give (192) in a 65% yield. The amine in the ß-carboline functionality was then Boc-protected to give (193) in a 92% yield. The acetonide protecting group was then removed using PPTSA to give the diol (194) in a 90% yield. The guanidine function was then introduced using the tri-boc protected guanidine in a 52% yield. The final step was to remove the boc protecting groups from the guanidine and ß-carboline functionalities using HCl to give tiruchanduramine (175) as its hydrochloride salt in a 63% yield.



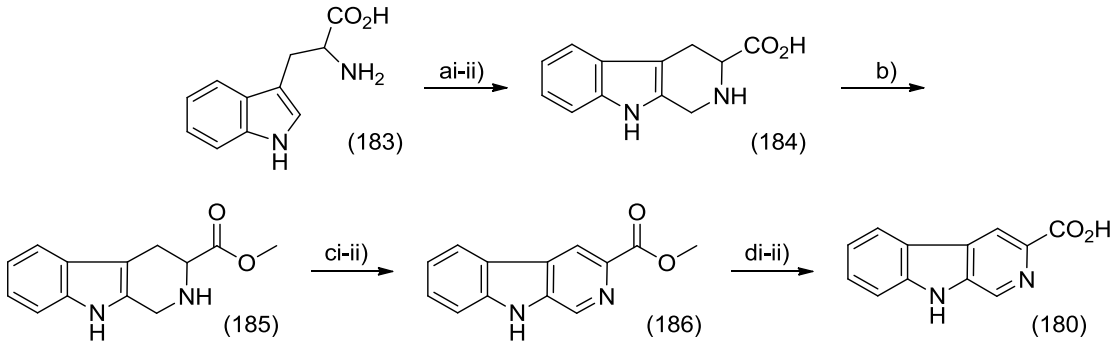
Scheme 54: Completion of the synthesis of (175). a) EDCI, HOBT, dry DMF, 65%, b) $(Boc)_2O$, Et_3N, dichloromethane, 92%, c) PPTSA, MeOH, 90%, d) tri-boc guanidine, TPP, DEAD, THF, 52%, e) 2M HCl, MeOH, rt, 4 h, 63%.

Ravinder *et al.* synthesised tiruchanduramine in 14 steps with the longest linear sequence being 10 steps with an overall yield of 7-8%. This synthesis can be improved as the main losses being incurred are found in the synthesis of the β-carboline-3-carboxylic acid fragment (180), the coupling of the two fragments (180) and (181) along with a low yield ~50% from the guanylation reaction. Ravinder reported that tiruchanduramine (175) showed promising α-glucosidase inhibitory activity (IC50 78.2 µg/mL) as compared with the acarbose, an anti-diabetic drug at 100 µg/mL as the standard.

Previous work in Murphy research group

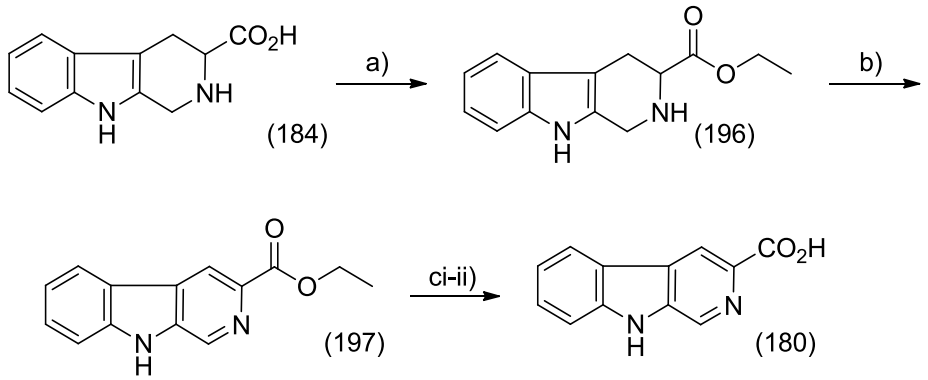
We were interested in the synthesis of tiruchanduramine and hoped to improve on the synthesis of Ravinder and to develop a convergent synthetic strategy. The initial work focused on the synthesis of the ß-carboline-3-carboxylic acid fragment (180).[88] Two routes to the ß-carboline carboxylic acid (180) were investigated both of which use tryptophan (183) as the starting material. The Pictet-Spengler of *L*-tryptophan with formaldehyde led to the carboxylic acid (184) in quantitative yield. In

the literature, the acid (184) was converted into the methyl ester (185) in 90% yield using gaseous hydrochloric acid. We substituted gaseous HCl for a methanolic solution of HCl, which was prepared by the addition of acetyl chloride to methanol. This method gave the ester (185) in a 65% yield. The next steps followed the literature method firstly involved the oxidation of (185) using lead tetra-acetate to give the β-carboline ester (186) in a 57% yield. Hydrolysis of the ester using aqueous sodium hydroxide followed by acidification gave the ß-carboline-3-carboxylic acid fragment (180) in 59% yield. Overall this method gave (180) in an overall yield of 21% from *L*-tryptophan, this compares well with the literature yield of 48%.



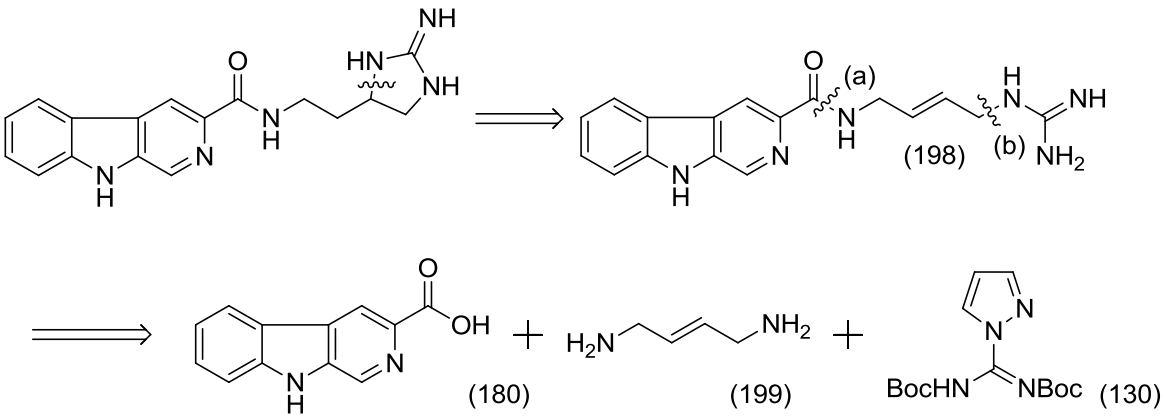
Scheme 55: synthesis of the ß-carboline fragment (180), a) i) NaOH, H_2O, formalin, 3.5 h, Δ, ii) HCl., b) methanolic HCl, MeOH, 44 h, c) i) AcOH, $Pb(OEt)_4$, ii) oxalic acid, 40 h, rt, d) i) NaOH, EtOH, 45 min, Δ, ii) HCl.

In an alternate route[89], the ester formation step was effected using ethanol with sulfuric acid as the catalyst to give the ethyl ester in an improved 84% yield. This step was followed by the aromatisation using manganese dioxide in benzene under reflux as an alternate to lead tetraacetate. This gave β-carboline ester (186) in a 19% yield compared which was lower than that reported in the literature, which was 30%. This lower yield was thought to be due to problems encountered in the purification of the product. The final step involved hydrolysis of the ester under basic conditions followed by hydrolysis to give to give the ß-carboline-3-carboxylic acid (180) in a 93% yield. Overall this method gave (180) in an overall yield of 14.8% from *L*-tryptophan (183), this compares well with the literature overall yield of 22.6%.[86],[87]



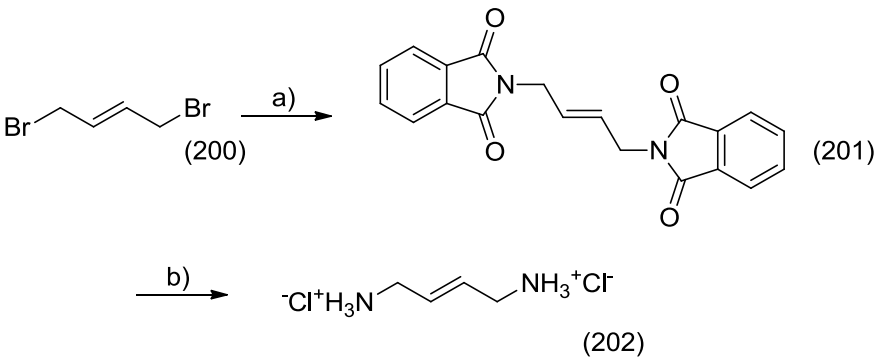
Scheme 56: Improved synthetic route to (180), a) H_2SO_4, EtOH, 48 h, Δ, b) benzene, MnO_2, 92 h, Δ, c) i) NaOH, EtOH, 60 min, Δ, ii) HCl.

Following the preparation of (180) the side chain of tiruchanduramine and its coupling to (180) was investigated. As previously discussed, tiruchanduramine (175) might be prepared in a more efficient manner by utilising a convergent synthetic strategy, rather than the linear one previously used. With this in mind, work in the research group focused on iodocyclisation reactions involving guanidines as and approache to 5-membered guanidine hetrocycles.[90] Thus the first synthetic strategy[91] was based on a retrosynthesis where the guanidine heterocycle was disconnected (a) to give the alkene (198). This could be disconnected (b) further to give the symmetric diamine (199), the commercially available guanidinylating reagent (130) and the ß-carboline-3-carboxylic acid (180).



Scheme 57: Initial retrosynthesis of tiruchanduramine.

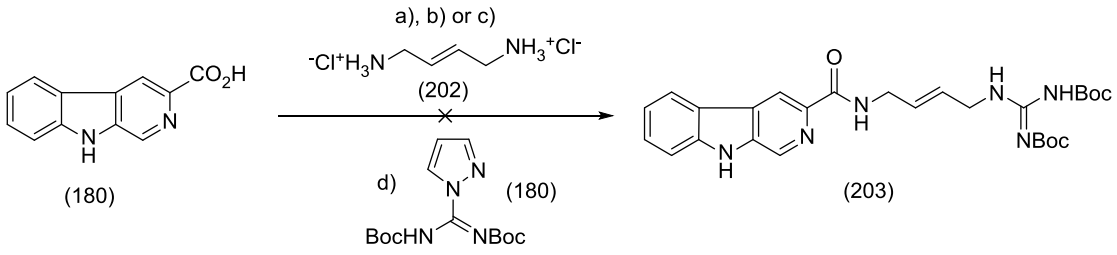
The required unsaturated diamine (199) which was reported in the literature to be available from *E*-1,4-dibromo-2-butene. Reaction of (200) with 2 equivalents of potassium phthalimide in DMF at 80°C gave (201) in a 92% yield after purification. Removal of the phtalimide groups was effected by treatment with excess aqueous hydrochloric acid and acetic acid under reflux over 65 h which gave the diamine (202) in a 20% yield.



Scheme 58: The Gabriel synthesis of the diamine (202).

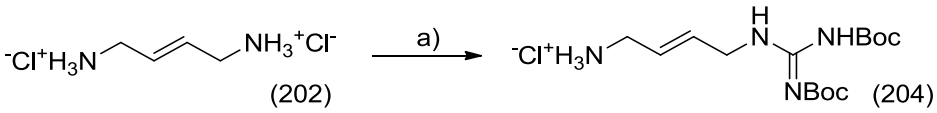
a) Phthalimide, PPh_3, THF, DIAD, 0°C, 48 h, b**)** HOAc, HCl, 65 h, 95°C.

Reaction of the diamine hydrochloride (202) with the ß-carboline (180) followed by the guanidinylating agent (130) proved very capricious, and under a variety of coupling conditions no appreciable amounts of (203) could be formed.



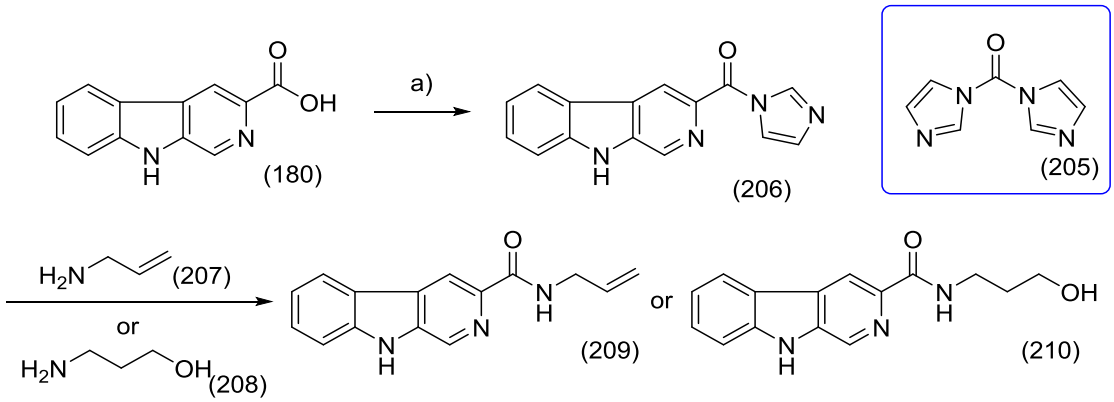
Scheme 59: Failed attempts at coupling fragments (180), (202) and (130), a) $SOCl_2$, 1 h, 80°C, DMF, then (202), b) DCCI, Et_3N, DMF, *N*-Hydroxysuccinimide, c) EDCI, DMAP, Et_3N, DMF, (130).

Instead amine (202) was coupled with the guanidinylating agent (130) to give (204) in a 33% yield. However attempts to couple (204) with ß-carboline (180) under the conditions reported by Ravinder[82] were also unsuccessful.



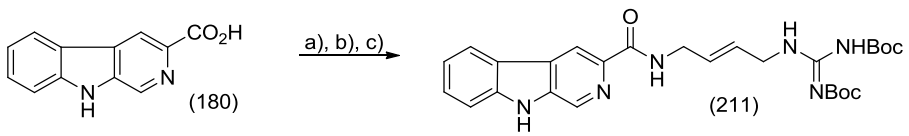
Scheme 60: a) Et_3N, MeCN, 60 h, (130).

Following this work[91] it was clear that the coupling step using the ß-carboline-3-carboxylic acid fragment (180) and a symmetrical diamine caused significant problems under the conditions reported and a new method was sought. Cook *et al*[92] reported that the coupling of (180) to various amines was possible by reacting it with carbonyldiimidazole (CDI) (205) to generate the intermediate imidazole activated amide.[92] In our hands, reaction with allyl amine (207) or 3-amino-1-propanol (208) gave the corresponding amides (209) and (210) in 72% and 53% yield respectively via the intermediate (206).



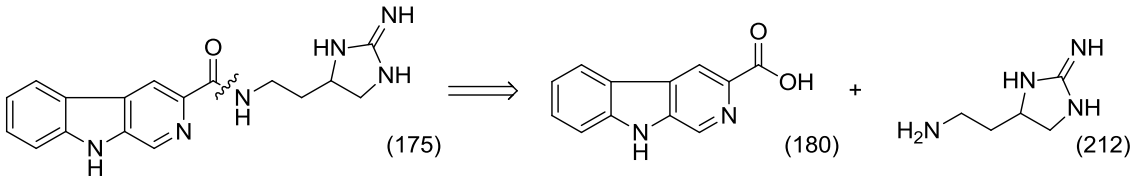
Scheme 61: Amine couplings with CDI (205), a) (205), DMF, 20 h, rt, then amine (207) or (208) 3 d, rt; (209), 72%; (210) 53%.

This methodology seemed to be an improvement upon previous methods and CDI was applied to the coupling of the acid (180) with diamine (202) followed by the addition of the guanidinylating agent (130). Under these conditions the derivative (211) was obtained in a disappointing 13% yield even on attempted optimisation.



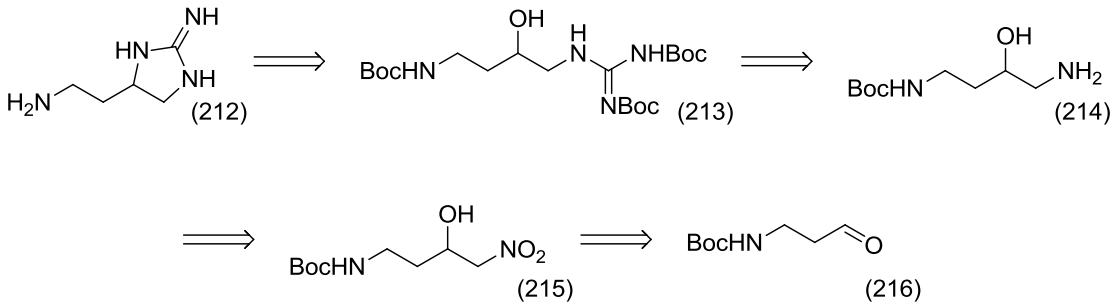
Scheme 62: Preparation of (211), a) CDI, DMF, 20 h, b) (202), Et_3N, 3 d, rt, c) (130), 5 d, rt. 13%.

The failure of this route led to a new retrosynthesis being considered in which tircunduramine is disconnected at the amide bond to give the guanidine amine (212) and the previously prepared acid (180).



Scheme 63: Modified retrosynthetic proposal.

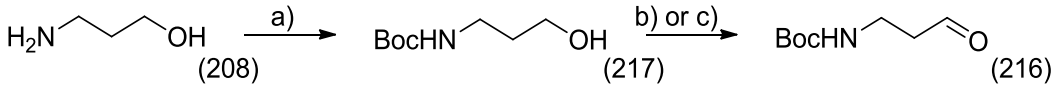
The retrosynthesis of the amine (212) was considered and a method via an Appel type cyclisation of the protected guanidine (213) was envisaged. This in turn should be accessed via guanidinylation of the corresponding amine (214) and the reduction of nitro alcohol (215). This in turn should be accessible via the aldehyde (216) via Henry reaction and reduction, which should be easily accessed via the alcohol (208) (Scheme 64).



Scheme 64: Retrosynthesis of guanidine amine (212)

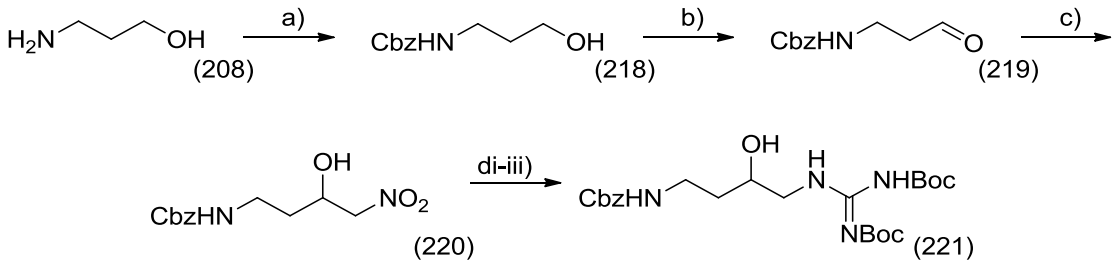
Initially the synthesis of the aldehyde (216) was attempted from the amino alcohol (208) by firstly Boc-protection to give (217) in 74% yield.[93],[94] Oxidation of

the alcohol under Swern conditions seemed to work very well and gave a good crude yield of (216). However, on column chromatography, the product decomposed considerably and the yield was dramatically reduced. PCC was used as an alternative oxidation method, however, this gave a very low yield of product and again upon column chromatography the yield was diminished. Attempts at using the unpurified aldehyde in the Henry reaction failed, and it was apparent that the Boc protecting group was unstable and not compatible with these oxidising conditions and column chromatography.



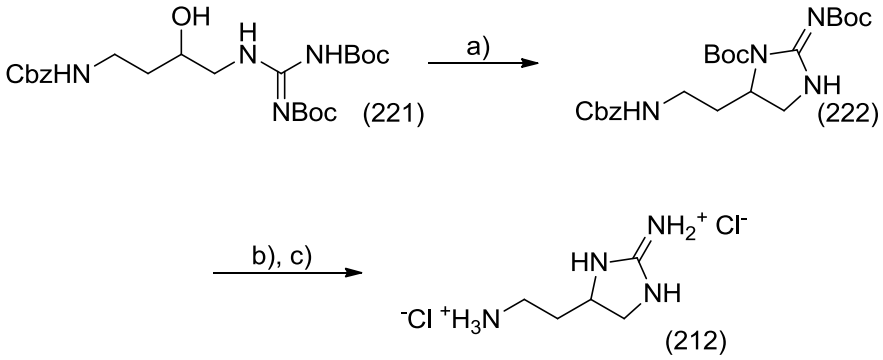
Scheme 65: Attempted synthetic route to aldehyde (216), a) Boc_2O, dichloromethane, 12 h, b) Oxalyl chloride, DMSO, dichloromethane, -78°C, Et_3N, or c) PCC, dichloromethane, 4 h.

The alternative Cbz-protected aldehyde (218)[14] was thus employed. The synthesis was realised from the amino alcohol (208) by Cbz protection in an 85% yield followed by PCC oxidation to give (219) in 68% yield. Henry reaction of (219) with nitromethane gave (220) in 82% yield which was reduced using nickel boride and then guanidinylated in situ with (130) to give the alcohol (221) in 60% yield (Scheme 66).



Scheme 66: synthetic route to (221). a) CbzCl, dichloromethane, 12 h, b) Oxalyl chloride, DMSO, dichloromethane, -78°C, Et_3N, c) $MeNO_2$, dichloromethane, DIPEA, rt, 48 h, d) i) $Ni(II)Cl_2 \cdot 6H_2O$, MeOH, $NaBH_4$, 30 min, ii) excess Et_3N, 1 h, iii) (130), 48 h.

With the alcohol (221) in hand, the Appel cyclisation reaction was attempted,[93] using triphenylphosphine and iodine, using conditions previously developed within the research group.[67] The reaction appeared to proceed well from crude NMR analysis, however the product (222) was difficult to isolate from the reaction by-product triphenylphosphine oxide, as it appeared to co-elute with this by-product. Repeated attempts at purification by precipitation and chromatography resulted in very low yields of impure material. The selective deprotection of crude (222) was attempted under hydrogenation conditions, however this also appeared to give a complex mixture of compounds. However, treatment with 3 M HCl led to the formation of the heterocycle (212) in reasonable material yield but contaminated with triphenylphosphine.



Scheme 67: synthetic route to (212), a) PPh_3, imidazole, I_2, dichloromethane, 2 h, then NH_4Cl, b) H_2, EtOAc, Pd/C 10%, 24 h, c) 3 M HCl, 24 h.

Attempts at coupling the impure amine (212) with the acid (180) were problematic leading to low yields and very complex mixture of products. Attempts at purifying (212) before coupling were not successful and this proved to be the major drawback in this early synthesis work.

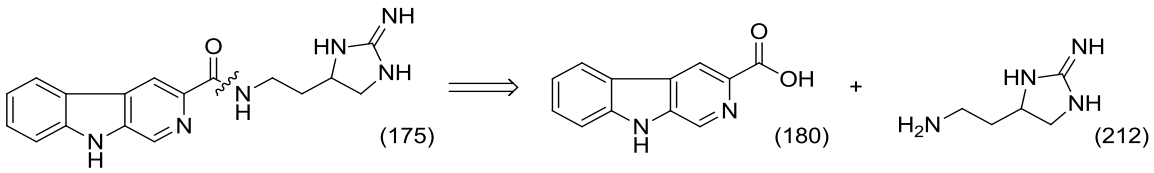
Aims

The previous work on this project identified three problems. There are problems associated with the preparation of the β-carboline heterocycle. The overall yields are somewhat low and the oxidation step to aromatise the tetrahydropyridine ring is problematic in both synthetic routes and employs the toxic lead tetraacetate in one case. The Appel cyclisation step involved in the formation of the guanidine

heterocycle (RHS) appears to be efficient but isolating it from the triphenylphosphine by-product oxide was problematic. Finally, the selective deprotection and the coupling reaction are not likely to be trivial and will need optimisation. Our aim was thus to study these three problems in turn, and the results discussed below.

Results and discussion

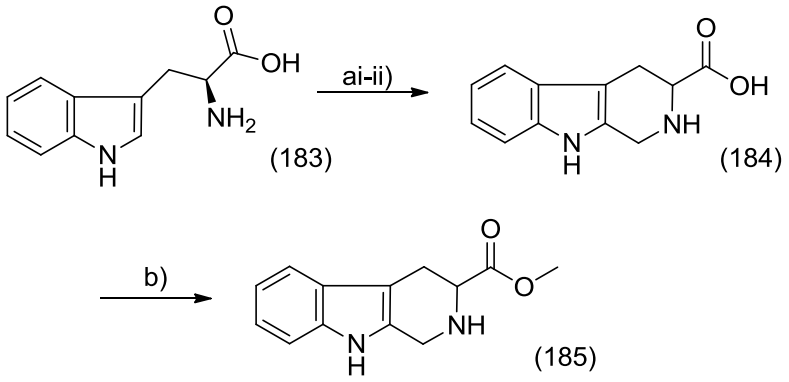
As proposed, the overall retrosynthesis (scheme 68) is to disconnect tiruchanduramine into two fragments *viz* the ß-carboline-3-carboxylic acid (180) and the cyclic guanidine side chain (212).



Scheme 68: retrosynthetic analysis of tiruchanduramine (175).

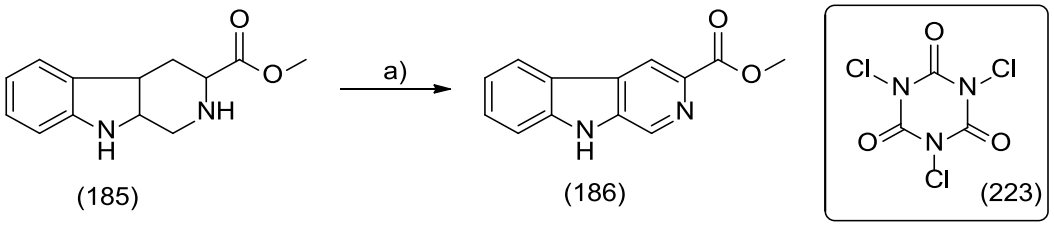
Preparation of ß-carboline-3-carboxylic acid (180)

To date, the best overall yield achieved for the preparation of ß-carboline-3-carboxylic acid (180) used a literature sequence, which gave an overall yield of 21.4%.[88] The first reaction in the synthetic sequence in previously reported methods is the Pictet-Spengler reaction of *L*-tryptophan (183) with formaldehyde which generally proceeds well and in our hands gave (184) in a 97% yield. The esterification step in previous syntheses was effected using either methanolic HCl (generated from acetyl chloride and methanol) or ethanol and concentrated sulfuric acid, which were reasonably efficient methods. Inspection of the literature,[92] suggested that using thionyl chloride in methanol might be a more efficient method. We attempted this method and on work up obtained a 60% yield of the methyl ester (185), which was lower than expected, but the method is quite straightforward and might be optimisable in the future (Scheme 69).



Scheme 69: Alternate synthesis of (185), a) i) NaOH, H_2O, formalin, 3.5 h, Δ, ii) HCl, dil., b) $SOCl_2$, MeOH, 44 h.

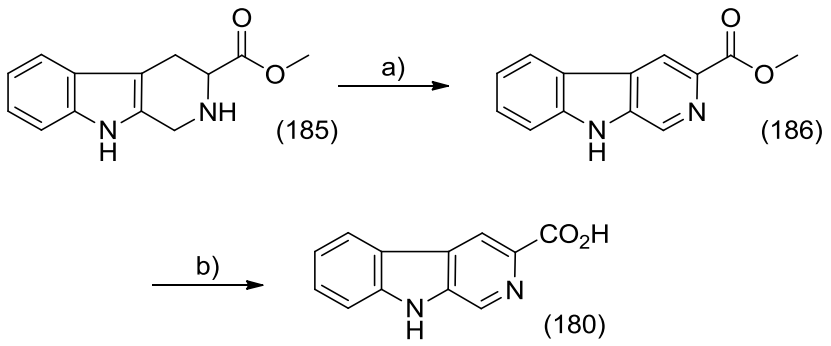
The most problematic steps in both previous sequences were the oxidation steps and it was felt that this was where the most improvement might be made. The current best method utilises lead tetraacetate and gives the aromatised heterocycle (186) in 57% yield. However one interesting literature method found was the aromatisation of (185) to give (186) using trichloroisocyanuric acid (223) which was reported to proceed in 97% yield. [96]



Scheme 70: Oxidation of (185):[96] a) (223), NEt_3, DMF, -20°C, 1 h, 97%.

We thus treated the ester (185) with a slight excess of trichloroisocyanuric acid (223) and triethylamine in DMF at -20°C to room temperature for 1 h. After work up, the aromatised product (186) was obtained in a 97% yield. This was a major improvement over the previous best of 57% yield, however this reaction was only attempted on a small scale and larger scale reaction were not attempted. The final step of the synthesis was the hydrolysis of the ester group in (186) under basic conditions which gave ß-carboline-3-carboxylic acid (180) in 48% yield. This yield was lower

than that that previously reported (59%) but this reaction might be open to optimisation in the future.

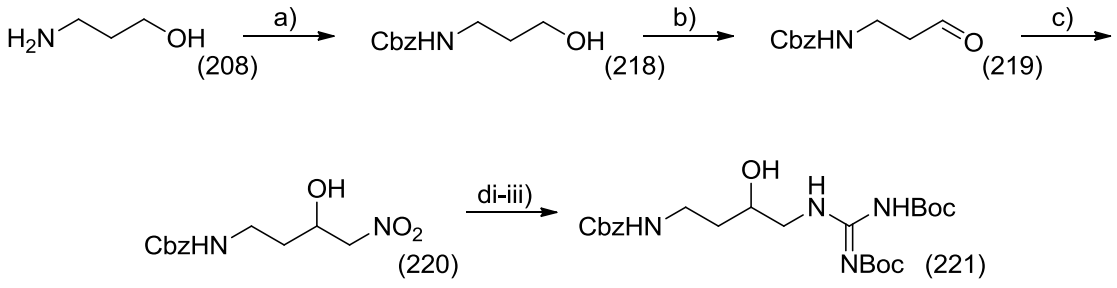


Scheme 71: Synthesis of (180), a) (223), NEt_3, DMF, -20°C, b) NaOH, EtOH, 45 h, Δ, then HCl.

This route resulted in an overall yield of the carboxylic acid (180) from *L*-tryptophan of 27%. This is an improvement on previous routes, however the esterification and hydrolysis steps are still problematic and future work could focus on optimisation of these steps to the high yields observed in the other two steps.

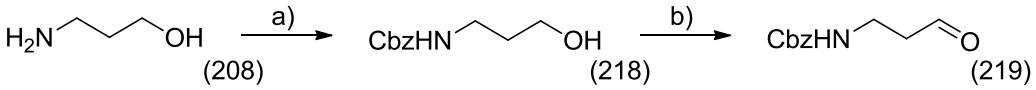
Preparation of the RHS fragment (212)

The next step in the sysntheis involved the optimisation of the synthesis and purification of the heterocycle (222). In order to achieve this, the synthesis of the guanidine (221) was repeated.



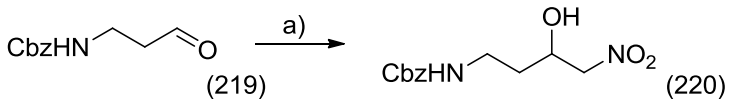
Scheme 72: synthetic route to (221), a) CbzCl, dichloromethane, 12 h, b) Oxalyl chloride, DMSO, dichloromethane, -78°C, Et_3N, c) $MeNO_2$, dichloromethane, DIPEA, rt, 48 h, d) i) $Ni(II)Cl_2 \cdot 6H_2O$, MeOH, $NaBH_4$, 30 min, ii) excess Et_3N, 1 h, iii) (130), 48 h.

The initial step in the synthesis is the Cbz-protection of the amine function of 3-amino-1-propanol (208), which was carried out using benzyl chloroformate in dichloromethane in the presence of sodium hydroxide. This gave alcohol (218) in an 80% yield, which compared well to the reported yield of 90%.[95] Oxidation was attempted using PCC in the presence of Celite©.[97] In our hands the yield for this method was at best 40%, which was attributed to difficulties in the work up. The PCC oxidation generates a large amount of black tar which prevents the easy isolation of the product. We found that performing the reaction in the presence of silica gel instead of Celite© greatly improved matters as the reaction mixture was easily absorbed directly onto a column of silica for purification. This process gave an improved yield of 60% for the aldehyde (219) as well as a much more convenient work up and reaction (Scheme 73).



Scheme 73: Preparation of aldehyde (219), a) CbzCl, dichloromethane, NaOH (aq., 1 M), 0°C, 1 h, b) PCC, dichloromethane, silica gel, 12 h.

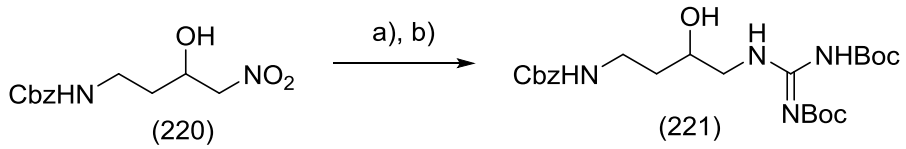
Aldehyde (219) was then treated with nitromethane in the presence of DIPEA in dichloromethane at 0°C to rt over 3 d.[93],[94] This led to the formation of the nitro-alcohol (220) in a 60% yield, with the recovery of significant quantities of the aldehyde (219). On repeating the reaction for 5 d an improved yield of (220) of 90% yield was achieved (Scheme 74).



Scheme 74: Henry reaction of (220), a) CH_3NO_2, DIPEA, dichloromethane, 0°C, 5 d.

The nitro alcohol (220) was then reduced and guanidinylated using the *bis*-Boc protected guanidinylating agent (130) in a two stage one pot process. The initial reduction step uses the reducing agent nickel boride which is prepared from the

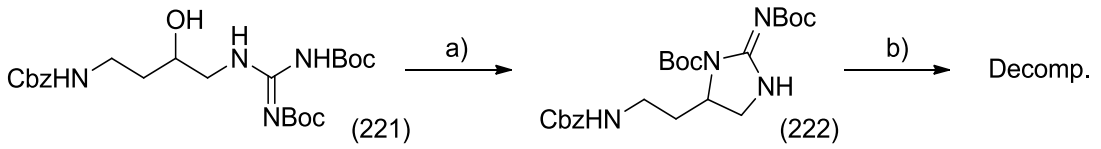
reduction of nickel chloride with sodium borohydride. This reaction is to be treated with caution as the reaction mixture can effervesce violently without warning so a slow addition of borohydride was required to form a suitable reagent. Once formed, the nitro alcohol (220) was added and the mixture stirred for 2 h and then triethylamine was added to neutralise the boronic acid by-products after which the guanidinylating agent is added and the mixture stirred for a further 48 h. On work up this gave the desired guanidine (221) in 50-60% (4 attempts) (Scheme 75). Repeating the reaction with sufficient triethylamine to raise the pH, the yield increased to 80%. Attempts to scale this reaction up (45.0 mmol scale) led to a poor yield of 40%. The reason for this is not apparent however, the concentration of the reaction or inefficient mixing might be a factor.



Scheme 75: Preparation of (221), a) Ni(II)$Cl_2 \cdot 6H_2O$, MeOH, $NaBH_4$, 30 min, b) (130), Et_3N, 48 h, H_2O.

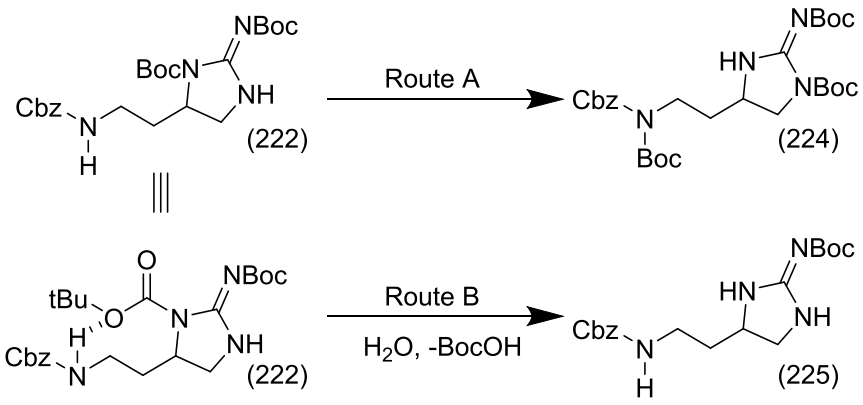
With the guanidine (221) in hand we next attempted to repeat the methods tried by the previous workers and thus cyclised (221) under Appel conditions. The compound together with imidazole and triphenylphosphine were dissolved in dichloromethane and solid iodine was added. After an aqueous work up the product was analysed by TLC and it was apparent that the product was difficult to visualise under UV light and did not stain in common reagent such as PMA, vanillin or potassium permanganate. After searching the literature[98] a stain used for alkaloids and guanidines was found known as Dragendorff's reagent. This is a freshly prepared solution of bismuth nitrate in glacial acetic acid and water and the second solution potassium iodide glacial acetic acid and water the two solutions added together and then diluted with water. This mixture gave dark orange-red spots on TLC when the plate was dipped and heated. We also tried the amino acid stain ninhydrin, which also gave positive results. The product spot eluted very closely to the by-product triphenylphosphine oxide but purification by column chromatography was attempted.

Elution of the compound from a silica gel column unfortunately resulted in the formation of a number of new spots eluting at different Rf values. None of these correlated to the desired product but they did give positive results with Dragendorff's reagent indicating the presence of a guanidine or amine group.



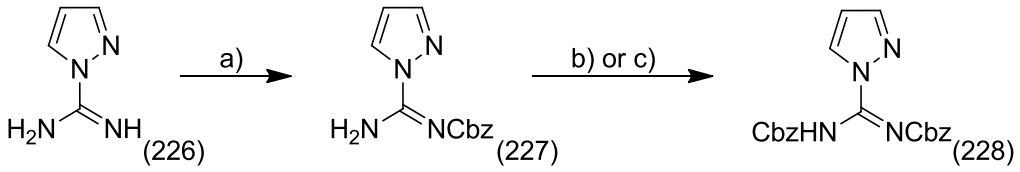
Scheme 76: Attempted preparation of (222), a) PPh_3, imidazole, I_2, dichloromethane, 2 h, then NH_4Cl, b) Silica gel chromatography.

It was apparent that the product (222) was undergoing decomposition and that the most likely cause was the loss or rearrangement of the Boc-protecting groups, a process which had been noted in other work within the group.[99],[100] In these cases the most sterically hindered Boc-group was known to either migrate to the other nitrogen of the guanidine (route A) or to undergo hydrolysis (route B). It is possible an anchimeric type assistance, which has been observed in similar systems studied, might be present where hydrogen bonding can lead to an accelerated hydrolysis of the Boc-group (route B). Route A is likely to be an intermolecular process and thus much less likely (Scheme 76).



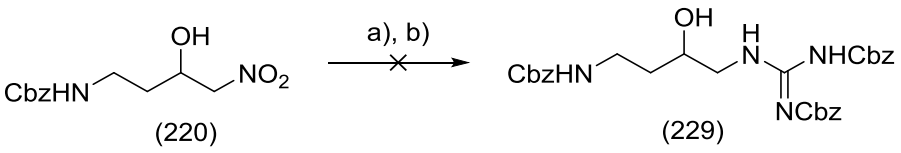
Scheme 77: Possible rearrangement or decomposition of (222).

It was known from previous work, that the corresponding *bis*-Cbz protected guanidinylating agent (228) was less prone to hydrolysis and rearrangement and we thus proposed to replace the bis-Boc-protected reagent (130) with (228). The reagent was prepared in two steps from 1H-pyrazole-1-carboximidamide (226)**,** by firstly reacting it with benzyl chloroformate under basic conditions, followed by reaction of the mono-protected compound (227) with sodium hydride and N-(benzyloxycarbonyloxy)succinimide (Z-OSu). In our hands the first step proceeded to give the mono-Cbz protected pyrazole (227) in a 84 % yield after recrystallization. However several problems were encountered in the second stage which was found to proceed to generate a range of by-products which co-eluted with the product on column chromatography and gave a yield of 49% after repeated chromatography and recrystallization. A search of the literature gave a patent[101] which suggested that the second Cbz-protecting group could be installed using sodium hydride and benzyl chloroformate. On attempting this reaction, it was found that after an aqueous work-up, purification by column chromatography greatly reduced the yield of the product. Circumbenting this was possible by triturating the crude reaction product with hexane followed by recrystallisation, which gave the pure compound in 77% yield.



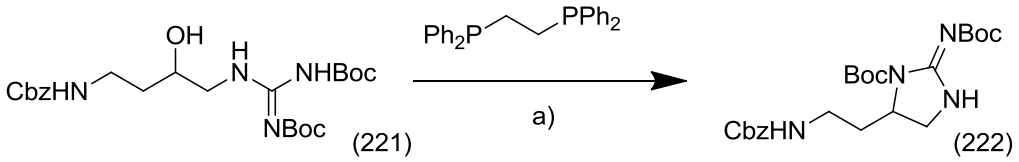
Scheme 78: preparation of (228), a) CbzCl, DIPEA, THF, 24 h, 84% yield, b) NaH, (Z-OSu), THF, 24 h, 49%, c) NaH. CbzCl, rt, THF, 20 h, 77%.

With (228) in hand the reduction of the nitro alcohol (220) was repeated and the product of this was then treated with (228) *in situ*. After stirring for 5 d the reaction was worked up and attempts made to isolate the desired product (229). Despite efforts to isolate (229) it was apparent that it was not formed under the conditions of the reaction and only the unreacted guanidinylating agent (228) was isolated in 27% yield. This reaction was repeated at higher concentration and with warming, however (229) was not isolated in either reaction.



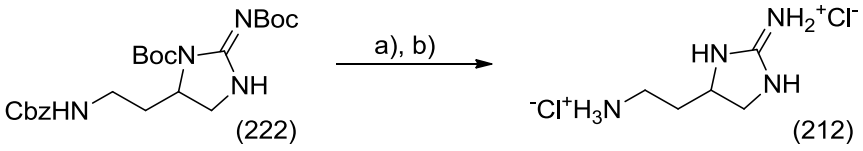
Scheme 79: Attempted preparation of (229), a) $Ni(II)Cl_2 \cdot 6H_2O$, MeOH, $NaBH_4$, 30 min, b) (228), Et_3N, 48 h.

It was theorised that the bis-Cbz protected guanylating agent (228) might be less reactive than the corresponding bis-Boc protected agent (130), however literature reports[102] suggests the opposite to this observation, so it is difficult to rationalise the failure of this reaction. With the lack of success in the formation of the Cbz-protected intermediate (229) it was decided to reinvestigate the cyclisation process and attempt to purify the cyclic guanidine (222). The problem with the process is the presence of the by-product triphenylphosphine oxide in the reaction product and the inability to separate this by chromatography. It has been reported[103] that 1,2-bis(diphenylphosphino)ethane (dppe) is a possible replacement for triphenylphosphine in many reactions as the by-products from the reaction are more polar than triphenylphosphine oxide and are easily removed by trituration or chromatography. We therefore attempted the reaction under the conditions previously utilised but replaced the triphenylphosphine with dppe. Thus dppe and imidazole were added to a cooled (-20°C) solution of (221) dissolved in anhydrous dichloromethane (20 mL), followed by the addition of finely powdered iodine. After 2 h, the reaction was worked up and the crude product dissolved in a dichloromethane and diethyl ether (100 mL) was added which effected the precipitation of the phosphine oxide by products. After storage at -20°C for 12 h, the solution was filtered and the filtrate evaporated to give (222) in quantitative yield which from NMR was 90-95% pure and was suitable for use in the further steps of the synthesis (Scheme 79).



Scheme 80: Preparation of (222) using dppe, a) (230), imidazole, I_2, -20 °C, dichloromethane, 2 h.

With (222) in hand, the selective deprotection of the terminal amine under hydrogenation conditions was attempted. We thus treated (222) with H_2 over Pd/C for 24 h and after filtration and evaporation, it was apparent from ^{1}H NMR that the Cbz–protecting group had been removed, however it also was apparent that a complex mixture of compounds had been formed. We speculated that this mixture might possibly have arisen from the intermediate amine undergoing protecting group migration as has been observed previously in related systems.[99] We thus took this mixture and removed the Boc-protecting groups by treatment with aqueous 3 M HCl for 24 h followed by filtration (to remove trace amounts of dppe by-products from the cyclisation step) to give the crude guanidine (212) as its bis-hydrochloride salt.



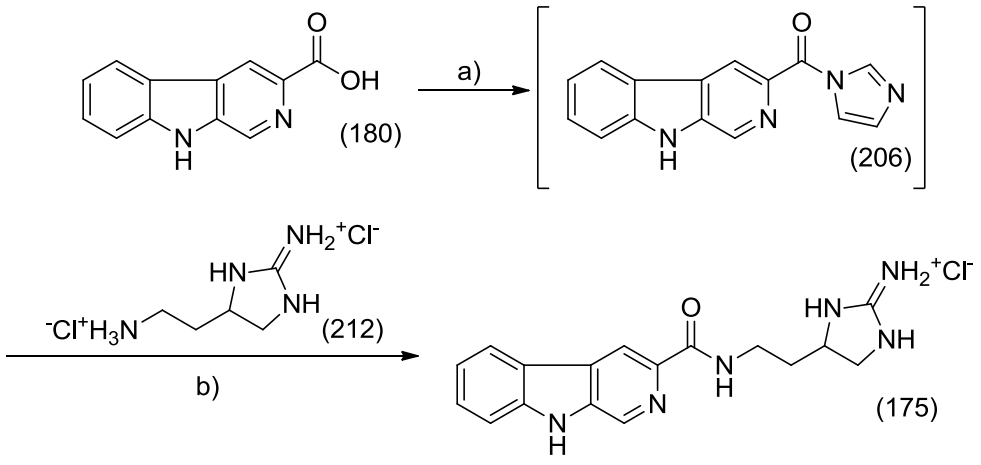
Scheme 81: Synthesis of (212), a) Pd/C 10%, MeOH, 24 h, b) HCl (3M), 24 h.

Analytical data for (212) included ^{1}H NMR signals in DMSO at δ_H 1.80-1.89 (2H, m, CH_2) and 2.75-2.91 (2H, m, CH_2) ppm for the 2 exocyclic methylenes and threes resonances at δ_H 3.23 (1H, dd *J* 6.5, 9.5 Hz, CH), 3.69 (1H, dd *J* 9.5, 9.5 Hz, CH) and 3.99-4.07 (1H, m, CH) for the three ring CH's. The N-H protons of the guanidinium and amine salt were observed at δ_H 7.89 (2H, s, NH_2), 8.05 (1H, s, NH), 8.29 (3H, br s, NH_3^+) and 8.49 (1H, s, NH) ppm. The compound gave the required 5 carbon resonances at δ_C 32.4, 35.3, 47.7, 52.3 and 159.2 ppm, the final signal being indicative of the guanidinium carbon. Finally analysis by high resolution mass spectrometry gave a $M+H^+$ ion at 129.1131 Daltons which was in close agreement with the required mass of 129.1135 for $C_5H_{13}N_4$ ($[M+H]^+$).

The conclusion of this stage of the synthesis is that the cyclisation reaction is a viable process but that the fully or part protected guanidine is prone to rearrangement of and that carrying through the intermediates to the salt (212) is the best strategy. The heterocycle (212) was thus prepared in 7 steps from (208) and the next phase of the synthesis was to couple this with the β-carboline carboxylic acid (180).

Coupling of the RHS (212) with ß-carboline-3-carboxylic acid (180)

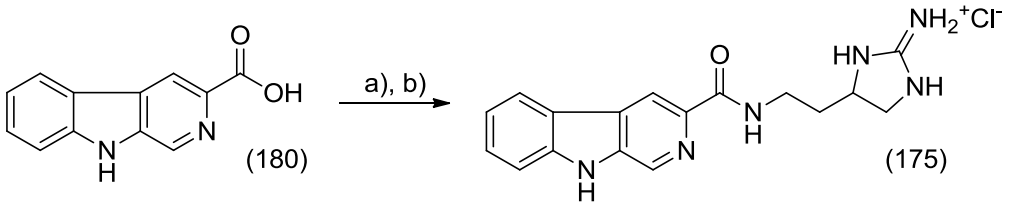
The final step of the synthesis was to couple the LHS of the molecule, the β-carboline carboxylic acid (180) and the RHS guanidinium heterocycle (212). Original attempts at forming the activated intermediate (206) followed the procedures of Cook *et al.*[92] who reported that ß-carboline-3-carboxylic acid (180) dissolved in DMF solution and when treated with CDI gave a purple coloured solution. We were unable to repeat this observation and noted that (180) did not dissolve very well in DMF and remained a suspension even after numerous days of stirring. When CDI was added to the mixture no visible signs of a reaction taking place such as a change in colour were observed and the reaction was attempted numerous times with various modifications such as gently heating to help the ß-carboline-3-carboxylic acid (180) dissolve, or the addition of an ecsess of CDI, but this didn not improve the activation reaction stage in any notable way. Further investigation of the literature a report was found in a patent[104] which reported the coupling of an amine with (180) using CDI in a 1:1 mixture of THF and DMF.



Scheme 82: a) (180), CDI, DMF/THF 1:1, b) (212), NEt_3, 1 week, 11.5%.

We attempted this modification and found that the ß-carboline-3-carboxylic acid (180) on addition to THF/DMF partially dissolves to give a light red colour an on addition of CDI, fully dissolve after stirring for 1 h. However instead of purple colour which was expected, we obtained a dark cherry red solution. This solution was then added via cannula to a cooled (0°C) solution of crude (212) and triethylamine

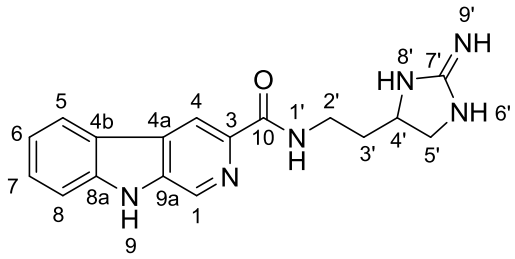
(2.2 eqv) in a 1:1 mixture of DMF and THF. The addition of triethylamine is intended to generate the free amine in situ from heterocycle (212) but should leave the guanidine, which has a higher pKa, largely protonated and hopefully allow selective amide formation to occur.



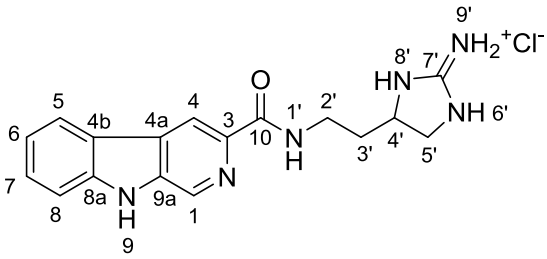
Scheme 83: Coupling reactions of (180) and (212), a) (180), CDI, DMF/THF 1:1, 1 h, b) (212), NEt_3, 1 week, 11.5%.

After stirring for 7 d, during which time the solution became orange in colour, methanol was added to hydrolyse any unreacted CDI or intermediate (206) and the mixture evaporated *in vacuo* (freeze-dried). Analysis of the crude product by NMR demonstrated the presence of a complex mixture of products, however it was apparent that the desired product (175) was present in this mixture. Purification of this mixture by chromatography in PE and methanol was unsuccessful as a method for purification as (175) co-eluted with several of the by-products. However it was possible to purify the tiruchanduramine as its hydrochloride salt from the mixture in reasonable purity by preparative TLC using PE and methanol in 8.5% yield over three steps from the alcohol (221) A sample was also purified by preparative HPLC (by Dr Rob Nash at Aberystwyth University) to give tiruchanduramine hydrochloride (175) in 11.5% yield over three steps.

The ^{1}H NMR data for synthetic (175) is shown in table 5 and demonstrates a very close agreement with the literature[82] data. The only major difference was that the aromatic C5-H proton which was reported at δ_H 8.20 (1H, d, *J* 8.0 Hz, CH) ppm in the literature was observed at δ_H 8.38 (1H, d, *J* 7.8 Hz, CH) ppm in our sample. This might be due to the differences in the counterions between synthetic and natural (175), however it might also be error in the original paper.



Tirunchduramine (175) (Ravinder)



Tirunchduramine (175) hydrochloride

Position[(i)]	Ravinder data	Our data	Ravinder data		Our data	
	δ_{13C}	**δ_C**	**δ_H**	**H, m, *J* (Hz)**	**δ_H**	**H, m, *J* (Hz)**
1	132.2	132.3	8.85	(1H, s)	8.89	(1H, d, 0.8 Hz)
3	139.5	139.6	-	-	-	-
4	113.9	114.0	8.81	(1H, s)	8.83	(1H, d, 0.8 Hz)
4a	128.2	128.1	-	-	-	-
4b	120.9	120.9	-	-	-	-
5	122.1	122.2	8.20	(1H, d, 8.0 Hz)	8.38	(1H, d, 7.9 Hz)
6	119.9	120.0	7.28	(1H, t, 7.6)	7.27–7.33	(1H, m)
7	128.5	128.6	7.58	(1H, t, 7.6)	7.57–7.62	(1H, m)
8	112.2	112.3	7.63	(1H, d, 8.0 Hz)	7.67	(1H, d, 8.2 Hz)
8a	141.0	141.1	-	-	-	-
9	-	-	12.10	(1H, br s)	12.05	(1H, br s)
9a	137.1	137.2	-	-	-	-
10	165.1	165.2	-	-	-	-
1′	-	-	8.84	(1H, s)	8.91	(1H, t, 6.2 Hz)
2′	35.2	35.3	3.42	(2H, m)	3.35–3.48	(2H, m)
3′	34.9	35.0	1.82	(2H, m)	1.82	(2H, app. q, 6.6 Hz)
4′	52.9	53.0	3.99	(1H, m)	3.93–4.01	(1H, m)
5′	47.9	48.0	3.25, 3.77.	(1H, dd, 8.2, 9.6), (1H, t, 9.6 Hz)	3.27, 3.73.	(1H, dd, 7.0, 9.5), (1H, app t, 9.5 Hz)
6′,8′,9′[(ii)]			7.80, 8.18.	(2H, br s), (1H, br s).	7.84, 7.99, 8.20.	(2H, br s), (1H, br s), (1H, br s).
7′	159.2	159.2	-	-	-	-

i) Numbering of rings and side chain as defined in Ravinders paper.[82]

ii) Signals differ as the reported data is for the free base, not the HCl salt.

Table 5: Comparison of data from Ravinder *et al.* and our data.

Unfortunately, we were unable obtain an original sample of (175) or copies of the NMR spectra from the author of the original paper so a direct comparison was not possible. Both the β-carboline and the amide NH signals are in close agreement with the literature apart from the observation of a coupling being present between the amide NH and the adjacent CH_2 which is not reported in the original paper. The other NH signals of the guanidine are different to those reported, however our compound is a hydrochloride salt and this will explain the difference. This might also explain the coupling observed in the amide NH and the differing resonance of the aromatic C5-H.

Proton spectroscopy is notoriously susceptible to changes in solvent temperature and minor acidic and basic impurities, however ^{13}C spectroscopy is less so. It was thus reassuring that the ^{13}C NMR data was again in near exact agreement with the literature data with correlation to ± 0.1 ppm (Table 5). Finally high resolution mass spectrometry gave an ion at 323.1609 Daltons, which is in close agreement with the required mass of 323.1615 Daltons for the formula $C_{17}H_{19}N_6O$ ($[M+H]^+$).

The conclusion from this part of the investigation is that the coupling of (212) with the acid (180) proved somewhat capricious but was eventually achieved by activation of (180) with 1,1′-carbonyldiimidazole (CDI) in DMF and THF,[21] followed by reaction of the activated amide with the free amine of (212). Overall this represents a synthesis of (175) in eight steps from 3-aminopropan-1-ol (208) in 4.5% yield.

Biological activity of synthetic (175)

Synthetic (175), the known carboxylic acid (180) and the guanidine (212) were submitted to assays on a panel of glycosidases at 143μg/mL and (175) was more inhibitory (over 50%) to Bacillus α-glucosidase than (180) 31% and (212) 11%. Compound (175) was also more inhibitory to yeast α-glucosidase (26%) than (180) 9% and (212) gave no inhibition. Synthetic (175) gave over 40% inhibition of β-glucosidase whereas (180) was a much weak inhibitor 14%. Heterocycle (180) was not inhibitory to jack bean hexosaminidase but (175) gave 37% inhibition. Both (175) 20% and (180) 37% inhibited the bovine hexosaminidase. β-Galactosidase was strongly inhibited 80% by (175) but (180) and (212) were not inhibitory to this enzyme. Compound (212) in fact weakly increased the activity of the bovine 11% and jack bean 15% hexosaminidases at the top concentration used. α-Galactosidase and α-mannosidase were not inhibited by any of the compounds. Our results confirm the α-glucosidase inhibition reported by Ravinder[82] although the compound is only a weak to moderate inhibitor of the two α-glucosidases tested here. It should be noted that acarbose used as the comparator for (175) by Ravinder is not a particularly potent inhibitor of glucosidases.[82] Compound (175) does, however, show a broad range of inhibition and also inhibits almond β-glucosidase, β-galactosidase and β-N-acetylglucosaminidase from Jack bean. For (175) to be suitable as an alternative to

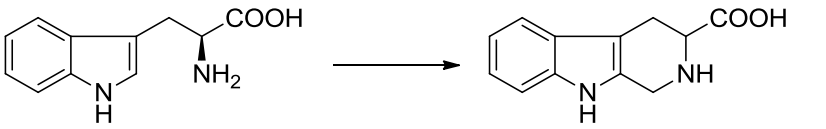
acarbose for diabetes, further modifications would be needed to make it more specific. However, hexosaminidase activity is elevated in many diseases including Alzheimer's[105] and so perhaps this inhibition is of more interest if selectivity can be improved. There are also indications that azasugars can improve the folding and function of glucohydrolases which can become aberrant in disease states including Alzheimer's.[105],[106]

Experimental

Column chromatography was carried out on silica gel (60A) and TLCs were conducted on precoated Kieselgel 60 F254 (Art. 5554; Merck) with the eluent specified in each case. All non-aqueous reactions were conducted in oven-dried apparatus under a static atmosphere of argon. Diethyl ether, THF and dichloromethane were dried by a Pure Solv MD-3 solvent purification system. Dry methanol and DMF was purchased from Aldrich. Chemical shifts are reported as δ values relative to chloroform (7.26/77.16 ppm), methanol (3.31/49.0 ppm) and DMSO (2.50/39.52 ppm) as internal standards. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 or 500 spectrometer with an internal deuterium lock at ambient temperature at 400 or 500 MHz with internal references of δ_H 7.26 and δ_C 77.16 ppm for $CDCl_3$, δ_H 3.31 and δ_C 49.0 ppm for CD_3OD and δ_H 2.54 ppm and δ_C 39.52 ppm for DMSO. Mass spectra data were obtained at the EPSRC Mass Spectrometry Service Centre at the University of Wales, Swansea. Low resolution Chemical Ionisation (CI) and Electrospray Ionisation (ESI) mass spectra were recorded on a Micromass Quattro II spectrometer and high resolution mass spectra were recorded on either a Finnigan MAT 900 XLT or a Finnigan MAT 95 XP. Infrared samples were prepared as thin films or solutions using sodium chloride plates or as KBr discs; spectra were recorded on a Bruker Tensor 37 FT-IR. Melting points (Mp.) were determined using a Gallenkamp MF370 instrument and are uncorrected.

Dragendorff's reagent[17] was prepared from two solutions, one of bismuth nitrate (0.17 g) in glacial acetic acid (2 mL) and water (8 mL) and the second of potassium iodide (4 g) in glacial acetic acid (10 mL) and water (20 mL). The two solutions were then added together and made up to 100 mL with water and the solution formed was storred in the fridge and used within 2-3 d.

2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylic acid (184)[86]



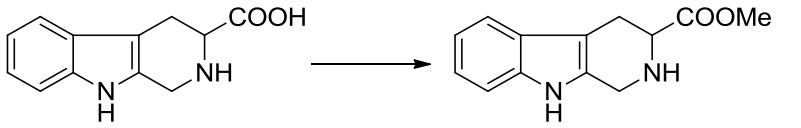
(183)
Chemical Formula: $C_{11}H_{12}N_2O_2$
Exact Mass: 204.09

(184)
Chemical Formula: $C_{12}H_{12}N_2O_2$
Exact Mass: 216.09

Tryptophan (183) (50.0 g, 0.245 mol) was added to a solution of NaOH (2M, 150 mL) and the mixture stirred for 30 min until the tryptophan had dissolved. Formaldehyde (21.4 g, 19.8 mL, 0.713.0 mol, 3.0 eqv.) was added and the solution was stirred at rt for 2 h and then heated at reflux for a further 3.5 h. The solution was then acidified with HCl (6M) and the product precipitated out of solution. The solid obtained was removed by filtration, washed with water (150 mL), methanol (100 mL) and dichloromethane (100 mL) and then dried under vacuum and over phosphorus pentoxide in a desiccator to constant weight to give (184) (50.96 g, 0.236 mol) as a light brown solid solid in 96 % yield. This product was used in the next step without further purification. Data was in agreement with the literature.[86]

Mp. 166-168°C (Lit.[86] 167-169°C); $\boldsymbol{\delta_H}$ (DMSO d_6) 2.83 (1H, dd, *J* 10.5, 16.0 Hz CH), 3.15 (1H, dd, *J* 16.0, 5.1 Hz, CH), 3.36 (2H, br s, OH, NH), 3.68 (1H, dd, *J* 5.1, 10.5 Hz, CH), 4.18 (1H, d, *J* 15.5 Hz, CH), 4.25 (1H, d, *J* 15.5 Hz, CH), 6.99 (1H, br t, *J* 7.5 Hz, CH), 7.07 (1H, dt, *J* 1.2, 7.5 Hz, CH), 7.33 (1H, d, *J* 8.0 Hz, CH), 7.45 (1H, br d, *J* 7.8 Hz, CH), 10.95 (1H, s, NH).

Methyl 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate (185)[86],[87]



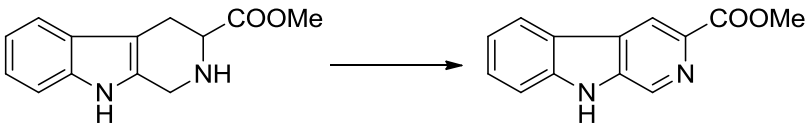
(184)
Chemical Formula: $C_{12}H_{12}N_2O_2$
Exact Mass: 216.09

(185)
Chemical Formula: $C_{13}H_{14}N_2O_2$
Exact Mass: 230.11

Carboxylic acid (184) (25.0 g, 0.116 mol) was dissolved in methanol (100 mL) and cooled (0°C) and thionyl chloride (71.6 g, 43.6 mL, 0.602 mol, 5.2 eqv.) was slowly added **(CAUTION! EXOTHERMIC REACTION!)** in a drop wise manner over 2 h. The mixture was stirred to rt over 3 d, then evaporated under reduced pressure and the resultant solid dissolved in dichloromethane (100 mL), washed with sodium carbonate (sat. 3 x 150 mL) and brine (3 x 100 mL). The solution was dried ($MgSO_4$) and evaporated under reduced pressure to give a crude product which was purified by column chromatography (10% ME in EA) to give (185) (21.5 g, 93.4 mmol) in 81 % yield as a light brown solid. Data was in agreement with the literature.[86],[87]

Mp. 191-193°C (Lit[86],[87] 187-189°C); δ_H (CD_3OD) 2.85 (1H, ddt, *J* 9.4, 15.2, 1.8 Hz CH), 3.06 (1H, ddd, *J* 15.2, 4.9, 1.8 Hz, CH), 3.74 (1H, dd, *J* 4.9, 9.4 Hz, CH), 3.77 (3H, s, Me), 3.98 (1H, dt, *J* 15.4, 1.8 Hz, CH), 4.06 (1H, br d, *J* 15.4 Hz, CH), 6.97 (1H, dt, *J* 1.2, 7.5 Hz, CH), 7.04 (1H, dt, *J* 1.2, 7.5 Hz, CH), 7.26 (1H, br d, *J* 8.1 Hz, CH), 7.45 (1H, br d, *J* 7.8 Hz, CH); δ_C (CD_3OD) 25.8, 42.6, 52.6, 56.8, 107.0, 111.8, 118.3, 119.8, 122.1, 128.4, 132.8, 137.9, 174.8.

Methyl 9*H*-pyrido[3,4-b]indole-3-carboxylate (186).[86],[87]



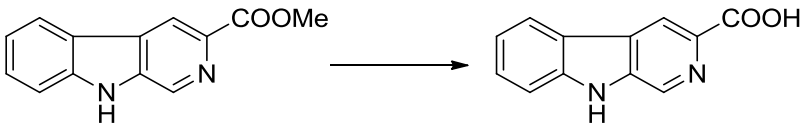
(185)
Chemical Formula: $C_{13}H_{14}N_2O_2$
Exact Mass: 230.11

(186)
Chemical Formula: $C_{13}H_{10}N_2O_2$
Exact Mass: 226.07

Ester (185) (2.0 g, 8.69 mmol) was dissolved in DMF (10 mL), triethylamine (2.3 g, 3.2 mL, 22.6, mmol, 2.6 eqv.) was added and the solution cooled (-20°C) and stirred for 5 min. Trichloroisocyanuric acid (2.22 g, 9.6 mmol, 1.1 eqv.) dissolved in DMF (10 mL) was then added to the reaction mixture over 30 min and the solution stirred to rt over 2 hrs. The reaction mixture was poured onto ice (100 mL) and after warming to rt the precipitate was removed by filtration and washed with water (ca. 50 mL). The solid was dried under vacuum over phosphorus pentoxide to constant weight to give (186) (1.91 g, 8.45 mmol) in 97 % yield which was used without further purification. Data was in agreement with the literature.[86],[87]

Mp. 240-241°C (Lit.[86],[87] 242-244°C); $\boldsymbol{\delta_H}$ (CD_3OD) 4.03 (3H, s, Me), 7.30-7.40 (1H, m, CH) 7.57-7.67 (2H, m, 2 x CH), 8.25 (1H, d, *J* 7.9 Hz, CH), 8.80-8.96 (2H, m, 2 xCH); $\boldsymbol{\delta_C}$ (MeOD) (partial data 4 x quaternary C not observed) 52.9, 113.3, 118.6, 121.8, 122.9, 130.2, 134.3, 143.0, 151.6.

9H-pyrido[3,4-b]indole-3-carboxylic acid (180).[86],[87]



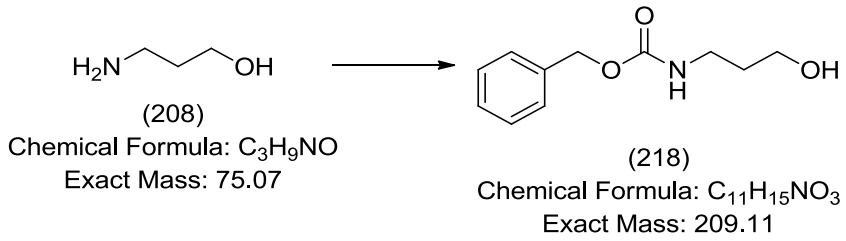
(186)
Chemical Formula: $C_{13}H_{10}N_2O_2$
Exact Mass: 226.07

(180)
Chemical Formula: $C_{12}H_8N_2O_2$
Exact Mass: 212.06

Ester (186) (1.91 g, 8.45 mmol) was dissolved in ethanol (25 mL), NaOH solution (aq., 2M, 25 mL) was added and the solution was refluxed for 4 h, following by TLC until the starting material spot had disappeared. The ethanol was then removed under vacuum and the solution acidified to pH 6 with dil HCl. The resultant precipitate was removed by filtration and washed with water (100 mL) and methanol (50 mL). The solid was dried under vacuum over phosphorus pentoxide to constant weight to give (180) (0.93 g, 4.3 mmol) in 51 % yield as a yellow solid. Data was in agreement with the literature.[86],[87]

Mp. 317-320°C (Lit.[86],[87] 316-318°C); $\boldsymbol{\delta_H}$ (DMSO d_6) 7.31 (1H, br t, *J* 7.4 Hz, CH), 7.60 (1H, t, *J* 7.4 Hz, CH), 7.67 (1H, br d, *J* 8.0, CH), 8.39 (1H, d, *J* 7.7 Hz, CH), 8.94 (1H, s, CH), 8.98 (1H, s, CH), 12.10 (1H, s, NH); $\boldsymbol{\delta_C}$ (DMSO d_6) 112.5, 117.4, 120.3, 120.9, 122.3, 127.9, 128.8, 133.2, 137.2, 137.5, 141.2, 166.9.

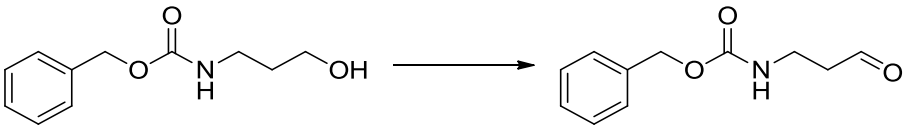
Benzyl (3-hydroxypropyl)carbamate (218)[95]



A stirred solution of 1-aminopropan-3-ol (208) (12.0 g, 133.3 mmol) in NaOH (aq. 1M, 40.0 mL, 3.0 eqv.) was cooled (0°C) and benzyl chloroformate (22.5 g, 133.3 mmol, 1.0 eqv.) was added drop wise over 10 min. After warming to rt the mixture was stirred for 1 h and dichloromethane (100 mL) was added and stirring continued for 16 h. The organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 50 mL) and EA (2 x 50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to yield the crude product as a pale yellow solid. Recrystallisation from EA (ca. 50 mL) gave (218) (22.5 g, 116.6 mmol) in an 88% yield as white crystals.

Mp. 49-50°C (Lit.[95] 50-51°C); Rf 0.13 (50 % EA in PE, PMA); $\boldsymbol{\delta_H}$ 1.70 (2H, app pentet, *J* 6.0 Hz, CH_2), 2.55 (1H, br s, OH), 3.25 (2H, apparent q, *J* 6.3 Hz, CH_2), 3.67 (2H, t, *J* 5.8 Hz, CH_2), 5.07 (1H, br s, NH), 5.10 (2H, s, CH_2), 7.28-7.39 (5H, m, Ph); $\boldsymbol{\delta_C}$ 32.7, 37.9, 59.7, 67.0, 128.2, 128.3, 128.7, 136.6, 157.5.

Benzyl (3-oxopropyl)carbamate (219) [107]



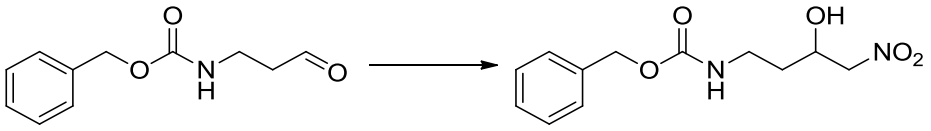
(218)
Chemical Formula: $C_{11}H_{15}NO_3$
Exact Mass: 209.11

(219)
Chemical Formula: $C_{11}H_{13}NO_3$
Exact Mass: 207.09

PCC (8.75 g, 40.6 mmol, 1.7 eqv.) and silica (9 g) were stirred in dichloromethane (50 mL) for 5 min after which alcohol (218) (5.0 g, 23.9 mmol) dissolved in dichloromethane (30 mL) was added. After 4 h the reaction was diluted with Et_2O (100 mL) and passed through a short pad of layered silica and Celite©. The remaining solids in the flask were dissolved/suspended in dichloromethane (25 mL) and precipitated with Et_2O (50 mL) and this mixture passed through the same pad. This process was repeated a further three times and the combined filtrates evaporated to give a yellow oil (4.98 g). Column chromatography (40% EA in PE) gave (219) as a colourless viscous oil (3.01 g, 14.5 mmol) in 61% yield. Data was in agreement with the literature.[107]

Rf 0.40 (50% EA in PE, PMA); $\boldsymbol{\delta_H}$ 2.77 (2H, t, *J* 5.8 Hz, CH_2), 3.52 (2H, app q, *J* 6.0 Hz, CH_2), 5.11 (2H, s, CH_2), 5.17 (1H, br s, HN), 7.30-7.41 (5H, m, Ar), 9.83 (1H, s, CHO); $\boldsymbol{\delta_C}$ 34.6, 44.2, 66.9, 128.2, 128.3, 128.7, 136.5, 156.4, 201.3; $\boldsymbol{\nu_{max}}$ 3445, 1704, 1645.

Benzyl (3-hydroxy-4-nitrobutyl)carbamate (220)



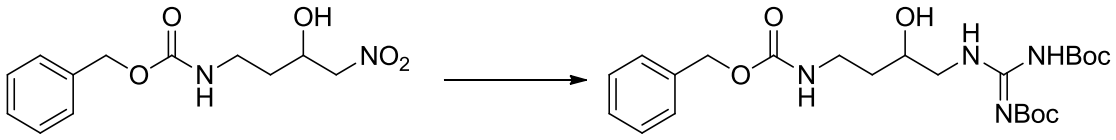
(219)
Chemical Formula: $C_{11}H_{13}NO_3$
Exact Mass: 207.09

(220)
Chemical Formula: $C_{12}H_{16}N_2O_5$
Exact Mass: 268.11

Aldehyde (219) (2.2 g, 10.23 mmol) and nitromethane (18.73 g, 16.5 mL, 0.307 mol, 30.0 eqv.) were dissolved in dichloromethane (20 mL) and cooled (0°C). DIPEA (3.7 g, 5.0 mL, 28.64 mmol, 2.8 eqv.) was added to the solution in a drop wise manner and the mixture stirred for 5 d. Ammonium chloride (saturated, 250 mL) was added and stirring continued for 10 min. The organic layer was separated and the aqueous layer extracted with dichloromethane (3 x 100 mL). The combined organic layers were dried ($MgSO_4$), evaporated and the crude product (4.81 g) purified by column chromatography (50% EA in PE) to give (220) (2.48 g, 9.24 mmol) in 90% yield as a clear colourless oil.

Rf 0.29 (50% EA in PE, PMA); $\boldsymbol{\delta_H}$ 1.56-1.73 (2H, m, CH_2), 3.22-3.29 (1H, m, CH), 3.54-3.66 (1H, m, CH), 3.85 (1H, br s, OH), 4.36-4.47 (3H, m, CH_2, CH), 5.05 (1H, br s, NH), 5.12 (2H, s, CH_2), 7.30-7.40 (5H, m, Ph); $\boldsymbol{\delta_C}$ 34.3, 37.1, 66.0, 67.4, 80.4, 128.3, 128.5, 128.8, 136.3, 157.7; $\boldsymbol{\nu_{max}}$ 3404, 3034, 1692, 1553; **HRMS** (CI) found 269.1134, $C_{12}H_{17}N_2O_5$ ($[M+H]^+$) requires 269.1132.

Benzyl (4-di-boc-guanidino-3-hydroxybutyl)carbamate (221)



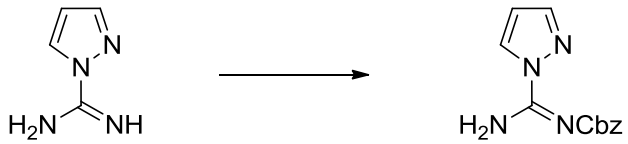
(220)
Chemical Formula: $C_{12}H_{16}N_2O_5$
Exact Mass: 268.11

(221)
Chemical Formula: $C_{23}H_{36}N_4O_7$
Exact Mass: 480.26

$NiCl_2 \cdot 6H_2O$ (5.31 g, 149.0 mmol, 3 eqv.) dissolved in methanol (400 mL) was cooled (0°C) and $NaBH_4$ (2.53 g, 67.05 mmol, 9 eqv.) was added slowly in portions and the mixture stirred for 45 min. Alcohol (220) (2.00 g, 7.45 mmol) dissolved in methanol (5 mL) was then added followed by the careful addition of further $NaBH_4$ (5.64 g, 149.0 mmol, 20.0 eqv.) in small portions over 30 min. After 2 h the mixture was filtered through a pad of Celite© which was washed with methanol (2 x 80 mL). Triethylamine (66.34 g, 92.1 mL, 0.65 mol, 88.0 eqv.) was added to the filtrate and the mixture stirred for 1 h. *N*,*N*′-Di-Boc-1H-pyrazole-1-carboxamidine (130) (2.77 g, 8.94 mmol, 1.2 eqv.) was then added and the mixture stirred for 3 d. The methanol was evaporated *in vacuo* and the resulting viscous oil dissolved in water (400 mL), which was then extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give crude (221) (4.63 g) as a viscous purple oil. Purification by column chromatography (35% EA in PE) gave (221) (2.9 g, 6.03 mmol) in 81% yield as a glassy solid containing trace amounts of ethyl acetate.

Rf 0.19 (40% EA in PE, PMA); $\boldsymbol{\delta_H}$ 1.46 (9H, s, 3 x Me), 1.48 (9H, s, 3 x Me), 1.53-1.66 (2H, m, CH_2), 3.20-3.39 (2H, m, 2 x CH), 3.40-3.60 (2H, m, 2 x CH), 3.75-3.85 (1H, m, CH), 5.08 (2H, s, CH_2), 5.41 (1H, br s, NH), 7.27-7.34 (5H, m, Ph), 8.68 (1H, br s, NH), 11.44 (1H, br s, NH); $\boldsymbol{\delta_C}$ 28.1, 28.3, 34.9, 38.0, 47.6, 66.8, 69.5, 79.7, 83.6, 128.2, 128.2, 128.6, 136.7, 153.1, 157.1, 157.3, 162.9; $\boldsymbol{\nu_{max}}$ 3681, 3415, 3331, 2984, 2935, 1725, 1638; **HRMS** (CI) found 481.2644, $C_{23}H_{37}N_4O_7$ ($[M+H]^+$) requires 481.2657.

(Z)-benzyl (amino(1H-pyrazol-1-yl)methylene)carbamate (227)[102]



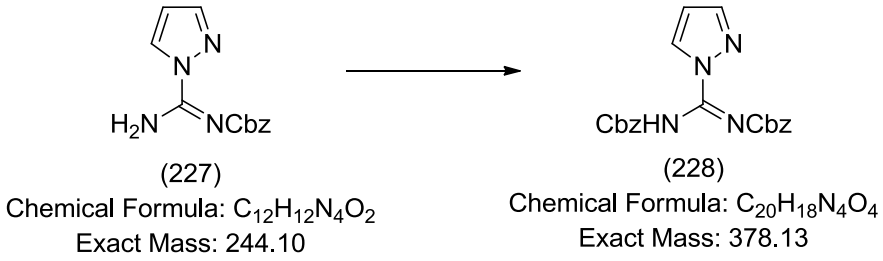
(226)
Chemical Formula: $C_4H_6N_4$
Exact Mass: 110.06

(227)
Chemical Formula: $C_{12}H_{12}N_4O_2$
Exact Mass: 244.10

1H-pyrazole-1-carboximidamide (226) (10.0 g, 68.0 mmol) was dissolved in anhydrous THF (40 mL) followed by the addition of benzyl chloroformate (17.4 g, 14.5 mL, 102.0 mmol, 1.5 eqv.). After stirring for 5 min, DIPEA (17.6 g, 24.0 mL, 136.0 mmol, 2.0 eqv.) was added in a drop wise manner over 15 min and the solution stirred for 24 h. Dichloromethane (60 mL) was then added and the reaction mixture washed with water (100 mL), $KHSO_4$ (aq. 5%, 100 mL) and then brine (150 mL). The organic layer was then dried ($MgSO_4$) and evaporated under reduced pressure to give a crude product which was recrystallised dichloromethane/hexane to give (227) (13.14 g, 57.0 mmol) as needle like crystals in 84% yield. Data was in agreement with the literature,

Mp. 106-108°C (Lit[102] 107-108°C); Rf 0.31 (30% EA in PE); $\boldsymbol{\delta_H}$ 5.23 (2H, br s, CH_2), 6.44 (1H, br s, CH), 7.36-7.43 (5H, m, Ph), 7.70-7.71 (2H, br s, CH, NH), 8.47 (1H, br s, CH), 9.06 (1H, br s, NH); $\boldsymbol{\delta_C}$ 67.6, 109.3, 128.1, 128.3, 128.5, 128.9, 136.3, 143.7, 155.5, 163.9; $\boldsymbol{\nu_{max}}$ 3457, 3018, 2360, 1628.

(Z)-benzyl((((benzyloxy)carbonyl)amino)(1H-pyrazol-1-yl)methylene)carbamate (228)



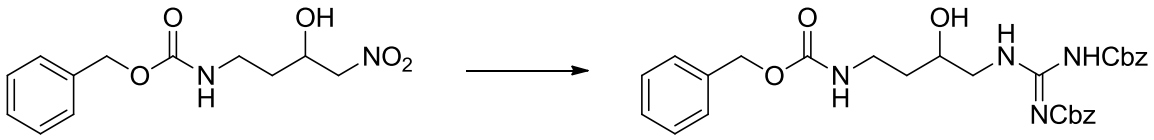
From N-(benzyloxycarbonyloxy)succinimide[102]*:* Pyrazole (227) (5.0 g, 20.47 mmol) was dissolved in anhydrous THF (25 mL) and cooled (0°C). The solution was stirred vigorously whilst NaH (1.72 g, 71.7 mmol, 3.5 eqv.) was added cautiously in portions over a period of 10 min. This mixture was stirred for 30 min whereuopon N-(benzyloxycarbonyloxy)succinimide (15.0 g, 61.41 mmol, 3.0 eqv.) was added and the reaction mixture stirred to rt overnight. The reaction mixture was cooled (0°C) then cautiously diluted with water (100 mL) and the resulting solution was then extracted with chloroform (3 x 100 mL). The combined organic extracts were washed with water (3 x 50 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give a crude brown oil. The brown oil was dissolved in chloroform (20 mL) and filtered through a short pad of silica which was washed with diethyl ether (250 mL). The filtrate was evaporated onto silica (15 g) and purified by column chromatography (0-50% EA in PE). The fractions containing the product eluted in 30-40% EA in PE, which co-eluted with an unidentified by-product, were combined and dissolved in 5mL of hot ethyl acetate which was then diluted with petroleum ether (20 mL). Crystals formed on storage in a freezer overnight, which were filtered, washed with petroleum ether (50 mL) to yield (228) (3.8 g, 10.04 mmol) in 49% yield as colourless needle shaped crystals

From benzyl chloroformate[104]*:* Pyazole (227) (10.0 g, 43.4 mmol) was dissolved in anhydrous THF (150 mL) and cooled to 0°C. The solution was stirred vigorously whilst NaH (5.2 g, 217.0 mmol, 5.0 eqv.) was added in small portions **(CAUTION! EXOTHERMIC REACTION!)** over a period of 15 min. After effervescence had ceased, benzyl chloroformate (8.88 g, 7.46 mL, 52.1 mmol, 1.2 eqv.) was then added drop wise over 30 min and the mixture stirred for 2 h, then

stirred to rt over 24 h. The reaction was then cooled (0°C) and a solution of sodium sulfate (Sat., 200mL) was cautiously added and the mixture stirred for 30 min. Ethyl acetate (200 mL) was then added and the mixture filtered through a Celite© pad. The organic phase was then separated, dried ($MgSO_4$) and evaporated under reduced pressure to give the crude product. This product was then dissolved in hot ethyl acetate (ca. 20 mL) and petroleum ether was added to the cloud point and the solution placed in the freezer overnight. The crystals that formed were filtered and washed with hexane to yield (228) (12.7 g, 33.6 mmol) in 77% yield as colourless needle shaped crystals. Further purification by column chromatography was attempted and but gave little improvement to the purity of the product but led to material losses (8.4 g, 22.2 mmol, 51% yield). Data was in agreement with the literature.

Mp. 89-93°C (Lit[104] 89-93°C); Rf 0.22 (30% EA in PE, PMA); δ_H 5.25 (4H, br s, 2 x CH_2), 6.47 (1H, br d, *J* 4.3 Hz, CH), 7.39 (10H, m, 2 x Ph), 7.65 (1H, br s, CH), 8.3 (1H, br d, *J* 2.8 Hz, CH), 9.36 (1H, br s, NH); δ_C 68.4, 68.7, 109.9, 110.3, 128.2, 128.4, 128.6, 128.7, 128.7, 128.8, 128.9, 128.9, 134.5, 135.7, 145.4, 150.7, 158.1

Attempted preparation of Benzyl (4-di-Z-guanidino-3-hydroxybutyl)carbamate (229)

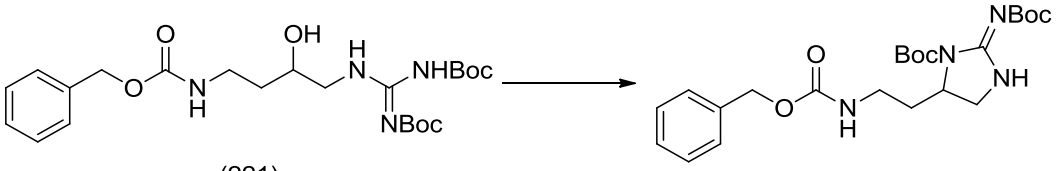


(220)
Chemical Formula: $C_{12}H_{16}N_2O_5$
Exact Mass: 268.11

(229)
Chemical Formula: $C_{29}H_{32}N_4O_7$
Exact Mass: 548.23

Ni(II)Cl$^-$6H_2O (4.24 g, 17.85 mmol, 3.0 eqv.) was dissolved in methanol (300 mL) and $NaBH_4$ (2.02 g, 53.55 mmol, 9.0 eqv.) was added slowly in portions and the mixture stirred for 45 min. Nitro alcohol (220) (1.5 g, 5.95 mmol) dissolved in methanol (5 mL) was then added followed by careful addition of further $NaBH_4$ (4.5 g, 119.0 mmol, 20.0 eqv.). After 2 h, the reaction mixture was filtered through a pad of Celite which was washed with methanol (2 x 80mL). Triethylamine (53.0 g, 73.6 mL, 523.6 mmol, 88.0 eqv.) was then added slowly to the filtrate and the mixture stirred for an hour. The solution was tested with pH paper to ensure it was basic and pyrazole (228) (4.5 g, 11.9 mmol, 2.0 eqv.) was added and the solution was stirred for 3 d. Excess solvent was removed under vacuum and the resulting viscous oil was dissolved in water (400 mL) which was then extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were dried ($MgSO_4$), evaporated under reduced pressure to give a crude product (1.70 g) as a viscous purple oil. Analysis by NMR and attempted chromatographic purification failed to yield any indication of the formation of (229).

(E)-tert-butyl-5-(2-(((benzyloxy)carbonyl)amino)ethyl)-2-((tert-butoxycarbonyl) imino)imidazolidine-1-carboxylate (222)



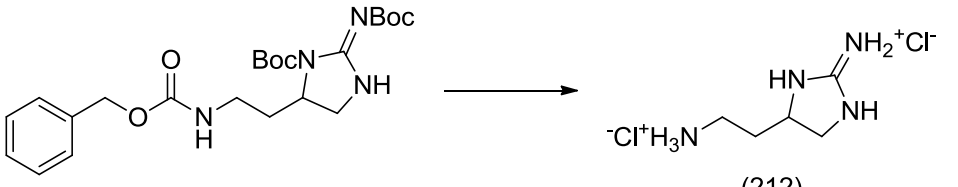
(221)
Chemical Formula: $C_{23}H_{36}N_4O_7$
Exact Mass: 480.26

(222)
Chemical Formula: $C_{23}H_{34}N_4O_6$
Exact Mass: 462.25

Dppe (1.24 g, 3.12 mmol, 1.5 eqv.) and imidazole (0.5 g, 7.28 mmol, 3.5 eqv.) were added to a cooled (-20°C) solution of (221) (1.0 g, 2.08 mmol) in anhydrous dichloromethane (20 mL) and the mixture stirred to ensure dissolution. At this point finely powdered iodine (0.8 g, 3.12 mmol, 1.5 eqv.) was added and the mixture stirred for 2 h. The reaction mixture was diluted with $CHCl_3$ (200 mL) then washed with saturated ammonium chloride (200 mL) and brine (200 mL). The organic layer was dried ($MgSO_4$) and evaporated *in vacuo* to give the crude product (2.68 g). This was dissolved in dichloromethane (10 mL) and diethyl ether (100 mL) was added which effected the precipitation of phosphine oxide by products. After storage at -20°C for 12 h, the solution was filtered and the filtrate evaporated to give crude (222) (1.06 g) which was used in the next step without further purification.

Rf 0.18 (40% EA in PE, PMA); $\boldsymbol{\delta_H}$ (partial data, NH not observed) 1.46 (9H, s, Boc), 1.49 (9H, s, Boc), 1.71-1.84 (1H, m, CH), 1.85-1.95 (1H, m, CH), 3.15-3.29 (2H, m, CH_2), 3.51 (1H, br d, *J* 12.3 Hz, CH), 3.88 (1H, dd, *J* 8.9, 12.3 Hz, CH), 4.19-4.29 (1H, m, CH), 5.06 (2H, s, CH_2), 5.25 (1H, br s, NH) 7.25-7.35 (5H, m, Ph); $\boldsymbol{\delta_C}$ (partial data, 2 x quaternary not observed) 28.1, 34.3, 36.9, 51.2, 54.5, 66.7, 81.4, 84.0, 128.1, 128.1, 128.5, 136.5, 150.7, 156.5; $\boldsymbol{\nu_{max}}$ 3332, 2982, 2933, 1712, 1645; **HRMS** (CI) found 463.2545, $C_{23}H_{35}N_4O_6$ ($[M+H]^+$) requires 463.2551.

2-(2-iminoimidazolidin-4-yl)ethanamine hydrochloride (212)



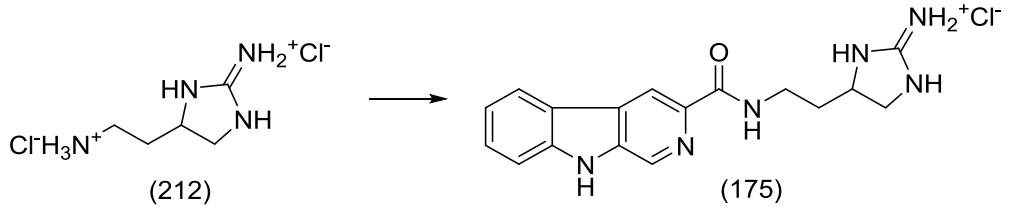
(222)
Chemical Formula: $C_{23}H_{34}N_4O_6$
Exact Mass: 462.25

(212)
Chemical Formula: $C_5H_{14}Cl_2N_4$
Exact Mass: 200.06

A solution of crude (222) (0.60 g) in anhydrous methanol (15 mL) together with 10% Pd/C (0.50 g) were stirred under an atmosphere of H_2 gas for 24 h. The reaction mixture was then filtered through a pad of celite© which was washed with further methanol. Evaporation of the filtrate gave a yellow viscous oil (0.42 g) to which aqueous HCl (3 M, 10 mL) was added, and the mixture stirred for 24 h. At this point the solution was filtered through a small pad of celite© (to remove final traces of phosphine oxide impurities) and evaporated *in vacuo*. The product was dried *in vacuo* (P_2O_5) to give crude (212) (0.257 g) which was used in the next reaction without further purification.

δ_H (DMSO d_6) 1.80-1.89 (2H, m, CH_2), 2.75-2.91 (2H, m, CH_2), 3.23 (1H, dd *J* 6.5, 9.5 Hz, CH), 3.69 (1H, dd *J* 9.5, 9.5 Hz, CH), 3.99-4.07 (1H, m, CH), 7.89 (2H, s, NH_2), 8.05 (1H, s, NH), 8.29 (3H, br s, NH_3^+), 8.49 (1H, s, NH); **δ_C** (DMSO d_6) 32.4, 35.3, 47.7, 52.3, 159.2; **ν_{max}** 3685, 3375, 1687, 1520, 1477, 1438, 1334, 1215, 1122, 1027; **HRMS** (CI) found 129.1131, $C_5H_{13}N_4$ ($[M+H]^+$) requires 129.1135.

Tiruchanduramine hydrochloride (175)



(212)
Chemical Formula: $C_5H_{14}Cl_2N_4$
Exact Mass: 200.06

(175)
Chemical Formula: $C_{17}H_{19}ClN_6O$
Exact Mass: 358.13

Carboxylic acid (180) (250.0 mg, 1.17 mmol, 1 eqv.) was suspended in DMF (15 mL) and anhydrous THF (15 mL) and stirred for 1 h. CDI (220.0 mg, 1.29 mmol, 1.1 eqv.) was then added and the mixture stirred for 7 h. This solution was then added via cannula to a cooled (0°C) solution of crude (212) (257 mg) and triethylamine (263.0 mg, 0.38 mL, 2.60 mmol, 2.2 eqv) in DMF/THF (1:1, 30 mL). The resulting mixture was warmed slowly to rt and stirred for 7 d, during which time the solution became orange in colour. Methanol (20 mL) was added and the mixture stirred for 45 min. After evaporation *in vacuo* (freeze-dried) a crude product (556.0 mg) was obtained as a dark brown solid. A sample (144.0 mg) of this was purified on an ACE 10 C18 250 x 21.8 mm column (HiChrom) using 80:20 water:acetronitrile with 0.01% TFA for 2 min increasing to 50:50 over 13 min at a flow rate of 15 mL/min (monitored at 250 nm) to give tiruchanduramine hydrochloride (175) (10.0 mg) in 11.5% yield over three steps. Preparative TLC (20% ME in dichloromethane) on a sample (205 mg) gave tiruchanduramine hydrochloride (175) (13.2 mg) as a solid in 8.5% yield over three steps (ca. 95% purity by ^{1}H NMR).

δ_H (DMSO d_6) 1.82 (2H, apparent q, *J* 6.6 Hz, CH_2), 3.27 (1H, dd, *J* 7.0, 9.5 Hz, CH), 3.35–3.48 (2H, m, CH_2), 3.73 (1H, apparent t, *J* 9.5 Hz, CH), 3.93–4.01 (1H, m, CH), 7.27–7.33 (1H, m, CH), 7.57–7.62 (1H, m, CH), 7.67 (1H, d, *J* 8.2 Hz, CH), 7.84 (2H, br s, 2 x NH), 7.99 (1H, br s, NH), 8.20 (1H, br s, NH), 8.38 (1H, d, *J* 7.9 Hz, CH) 8.83 (1H, d, *J* 0.8 Hz, CH), 8.89 (1H, d, *J* 0.8 Hz, CH), 8.91 (1H, br t, *J* 6.2 Hz, NH), 12.05 (1H, br s, NH); **δ_H** (CD_3OD) 1.97–2.01 (2H, m, CH_2), 3.45 (1H, dd, *J* 6.8, 9.5 Hz, CH), 3.56–3.67 (2H, m, CH_2), 3.87 (1H, apparent t, *J* 9.5 Hz, CH), 4.12–4.18 (1H, m, CH), 7.41–7.44 (1H, m, CH), 7.70–7.73 (2H, m, 2 x CH) 8.33 (1H, d, *J* 8.0 Hz, CH), 8.99 (1H, s, CH), 9.01 (1H, s, CH); **δ_C** (DMSO d_6) 35.0, 35.3, 48.0, 53.0, 112.3, 114.0, 120.0, 120.9, 122.2, 128.1, 128.6, 132.3, 137.2, 139.6, 141.1, 159.2, 165.2; **ν_{max}** 3304, 2970, 2919, 1681, 1656, 1648, 1560, 1501, 1462, 1254,

1203, 1124, 1073, 845, 750 cm^{-1}; **HRMS** (CI) found 323.1609, $C_{17}H_{19}N_6O$ ($[M+H]^+$) requires 323.1615.

Method for determining activity[108]

All enzymes and *para*-nitrophenyl substrates were purchased from Sigma. Enzymes were assayed at 27°C in 0.1 M citric acid / 0.2 M disodium hydrogen phosphate buffers at the optimum pH for the enzyme. The incubation mixture consisted of 10 μl enzyme solution, 10 μl of 1 mg/mL aqueous inhibitor solution and 50 μl of the appropriate 5 mM *para*-nitrophenyl substrate made up in buffer at the optimum pH for the enzyme. The reactions were stopped by addition of 70 μl 0.4 M glycine (pH 10.4) during the exponential phase of the reaction, which had been determined at the beginning using uninhibited assays in which water replaced inhibitor. Final absorbances were read at 405 nm using a Versamax microplate reader (Molecular Devices). Assays were performed in triplicate.

Section C:

C: A Synthetic approach to nitensidine E.

Introduction

The Nitensidines alkaloids

Nitensidines A-E are a group of guanidine alkaloids found in the South American legume, *pterogyne nitens*. (Figure 12). There are 5 members of the family with nitensidines A-C (230-232) being isolated in 1995[109] and nitensidines D (233) and E (234) in 2008.[110] Nitensidine E (234) represents the first reported natural occurrence of a cyclic monoterpene derivative containing a guanidine moiety. This legume also contains two other similar guanidine containing alkaloids, pterogynine (235) and pterognidine (236) [110] (Figure 13).

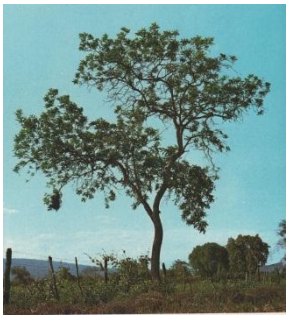


Figure 12: *pterogyne nitens.* (Picture from google images).

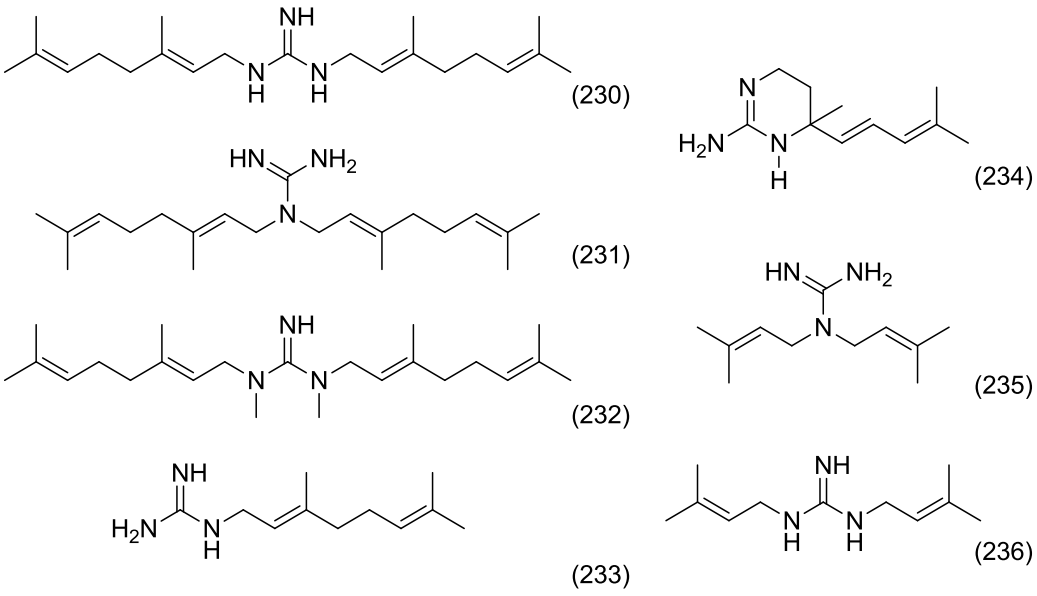
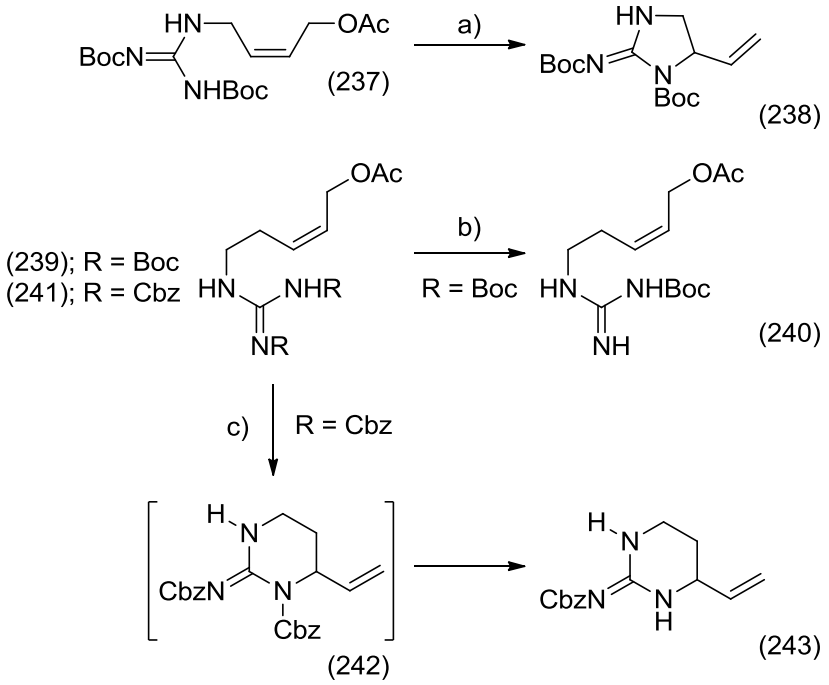


Figure 13: The nitensidine alkaloids.

The nitensidines A-C gave moderate cytotoxic activity,[109] however nitensidines D (233) and E (234) gave a higher range of cytotoxicity against a number of human cancer cell lines such as leukemia, colon, melanoma and glioblastoma, with nitensidine E showing the widest range of activity.[110] In 2010 Bolzani *et al.*[111] reported that nitensidines A-C (230-232) displayed moderate activity against opportunistic fungi and in comparison with the known antifungal agent fluconazole, they were better at inhibiting the growth of *candida krusei*, an emerging hospital acquired infection in immune-compromised patients. This may be of significance, as the treatment of these infections is very difficult as patients who contract these types of infections are already in a weakened state due to impaired immune function. Following this report Bolzani *et al.*[112] also found that the nitensidines A-C (230-232) were active against a number of bacterial infections including MRSA.

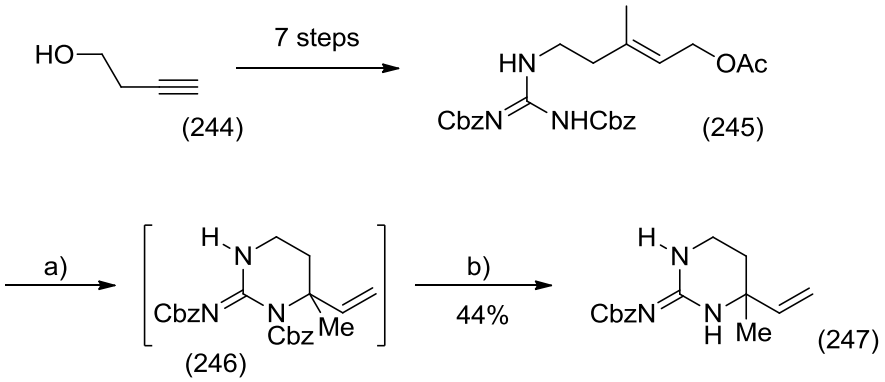
A synthetic approach to Synthesis of nitensidine E; aims and background work

This part of the thesis is directed toward the synthesis of Nitensidine E (234) using π-allyl palladium cyclisation developed within the research group. Previous to this study π-allyl palladium cyclisations had been investigated in the research group[113] and the cyclisation of the substrate (237) and (239) were investigated. The cyclisation of (237) was achieved by treatment with $Pd(OAc)_2$ and PPh_3, in THF to give the 5-membered guanidine (238) in an excellent 90% yield. However, cyclisation of (239) using $Pd(OAc)_2$ and PPh_3 in either THF or CH_3CN failed to give any cyclisation. Prolonged reaction time lead to the loss of the protecting group was observed and (240) was the only product isolated, which also did not undergo cyclisation. Different palladium catalysts were tried, however, without success. It was concluded that the presence of the bulky Boc-protecting groups was inhibiting cyclisation and the cyclisation of the less sterically hindered Z-protected analogue (241) was investigated. Reaction of (241) with $Pd(PPh)_4$ in acetonitrile led rapidly to the cyclised compound (242), however on work up and chromatography the mono-Cbz-protected guanidine (243) was formed (Scheme 84), which unfortunately co-eluted with the Ph_3PO by-product of the reaction. When the reaction was repeated using $Pd(dppe)_2$ the product (243) was formed in 84% yield (Scheme 84).



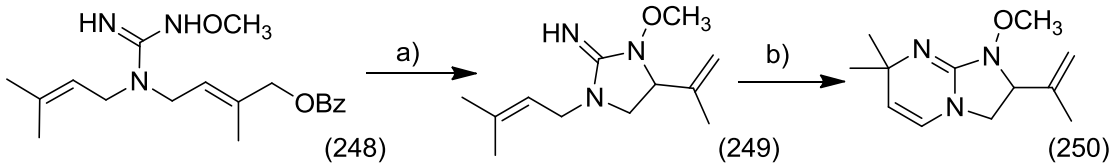
Scheme 84: a) $Pd(OAc)_2/PPh_3$, THF, reflux, 3 h, 90%, b) $Pd(OAc)_2/PPh_3$, THF, reflux, c) See text.

This methodology was applied to a model study of nitensidine E[114] and thus, the allylic acetate guanidine (245) was prepared from the alcohol (244) in seven steps. Cyclisation of this substrate gave the mono-protected 6-membered guanidine (247) in 44% yield (Scheme 85). This was an encouraging result however, attempted deprotection of the Cbz-protecting groups under a wide range of conditions (basic and acidic), generally led to complex mixtures of products.



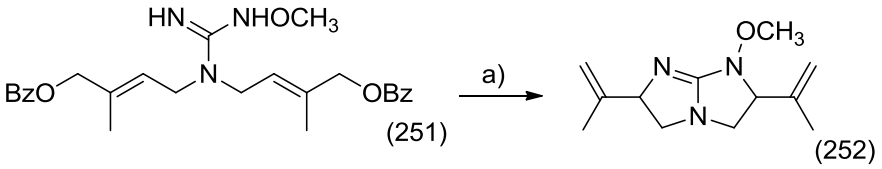
Scheme 85: a) THF, $Pd(dppe)_2$, Et_3N, Δ, 48 h, b) TFA, MeOH, rt, 48 h, 44%.

Prior to our initial work, cyclisations of this type using simple guanidines were largely unknown. A related cyclisation had been reported by Büchi *et al*.[115] in their 1989 approach to the synthesis of (±)-alchorneine (250) and (±)-isoalchorneine (252). They found that treatment of the *N*-methoxyguanidine (248) with $Pd(PPh_3)_4$ in CH_3CN at 50°C for 3 h in the presence of NEt_3 gave the 5-membered guanidine (249) in 81% yield. This was probably formed either *via* an intermediate π-allyl Pd(II) complex or *via* a *cis*-amidopalladation. Further reaction of (249) under oxidative conditions using two equivalents of $PdCl_2(CH_3CN)_2$ in dichloromethane at 40°C for 48 h gave (±)-alchorneine (250) in 46% yield (Scheme 86).



Scheme 86: a) $Pd(PPh_3)_4$, Et_3N, CH_3CN, 81%, b) $PdCl_2(CH_3CN)_2$, dichloromethane, 40°C, 46%.

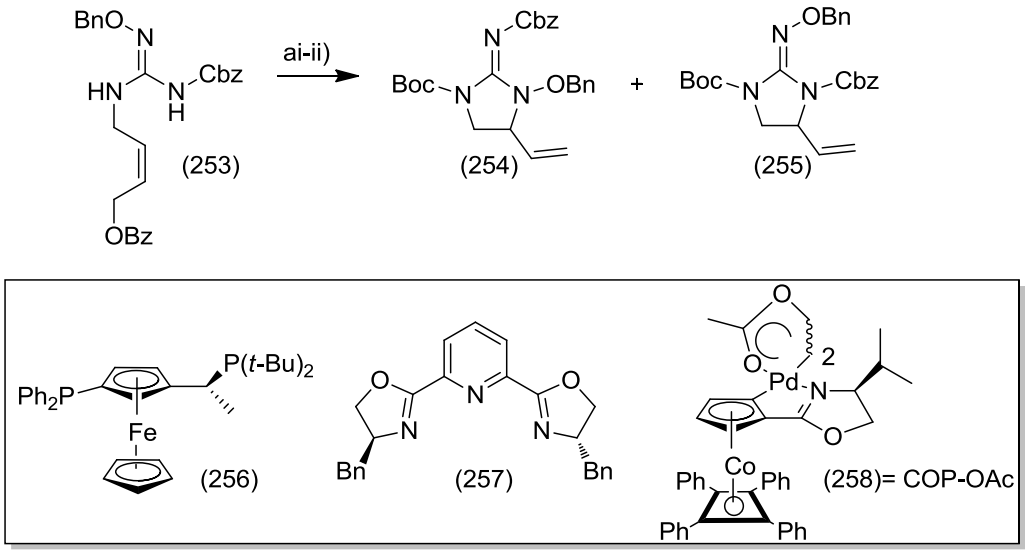
They similarly reported that, cyclisation of the *N*-methoxyguanidine (251) with $Pd(PPh_3)_4$ (0.2 equiv.) in CH_3CN at 50°C for 24 h in the presence of NEt_3 gave a 1:l mixture of the two diastereoisomers of (±)-isoalchorneine (252) (Scheme 87).[115]



Scheme 87: a) $Pd(PPh_3)_4$, Et_3N, CH_3CN, 50°C.

Subsequent to this work, Buck and Wipf reported[116] the cyclisation of allylic N-benzyloxy-guanidine (253) under Pd(II) catalyzed conditions (Scheme 88, Table 6). They reported that using $Pd_2(dba)_2$ (0.5 equiv.) as the catalyst gave a highly regioselective synthesis of (254), however in a poor 14% yield (Entry 1). The use of $Pd(OAc)_2$ in THF (0.05 M) gave a higher yield of 57% but a complete loss of

regioselectivity (Entry 2). However, changing the catalyst to $PdCl_2(CH_3CN)_2$ in THF afforded (254) in 84% yield with complete regioselectivity (Entry 3). Attempts to use chiral catalysis in this reaction were disappointing. The use of the ligands (256), (257), sparteine or Overman's chiral palladium (II) catalyst COP-OAc[117] (258) gave mixed results (Entries 4-7), with only the last catalyst being effective giving a 50:50 mixture of (254) and (255) in a 70% combined yield with a 48% ee for (254) being obtained.

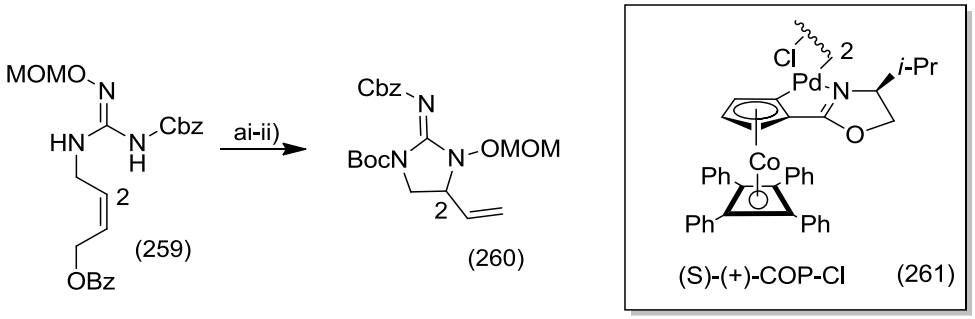


Scheme 88: a) i) See text, ii) Boc_2O, DMAP, Et_3N, THF.

Entry	Conditions	Ligand	Ratio (254):(255)	Result
1	$Pd_2(dba)_2$ (0.5 equiv.), Et_3N (1.1 equiv.), dichloromethane (0.05 M)	-	100:0	14%
2	$Pd(OAc)_2$ (0.2 equiv.), THF (0.05 M)	-	50:50	57%
3	$PdCl_2(CH_3CN)_2$ (0.2 equiv.), THF (0.2 M)	-	100:0	84%
4	(R)-(-)-COP-OAc (258) (0.1 equiv.), dichloromethane (0.6 M)	-	50:50	70% ((254): 48% ee)
5	$PdCl_2(CH_3CN)_2$ (0.2 equiv.), DCE (0.5 M), 85^oC	(256) (0.4 equiv.)	-	NR
6	$PdCl_2(CH_3CN)_2$ (0.2 equiv.), THF (0.5 M)	(-)-sparteine	100:0	55% (< 1% ee)
7	$PdCl_2(CH_3CN)_2$ (0.2 equiv.), DCE (0.5 M), 85^oC	(257) (0.4 equiv.)	100:0	67% (< 2% ee)

Table 6: Showing conditions for key amidoalkylation of guanidine (253).[116]

They also reported that the related guanidine derivative (259) underwent Pd(II)-mediated cyclisation using $PdCl_2(CH_3CN)_2$ in THF (0.2 M) to give exclusively (260) in 95% yield (Entry 1). The use of (*S*)-(+)-COP-Cl as a chiral catalyst gave the same selectivity for the formation of (260) which was obtained in 76% yield with a 3:1 e.r (50% ee) (Entry 2). The use of the opposite enantiomer (*R*)-(-)-COP-Cl gave a similar yield but with the enantioselectivity reversed (Entry 3) (Scheme 89, Table 7).[116]



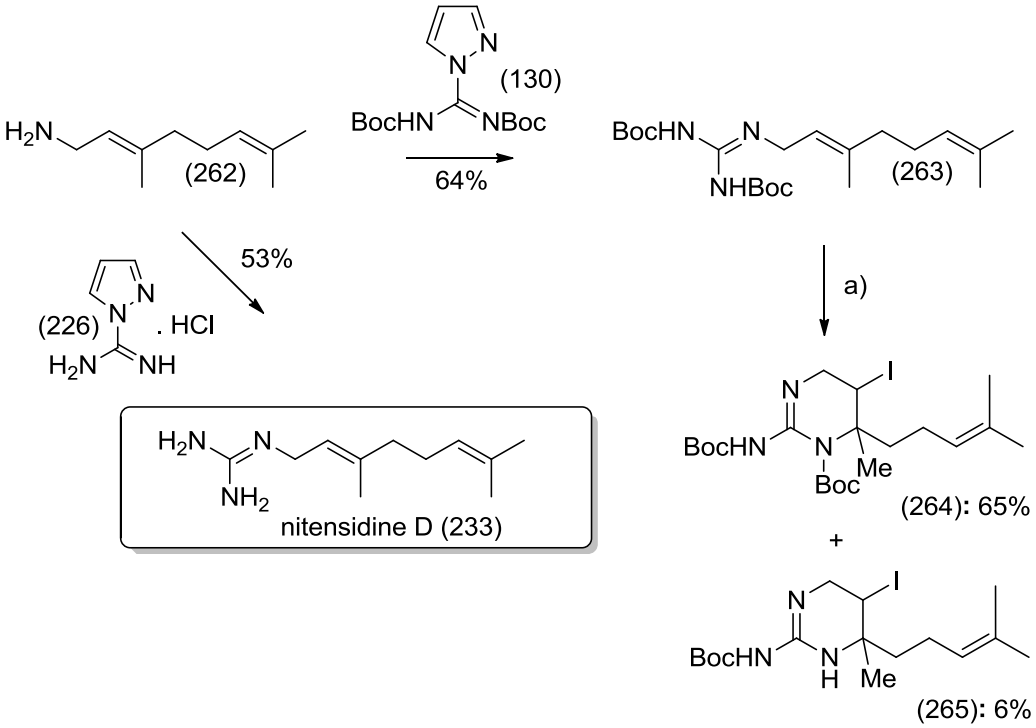
Scheme 89: a) i) Conditions, ii) Boc_2O, DMAP, Et_3N, THF.

Entry	Conditions	Yield (er)
1	$PdCl_2(CH_3CN)_2$ (0.2 equiv.), THF (0.2 M)	95%
2	(*S*)-(+)-COP-Cl (0.025 equiv.), dichloromethane (0.6 M)	76% (3:1)
3	(*R*)-(-)-COP-Cl (0.025 equiv.), dichloromethane (0.6 M)	74% (1:3)

Table 7: Pd(II)-mediated cyclization of MOM-derivative (259).

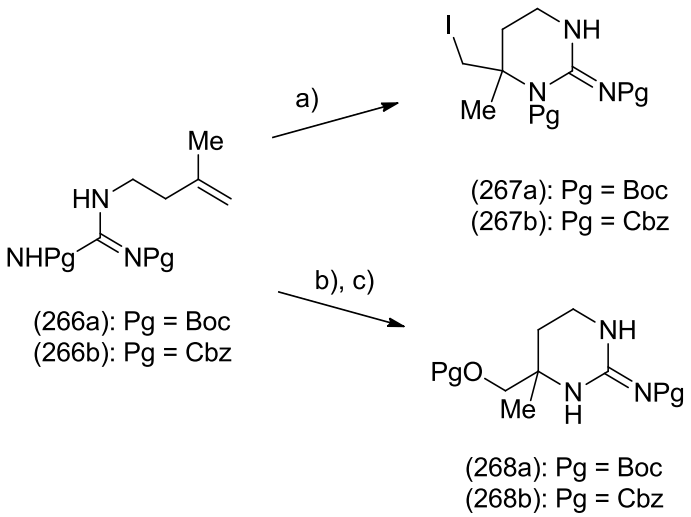
A few similar cyclisations have been reported using structurally related ureas[118],[119],[120] and carbamates,[117] whilst intramolecular diamination of alkenes[121],[122] have been reported for the preparation of guanidines.

Several other strategies for the synthesis of nitensidine D (233) and nitensidine E (234) were investigated in the previous study.[123] Indeed, nitensidine D (233) was prepared[124] in 53% from commercially available geranylamine (262) on guanidinylation with (226). A similar guanidinylation with (130) gave the protected guanidine (263) in 64% yield, which on iodocyclisation gave the bis-protected (264) and mono-cyclic guandines (264) and (265) in 65% and 6% yields respectively (Scheme 90). These were considered to be models of the natural product (234), however attempted deiodination was unsuccessful and attempted acidic deprotection of these compounds led to considerable decomposition (Scheme 90).



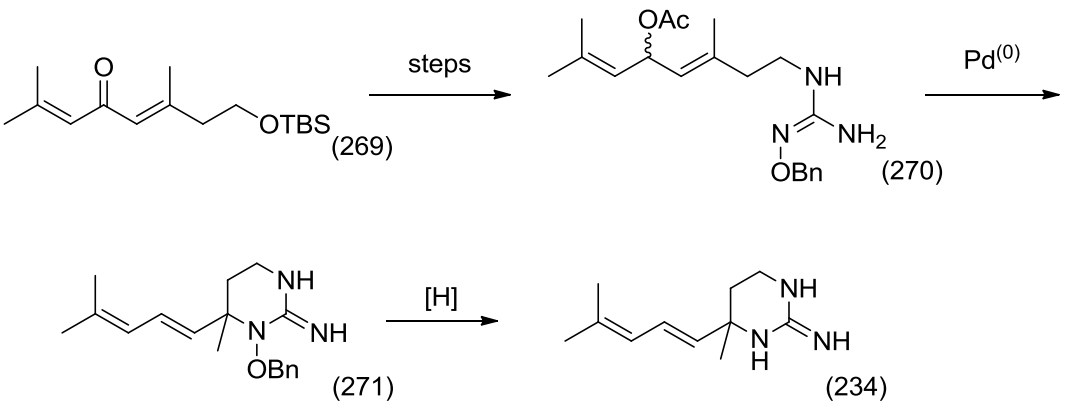
Scheme 90: Preparation of the natural product nitensidine D and the iodocyclisation of guanidine (263), a) I_2, CH_3CN, -15°C-rt, 16-24 h, (256): 65%; (257): 6%.

Several other approaches to the core of nitensidine E (234) were also attempted[86],[90] including the iodocyclisations of guanidines (266a) and (266b) to give the cyclic guanidines (267a) and (267b) in 71% and 68% yields and the corresponding oxidation (and subsequent protecting group migration) with DMDO to give the cyclic guanidines (268a) and (268b) in 72% and 95% yields. Attempted modification of these compounds to nintesidine E (234) was unsuccessful and deprotection of these compounds was again problematic (Scheme 91).



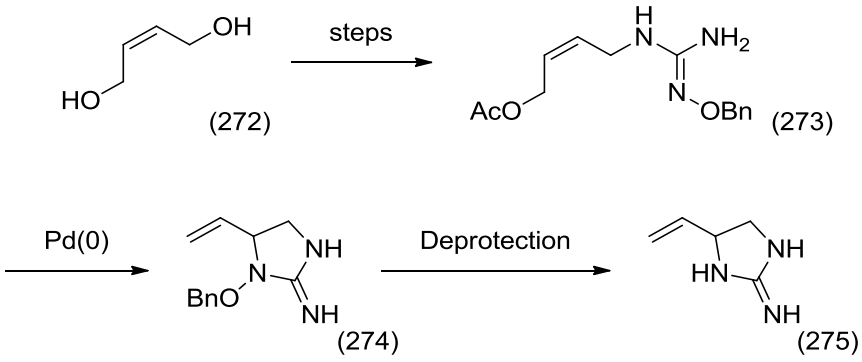
Scheme 91: a) I_2 K_2CO_3, CH_3CN, -15°C-rt, 16-24 h, b) DMDO, acetone 1-5 d, c) (iii) MeOH, TFA, rt, 16-24 h.

It is apparent that of the methodologies adopted, the one that has shown the greatest success is the palladium-catalysed π-allyl cyclisation methodology. However, the use of protecting groups in these cyclisations is problematic as the cyclisation is not successful for 6-membered systems using Boc-protecting groups. Similarly the Cbz-protecting group presents problems as it is hard to remove under acidic or basic conditions and hydrogenation is no possible as this will lead to problems with the unsaturated side-chain of nintesidine E (234). We were intrigued by the reports of Büchi[115] and Buck[116] who used *N*-methoxy- and *N*-benzyloxy-guanidines in palladium-catalysed π-allyl cyclisations with considerable success. We were thus interested in the investigation of a similar cyclisation to give 6-membered guanidines with the goal of the synthesis of nintesidine E (234). A proposed synthesis is shown in (Scheme 92) from the ketone (269) which was prepared in the previous investigation.[123]



Scheme 92: Proposed synthesis of (234).

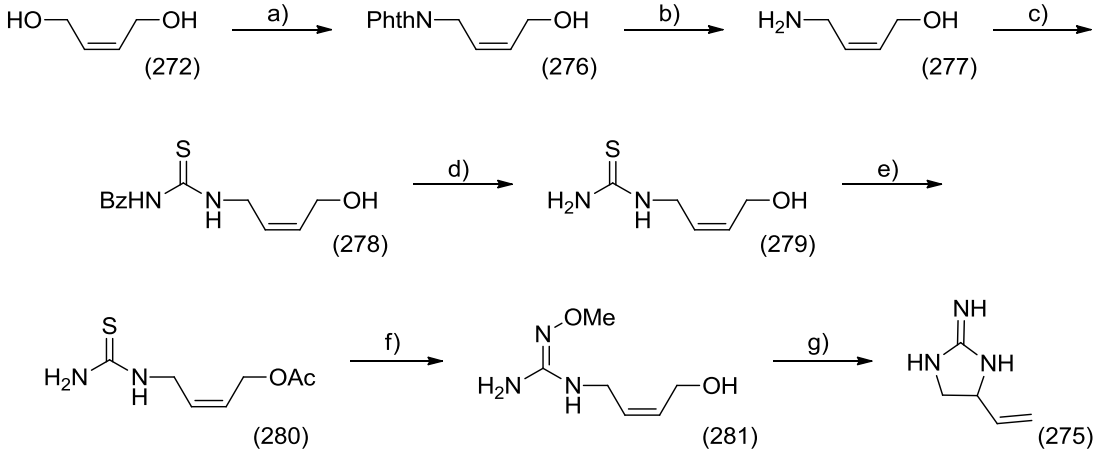
Conversion of (269) to the guanidine (270) should enable the cyclisation to the N-O derived compound (271). Removal of the protecting group from this should be possible using a selective dissolving metal reduction. As the synthesis of substrate (271) is a complex process a cyclisation reaction was initially envisaged on the model system (273). The cyclic intermediate (274) should then be a good model for the investigation of the selective deprotection of the guanidine in the presence of the alkene function.



Scheme 93: Proposed synthesis of model system to study cyclization and deprotection conditions.

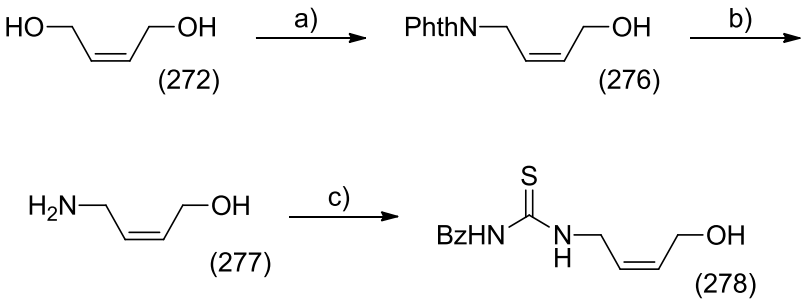
Results and discussion

The initially planned route to substrate (273) began with the Gabriel synthesis of the amine (277) from *cis*-1,4-but-2-ene diol (272) via the phthalimide (276). Reaction of (276) with benzoyl isothiocyanate should give thiourea (278) which on hydrolysis should give the thiourea (279). Acetylation of the free alcohol (279) should give (280) which on coupling with *o*-methylhydroxylamine mediated by EDCl and DIPEA should give the desired substrate (281) (scheme 94).



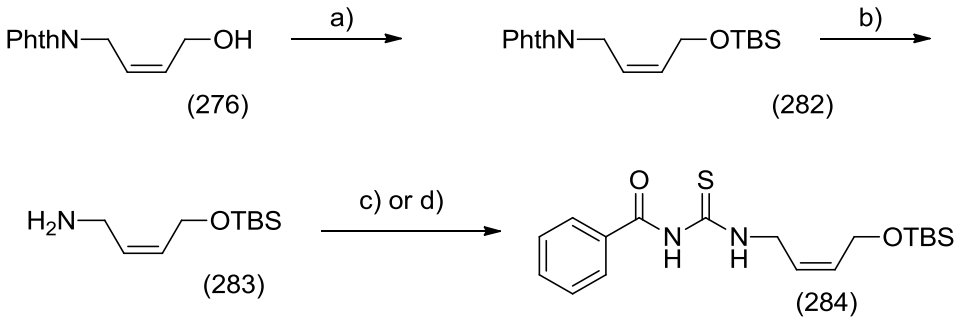
Scheme 94: Proposed route to (281), a) PPh_3, phthalimide, THF, DIAD, 0°C, 48 h, b) ethylenediamine, EtOH, Δ, c) Acetone, NEt_3, PhCONCS, d) NaOH, 70°C, 20 min, dil HCl, e) Ac_2O, dichloromethane, DMAP, 0°C, f) $MeONH_2$, dichloromethane, DIPEA, 0°C, EDCI, 48 h, g) $Pd(PPh_3)_4$, THF, Δ.

We thus treated *cis*-1,4-but-2-ene diol (272) with triphenylphosphine, DIAD and phthalimide[113] which gave the phthalimide (276) in a 69% yield. Deprotection of the amine was effected by heating the phthalimide (276) with ethylenediamine in ethanol which after filtration and evaporation gave the crude amine (277). The benzoyl isothiocyanate, required for the next step was prepared by refluxing a mixture of ammonium thiocyanate, triethylamine and benzoyl chloride in dry acetone and was used without further work up. Treating crude amine (277) with the benzoyl isothiocyanate did not form the expected product according to NMR of the crude reaction mixture. The experiment was repeated with commercially obtained benzoyl isothiocyanate which again did not yield any (278).



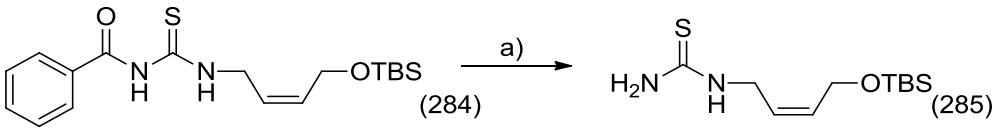
Scheme 95: Attempted preparation of (278), a) PPh_3, phthalimide, THF, DIAD, 0°C, 48 h, b) ethylenediamine, EtOH, Δ. 24 h, c) Acetone, NEt_3, PhCONCS, 24 h.

There were concerns about the hygroscopic nature of amine (277) and that the ethylene diamine was reacting preferentially to our substrate. We thus decided to protect the alcohol as a silyl ether (282) and to switch from ethylene diamine to hydrazine.[113] Thus silylation of phthalimide (276) using TBDMSCl and imidazole gave the silyl ether (282) in a 81% yield. Deprotection of the phthalimide group was realised using hydrazine in refluxing ethanol after which filtration and evaporation gave the crude amine (283). Following this, commercially available benzoyl isothiocyanate was added to the reaction and after work up and chromatography then benzoyl protected thiourea (284) was obtained in a 26% yield over 2 steps. A repeat of this reaction using the benzoyl isothiocyanate generated in situ using the same method as previously employed, but using anhydrous dichloromethane instead of acetone gave (284) in a slightly improved 31% yield.



Scheme 96: new route, a) *t*BDMSCl, dichloromethane, imidazole, DMAP, 0°C-rt, 3d, b) hydrazine hydrate, EtOH, Δ, 24 h, c) Acetone, NEt_3, PhNCS, rt, 24 h, 26%, or d) dichloromethane, NEt_3, PhNCS, rt, 24 h, 31%.

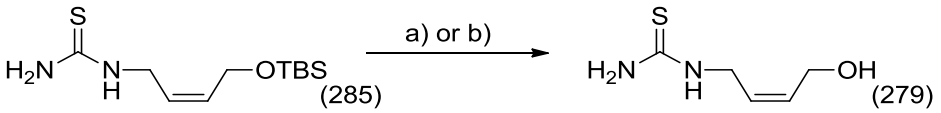
With the benzoyl-thiourea (284) hand the next step in the synthesis was the removal of the benzoyl protecting group to give the thiouurea (285). It was reported in the literature[125] that the benzoyl group could be removed by hydrolysis with sodium hydroxide solutions in methanol for up to 24 h, however yields for (285) could be poor. An alternative method[126] was to use potassium carbonate in methanol which appears to be a milder method. We thus stirred a methanolic solution of (284) with potassium carbonate for 3 h and after work up and chromatography obtained the deprotected thiourea (285) in a 96% yield. The H^1 NMR indicated that the aromatic signals at 7.49 ppm, 7.61 ppm and 7.83 ppm are gone which would indicate that the benzoyl group had been removed successfully.



Scheme 97: Deprotection of (284), MeOH, K_2CO_3, 3 h, rt, 96%.

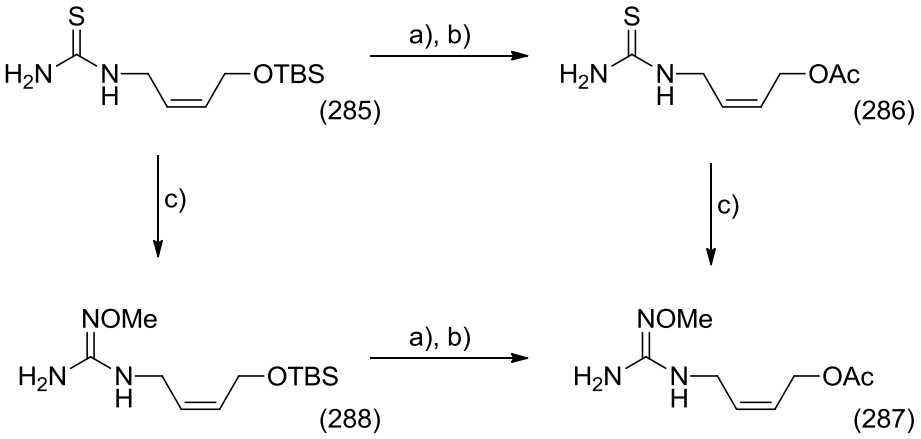
The next step in the synthesis was the removal of the silyl protecting group from (285) to give the alcohol (286). This was attempted using TBAF in THF however this proved problematic. On addition of TBAF the starting material was rapidly consumed and after work-up, NMR spectroscopy did not give any signals that were indicative of the desired product. It was felt that the product might be water soluble and we attempted to re-extract the aqueous phase but no product was obtained. The reaction was repeated under different work up conditions such as non aqueous conditions and again we were unable to identify any likely compound by TLC or by NMR spectroscopy. It was felt that the tetra-*N*-butyl counter ions from the TBAF might be a problem in this reaction as they are polar and might be masking the product, which was not clearly visible on TLC. The removal of the silyl protecting group was then attempted using pyridinium *p*-toluene sulfonate which appeared was promising as a new spot was formed on TLC (50% EA in PE) which after complete consumption of the starting material. The NMR analysis of the crude sample did seem to indicate that the Silyl group had been removed, however on attempted chromatography this spot was lost. The conclusion was that this compound was

highly polar and was retained on the chromatography column even in highly polar solvents such as methanol or that the compound was unstable on silica gel.



Scheme 98: Attempted desilylation of (285), a) TBAF, THF, rt, 24 h, or b) PPTS, MeOH, rt, 24 h.

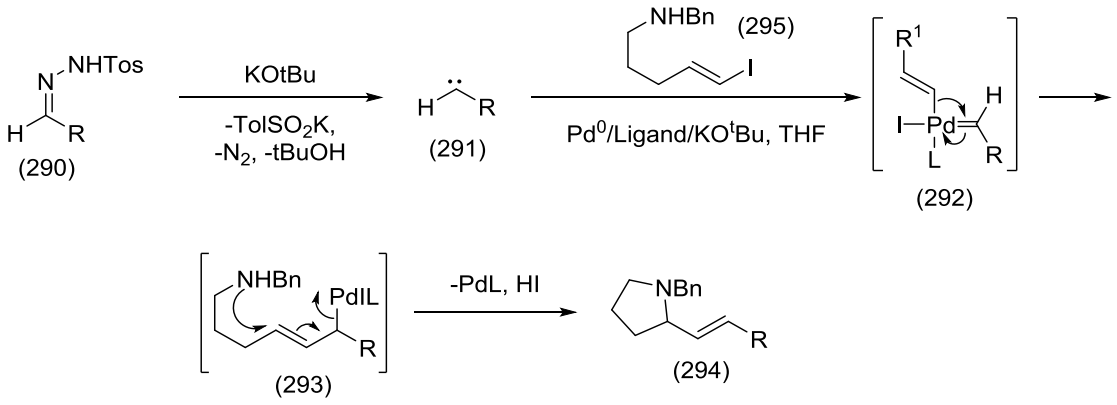
We reappraised the synthetic approach to nitensidine E (234) and felt that a possible solution might be to acetylate the crude product (279) *in situ* to give (286), which could be converted into guanidine (287). Another possibility was to reverse the order of these reactions and to convert the thiourea (285) into the methoxy guanidine (288) which could be hydrolysed to give an alcohol which could be acetylated to give (287).



Scheme 99: Planned deprotection and acetylation of (285), a) TBAF, THF, rt, 24 h, b) Ac_2O, pyr, DMAP, 24 h, c) EDCI, *O*-Methoxyhydroxylamine, DIPEA, dichloromethane, 0°C, 41 h.

Before we were able to embark upon this modified study a report appeared by Vranken *et al.*[127] which reported the first total synthesis of nitensidine E (234) which used a Pd^0 catalysed cyclisation. Vranken and co-workers had developed[128] a novel reaction in which *N*-tosylhydrazones underwent intramolecular carbenylative

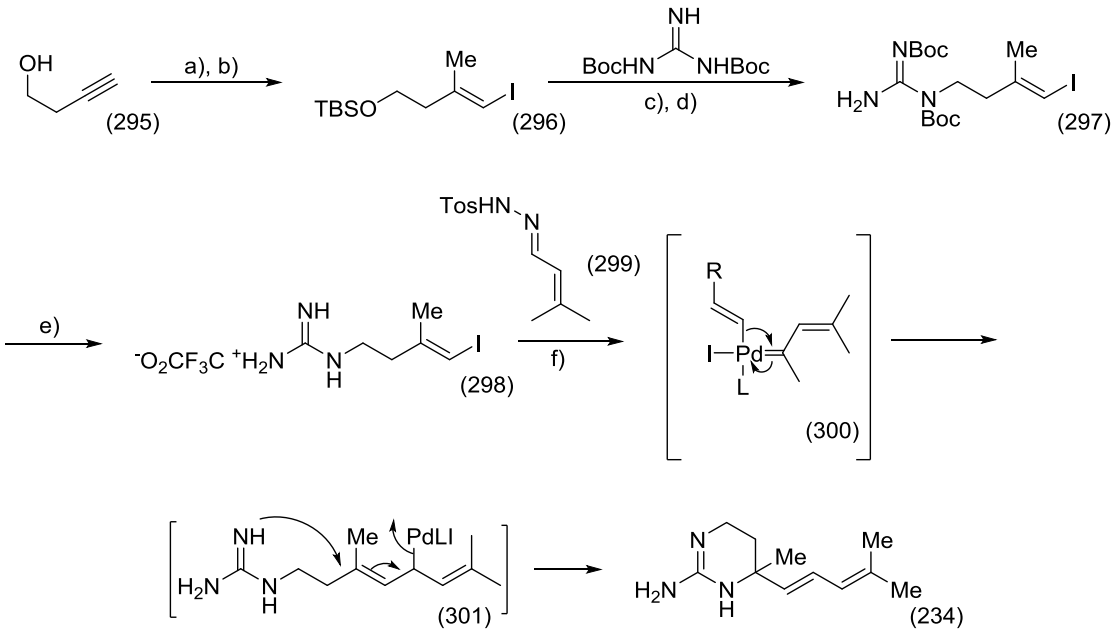
amination reactions to generate pyrrolidines and piperidines (Scheme 100). In this process the *N*-tosylhydrazones (289) are treated with potassium t-butoxide which generates an intermediate carbene (290) which in the presence of Pd^0 and the vinyl iodide (294) forms the intermediate carbene complex (291). This results in an overall carbene insertion to give the allyl intermediate (292) which is subsequently trapped in an intermolecular manner to give the pyrrolidines (293) in good yields.



Scheme 100: Carbenylative amination to generate cyclic amine (294), R = alkyl, aryl; $R^1 = -(CH_2)_3NHBn$.

Vranken *et al.* then applied this methodology to the total synthesis of nitensidine E (234). The required vinyl iodide (294) was prepared from but-3-yn-1-ol, by silylation then a Negishi's methylzirconation/iodination sequence[129] gave the to give the volatile vinyl iodide (294) as a mixture of regioisomers (ca. 9:1 ratio). Deprotection gave the corresponding alcohols which were converted to the Boc-*N*-alkyl-*N*,*N*′-bis-Boc guanidine (295) in 82% yield using a Mitsunobu reaction.[130] The regiochemistry of this reaction was determined from the NH chemical shifts (9.32 and 9.11 ppm) of adduct (295) as the regioisomeric *N*-alkyl-*N*′,*N*″-bis-Boc guanidines typically exhibit very different NH chemical shifts (below 9 ppm and greater than 11 ppm). The Boc groups were next removed with trifluoroacetic acid to afford the *N*-alkylguanidine (296) as a trifluoroacetate salt in 94% yield. This guanidine was subjected to carbenylative amination using *N*-tosylhydrazone (297) which generates intermediate (298) followed by the allylic palladium species (299), which undergoes cyclisation to give a 29% yield of nitensidine E (234) (50% yield by NMR). This reaction was interesting as it was the first example of an un-protected guanidine

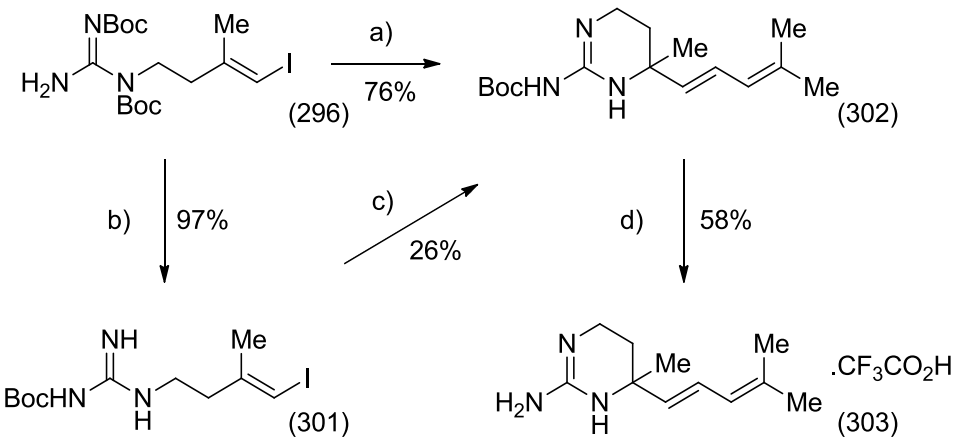
moiety participating in a palladium-catalyzed allylic alkylation, which from our perspective was encouraging as it meant our approach was likely to succeed and was not without merit.



Scheme 101: Vranken's total synthesis of nitensidine E (234), a) TBDMSCl, imidazole, dichloromethane, b) i) Me_3Al, Cp_2ZrCl_2, dichloromethane, 23- 45°C, 17 h, ii) I_2¸ THF, 0°C, 56%, c) TBAF, THF, 23°C, 1 h, d) $HN=C(NHBoc)_2$, Ph_3P, DEAD, PhMe, 23°C, 15 h, 82%, e) CF_3CO_2H, dichloromethane, rt, 14 h, 94%, f) 0.1 eqv $Pd(OAc)_2$, 0.3 eqv PPh_3, 3.0 eqv. *t*BuOLi, THF, 65°C, 6 h, 29%.

The cyclisation of the bis-Boc protected guanidine (296) was also investigated and this cyclisation gave a much higher yield of 76% of the cyclised product *N*-Boc-nitensidine (302) in which it was found that one of the Boc groups was missing. This result correlates well with the work reported by the previous worker in the Murphy group[123] who observed a similar loss of protecting groups during Pd^0. The researchers in this work were unclear as to whether the Boc- group was lost before or after cyclization, but noted was that the same Boc-group was lost if (296) was treated with camphorsulfonic acid to give exclusively (301). Cyclisation of this mono-protected guanidine (301) was however much less successful and only a 26% yield was obtained under the conditions previously employed. This might suggest Boc group is lost post cyclisation and the authors noted that the results did not support the

idea that deprotection of one of the Boc groups facilitates the allylic alkylation step. The mono-protected *N*-Boc-nitensidine (302) could be deprotected using TFA in dichloromethane as its trifluoroacetate salt (303) in 58% yield.



Scheme 102: Further cyclisation reactions: a) 0.1 eqv $Pd(OAc)_2$, 0.3 eqv PPh_3, 5.0 eqv. *t*BuOLi, THF, 65°C, 6 h, 76%, b) CSA, PhMe, 80°C, 97%, c) 0.1 eqv $Pd(OAc)_2$, 0.3 eqv PPh_3, 3.0 eqv. *t*BuOLi, THF, 65°C, 6 h, 26%, d) CF_3CO_2H, dichloromethane, 14 h, rt, 58%.

The publication of the total synthesis of the target molecule (234) was unfortunate, however as no further time was available to investigate this project at this point the investigation was stopped.

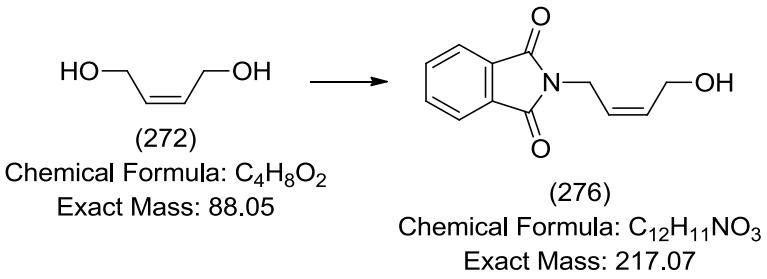
Conclusion

The work of Vranken offers some support to our methodology and indeed the use of a completely unprotected guanidine could be investigated as a model study. From our work it is apparent the initial steps of the reaction were effective methods for forming the desired intermediates, however the latter compounds such as thiourea (286) are highly polar and were not easy to purify from crude reaction mixtures.

Experimental

Column chromatography was carried out on silica gel (60A) and TLCs were conducted on precoated Kieselgel 60 F254 (Art. 5554; Merck) with the eluent specified in each case. All non-aqueous reactions were conducted in oven-dried apparatus under a static atmosphere of argon. Diethyl ether, THF and dichloromethane were dried by a Pure Solv MD-3 solvent purification system. Dry methanol and DMF was purchased from Aldrich. Chemical shifts are reported as δ values relative to chloroform (7.26/77.16 ppm), methanol (3.31/49.0 ppm) and DMSO (2.50/39.52 ppm) as internal standards. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 or 500 spectrometer with an internal deuterium lock at ambient temperature at 400 or 500 MHz with internal references of δ_H 7.26 and δ_C 77.16 ppm for $CDCl_3$, δ_H 3.31 and δ_C 49.0 ppm for CD_3OD and δ_H 2.54 ppm and δ_C 39.52 ppm for DMSO. Mass spectra data were obtained at the EPSRC Mass Spectrometry Service Centre at the University of Wales, Swansea. Low resolution Chemical Ionisation (CI) and Electrospray Ionisation (ESI) mass spectra were recorded on a Micromass Quattro II spectrometer and high resolution mass spectra were recorded on either a Finnigan MAT 900 XLT or a Finnigan MAT 95 XP. Infrared samples were prepared as thin films or solutions using sodium chloride plates or as KBr discs; spectra were recorded on a Bruker Tensor 37 FT-IR. Melting points (Mp.) were determined using a Gallenkamp MF370 instrument and are uncorrected.

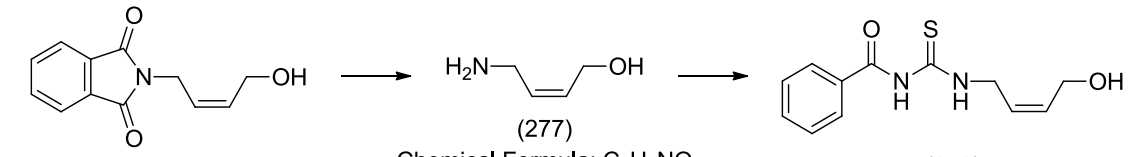
(Z)-2-(4-Hydroxybut-2-en-1-yl)isoindoline-1,3-dione (276)[113]



But-2-en-1,4-diol (272) (10.0 g, 9.35 mL, 114.0 mmol), triphenylphosphine (35.7 g, 136.0 mmol, 1.2 eqv.) and phthalimide (16.7 g, 125.0 mmol, 1.0 eqv.) were dissolved in anhydrous THF (300 mL) and stirred until all the solids had dissolved. The reaction mixture was then cooled (0°C) and DIAD (25.3 g, 24.6 mL, 125.0 mmol, 1.1 eqv.) was added in a dropwise manner over 15 min. After stirring to rt over for 48 h, silica gel (60.0 g) was added and the reaction evaporated to give a free flowing powder. This powder was added to a silica gel column which was eluted with 0-50% EA in PE. Fractions containing the product (eluting in ca. 30% EA in PE) were combined and recrystallised from hot ethyl acetate to give (276) (17.0 g, 78.3 mmol) in a 69% yield as a white crystalline solid. Data was in agreement with the literature.[113]

Rf 0.30 (30% EA in PE); $\boldsymbol{\delta_H}$ 2.31 (1H, br s, OH), 4.38 (2H, dd, *J* 0.8, 7.3 Hz, CH_2), 4.41 (2H. dd, *J* 1.2, 7.7 Hz, CH_2), 5.51-5.60 (1H, m, CH), 5.86-5.94 (1H, m, CH), 7.72-7.74 (2H, m, 2 x CH), 7.84-7.86 (2H, m, 2 x CH); $\boldsymbol{\delta_C}$ 34.6, 58.2, 123.5, 125.1, 132.2, 133.4, 134.3, 168.3; ν_{max} 3427, 3020, 1714.

Attempted synthesis of (Z)-N-((4-hydroxybut-2-en-1-yl)carbamothioyl)benzamide (278) via (Z)-4-Aminobut-2-en-1-ol (277)



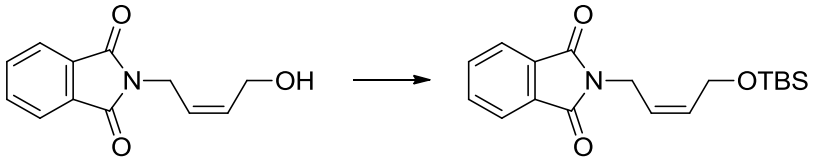
(276)
Chemical Formula: $C_{12}H_{11}NO_3$
Exact Mass: 217.07

(277)
Chemical Formula: C_4H_9NO
Exact Mass: 87.07

(278)
Chemical Formula: $C_{12}H_{14}N_2O_2S$
Exact Mass: 250.08

The alcohol (276) (2.0 g, 9.2 mmol) was dissolved in absolute ethanol (50 mL) and stirred for 5 min. Ethylene diamine (0.6 g, 0.7 mL, 10.2 mmol, 1.1 eqv.) was added and the solution refluxed for 24 h whereupon a solid precipitate formed. The reaction mixture was then filtered through Celite© and the filter pad washed with ethanol (50 mL). The solution was evaporated to give the crude amine (277) (1.3 g). Benzoyl chloride (2.6 g, 2.2 mL, 18.4 mmol, 2.0 eqv.) was added in a dropwise manner to a stirred solution of ammonium thiocyanate (1.5 g, 19.3 mmol, 2.1 eqv.) dissolved in dry acetone (50 mL) at rt, The solution was then refluxed for 20 min and then cooled (0°C) and was then added by cannula to a stirred a solution of the amine (277) (1.3 g, 14.9 mmol) and triethylamine (3.8 g, 5.2 mL, 36.8 mmol, 4.0 eqv.) dissolved in dry acetone (50 mL). After stirring to rt over 24 h, the reaction was poured onto ice (500 g), allowed to melt and then the aqueous phase extracted with dichloromethane (3 x 100 mL) the combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give a crude product (4.2 g) as a dark brown oil. Attempted purification failed to yield the desired product.

(Z)-2-(4-((tert-butyldimethylsilyl)oxy)but-2-en-1-yl)isoindoline -1,3-dione (282)



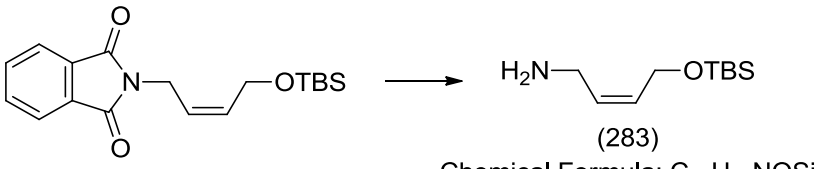
(276)
Chemical Formula: $C_{12}H_{11}NO_3$
Exact Mass: 217.07

(282)
Chemical Formula: $C_{18}H_{25}NO_3Si$
Exact Mass: 331.16

TBDMSCl (3.8 g, 25.0 mmol, 1.1 eqv.) was added in small portions over 10 min to a cooled to (0°C) solution of the alcohol (276) (5.0 g, 23.0 mmol), imidazole (3.1 g, 46.0 mmol, 2.0 eqv.) and DMAP (ca 100 mg) dissolved in dichloromethane (100 mL). After stirring to rt over 3 d, the reaction was washed with $NaHCO_3$ (aq., 2 x 100 mL), brine (2 x 100 mL) and the organic layer dried ($MgSO_4$). After evaporation under reduced pressure, the crude product was purified by column chromatography (10% EA in PE) to give (282) (5.9 g, 18.7 mmol) as a pale yellow oil in 81% yield.

Rf 0.59 (30% EA in PE, PMA); $\boldsymbol{\delta_H}$ 0.10 (6H, br s, 2 x Me), 0.90 (9H, br s, 3 x Me), 4.31 (2H, br d, *J* 7.0 Hz, CH_2), 4.44 (2H, dd, *J* 1.6, 6.0 Hz, CH_2), 5.45-5.54 (1H, m, CH), 5.67-5.75 (1H, m, CH), 7.66-7.71 (2H, m, 2 x CH), 7.80-7.84 (2H, m, 2 x CH); $\boldsymbol{\delta_C}$ -5.1, 26.1, 35.1, 59.5, 123.3, 123.7, 132.3, 134.0, 134.1, 167.9; ν_{max} 3375, 2960, 2919, 2850, 1770, 1716, 1428, 1088, 838, 779; **HRMS** (CI) found 332.1673, $C_{18}H_{25}NO_3Si$ ($[M+H]^+$) requires 332.1676.

(Z)-4-((tert-butyldimethylsilyl)oxy)but-2-en-1-amine (283)



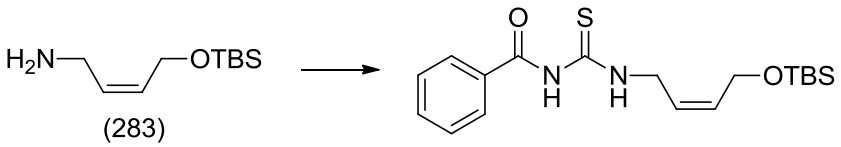
(282)
Chemical Formula: $C_{18}H_{25}NO_3Si$
Exact Mass: 331.16

(283)
Chemical Formula: $C_{10}H_{23}NOSi$
Exact Mass: 201.15

The silyl ether (282) (1.0 g, 3.2 mmol,) was dissolved in absolute ethanol (50 mL) and hydrazine hydrate (0.6 g, 0.6 mL, 12.7 mmol, 4.0 eqv.) was added and the solution was stirred for 24 h. A white precipitate was formed which was removed by filtration through a sintered funnel using a vacuum, the filter pad was washed with ethanol (2 x 10 mL) and the reaction mixture was then evaporated under reduced pressure. The product was re-dissolved in absolute ethanol (100 mL) and then the solution was again evaporated to dryness. This process was repeated a further 2 times then dichloromethane (80 mL) was added to the crude product and the mixture stirred for 5 min. The mixture was then dried using magnesium sulfate and filtered to remove insoluble impurities. Evaporation under reduced pressure gave the crude amine (283) (1.0 g) which was used in the next stage without further purification.

δ_H 0.07 (6H, br s, Me), 0.90 (9H, br s, 3 x Me), 1.54 (2H, br s, NH) 3.25-3.34 (2H, m, CH_2), 4.16-4.24 (2H, m, CH_2), 5.48-5.67 (2H, m, 2 x CH).

(Z)-N-((4-((tert-Butyldimethylsilyl)oxy)but-2-en-1-yl)carbamothioyl)benzamide (284)



(283)
Chemical Formula: $C_{10}H_{23}NOSi$
Exact Mass: 201.15

(284)
Chemical Formula: $C_{18}H_{28}N_2O_2SSi$
Exact Mass: 364.16

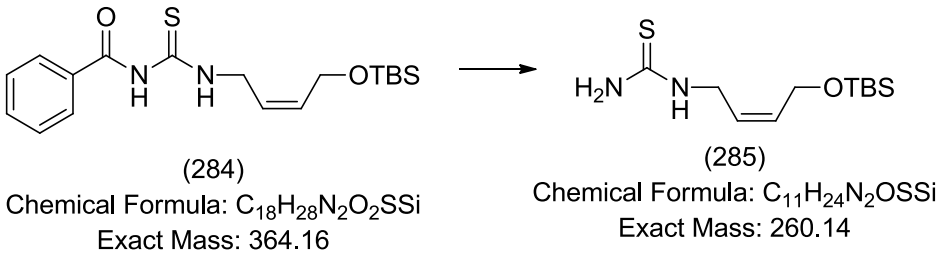
Using commercial Benzoyl isothiocyanate. Benzoyl isothiocyanate (1.0 g, 0.9 mL, 6.3 mmol, 2.0 eqv), was added in a dropwise manner to a cooled (0°C) solution of the amine (283) (0.6g, 3.2mmol) and triethylamine (0.5 g, 0.6 mL, 4.6 mmol, 1.5 eqv.) dissolved in dichloromethane (50 mL). After 24 h, the reaction mixture was poured onto ice (250 g) and once melted the aqueous layers was separated and extracted with dichloromethane (3 x 100 mL). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated under reduced pressure to give the crude product as a dark orange oil. Purification by column chromatography (15 % EA in PE) gave (284) (304.0 mg, 0.8 mmol) as orange viscous oil in a 26% yield.

Using prepared Benzoyl isothiocyanate. Ammonium thiocyanate (4.8 g, 62.6 mmol, 2.1 eqv.) was suspended in anhydrous dichloromethane (200 mL) at rt and benzoyl chloride (8.4 g, 7.0 mL, 60.0 mmol, 2.0 eqv.) was added in a dropwise manners over 10 min. The mixture was then refluxed for 30 min, cooled to rt and filtered through a pad of Celite© to give an orange solution of benzoyl isothiocyanate which was added via cannula to a cooled (0°C) solution of the amine (283) (6.0g, 29.8 mmol, prepared using the hydrazine method using (282) (10.0 g, 31.7 mmol)) and triethylamine (4.5 g, 6.2 mL, 44.7 mmol, 1.5 eqv.) in anhydrous dichloromethane (200 mL). After stirring to rt over 24 h, the reaction mixture was poured onto ice (500 g) and once melted the aqueous layers was separated and extracted with dichloromethane (3 x 100 mL). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated under reduced pressure to give the crude product as a dark orange oil. Purification by column chromatography (15% EA in PE) gave (284) (3.4 g, 9.3 mmol) as an orange viscous oil in a 31% yield.

Rf 0.35 (15% EA in PE); $\boldsymbol{\delta_H}$ 0.09 (6H, br s, 2 x Me), 0.91 (9H, br s, 3 x Me), 4.33 (2H, d, *J* 5.8Hz, CH_2), 4.38 (2H, br t, *J* 6.1 Hz, CH_2), 5.57-5.64 (1H, m, CH),

5.74-5.81 (1H, m, CH), 7.51 (2H, br t, *J* 8.4 Hz, 2 x CH), 7.62 (1H, br t, *J* 7.5 Hz, 2 x CH) 7.82 (2H, d, *J* 8.8 Hz, CH), 9.02 (1H, br s, NH), 10.7 (1H, br s, NH); $\boldsymbol{\delta_C}$ -5.1, 26.1, 43.3, 59.7, 62.6, 123.9, 127.5, 129.3, 131.9, 133.7, 134.3, 166.9, 179.9; ν_{max} 3250, 2927, 2135, 1716, 1656, 1621, 806, 750; **HRMS** (CI) found 365.1732, $C_{18}H_{29}N_2O_2SSi$ ($[M+H]^+$) requires 365.1734.

(Z)-1-(4-Hydroxybut-2-en-1-yl)thiourea (285)



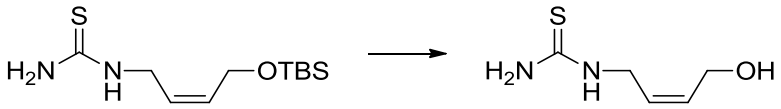
(284)
Chemical Formula: $C_{18}H_{28}N_2O_2SSi$
Exact Mass: 364.16

(285)
Chemical Formula: $C_{11}H_{24}N_2OSSi$
Exact Mass: 260.14

Anhydrous potassium carbonate (70.0 mg, 0.5 mmol, 1.9 eqv.) was added to a solution of thiourea (284) (100.0 mg, 0.27 mmol) dissolved in methanol (1.0 mL) and the mixture stirred for 3 h. Excess solvent was removed under reduced pressure and the residue dissolved in chloroform (30 mL) and the solution washed with water (30 mL) and brine (30 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated under reduced pressure to give (285) (63.9 mg, 0.25 mmol) in 91% yield as a waxy solid.

Rf 0.35 (80% EA in PE, PMA); $\boldsymbol{\delta_H}$ 0.10 (6H, br s, 2 x Me), 0.91 (9H, br s, 3 x Me), 3.89-4.09 (2H, br m, CH_2), 4.27 (2H, br d, *J* 5.9 Hz, CH_2), 5.46-5.57 (1H, m, CH), 5.71-5.87 (1H, m, CH), 6.40 (2H, br s, NH_2), 6.72 (1H, br s, NH); $\boldsymbol{\delta_C}$ -5.2, 21.1, 26.1, 59.6, 60.4, 175.8; ν_{max} 3295, 2980, 2166, 1753, 1585, 832, 812; **HRMS** (CI) found 261.1543, $C_{11}H_{25}N_2OSSi$ ($[M+H]^+$) requires 261.1547.

Attempted preparation of (Z)-1-(4-Hydroxybut-2-en-1-yl)thiourea (286)



(285)
Chemical Formula: $C_{11}H_{24}N_2OSSi$
Exact Mass: 260.14

(286)
Chemical Formula: $C_5H_{10}N_2OS$
Exact Mass: 146.05

With TBAF: TBAF (0.27 mL, 0.27 mmol, 1.1 eqv.) was added to a solution of thiourea (285) (63.9 mg, 2.45 mmol) dissolved in anhydrous THF (2.5 mL). After 24 h, ammonium chloride (30 mL) was added and the layers were then separated. The aqueous phase was extracted with further dichloromethane (2 x 30 mL) and the combine organic layers, dried ($MgSO_4$) and evaporated under reduced pressure to give a crude product (72.3 mg). Analysis by NMR failed to indeicate the presence of (286), and re-extraction of the organic layer with ethyl acetate gave no significant further material.

With PPTS: Thiourea (285) (1.13 g, 4.35 mmol) was dissolved in methanol (30 mL) and PPTS (0.31 mg, 1.30 mmol, 0.3 eqv.) was added and the solution was refluxed over 24 h. Excess solvent was then removed and the resultant residue was then dissolved in chloroform (50 mL) and washed with brine (50 mL) and water (50 mL). The organic layer was then dried ($MgSO_4$) and evaporated under reduced pressure. No material was obtained on evaporation and re-extraction of the aqueous washes failed to give significant amounts of organic material.

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