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Synthetic steps towards the cylindrospermopsin alkaloids

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Synthetic Steps Towards the Cylindrospermopsin Alkaloids

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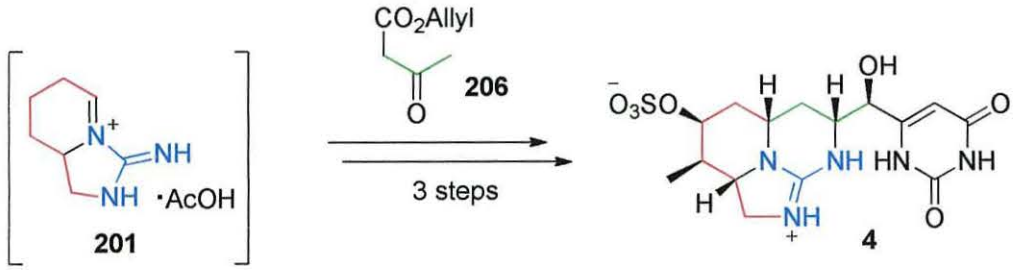
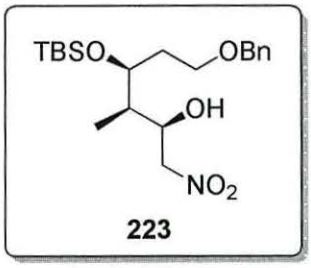
Abstract

Detailed herein is the tethered Biginelli condensation between iminium ion **201** and β-keto ester **206** leading to a model tricyclic ring system representative of the guanidinium core of cylindrospermopsin alkaloids. This was achieved in a biosynthetically-inspired manner in 12 steps and 8.3% overall yield from simple, commercially available 1,5-pentanediol **172**. Also discussed is the adaption of this methodology to allow for the highly efficient stereoselective synthesis of all 3 of the cylindrospermopsin alkaloids and the preparation of the advanced synthetic intermediate nitro-alcohol **223**.





Abbreviations

General

ACP	Acyl carrier protein
conc.	Concentrated
DNA	Deoxyribonucleic acid
HPLC	High Performance Liquid Chromatography
HSQC	Heteronuclear Single Quantum Coherence
HWE	Horner-Wadsworth-Emmons
MS	Molecular sieves
NMR	Nuclear Magnetic Resonance
nOe	Nuclear Overhauser effect
NOESY	Nuclear Overhauser Effect Spectroscopy
ORTEP	Oak Ridge Thermal Ellipsoid Plot Program
rt	Room temperature
TLC	Thin Layer Chromatography

Reagents

Boc_2O	Di-*tert*-butyl dicarbonate
BOMCl	Benzyl chloromethyl ether
CbzCl	Benzyl chloroformate
CDI	Carbonyldiimidazole
CSA	Camphorsulfonic acid
DEAD	Diethyl azodicarboxylate
DIAD	Diisopropyl azodicarboxylate
DIBAL	Diisobutylaluminium hydride
DIPA	Diisopropylamine
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMDO	Dimethyldioxirane
DMP	Dess-Martin periodinane

DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DMS	Dimethyl sulfide
DMSO	Dimethyl sulfoxide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
LDA	Lithium diisopropylamide
m-CPBA	*m*-Chloroperoxybenzoic acid
(+)-MeOB(Ipc)$_2$	(+)-*B*-methoxy *bis*(isopinocampheyl)borane
MeOTf	Methyl trifluoromethansulfonate
MOMCl	Methyl chloromethyl ether
Ms_2O	Methanesulfonic anhydride
NaHMDS	Sodium *bis*(trimethylsilyl)amide
NMO	*N*-Methylmorpholine-*N*-Oxide
PIDA	Phenyliodonium diacetate
PMBCl	*p*-Methoxybenzyl chloride
p-NBA	*p*-Nitro Benzoic Acid
p-TsOH	p-Toluenesulfonic acid
(Sia)$_2$BH	*Bis*(1,2-dimethylpropyl)borane)
TBAF	Tetra-*n*-butylammonium fluoride
TBAI	Tetrabutylammonium iodide
TBD	Triazabicyclodecene
TBSCl	*t*-Butyldimethylsilyl chloride
TBSOTf	*t*-Butyldimethylsilyl trifluoromethanesulfonate
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TESOTf	Triethylsilyl trifluoromethanesulfonate
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
Tf_2O	Trifluoromethanesulfonic anhydride
TMSCl	Trimethylsilyl chloride
TMSI	Trimethylsilyl iodide
TPAP	Tetrapropylammonium perruthenate
TrCl	Triphenylmethyl chloride
TrocCl	2,2,2-Trichloroethyl chloroformate

Functional Groups

Ac	Acetyl
Bn	Benzyl
Boc	*t*-Butoxycarbonyl
Cbz	Carbobenzyloxy
Et	Ethyl
i-Bu	*i*-Butyl
i-Pr	*i*-Propyl
Me	Methyl
MOM	Methoxymethyl
Ms	Mesyl
PBB	*p*-Bromobenzyl
Ph	Phenyl
PMB	*p*-Methoxybenzyl
PNB	*p*-Nitro Benzyl
TBS	*t*-Butyldimethylsilyl
TES	Triethylsilyl
Tf	Triflyl
TMS	Trimethylsilyl
Tr	Trityl
Troc	2,2,2-Trichloroethyl

Solvents

DCM	Dichloromethane
DMF	Dimethylformamide
THF	Tetrahydrofuran
EtOAc	Ethyl acetate

Introduction

Guanidine is one of the most basic neutral nitrogen compounds known; having a pK_a of 13.6 in water.[1] This high level of basicity results from the extensive resonance stabilisation of the guanidinium cation (Figure 1).[2]

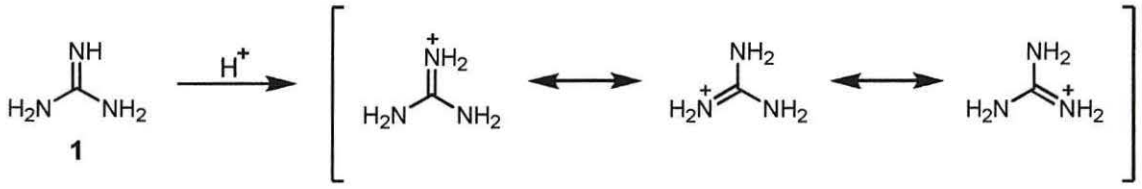


Figure 1:- The three resonance forms of the guanidinium ion.

The guanidine functional group is prevalent throughout nature being present in potent toxins produced by various species of the animal kingdom,[3,4] as well as the active sites of enzymes[5] and in the human body in the form of arginine **2** and one of its important derivatives creatine **3** (Figure 2).[6] The guanidinium ion has six potential hydrogen bond donor sites allowing the unit to engage in patterns of hydrogen bonding that are rare if not unique.[7,8] This may account for the evolutionary incorporation of arginine into the twenty proteinogenic amino acids.

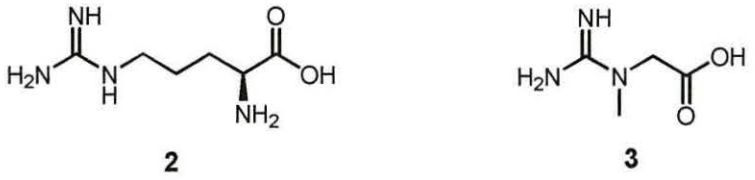


Figure 2:- Important guanidine species present in the human body.

Several natural and synthetic guanidine derivatives have generated great interest primarily as a result of their pronounced biological activities.[9] However guanidine derivatives have also been shown to have applications in other fields such as catalysis,[10,11] peptidomimetics[12] and adhesives[13] as well as antifouling agents[14] and pharmaceuticals.[15]

The Murphy Group has a long standing interest in guanidine containing natural products[16-23] and their analogues,[24] of particular interest is the marine natural product

cylindrospermopsin **4** and the related metabolites 7-*epi*-cylindrospermopsin **5** and 7-*deoxy*-cylindrospermopsin **6** (Figure 3).[25-28] The unique structural features of these compounds, comprising of a sulfonated tricyclic guanidine moiety joined to a uracil ring system combined with their potent biological activity have made them a synthetic target of considerable interest.

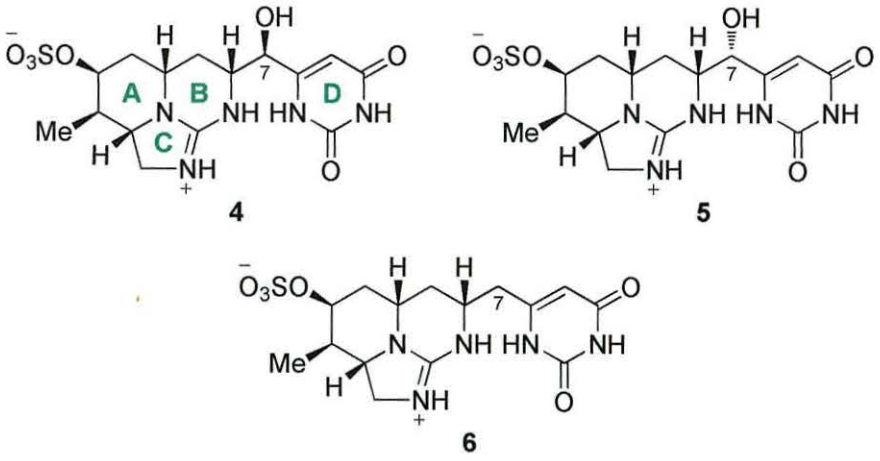


Figure 3:- The cylindrospermopsin alkaloids.

Isolation and Characterisation

Cylindrospermopsin was initially isolated from the cyanobacterium *Cylindrospermopsis raciborskii* and characterised by Moore and co-workers in 1992 using mass spectrometry and a combination of 1D and 2D NMR techniques.[29] It was this toxin that was retrospectively identified as the causative agent of an outbreak of hepatoenteritis on Palm Island Australia 13 years earlier; the outbreak affected 148 inhabitants of the island with the large majority of those being children requiring hospital care.[30] The outbreak occurred a few days after the treatment of a dense algal bloom with copper sulfate on the town's water supply Solomon dam. It is believed that the copper sulfate treatment commonly used to treat nuisance algal blooms caused the lysis of the cyanobacterial cells and the release of the toxin into the drinking water supply, although conjecture was raised that the illness the towns' folk were suffering from was in fact acute copper sulfate poisoning.[31]

An intense interest surrounding this newly characterised toxin led to the discovery and isolation of 7-*deoxy*-cylindrospermopsin which had been routinely observed by

HPLC during the isolation of cylindrospermopsin from *C. raciborskii* but had not been isolated and characterised until 1999.[32] The group who isolated this metabolite believed that it exhibited the tautomeric forms **7** and **8** as the vinylic proton of the uracil ring was not observed in the ^{1}H NMR spectrum of the isolated material (Figure 4). However the absorbance maximum (λ_{max}) of **7** was consistent with the presence of a uracil group and through total synthesis it is most likely represented by structure **6** (Figure 3). Although it was clear that the natural material was a mixture of components, it was not possible to ascertain whether **6** was a minor component of that mixture.[33]

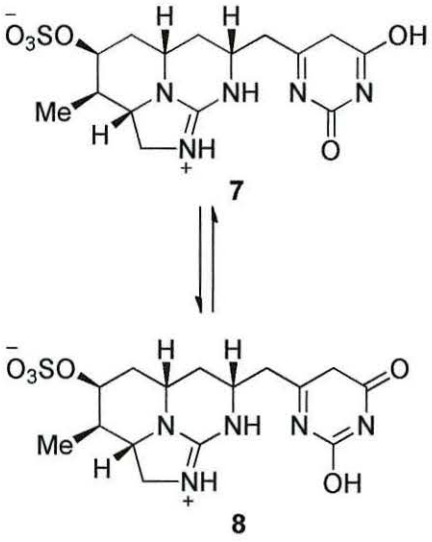


Figure 4:- Proposed tautomeric forms of 7-*deoxy*-cylindrospermopsin.

The C-7 epimer of cylindrospermopsin, 7-*epi*-cylindrospermopsin was initially found to be produced by a different species of cyanobacterium, *Aphanizomenon ovalisporum* in Israel in the year 2000.[34] The initial assignment of the relative stereochemistry of cylindrospermopsin with particular regard to the C-7 hydroxyl group was made on the basis that the uracil D ring existed as an unusual enol tautomer which was intramolecularly hydrogen bonded to a nitrogen terminus of the guanidine moiety as shown in structure **9**. In this configuration the molecule satisfied the NMR evidence obtained by the group leading them to propose structure **9** as the most likely representation of cylindrospermopsin (Figure 5). This in turn led to the belief that the C-7 epimer 7-*epi*-cylindrospermopsin displayed a conformation similar to **9** and thus was assigned the structure **10** (Figure 5). However, upon stereoselective total synthesis it became clear that the stereochemical nature of the C-7 hydroxyl group was in fact

reversed,[35] nullifying the theory that these alkaloids existed as uracil tautomers and establishing with certainty that cylindrospermopsin was accurately represented by **4** and its epimer by structure **5** (Figure 3).

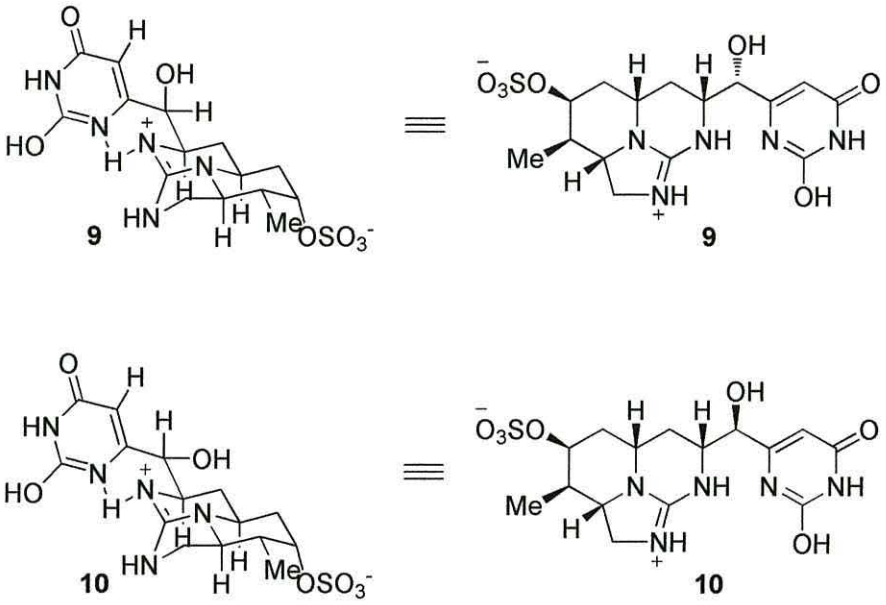


Figure 5:- Proposed tautomeric structures of cylindrospermopsin and 7-*epi*-cylindrospermopsin.

Occurrence and Production of Cylindrospermopsin

Since the initial isolation of cylindrospermopsin from *C. raciborskii* in 1992 an ever evolving collection of cyanobacterial species have been found to produce the toxin throughout geographically diverse locations encompassing all 5 continents of the globe (Figure 6).[29,32,36-58] Cylindrospermopsin has been detected in water bodies at concentrations ranging from 0.01 to 126 μg L^{-1} and the biosynthetic intermediate 7-*deoxy*-cylindrospermopsin has been observed at concentrations of 42 μg L^{-1}.[38,46,56] Data relating to the concentration of 7-*epi*-cylindrospermopsin in water bodies has not been reported, however, laboratory cultures of *Oscillatoria sp.* have produced the metabolite in concentrations up to 690 μg L^{-1}.[59]

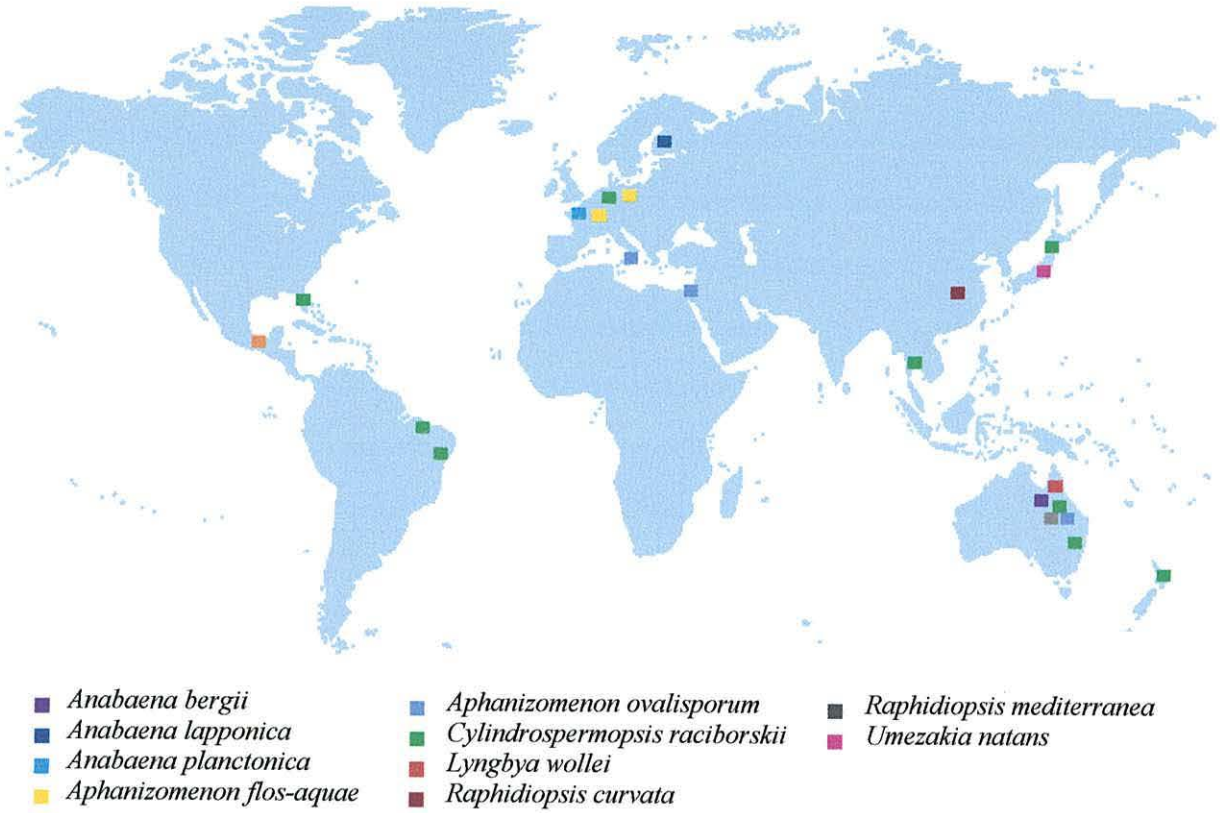


Figure 6:- Geographic distribution of confirmed cylindrospermopsin producers.

Detection

Cylindrospermopsin and cyanobacteria capable of producing the toxin are a prevalent problem throughout the world, one which is likely to be intensified by the effects of climate change.[60] This makes the detection and removal of the toxin as well as the producing species of cyanobacteria of paramount importance.

The presence of cylindrospermopsin and related metabolites can readily be confirmed chromatographically through the use of HPLC in conjunction with several different detectors. One of the first methods employed in the HPLC detection of the toxin was a photodiode array detector (HPLC-PDA) which proved successful in identifying cylindrospermopsin from lyophilised cyanobacterial cells[61] and confirming the presence of the toxin in purified samples.[50] However, results from the analysis of environmental samples have been shown to suffer from considerable matrix background effects with peaks eluting close to cylindrospermopsin and in some cases completely masking the signal of the toxin.[62]

Both HPLC and hydrophilic interaction liquid chromatography (HILIC) have been used in conjunction with single stage mass spectrometric detectors to confirm the presence of the toxin, with the latter being capable of detecting cylindrospermopsin in environmental samples containing other cyanobacterial toxins, without requiring a clean up or preconcentration step.[63,64] The use HPLC tandem mass spectrometry (HPLC-MS^2) has allowed for the accurate and reproducible detection of cylindrospermopsin in surface water samples at concentrations as low as 1 μg L^{-1},[65] this level of sensitivity has been further improved by the use of the mass filtering capacity to reduce background noise allowing for detection at concentrations as low as 0.5 μg L^{-1} in directly injected aqueous samples.[66] The further addition of ion trap analysis has resulted in the lowest published limits of quantification of 0.10 ng mL^{-1} and 1.0 ng g^{-1} for detecting the toxin in surface water and fish muscle respectively, which is hoped, will prove useful in monitoring early stages of contamination and bioaccumulation.[67]

Due to the worldwide problem cylindrospermopsin exposure poses, alternative assays for the toxins detection avoiding the use of expensive HPLC equipment have been investigated. These include commercially available test kits based on the rabbit reticulocyte translation system, the brine shrimp (*Artemia salena*) and the larvae of the beavertail fairy shrimp (*Thamnocephalus platyurus*) which have all demonstrated an ability to quickly and cost effectively detect cylindrospermopsin with comparable sensitivity to HPLC analysis.[68-70] Similar sensitivity has also been reported using cell substrate impedance sensing (ECIS) in conjunction with the *Chinese hamster ovary* (CHO) cell line were an $ECIS_{50}$ of ~2 μg mL^{-1} was observed after 20h.[71] Currently the accepted alternative to HPLC detection is the enzyme linked immunosorbent assay (ELISA), of which commercially produced test kits are available and have demonstrated good qualitative agreement with conventional HPLC methods.[56] Recently a real-time PCR assay has also been employed to rapidly detect cylindrospermopsin producing cyanobacterial species both in the lab and in the field.[72,73]

Removal

There are several routes of exposure to cyanobacterial toxins such as cylindrospermopsin[74,75] with the main being consumption of tainted drinking water.[76] Traditional methods of water treatment such as flocculation, sedimentation and filtration

have been shown to be successful in the removal of cyanobacterial cells but ineffective in the removal of dissolved cyanobacterial toxins,[77-79] and may even facilitate the release of intracellular material leading to higher observed toxin concentrations.[79,80]

The concentration of cylindrospermopsin in water bodies varies throughout the world with reported toxin concentrations ranging from 0.01 to 126 μg L^{-1}.[46,56] Recognising the threat this metabolite poses to human safety some countries already employ guideline values for its presence in drinking water, these include Brazil (15 μg L^{-1}), New Zealand (2.0 μg L^{-1}) and the European Union (0.1 μg L^{-1}).[81] However, Research to determine a definitive safe water quality concentration is on-going with a practical guideline value of 1.0 μg L^{-1} recently being proposed.[82]

The addition of free chlorine (OCl^-) in the form of sodium hypochlorite (2 mg L^{-1}) has demonstrated success in the removal of pure cylindrospermopsin. However greater concentrations of chlorine (4 mg L^{-1}) are required for the removal of the toxin in the presence of its cell free extract as well as the inactivation of the producing species of cyanobacteria.[83,84] The effectiveness of weaker chlorinated oxidants such as chlorine dioxide (ClO_2) and monochloramine (NH_2Cl) have also been investigated,[84-87] but have been shown to be ineffective in the removal of cylindrospermopsin and display only a limited ability to inactivate *C. raciborskii*.[84,86] Currently two by-products of cylindrospermopsin chlorination have been characterised, namely 5-*chloro*-cylindrospermopsin **11** and cylindrospermic acid **12** (Figure 7) both of which have been found to be virtually non-toxic by mouse bioassay (10 mg kg^{-1}) with no toxic symptoms being observed within ten days of treatment.[87]

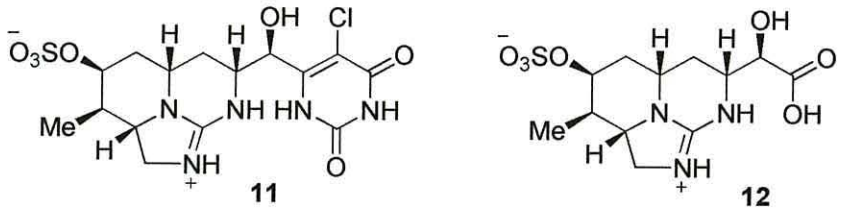
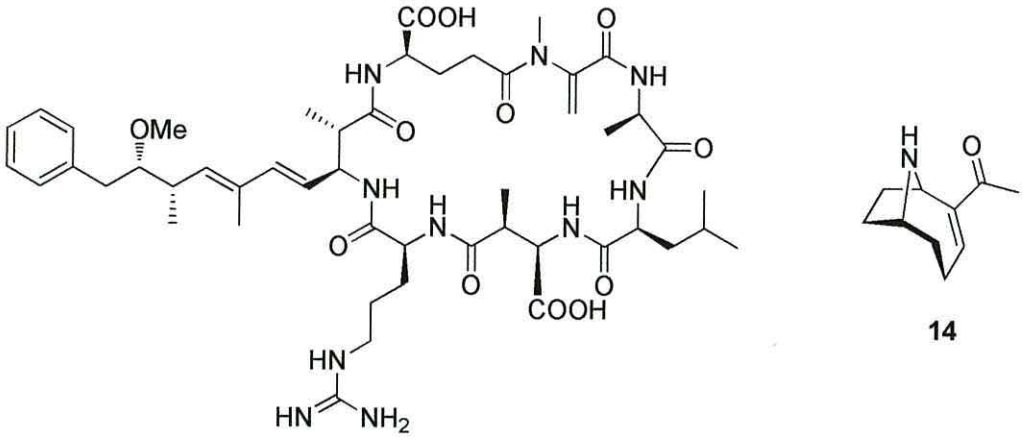


Figure 7:- Characterised cylindrospermopsin chlorination by-products 5-*chloro*-cylindrospermopsin and cylindrospermic acid.

Ozonolysis has also been shown to be an effective process for the removal of cylindrospermopsin which displays a half-life of 0.1 second when treated with the oxidant (1 mg L^{-1}).[85] Treatment with ozone has also been shown to cause complete inactivation of *C. raciborskii*, combined with no observable build-up of the toxin in solution, either as it is not released from the cyanobacterial cells or that it is almost instantly oxidised. Therefore, treatment with ozone poses a highly effective method for the removal of cylindrospermopsin and the disinfection of the producing cyanobacterial species.[84,88]

Permanganate (MnO_4^-) is used as an alternative oxidant in the drinking water treatment process, but this far its ability to remove cylindrospermopsin has not been as rigorously investigated as that of chlorine or ozone.[84-86] A dose of 1.5 mg L^{-1} of MnO_4^- has been shown to be effective in removing only 10% of the toxin while this level of dosing is capable of fully removing other cyanobacterial toxins such as microcystin-LR **13** and anatoxin-a **14** (Figure 8) suggesting that permanganate is ineffective for the removal of cylindrospermopsin in the drinking water treatment process.[85]



13 **14**

Figure 8:- Cyanobacterial toxins microcystin-LR and anatoxin-a.

As a green alternative to chemical treatments the photodegradation of cylindrospermopsin has also been investigated. At low concentrations (4 mg L^{-1}) the toxin has been shown to degrade rapidly having a half-life of only 1.5 hours,[89] a phenomenon that has been shown to be highly dependent on UV-A radiation (320-400 nm).[90] UV light is commonly used in the water treatment process and has been shown to degrade

cylindrospermopsin and the producing species of cyanobacteria, unfortunately the levels of UV radiation required were much greater than those currently used in water treatment disinfection.[84] Titanium dioxide (TiO_2) has shown promise as an effective photocatalyst for the degradation of cylindrospermopsin with UV light, in the presence of 0.1 g L^{-1} TiO_2 the half-life of the toxin has been shown to be only be 0.7 minutes.[91]

Significant interest has also been expressed towards the exploitation of microorganisms as an environmentally friendly technique for the removal of cylindrospermopsin from drinking water supplies.[92-94] The large ciliate *Paramecium caudatum* has shown promise as a grazer of toxin producing *C. raciborskii* being capable of consuming 100 cyanobacterial cells $animal^{-1}$ $hour^{-1}$ which was observed in conjunction with a reduction in toxin concentration.[94] Several strains of probiotic bacteria have also shown promise in this area with the most efficient species; *Bifidobacterium longum* 46, being capable of removing 31.6% of the toxin over a 24 hour period.[95]

Nanofiltration membranes have also shown promise in treating drinking water containing a variety of cyanobacterial toxins including cylindrospermopsin.[96-99] The ability of several different molecular weight cut off (MWCO) membranes to remove cylindrospermopsin from both ultrapure and conventionally treated water have been investigated.[98,99] It was found that membranes having a MWCO of 300 Da or less were effective in removing between 90% and 100% of the toxin from both water sources, demonstrating the feasibility of this approach for water treatment.[99]

Another technique that has been investigated for its ability to safely remove cylindrospermopsin from drinking water is adsorption.[100-104] The first suitable medium to be investigated was powdered activated carbon (PAC) which was capable of reducing the concentration of cylindrospermopsin to below 1 μg L^{-1} at doses ranging from 25 mg L^{-1} to 50 mg L^{-1}, the efficiency of this process was shown to largely be dependent on the dissolved organic carbon (DOC) content of the feed water used.[100] Another adsorbent of interest is sediments, one of the most important parameters of a suitable sediment for the adsorption of cylindrospermopsin is the organic carbon content as sediments with the highest organic carbon content (44.5% organic carbon) demonstrated the greatest adsorption of the toxin (0-360.5 μg kg^{-1}).[101] Once adsorbed the most efficient degradation

of cylindrospermopsin was observed in 4.7 days with the time required rising to 8 days when a feed water with an increased DOC content was used.[102]

Toxicity

Cylindrospermopsin is a potent hepatotoxin and has been implicated in the death of livestock[105,106] as well as a 1979 outbreak of hepatoenteritis on Palm Island Australia which led to its discovery.[29,30] The toxic modes of action of cylindrospermopsin are not fully understood, however the metabolite has been shown to affect several organ systems, primarily the liver but also the kidneys, thymus, heart and spleen[107-109] and as a result is under continuing research due to the public health implications. The potency of the toxin has been quantified, with several LD_{50} values being reported from varying toxin isolates and exposure routes in mouse models (Table 1).

Metabolite	LD_{50}	Time	Dosing method	Ref
4	64 mg kg^{-1}[a]	24 h	IP	57
4	116 mg kg^{-1}[a]	24 h	IP	30
4	50-110 mg kg^{-1}[a] 20-65 mg kg^{-1}[a]	24 h 7 days	IP	129
4	52 mg kg^{-1}[a] 32 mg kg^{-1}[a]	24 h 7 days	IP	15
4	4.4-6.9 mg kg^{-1}[a]	2-6 days	Oral	56
5	200 μg kg^{-1}[b]	5 days	IP	100
6	>800 μg kg^{-1}[b]	5 days[c]	IP	6
11	>10000 μg kg^{-1}[b]	5 days[c]	IP	100
12	>10000 μg kg^{-1}[b]	5 days[c]	IP	100

[a]Cell free algal extract [b]Purified material (HPLC) [c]No toxicity observed.

Table 1:- Reported half lethal doses for cylindrospermopsin and related metabolites.

The cytotoxic effects of cylindrospermopsin have been shown to be mediated through the toxins metabolism by cytochrome P450 enzymes (CYP450) in both animal and cell line models.[110-113] Mice treated with the CYP450 inhibitor piperonyl butoxide prior to the administration of a 200 μg kg^{-1} dose of cylindrospermopsin displayed an increase in the 7 day survival rate to 100% compared to 40% in the control group, the

same protective effect was also observed with toxin doses up to 800 μg kg^{-1} confirming the importance of CYP450 activation in living systems.[110] Significant concentration dependent cytotoxicity has also been observed towards primary mouse hepatocytes at cylindrospermopsin concentrations ranging from 1-5 μM causing between 52% and 82% cell death. Again treatment with the potent CYP450 inhibitors proadifen and ketoconazole at 50 μM diminished the cytotoxicity of cylindrospermopsin confirming the involvement of CYP450 metabolites in its cytotoxicity.[111]

In-vitro studies have shown that cells exposed to cylindrospermopsin display a concentration and time dependent increase in the inhibition of protein synthesis, which has been shown to be irreversible even after toxin removal.[111,114] Investigations utilising primary mice hepatocyte cultures have shown that toxin exposure at concentrations of between 0.5 μM and 5.0 μM demonstrated inhibition of between 74% and 88%, with inhibition being maximal at 4 hours.[111] Cylindrospermopsin exposure also induces inhibitory effects towards protein synthesis in other systems including the commercially available rabbit reticulocyte lysate assay[115] as well as fish liver derived cell lines[114] and even in *E. coli* 70S extracts.[116] Unlike the toxins cytotoxicity, the ability to inhibit protein synthesis is not effected by treatment with CYP450 inhibitors, highlighting the importance of protein synthesis inhibition in cells deficient of CYP450 enzymes.[111]

Evidence of genotoxic effects have also been observed from exposure to cylindrospermopsin.[117,118] The toxin or one of its metabolites has been shown to form covalent adducts with the liver DNA of mice sacrificed between 24 hours and 96 hours after treatment with a single 1 μg kg^{-1} IP dose of the toxin,[117] with higher doses of 200 μg kg^{-1} being found to cause strand breaks in the liver DNA of effected animals.[118] The *in-vitro* genotoxic effects of cylindrospermopsin have been observed in primary mouse hepatocytes, however, these effects could be prevented by treatment with the CYP450 inhibitors proadifen (50 μM) and omeprazole (100 μM) suggesting that cylindrospermopsin is pro-genotoxic requiring metabolic activation.[113] Equally the genotoxic effects of the toxin have not been observed in undifferentiated human liver (HepaRG) and Chinese hamster ovary (CHO-K1) derived cell lines, a phenomenon which has been attributed to a reduced number of the appropriate metabolic systems needed to convert the toxin to its active form.[119,120]

Although limited, experimental evidence suggests that cylindrospermopsin also acts as a carcinogen *in-vivo*. An initial investigation orally dosed (gavage) 53 mice with extracts of *C. raciborskii* containing cylindrospermopsin (5.5 mg g^{-1} by HPLC), animals were either given two 1500 mg kg^{-1} doses or two doses each consisting of three 500 mg kg^{-1} portions two weeks apart. Unfortunately the experimental results did not prove to be statistically significant with only five test subjects developing tumours; however the calculated relative risk factor of 6.2 suggests that further investigation is needed in this area.[121] The Syrian hamster embryo (SHE) assay has been used to evaluate the carcinogenic potential of cylindrospermopsin *in-vitro*. The assay demonstrated the cell transforming potential of the toxin following a 7 day continuous treatment with non-cytotoxic or genotoxic doses of cylindrospermopsin ($1x10^{-7}$ to $1x10^{-2}$ ng mL^{-1}) and indicated the toxin is a carcinogenic hazard at very low doses.[122]

Investigations have also been undertaken to equate the toxic action of cylindrospermopsin to parts of the metabolites molecular structure; initial findings have indicated that the uracil moiety of the toxin is partially responsible for the potent biological activity it displays, possibly as a result of competitive or inhibitory binding to a catalytic site.[87] Further investigations have probed the significance of the various functionalities of cylindrospermopsin, investigating the biological properties of synthetic analogues cylindrospermopsin diol **15**, 7-*epi*-cylindrospermopsin diol **16** as well as the AB model **17**, uracil model **18** and functionalised AC model **19** (Figure 9).[123]

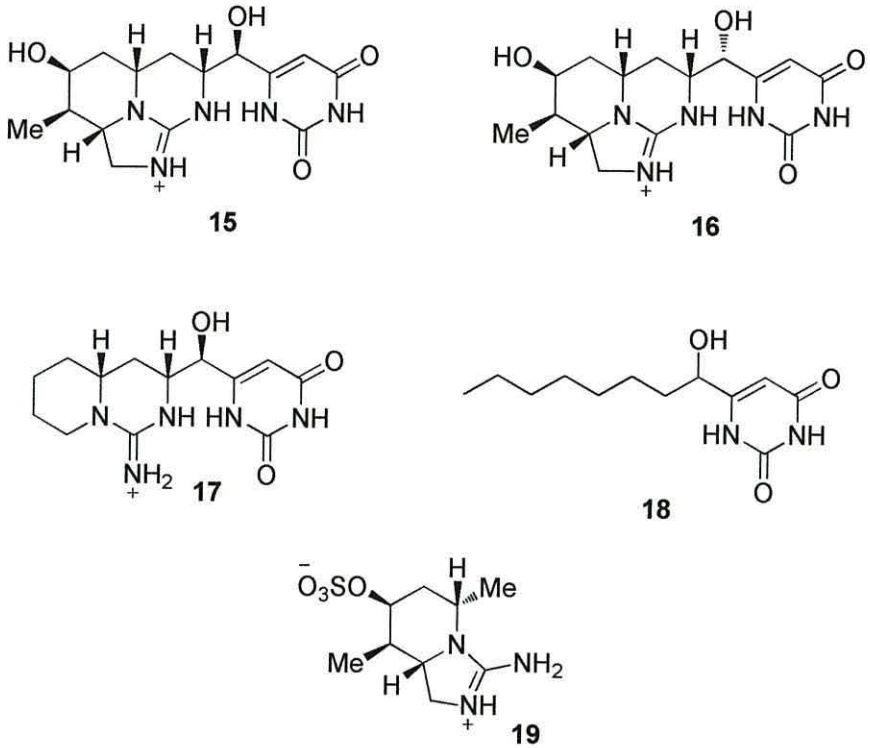


Figure 9:- Synthetic analogues used to investigate the biological properties of cylindrospermopsin.

Comparisons of synthetic (±)-cylindrospermopsin to that of native 7-*epi*-cylindrospermopsin suggest that the orientation of the hydroxyl group at C-7 has no impact on the biological activity or transport of toxin. However the presence of this functionality appears necessary for the toxic action of these natural products as the intermediary metabolite 7-*deoxy*-cylindrospermopsin has been shown to elicit no toxic effects *in-vivo* at doses up of 800 μg kg^{-1}.[32] However, clear dose response curves have been obtained with 7-*deoxy*-cylindrospermopsin against four mammalian cell lines were a comparable toxicity to that of cylindrospermopsin was observed. Both metabolites also display similar levels of protein synthesis inhibition having IC_{50} values of 340 nM and 220 nM respectively.[124] Comparisons have also been made between (±)-cylindrospermopsin and 7-*epi*-cylindrospermopsin and their corresponding diols, compounds **±15** and **±16**, investigations have shown that cylindrospermopsin and the corresponding diol **±15** both inhibited protein synthesis with comparable IC_{50} values of 0.20 μM and 0.21 μM. In addition, 7-*epi*-cylindrospermopsin and the corresponding diol **±16** also both depleted cell GSH by similar amounts, suggesting that the sulfate group does not play a significant role in the biological activity or uptake of the toxin.

The AB model compound **±17** has also demonstrated an inhibitory effect on protein synthesis but at concentrations 500 to 1000 fold higher than that of natural cylindrospermopsin, whilst the uracil **±18** and AC model **±19** proved devoid of biological activity with no inhibitory effect on protein synthesis being observed at concentrations up to 800 μM and 2000 μM respectively. These findings suggest that an intact tricyclic ABC ring system plays a key role in the biological activity of these natural products.[123]

Biosynthesis

Initial investigations regarding the biosynthesis of cylindrospermopsin determined that the toxin was of polyketide origin, with guanidino acetic acid serving as the starter unit for the formation of the polyketide chain.[125] ^{13}C labelled acetate feeding experiments have indicated that C-4 through to C-13 and the oxygen atoms attached to C-4 and C-12 arise from five contiguous acetate units attached head to tail. Glycine feeding experiments indicate that C-14, C-15 and N-16 are the result of the incorporation of one intact glycine unit, with the methyl group attached to C-13 also of glycine origin (Figure 10).[125]

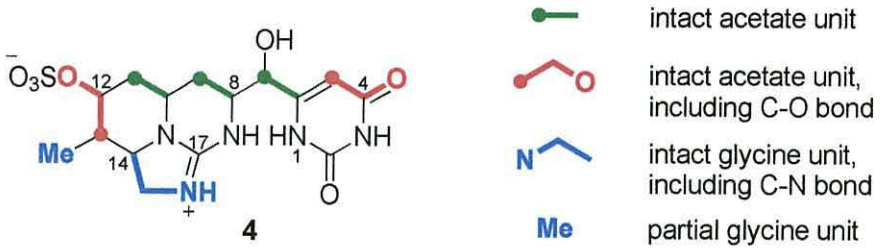
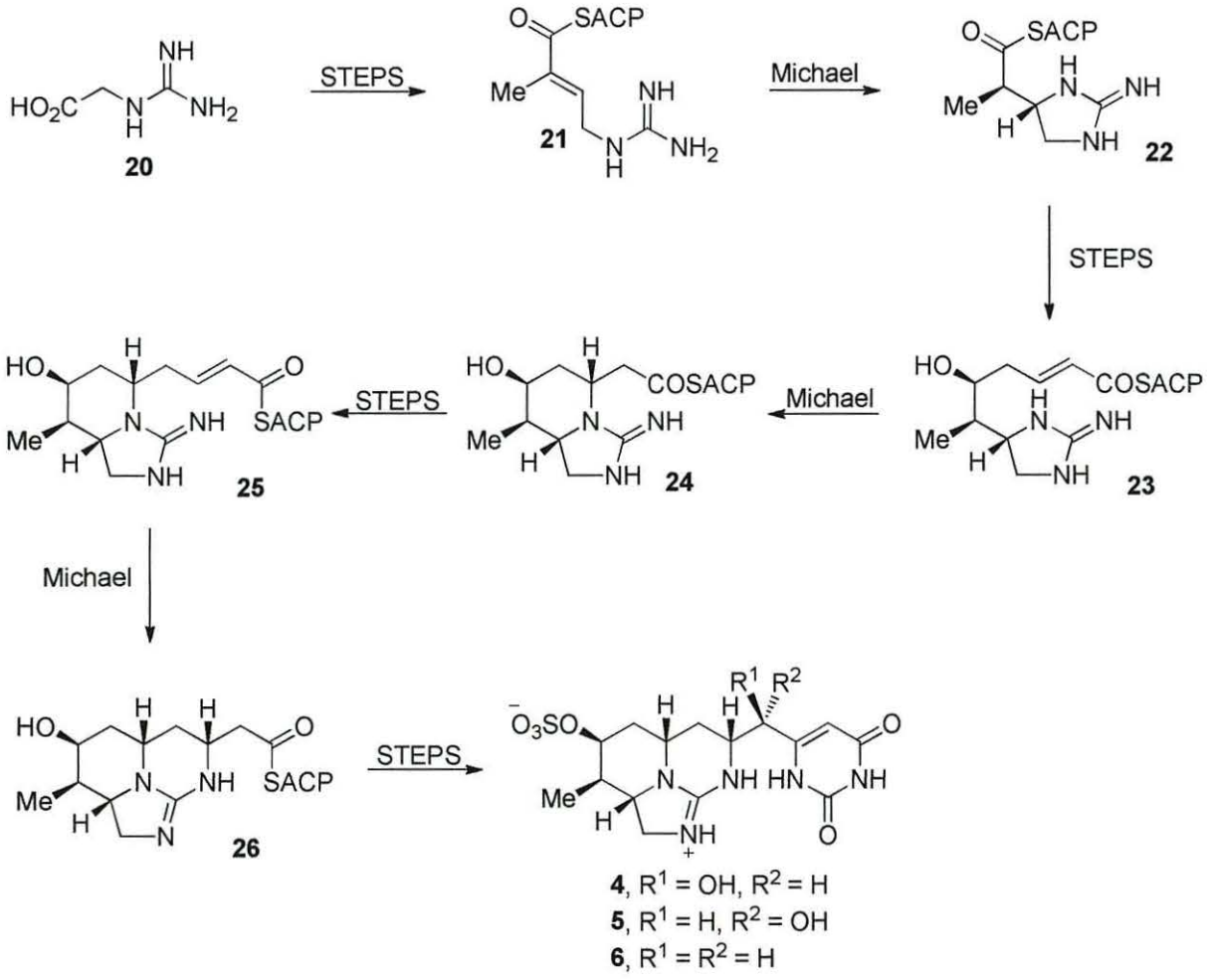


Figure 10:- Incorporation of labelled precursors into cylindrospermopsin.

Recent advances have led to the characterisation of the gene cluster responsible for the biosynthesis of cylindrospermopsin in *C. raciborskii* strain AWT205.[126] As previously indicated the starter unit for the polyketide chain is guanidino acetate **20** which has been shown to be derived from a novel L-arginine:glycine amidino transferase enzyme.[127,128] It is believed that the tricyclic guanidinium core of this family of natural products is constructed in a stepwise manner from the guanidino acetate starter unit, with sequential polyketide extensions and Michael type ring closures generating in turn the C ring (**21**→**22**), the A ring (**23**→**24**) and the B ring (**25**→**26**) (Scheme 1). Formation of the uracil moiety is achieved through the addition of a second guanidinium donor such as arginine or urea followed by dehydration and a further addition reaction. Having

constructed the core ring systems of this family of natural products two further tailoring reactions are required to complete their synthesis. The first involves the sulfonation of the C-12 hydroxyl group which is achieved by a sulfotransferase enzyme and gives rise to the biosynthetic intermediate 7-*deoxy*-cylindrospermopsin, and the second and final step in the biosynthesis involves the hydroxylation of the bridging C-7 carbon atom.[126] the stereochemical outcome of this final step has been shown to vary between different strains of cyanobacteria giving rise to either cylindrospermopsin or 7-*epi*-cylindrospermopsin.[129]

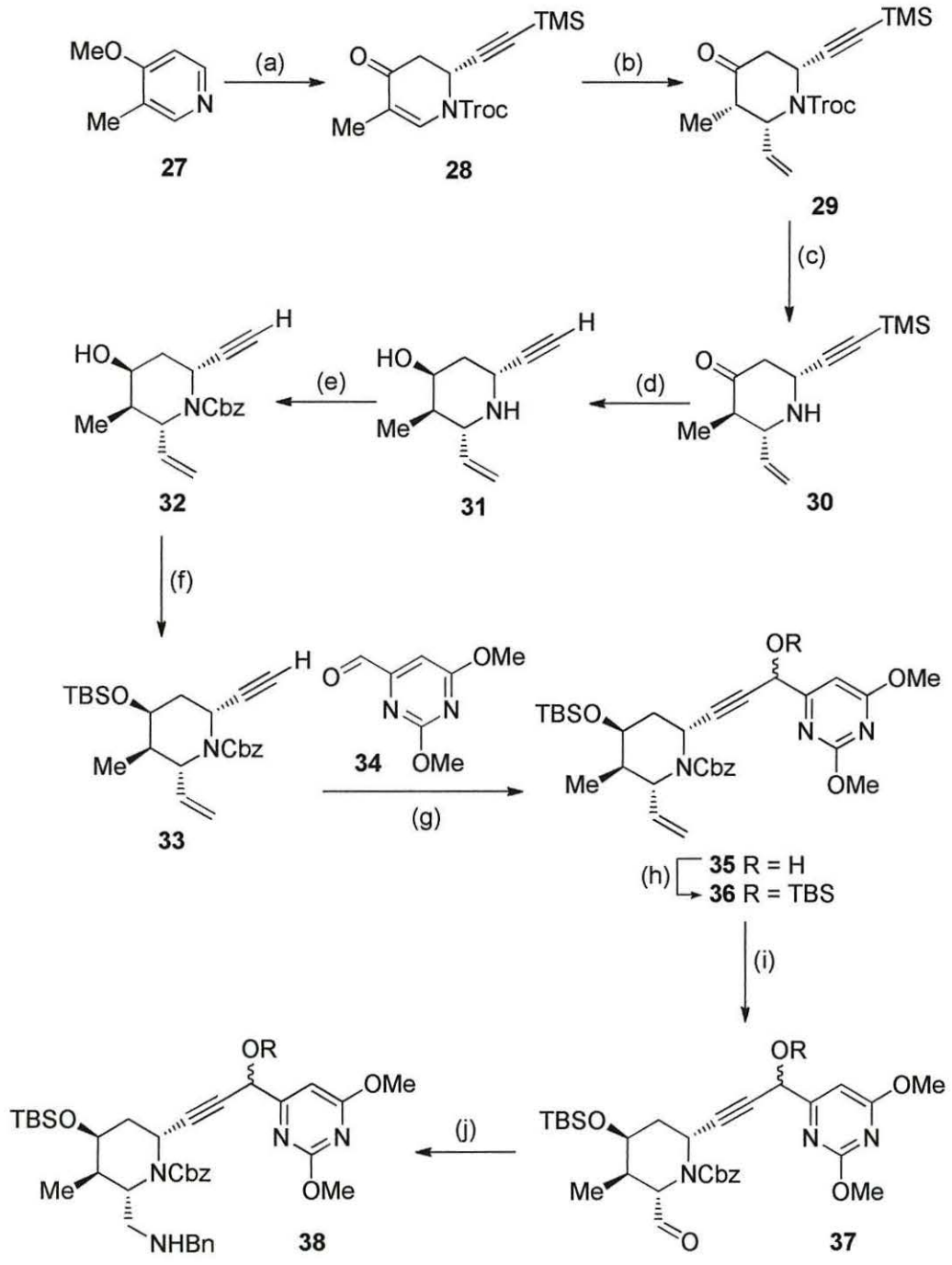


Scheme 1:- Proposed biosynthesis of cylindrospermopsin with sequential polyketide extensions and subsequent cyclisations.

Total Syntheses

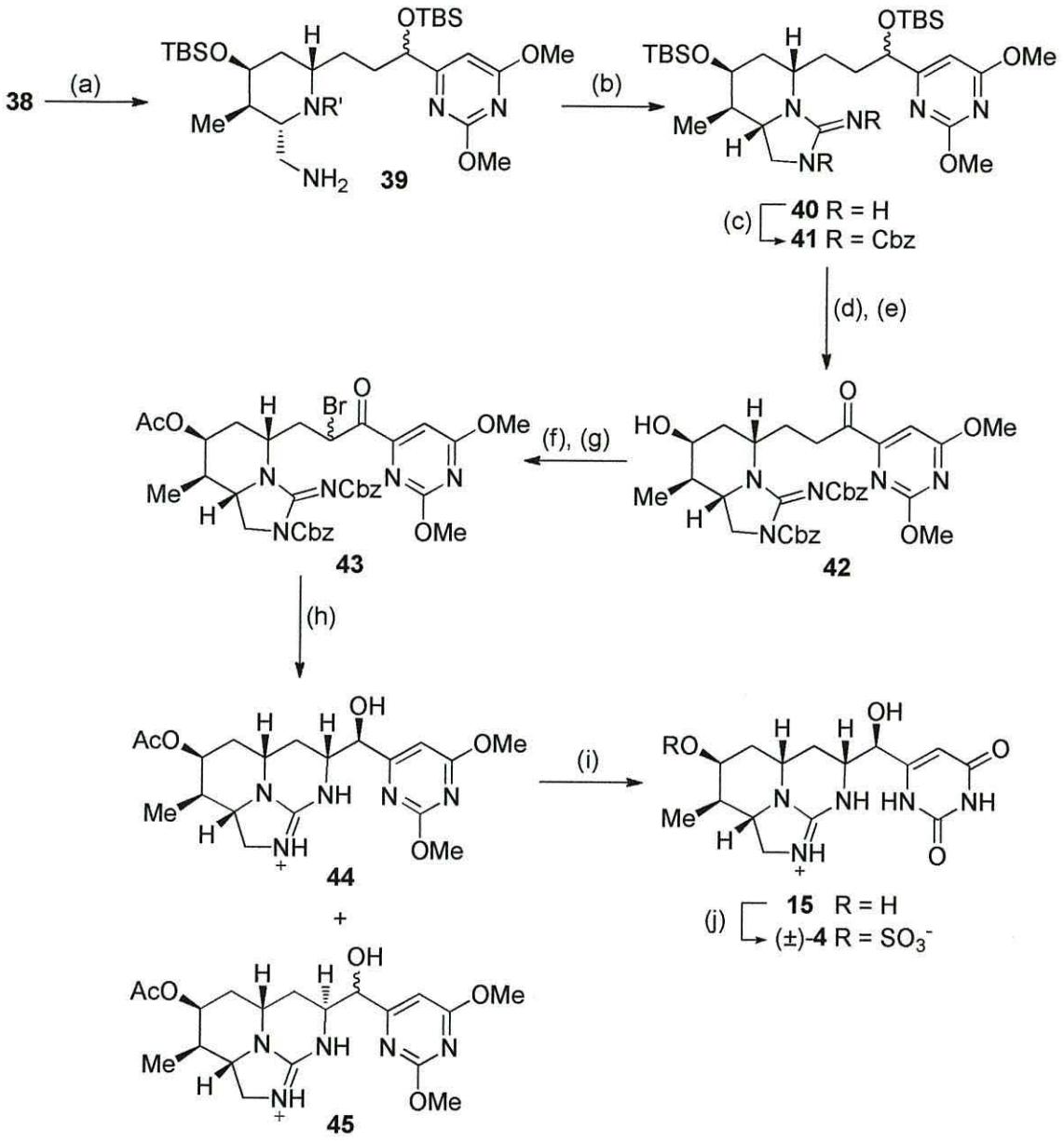
Currently four different research groups have reported total syntheses pertaining to this family of natural products.[35,130-135] The first total synthesis was published by the Snider research group in 2000 who detailed the first total synthesis of (±)-cylindrospermopsin.[130,136,137]

The group's synthesis began with the known pyridine, 4-methoxy-3-methylpyridine **27** which was prepared from 3-picoline in three steps via a modified literature procedure. The disubstituted pyridine **27** was then treated with TrocCl followed by trimethylsilylethynylmagnesium bromide to give dihydropyridine **28** in 49% yield (87% based on recovered **27**). A cuprate-catalysed conjugate addition of vinylmagnesium bromide to the enone of **28** successfully generated piperidone **29** in 92% yield. Cleavage of the Troc protecting group from **29** was achieved by treatment with zinc dust in acetic acid affording the free piperidone, in which the methyl group adjacent to the ketone had equilibrated under the acidic conditions to give the more thermodynamically stable isomer **30** with all three substituents equatorial. Reduction of **30** with L-Selectride in THF at -78 °C followed by basic hydrolysis generated the desired axial alcohol and cleaved the alkynylsilane furnishing the desired piperidine **31** with all four stereogenic centres of the A ring of cylindrospermopsin in place. The amino group of **31** was then protected by treatment with CbzCl generating carbamate **32** followed by conversion of the remaining hydroxyl function to the corresponding silyl ether giving alkyne **33**. Alkyne **33** was then coupled with aldehyde **34** via the corresponding alkynylmagnesium bromide giving alcohol **35** as a 1:1 mixture of diastereomers in 83% yield. The newly formed alcohol function of **35** was then protected by treatment with TBSCl, imidazole and a catalytic amount of DMAP giving **36** in 88% yield. Ozonolysis of the alkene substituent of **36** in DCM followed by reductive workup with Me_2S gave aldehyde **37** which subsequently underwent reductive amination providing benzylamine **38** in 68% yield (Scheme 2).



Scheme 2:- (a) TrocCl, THF, -30 °C, TMSC≡CMgBr, 87%; (b) CuBr•SMe_2, -78 °C, vinylmagnesium bromide, TMSCl, THF, 92%; (c) Zn, AcOH; (d) L-Selectride 90% (2 steps from **29**); (e) CbzCl, Na_2CO_3, THF, 96%; (f) TBSCl, imidazole, DMAP, DCM, 89%; (g) EtMgBr, THF, 0 °C, **34**, 83%; (h) TBSCl, imidazole, DMAP, DCM, 88%; (i) O_3, DMS, DCM, -78 °C, 72%; (j) NH_2Bn, AcOH, benzene; (k) $NaBH_3CN$, MeOH, 68% (2 steps from **37**).

Hydrogenation (1 atm) of **38** over 5% Pd/C in methanol reduced the alkyne bond and cleaved the benzyl and Cbz groups generating the unpurified diamine **39** in 65-75% yield. Formation of the desired guanidine proved difficult but was successfully achieved by the slow addition of 1 molecular equivalent of cyanogen bromide to a dilute benzene solution of **39** to give a primary cyanamide, which spontaneous cyclised to give guanidine **40** in which both the A and C rings of cylindrospermopsin had been established. The newly formed guanidine function was then protected as the corresponding carbamate by treatment with CbzCl to afford protected guanidine **41** in 45% yield from benzylamine **38**. Treatment with TBAF in THF at room temperature removed the silyl protecting groups allowing for the selective oxidation of the benzylic hydroxyl group with MnO_2 in DCM to afford ketone **42** in high yield. Bromination of ketone **42** could not be accomplished; therefore the remaining alcohol group was acetylated under standard conditions followed by successful bromination with $CuBr_2$ in EtOAc to give an unstable mixture of α-bromo ketones **43**. Immediate hydrogenation of this mixture over 5% Pd/C in methanol liberated the free guanidine which, in turn, underwent an S_N2 cyclisation, with concomitant ketone reduction to give an easily separable 3:2 mixture of tricyclic guanidines **44** and **45** in 70% yield over two steps. Hydrolysis of **44** was achieved in concentrated HCl at 100 °C for 6 hours affording uracil diol **15** in 95% yield. The final step of the synthesis involved the monosulfonation of the C-12 hydroxyl group of **15**, which was achieved by exposure to $SO_3 \cdot DMF$ in pyridine and DMF successfully generating (±)-cylindrospermopsin in yields of 60-80% (Scheme 3).



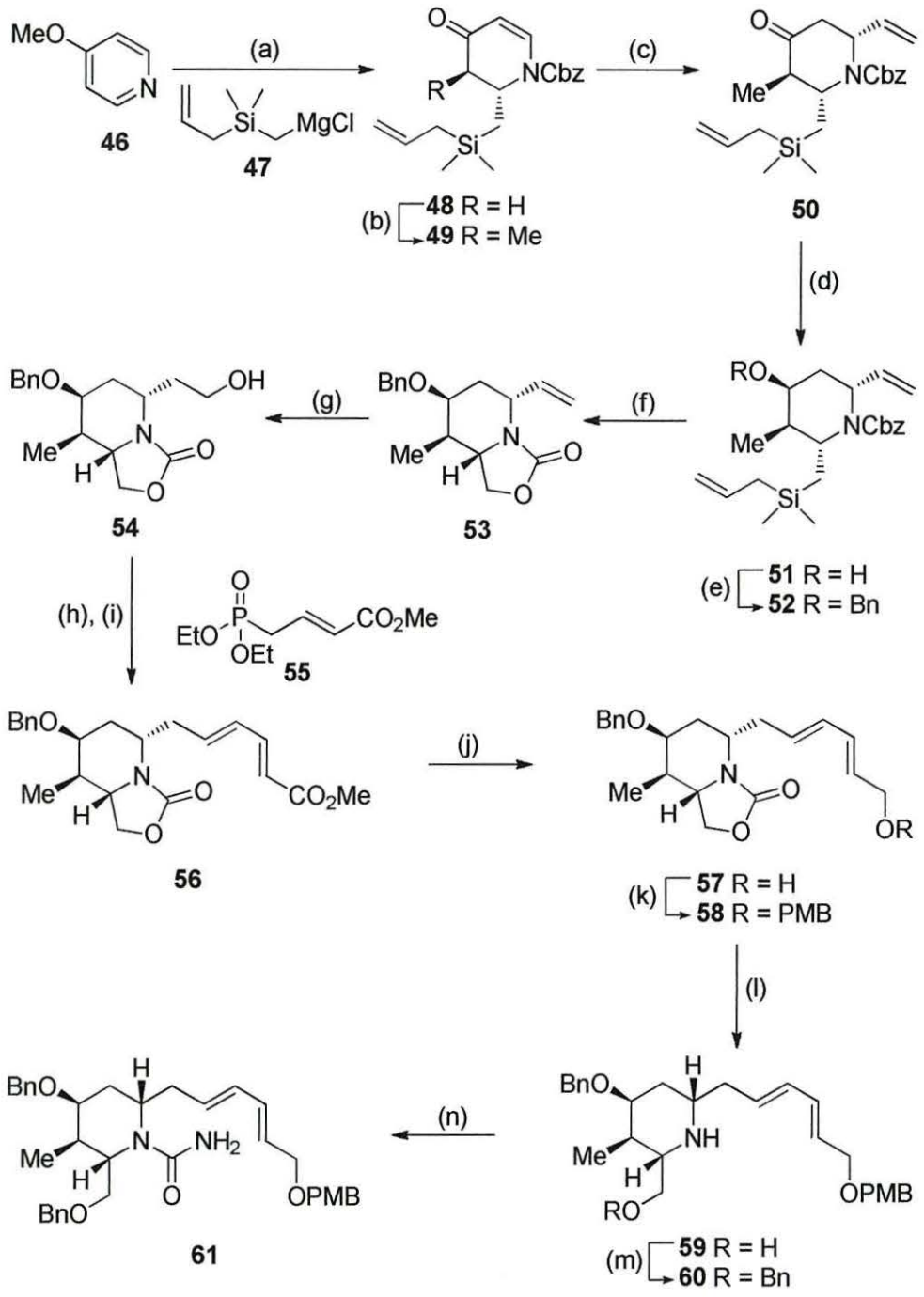
Scheme 3:- (a) 5% Pd/C, H_2, MeOH; (b) CNBr, benzene; (c) NaH, CbzCl, THF, 45% (3 steps from **38**); (d) TBAF, THF, 83%; (e) MnO_2, DCM, 87%; (f) Ac_2O, pyridine, rt, 87%; (g) $CuBr_2$, EtOAc, rt, 30 min; (h) $Pd(OH)_2$, H_2, MeOH, 42% (2 steps); (i) conc. HCl, Δ, 95%; (j) SO_3·DMF, pyridine, DMF, 60-80% (**4**).

The Snider group's synthesis represents the first reported total synthesis of (±)-cylindrospermopsin which was achieved in 1.5-2.0% overall yield from 4-methoxy-3-methylpyridine in 20 steps. Unfortunately the group were unable to establish conclusively the C-7 stereochemistry of any of their intermediates. However, they noted that the chemical shift of the H-7 proton of intermediate **44** δ 4.68 (d, J = 3.7 Hz) corresponded

closely to that initially reported for that of the natural product δ 4.70 (d, J = 3.9 Hz) suggesting that their synthetic material displayed the same C-7 stereochemistry. The key step in the group's synthesis involved α-bromination coupled with an intramolecular nucleophilic substitution to successfully install the cylindrospermopsin B ring and complete the tricyclic portion of the molecule (**42**→**44**).

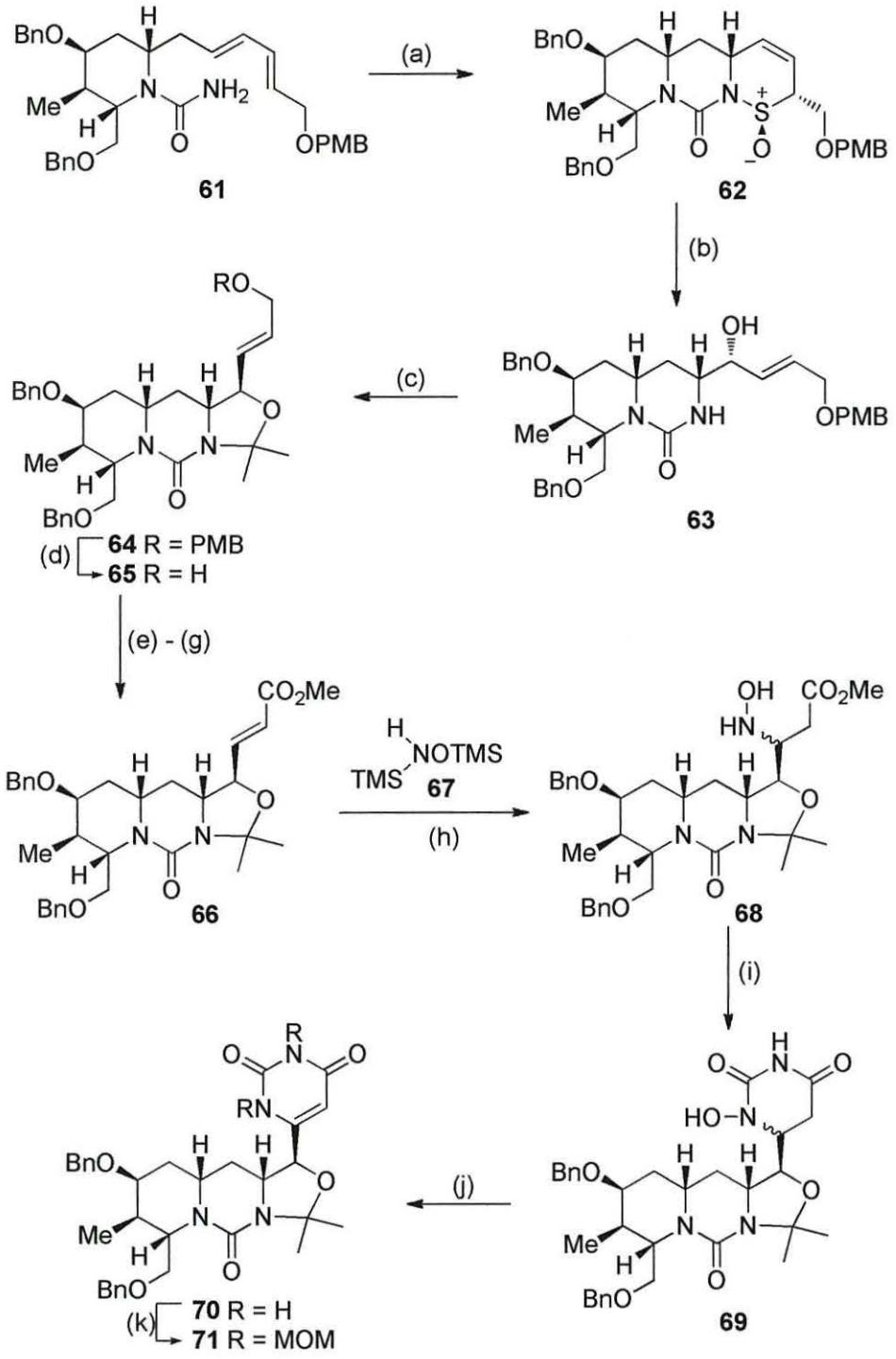
Following the work of Snider, the Weinreb group reported the total synthesis of both cylindrospermopsin and 7-*epi*-cylindrospermopsin.[35,131,138-140] The group's work also led to the stereochemical reassignment of the bridging hydroxymethylene group, showing that cylindrospermopsin was correctly represented by structure **4** and not structure **5** as detailed in the original structural assignment.[29]

The starting point for the group's synthesis was 4-methoxypyridine **46**, which was treated with CbzCl, followed by the addition of a hydroxymethyl anion equivalent in the form of Grignard reagent **47** to give dihydropyridinone **48** in 94% yield. Generation of the enolate anion of **48** with NaHMDS followed by treatment with MeI afforded the desired *trans* product **49**. A cuprate-catalysed conjugate addition of vinylmagnesium bromide to **49** gave the requisite adduct **50** in very high yield. Reduction of ketone **50** with L-Selectride in THF gave the corresponding alcohol **51** having the four correct A ring stereocenters displayed by this family of natural products. Alcohol **51** was then protected to give benzyl ether **52**, with subsequent Tamao oxidation of the silane group leading directly to cyclic carbamate **53**. Elaboration to the required side chain began with the hydroboration of alkene **53** with disiamylborane to successfully generate primary alcohol **54** in near quantitative yield. Oxidation of **54** to the corresponding aldehyde was achieved under Swern conditions with a subsequent HWE olefination with phosphonate **55** cleanly generating the desired (*E*,*E*)-diene ester **56**. The ester group of **56** was then reduced with DIBAL providing allylic alcohol **57** which was protected as the *p*-methoxybenzyl ether **58**. Hydrolysis of the cyclic carbamate functionality of **58** was achieved by treatment with ethanolic sodium hydroxide generating primary alcohol **59** quantitatively, with subsequent protection of the hydroxyl group with BnBr furnishing the dibenzyl compound **60** in 65% yield. Finally conversion of **60** to the corresponding urea successfully generated the required Diels-Alder precursor **61** in 85% yield (Scheme 4).



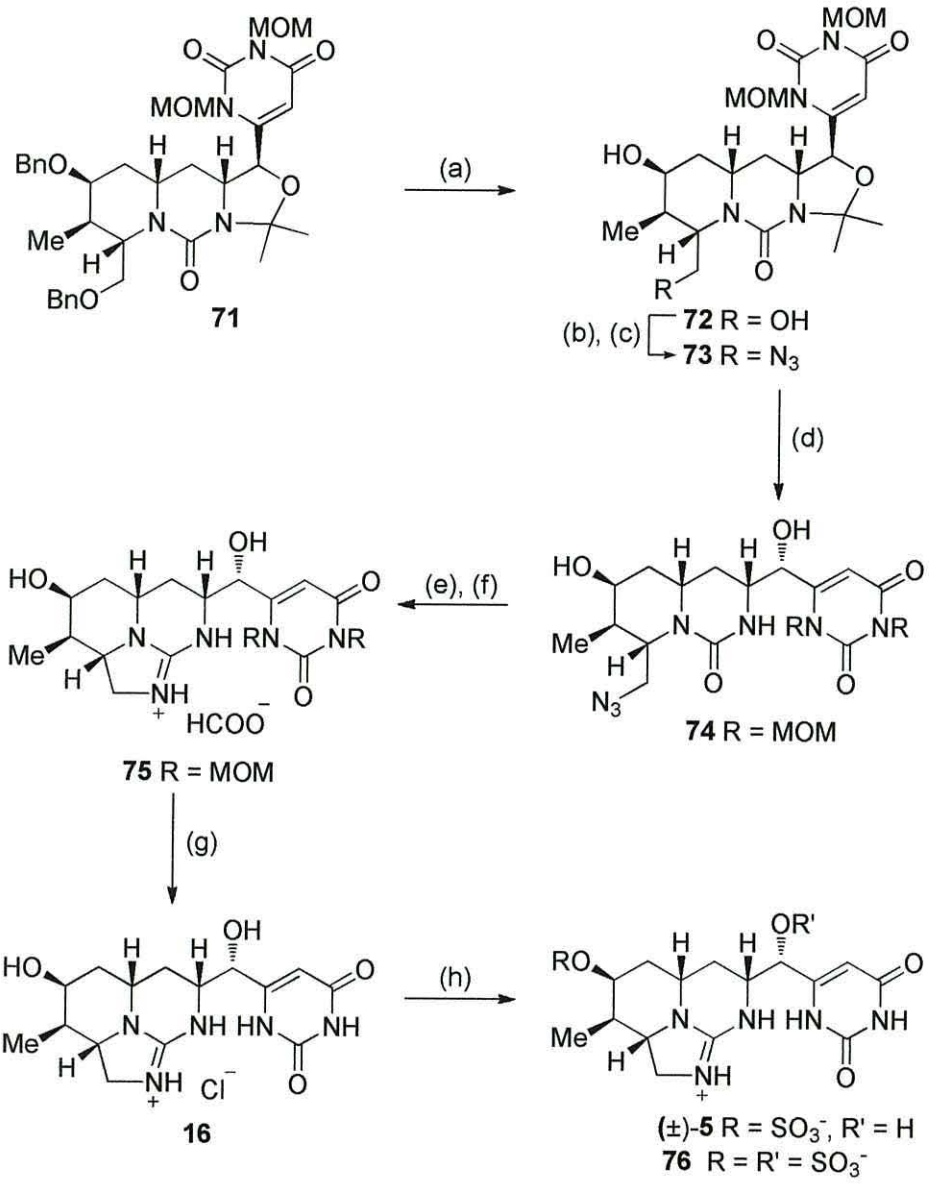
Scheme 4:- (a) i) CbzCl, THF, -20 °C, ii) **47**, Et_2O, -20 °C, 94% (2 steps from **46**); (b) NaHMDS, MeI, THF, -78 °C, 88%; (c) vinylmagnesium bromide, CuI, THF, -78 °C to -20 °C, 98%; (d) L-Selectride, THF, 80%; (e) NaH, BnBr, TBAI, THF, Δ, 95%; (f) i) KHF_2, $CHCl_3$, TFA, ii) MeOH, $NaHCO_3$, THF, 30% H_2O_2, 88% (2 steps); (g) i) $(Sia)_2BH$, THF, ii) H_2O_2, NaOH, -20 °C to rt, 97% (2 steps from **52**); (h) $(COCl)_2$, DMSO, NEt_3, DCM, -55 °C to rt, 84%; (i) **55**, LiOH-H_2O, 4 Å MS, THF, Δ, 80%; (j) DIBAL, $BF_3 \cdot Et_2O$, DCM, -78 °C, 83%; (k) NaH, PMBCl, TBAI, THF, Δ, 96%; (l) NaOH, H_2O, EtOH, Δ, 100%; (m) NaH, BnBr, TBAI, THF, 0 °C to rt, 65%; (n) KOCN, AcOH, pyridine, NEt_3, 85%.

Treatment of (*E*,*E*)-diene urea **61** with thionyl chloride and imidazole in DCM at -78 °C followed by slowly warming to room temperature led to a single stereoisomeric cycloadduct **62** in excellent yield. At this juncture the group were able to remove the PMB protecting group from **62** by treatment with DDQ in order to generate the corresponding alcohol whose structure and stereochemistry could be firmly established by X-ray crystallography, allowing them to unambiguously continue with their synthesis. Dihydrothiazine oxide **62** then underwent a stereospecific ring opening/[2,3]-sigmatropic rearrangement to afford bicyclic urea **63** having all six stereocenters of the target natural product in place, the stereochemistry of **63** was verified by the X-ray crystallography of its MOM protected derivative. In order to elaborate the uracil moiety, protection of the newly introduced alcohol function was required, this was achieved by converting bicyclic urea **63** into cyclic acetonide **64**. Subsequent removal of the PMB group gave allylic alcohol **65** which was converted via a short sequence to the required α,β-unsaturated methyl ester **66** in 81% yield. Treatment of ester **66** with *N*,*O*-bis-(trimethylsilyl)hydroxylamine **67** provided the desired conjugate addition product **68**; subsequent exposure of which to phenyl chloroformate, followed by ammonium hydroxide furnished *N*-hydroxydihydrouracil **69**. Finally dehydration of **69** with triflic anhydride completed the installation of the uracil D ring **70**, with subsequent exposure to MOMCl generating the bis-*N*-MOM protected derivative **71** (Scheme 5).



Scheme 5:- (a) $SOCl_2$, imidazole, DCM, -78 °C to rt, 81%; (b) i) PhMgBr, THF/DCM, -55 °C, ii) $(MeO)_3P$, MeOH, 50 °C, 84%; (c) $Me_2C(OMe)_2$, acetone, CSA, Δ, 93%; (d) DDQ, H_2O, DCM, 78%; (e) Dess-Martin periodinane, DCM; (f) $NaClO_2$, *t*-BuOH, H_2O; (g) DIPEA, MeI, DMF, 81% (3 steps from **65**); (h) THF, EtOH, 82%; (i) i) PhOCOCl, NEt_3, THF, ii) NH_4OH, *i*-PrOH, 65% (2 steps from **68**); (j) Tf_2O, pyridine, DCM, 73%; (k) TMSCl, MOMCl, DIPEA, DCM, 80%.

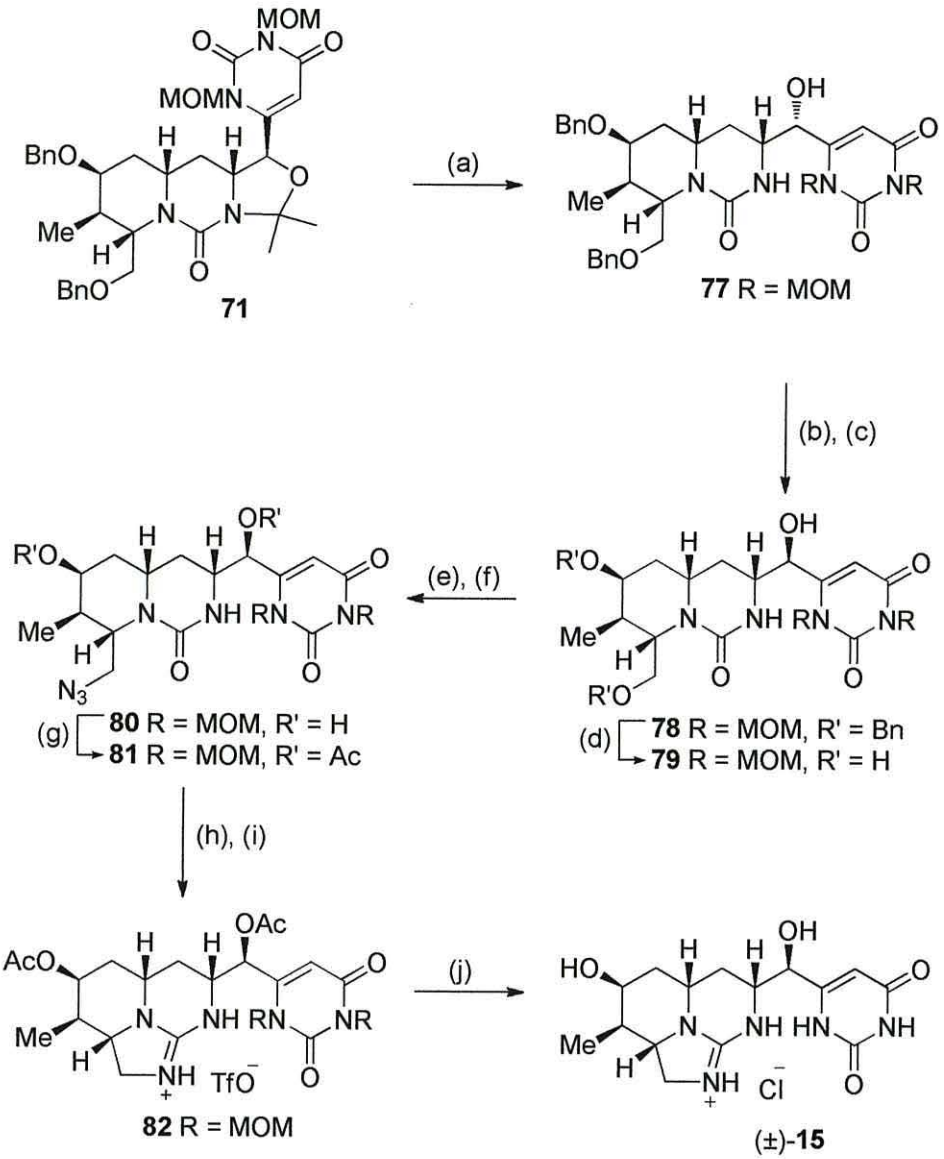
The final challenge for the group to overcome was the construction of the 5-membered C ring. This sequence began with the hydrogenation of **71** over Pearlman's catalyst furnishing the crystalline diol **72**, the X-ray analysis of which further verified the structures of the group's late stage synthetic intermediates. The primary alcohol group of **72** was then converted to the corresponding azide by treatment with triphosgene followed by exposure to NaN_3 giving the requisite azide **73** in 86% yield. Selective acid hydrolysis of the acetonide protecting group of **73** was achieved by treatment with dilute HCl producing urea diol **74**. Subsequent activation of the urea moiety of **74** with methyl triflate followed by catalytic hydrogenation of the azide over 10% Pd/C proceeded smoothly, leading directly to the tetracyclic guanidinium compound **75**. Deprotection of the uracil D ring of **75** was achieved with vigorous acid hydrolysis to afford guanidinium diol **16** in 43% yield from urea **74**. The spectroscopic properties of **16** were found to be incongruous to the corresponding intermediate in the Snider group's total synthesis of (±)-cylindrospermopsin, in particular the H-7 proton of intermediate **16** displayed δ 4.50 (d, J = 6.6 Hz) which in line with that reported for 7-*epi*-cylindrospermopsin, versus δ 4.70 (d, J = 4.0 Hz) for both Sniders synthetic diol ±**15** and natural cylindrospermopsin. Moreover the monosulphate ±**5** which was prepared in high yield and isolated along with 25% of the corresponding *bis* sulphate **76**, provided NMR spectra identical to those of natural 7-*epi*-cylindrospermopsin and significantly different from cylindrospermopsin (Scheme 6).



Scheme 6:- (a) $Pd(OH)_2$, H_2, EtOH, 71%; (b) triphosgene, THF, rt; (c) NaN_3, DMF, 65 °C, 86%; (d) dil. HCl, THF, H_2O, 85 °C, 72%; (e) MeOTf, 2,6-di-*tert*-butylpyridine, DCM, -78 °C to rt; (f) 10% Pd/C, H_2, EtOH; (g) 12 M HCl, 95 °C, 43% (3 steps from **74**); (h) SO_3·DMF, pyridine, Na_2SO_4, DMF, rt, 70% (±**5**).

A subsequent publication by Weinreb detailed the conversion of a late synthetic intermediate, compound **71** into the newly assigned cylindrospermopsin structure **4**.[131] Firstly, the acetonide functionality of **71** was removed under acid hydrolysis to afford alcohol **77** in 85% yield, followed by inversion of the C-7 hydroxyl group via a Mitsunobu process to generate the desired epimer **78**. Removal of the two benzyl groups of **78** by hydrogenation over Pearlman's catalyst provided triol **79** which upon treatment

with triphosgene followed by NaN_3 in DMF cleanly gave the azide diol **80**, whose structure and stereochemistry were verified by X-ray crystallography. Conversion of diol **80** to the corresponding diacetate **81** allowed for activation of the urea functionality with methyl triflate followed by hydrogenation to directly furnish guanidinium salt **82**. Finally, acidic hydrolysis of the acetyl and MOM groups afforded the tetracyclic diol ±**15** in 61% overall yield from diacetate **81**, which had spectra identical to those reported by Snider in his total synthesis of cylindrospermopsin,[130] thus confirming that the toxin should be represented by structure **4** (Scheme 7). In light of this it also seems unlikely that the toxins exist as uracil tautomers as previously believed, which contributed to the incorrect stereochemical assignment of the C-7 hydroxyl group.



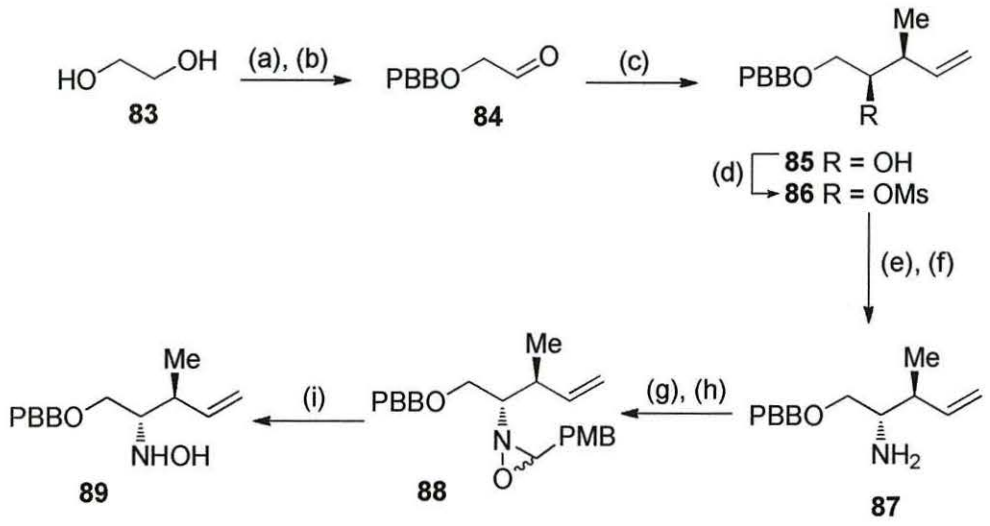
Scheme 7:- (a) dil. HCl, THF, H_2O, 85%; (b) PPh_3, *p*-NBA, DEAD, benzene; (c) MeOH, K_2CO_3, 61% (2 steps form **77**); (d) $Pd(OH)_2$, EtOH, cyclohexene, 95%; (e) triphosgene, THF; (f) NaN_3, DMF, Δ, 70% (2 steps from **79**); (g) Ac_2O, pyridine, DMAP, 78%; (h) MeOTf, 2,6-di-*tert*-butylpyridine, DCM; (i) 10% Pd/C, H_2, EtOH; (j) 12 M HCl, Δ, 61% (3 steps from **81**).

The Weinreb group's synthesis represents the first synthesis were the C-7 stereochemistry was definitively assigned, via the X-ray crystallography of their late stage synthetic intermediates. This led to the reassignment of the orientation of the C-7 hydroxyl group meaning that cylindrospermopsin was represented by **4** and its epimer by structure **5**. The key steps in the groups synthesis involve an *N*-sulfinyl Diels Alder cyclisation they had developed to install the B ring, followed by a Grignard ring

opening/allylic sulfoxide [2,3]-sigmatropic rearrangement to stereoselectively install the C-7 hydroxyl group (**61**→**63**). This led them to report the first racemic synthesis of 7-*epi*-cylindrospermopsin in 33 steps and 0.26% overall yield, as well as a formal synthesis of racemic cylindrospermopsin from a common late synthetic intermediate in 36 steps and 0.2-0.25 % overall yield.

The first asymmetric synthesis of a metabolite from this family of natural products was reported by White and Hansen who detailed the asymmetric total synthesis of 7-*epi*-cylindrospermopsin, which became their natural target after the toxin's stereochemistry was reassigned by Weinreb.[35] They employed a convergent approach splitting the target molecule into two fragments namely hydroxylamine **89** termed the "western" fragment and pyrimidine aldehyde **98** termed the "eastern" fragment.[132,133]

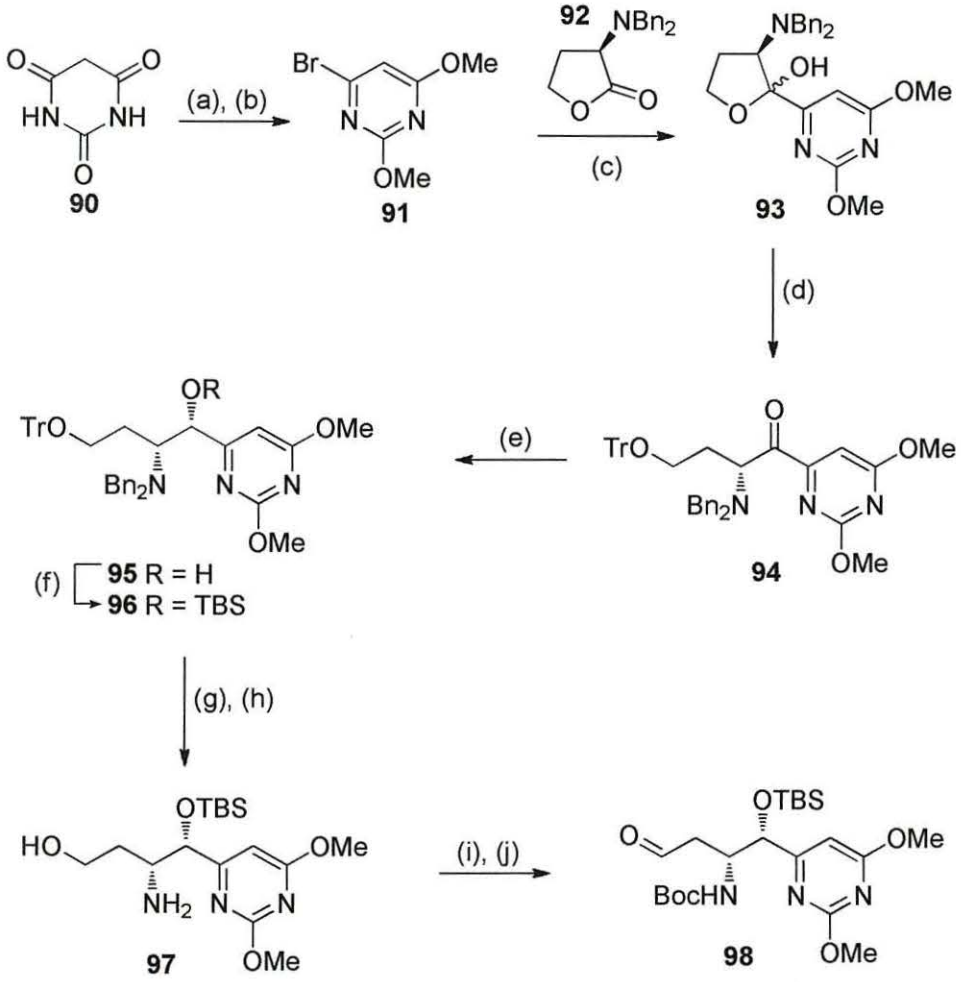
The preparation of the group's first substrate began with the mono *p*-bromobenzyl protection of ethylene glycol **83** followed by oxidation of the residual alcohol group with Dess-Martin periodinane to afford aldehyde **84** in 90% yield. Aldehyde **84** successfully underwent asymmetric crotylation to give the *syn* homoallylic alcohol **85** in 94% enantiomeric excess. Treatment of alcohol **85** with methanesulfonic anhydride and pyridine in DCM generated the corresponding mesylate **86** quantitatively. Displacement of the mesylate functionality of **86** with sodium azide, followed by a Staudinger reduction with triphenylphosphine afforded the inverted primary amine **87** in 56% yield. Condensation of amine **87** with *p*-anisaldehyde, followed by *in-situ* oxidation of the resulting imine with *m*-chloroperbenzoic acid gave oxaziridine **88**. Finally treatment of oxaziridine **88** with hydroxylamine hydrochloride furnished the groups western fragment **89** in 60% yield from amine **87** (Scheme 8).



Scheme 8:- (a) NaH, *p*-$BrC_6H_4CH_2Br$, THF, 65%; (b) Dess-Martin periodinane, DCM, 90%; (c) *cis*-2-butene, *t*-BuOK, *n*-BuLi, (+)-$MeOB(Ipc)_2$, Et_2O/THF, 45%; (d) Ms_2O, pyridine, DCM; (e) NaN_3, DMF, 85 °C; (f) Ph_3P, THF/H_2O, 56% (3 steps from **85**); (g) *p*-anisaldehyde, Na_2CO_3, MeOH, 60 °C; (h) *m*-CPBA, DCM, 0 °C to rt; (i) $HONH_2 \cdot HCl$, MeOH, 0 °C to rt, 60% (3 steps from **87**).

The duo prepared their eastern fragment from barbituric acid **90**, treatment of which with phosphorus oxybromide and triethylamine generated the corresponding tribromopyrimidine which upon exposure to two equivalents of sodium methoxide furnished the desired 4-bromo-2,6-dimethoxypyrimidine **91** in high yield. Preparation of γ-lactone **92** was achieved by treatment of (*R*)-(–)-methionine with excess benzyl bromide which after recrystalisation allowed the desired product to be isolated in >98% enantiomeric excess. Subsequent halogen–metal exchange of bromopyrimidine **91** by treatment with *n*-BuLi followed by exposure of the resulting lithio pyrimidine to **92** in the presence of cerium trichloride gave lactol **93** quantitatively as a mixture of stereoisomers. Treatment of **93** with trityl chloride gave the primary trityl ether **94**; subsequent reduction of the ketone functionality of **94** with L-Selectride at -78 °C gave the major *syn* amino alcohol **95** and its *anti* epimer in the ratio 12:1 respectively. The newly formed hydroxyl group was protected as the corresponding silyl ether **96** in 87% yield. Treatment of **96** with formic acid followed by hydrogenation over Pearlman's catalyst removed both the trityl and benzyl protecting groups to afford primary alcohol **97**. Boc protection of the newly unmasked secondary amine group followed by TPAP oxidation generated the

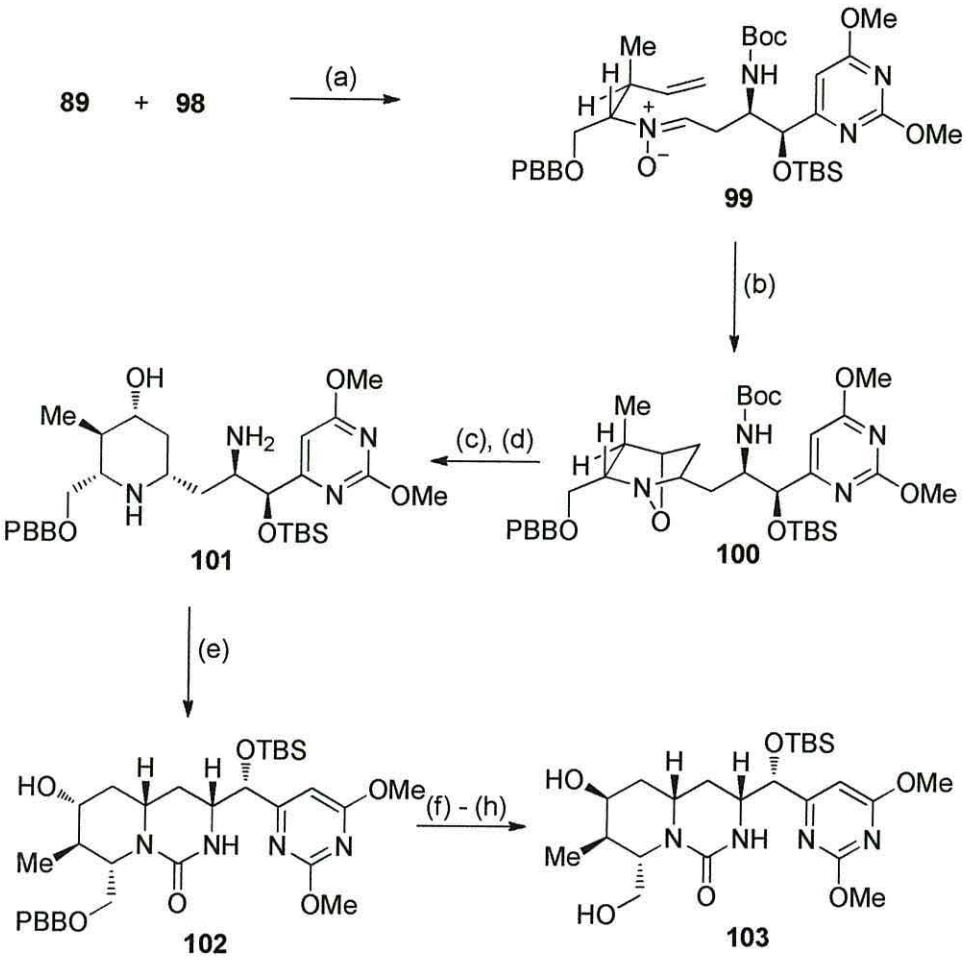
desired pyrimidine aldehyde **98** in high yield, completing the synthesis of the eastern fragment (Scheme 9).



Scheme 9:- (a) $POBr_3$, NEt_3, toluene, Δ, 99%; (b) NaOMe, MeOH, 84%; (c) *n* -BuLi, **92**, $CeCl_3$, Et_2O/THF, -78 °C to rt, 97%; (d) TrCl, NEt_3, DMAP, DCM, 93%; (e) L-Selectride, THF, -78 °C, 84%; (f) TBSOTf, NEt_3, THF, 87%; (g) HCO_2H, THF, 100%; (h) $Pd(OH)_2$, H_2, EtOH, 81%; (i) Boc_2O, NEt_3, DCM, 68%; (j) TPAP, NMO, MS, DCM, 91%.

The coupling of the eastern and western fragments **98** and **89** proceeded efficiently in refluxing methanol in the presence of 3 Å molecular sieves to remove the water of condensation; under these conditions the (*Z*)-nitrone **99** was obtained as a single isomer. Substantial effort was devoted to optimising the reaction conditions of the following key 1,3-dipolar cycloaddition, it was found that toluene was the optimum reaction solvent and that the reaction proceeded in a narrow temperature window. Above

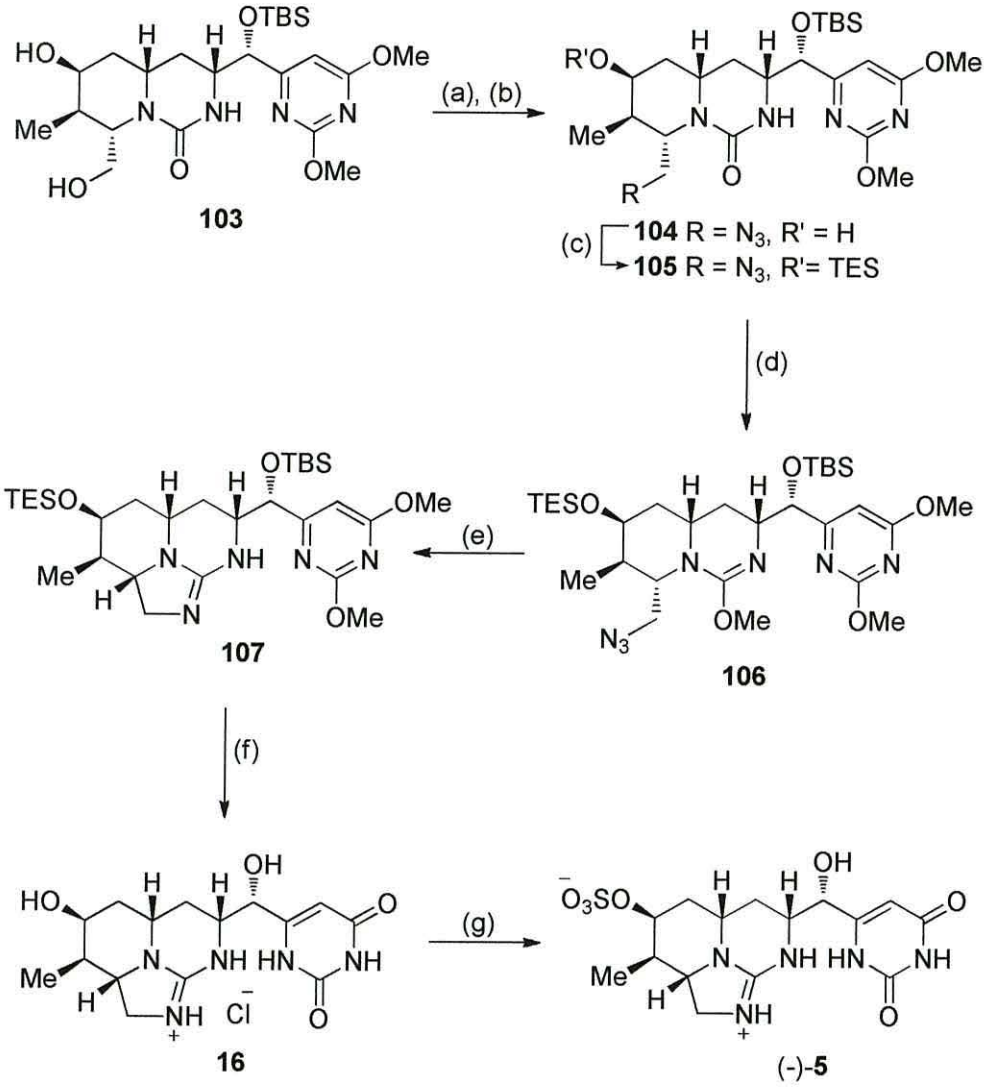
110 °C nitrone **99** decomposed rapidly, whereas below 95 °C there was little reaction, within this temperature range isomerisation of the (*Z*)-nitrone to the (*E*)-isomer was observed, a process which was accelerated by Lewis acid catalysts such as lithium perchlorate, with no benefit to the cycloaddition process. The optimised conditions for intramolecular cycloaddition of **99** gave oxazabicyclo[2.2.1]heptane **100** as a 2:1 mixture of isomers which arose from *exo* and *endo* cycloaddition respectively. The *in-situ* reduction of **100** with zinc and ammonium chloride followed by acidic removal of the Boc group furnished piperidine **101** in 68% from nitrone **99**. Treatment of piperidine **101** with carbonyldiimidazole successfully bridged the piperidine nitrogen and C-8 amine groups generating bicyclic urea **102** in high yield. A short sequence involving oxidation of the piperidyl hydroxyl group of **102** with Dess-Martin periodinane followed by reduction with L-Selectride successfully inverted the hydroxyl group to the correct configuration required for the target molecule. Subsequent cleavage of the *p*-bromobenzyl ether by hydrogenation over palladium hydroxide gave diol **103** containing all six stereocenters of 7-*epi*-cylindrospermopsin, as was confirmed by X-ray crystallography (Scheme 10).



Scheme 10:- (a) MeOH, 3 Å MS, Δ, 60%; (b) toluene, 3 Å MS, Δ; (c) Zn, NH_4Cl, THF/H_2O, Δ; (d) HCl, MeOH, 68% (3 steps from **99**); (e) i) CDI, DCM, ii) K_2CO_3, MeOH, 85%; (f) Dess-Martin periodinane, DCM, 98%; (g) L-Selectride, THF, 85%; (h) $Pd(OH)_2$, H_2, EtOH, 76%.

Treatment of diol **103** with triphosgene followed by exposure to NaN_3 in hot DMF gave azide **104** in appreciable yield. Subsequent protection of the remaining alcohol function was achieved by treatment with triethylsilyl triflate giving silyl ether **105** quantitatively. The urea functionality of **105** was then converted to the *O*-methylisourea derivative **106** which was hydrogenated immediately over palladium on carbon, reducing the azide functionality to the corresponding primary amine which spontaneously cyclised to afford tricyclic guanidine **107**. Global deprotection of guanidine **107** was achieved with vigorous acid hydrolysis in concentrated HCl producing tetracyclic diol **16** in 21% overall yield from azide **105**. The final step in the synthesis involved the installation of the

sulfonic acid moiety which was achieved via known chemistry to afford 7-*epi*-cylindrospermopsin in a 2.5:1 ratio with the corresponding *bis* sulfate (Scheme 11).

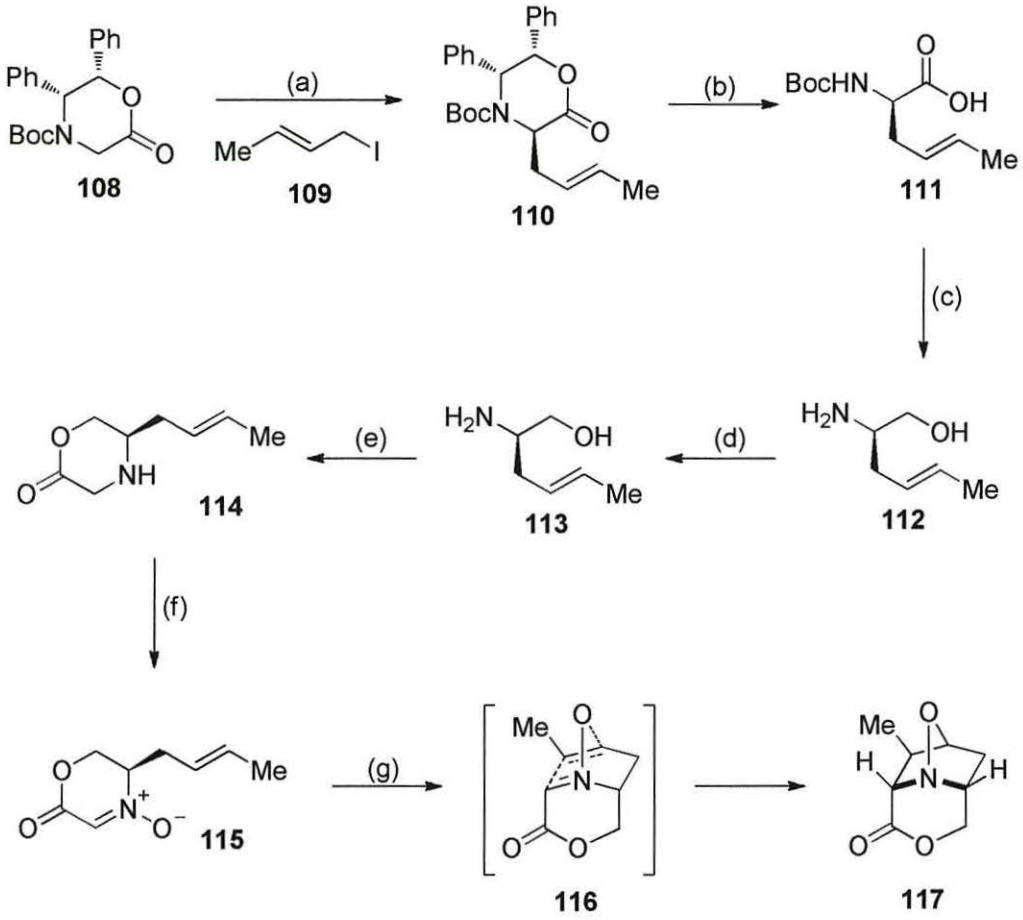


Scheme 11:- (a) triphosgene, THF; (b) NaN_3, DMF, 49% (over 2 steps from **103**); (c) TESOTf, NEt_3, THF, 99%; (d) KHMDS, Me_3OBF_4, DCM, 0 °C to rt; (e) Pd/C, H_2, EtOH; (f) conc. HCl, Δ, 21% (3 steps from **105**); (g) SO_3·pyridine, DMF, 63%.

White and Hansen reported the asymmetric total synthesis of 7-*epi*-cylindrospermopsin in 25 steps (longest linear sequence) and 0.39% overall yield using a convergent methodology. The pivotal step in the synthesis was an intramolecular nitrone 1,3-dipolar cycloaddition arising from the coupling of the eastern and western fragments (**99**→**100**). The synthesis allowed them to assign the absolute stereochemistry of **5** as 7*S*, 8*R*, 10*S*, 12*S*, 13*R*, 14*S*.

The most recent synthetic work regarding the cylindrospermopsin alkaloids has been reported by the Williams research group and details the asymmetric synthesis of cylindrospermopsin and 7-*epi*-cylindrospermopsin as well as the synthesis of racemic 7-*deoxy*-cylindrospermopsin.[33,134,135,141]

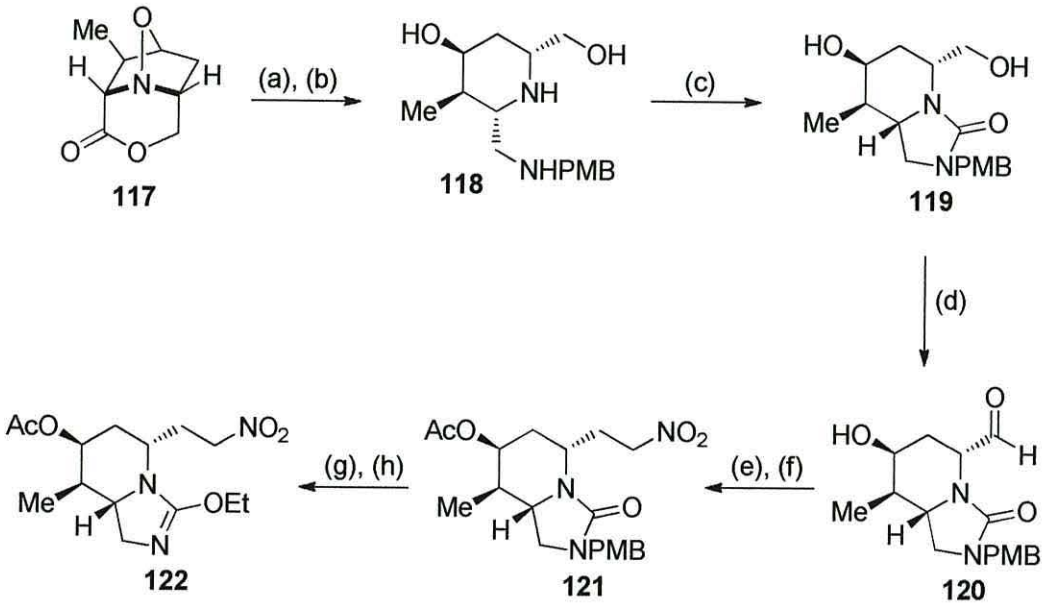
Initially Williams and Looper investigated the utility of a 1,3-dipolar cycloaddition to stereoselectively construct a suitable A ring synthon for use in the synthesis of the cylindrospermopsin alkaloids.[141] The group began with oxazinone **108** which was alkylated with crotyl iodide **109** to give **110** as a single diastereomer in 92% yield, followed by removal of the auxiliary with lithium in ammonia to deliver the Boc-protected crotyl glycine derivative **111** with very high enantioselectivity. The carboxylic acid function of **111** was then reduced with $LiAlH_4$ at 0 °C to give the corresponding alcohol **112** which upon removal of the Boc protecting group with boron trifluoride etherate afforded amino alcohol **113**. Treatment of the resulting amino alcohol **113** with phenyl bromoacetate furnished oxazin-2-one **114** in moderate yields. As **114** was prone to dimerisation, oxidation was carried out immediately after isolation using Davis oxaziridine or *m*-CPBA leading to the conjugated oxazinone-*N*-oxide **115** in 75% or 84% yield respectively. In contrast to **114**, the *N*-oxide **115** was surprisingly stable and no dimerisation or spontaneous cyclisation was observed. Subsequent exposure of oxazinone-*N*-oxide **115** to elevated temperatures cleanly affected the 1,3-dipolar cycloaddition. It was postulated that the nitrone added suprafacially to the alkene predominantly through the chair like *exo*-transition state **116** to give the tricyclic isoxazolidine **117** in 78% yield as a 10:1 mixture of regioisomers (Scheme 12). Isoxazolidine **117** could also be prepared in the improved ratio of 12:1 by treatment of **114** with scandium triflate although the reaction took 3 days to reach completion at ambient temperatures.[135] The stereochemistry of **117** was confirmed by X-ray crystallography.[141]



Scheme 12:-(a) KHMDS, **109**, THF, -78 °C to rt, 92%; (b) Li, NH_3, THF, EtOH, 68-87%; (c) $LiAlH_4$, 0 °C, THF, 62-83%; (d) $BF_3 \cdot Et_2O$, DCM, 5 eqv 1,3-dimethoxybenzene; (e) phenyl bromoacetate, DIPEA, MeCN, 63% (2 steps from **112**); (f) Davis oxaziridine, THF, 0 °C, 75%, or *m*-CPBA, Na_2HPO_4, DCM, -78° C, 84% (g) toluene, 200 °C, sealed tube, 78%.

Reduction of isoxazolidine **117** with DIBAL gave the corresponding lactol which underwent reductive amination with *p*-methoxybenzylamine furnishing diol **118**. Conversion of **118** to bicyclic urea **119** was achieved in 67% yield by treatment with bis-*p*-nitrophenyl carbonate in acetonitrile. Oxidation of the primary alcohol group in **119** to the corresponding aldehyde **120** proved problematic but could be achieved in high yield by treatment with TEMPO and 1.5 equivalents of PIDA in the presence of 1 mol% methanesulfonic acid. Homologation of the resultant aldehyde **120** by addition of lithiated nitromethane produced a scalemic mixture of nitro alcohols which could be dehydrated by treatment with acetic anhydride, concomitant to acetylation of the piperidyl alcohol group. The newly formed nitroalkenes were then reduced with $NaBH_4$ without

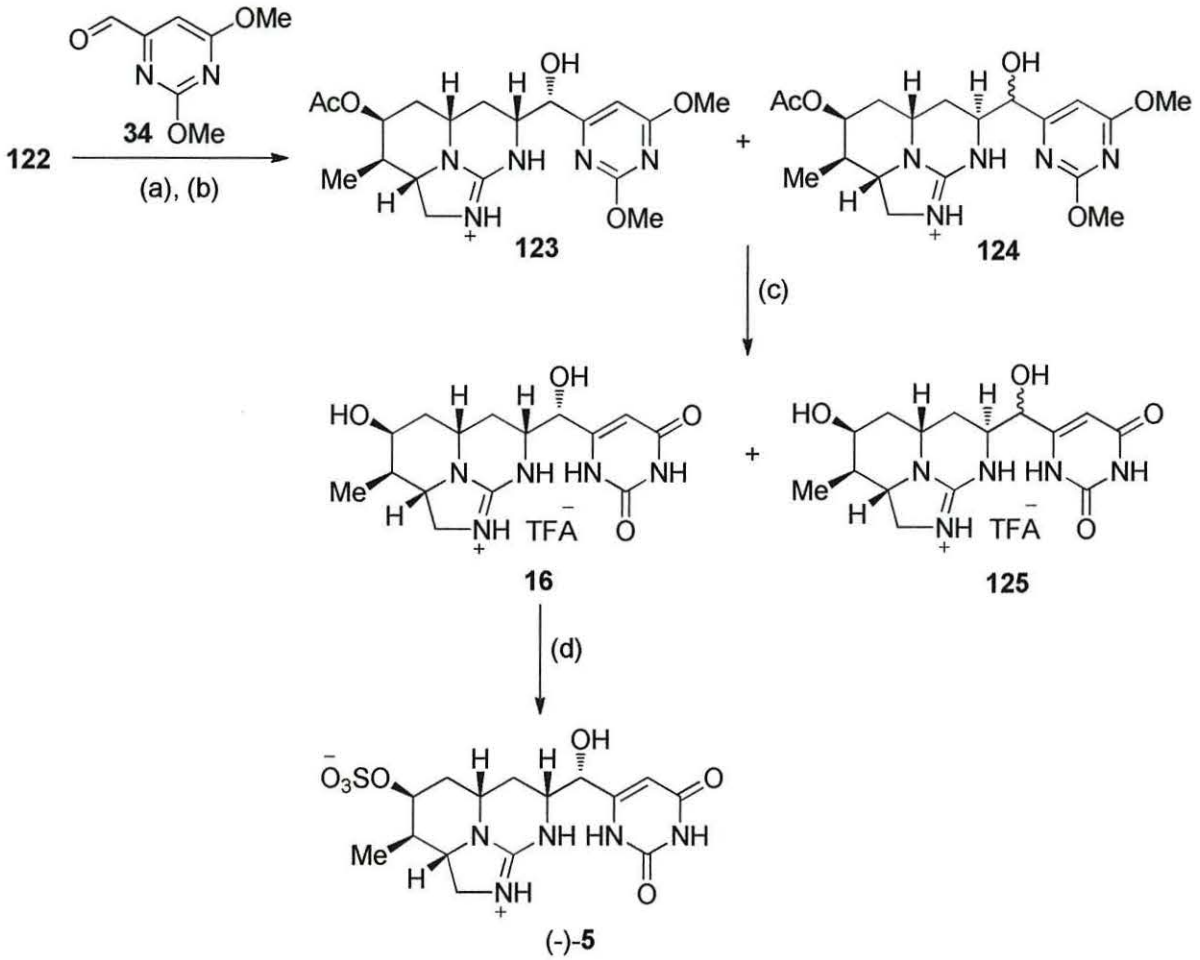
purification furnishing nitroalkane **121** in 56% yield from aldehyde **120**. Finally removal of the *p*-methoxybenzyl protecting group by treatment with refluxing TFA, followed by exposure to triethyloxonium tetrafluoroborate afforded the key reductive guanylation substrate, activated *O*-ethylisourea **122** in 62% yield (Scheme 13).



Scheme 13:- (a) DIBAL, -78 °C; (b) 10% Pd/C, H_2 (1 atm), $PMBNH_2$, EtOAc; (c) (p-$O_2NC_6H_4O)_2CO$, MeCN, 67% (3 steps from **117**); (d) 40 mol% TEMPO, 1.5 eqv $PhI(OAc)_2$, 1 mol% $MeSO_3H$, $CDCl_3$, 75%; (e) $MeNO_2$, *n*-BuLi; (f) Ac_2O, DMAP then $NaBH_4$, EtOH, 56%, (2 steps from **120**); (g) TFA, Δ; (h) Et_3OBF_4, Cs_2CO_3, 62% (2 steps from **121**).

With the *O*-ethylisourea **122** in hand the construction of the C7-C8 bond of the target was undertaken via a nitro-aldol (Henry) reaction. Thus **122** was reacted with dimethoxypyrimidine-4-carbaldehyde **34**, in the presence of 2 molecular equivalents of TBAF for 15 minutes, followed by an acidic quench and reductive guanylation using $Pd(OH)_2/H_2$ in methanol. It was found that short reaction times gave the best selectivity in the nitro-aldol reaction, furnishing the tetracyclic guanidines **123** and **124** as an inseparable 1:0.8 mixture, which favoured **123**, the diastereoisomer required for the synthesis of 7-*epi*-cylindrospermopsin. The acidic quench was also found to be imperative as omitting this step gave an approximate 1:1:1:1 mixture of the four possible diastereomers, indicating that the reaction was highly reversible. Acidic hydrolysis of pyrimidines **123** and **124** furnished a separable mixture of **16** and **125** isolated in 32% and

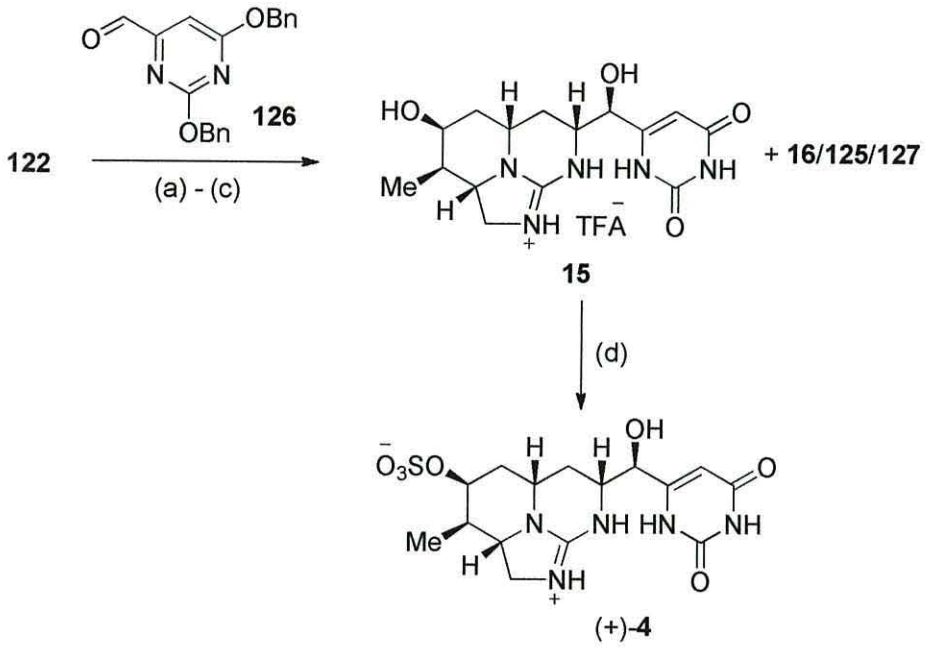
29% yield respectively from **122**. The synthesis was completed by treatment of **16** with sulfur trioxide pyridine complex in the presence of 3 Å molecular sieves reproducibly giving (-)-7-*epi*-cylindrospermopsin in 59% yield (Scheme 14).



Scheme 14:- (a) **34**, 2.0 eqv TBAF, THF, -15 °C, 15 min; (b) $Pd(OH)_2$, H_2, MeOH, 5% AcOH; (c) conc. HCl, Δ, 12 h, 32% (**16**), 29% (**125**) (3 steps from **122**); (d) SO_3·pyridine, 3 Å MS, DMF, 59%.

The group then adapted this methodology for the synthesis of cylindrospermopsin, in this sequence the uracil precursor was constructed in such a way that it would undergo simultaneous deprotection when subjected to the group's reductive guanylation conditions.[135] Thus treatment of *O*-ethylisourea **122** with the dibenzyloxypyrimidine-4-carbaldehyde **126** in the presence of 1 molecular equivalent of TBAF for 30 minutes, followed by reductive guanylation led to a mixture of diastereomers. Partial cleavage of the acetate protecting group was observed under the reaction conditions; unfortunately the

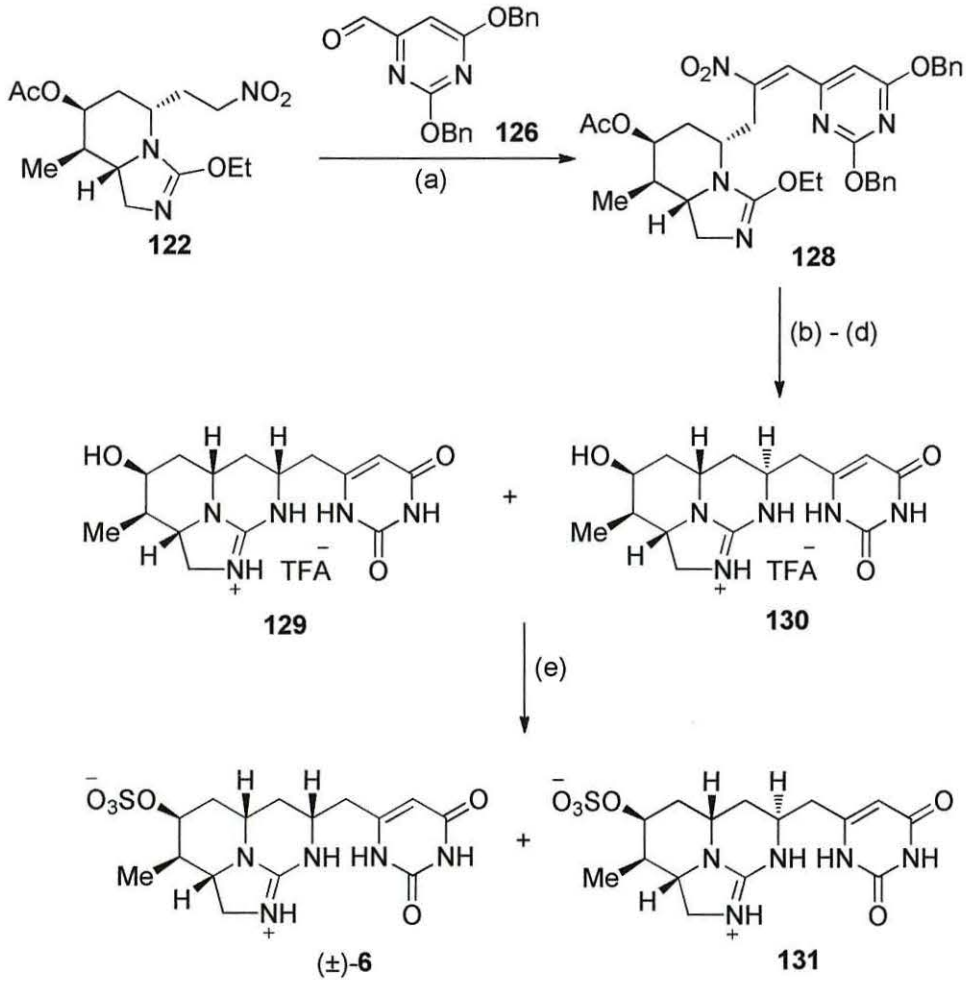
group were unable to drive this to completion, therefore the mixture was exposed to concentrated HCl briefly to fully remove the acetate functionality. Although this three step sequence produced an approximate 1:1:1:0.5 mixture of the four possible diastereoisomers **15:16:125:127**, the overall chemical yield was excellent and the cylindrospermopsin diol **15** was isolated in 20% overall yield. Finally, sulfonation under previously reported conditions afforded (+)-cylindrospermopsin in 60% yield, representing the first enantioselective synthesis of this metabolite (Scheme 15).



Scheme 15:- (a) **126**, 1.0 eqv TBAF, -15 °C, 0.5 h; (b) $Pd(OH)_2$, H_2, MeOH, 5% AcOH; (c) conc. HCl, Δ, 30 min, 20% (**15**) (3 steps from **122**); (d) SO_3·pyridine, 3 Å MS, DMF, 60%.

Having completed the synthesis of the two C-7 hydroxylated cylindrospermopsin alkaloids, effort was directed toward the synthesis of the previously unprepared metabolite 7-*deoxy*-cylindrospermopsin.[33,135] Treatment of racemic **122** with dibenzyloxypyrimidine-4-carbaldehyde **126** in the presence of acetic anhydride and excess caesium fluoride coupled the two units together and allowed dehydration to occur in a single operation, thus affording nitroalkene **128** in 67% yield. Attempts to reduce **128** directly to **129** via the group's previously utilised reductive guanylation conditions returned a complex mixture, containing products arising from hydrolysis of a supposed enamine intermediate. This hydrolysis step was circumvented by subjecting nitroalkene

128 to a two stage conjugate reduction/reductive guanylation using sodium borohydride followed by hydrogenation over Pearlman's catalyst and a brief HCl deprotection leading to a 1:1 mixture of diastereomers **129** and **130**. The final sulfonation step was simplified by the lack of a C-7 hydroxyl group affording (±)-7-*deoxy*-cylindrospermopsin and **131** in a combined yield of 66% (Scheme 16).



Scheme 16:- (a) CsF, Ac_2O, MeCN, 67%; (b) $NaBH_4$, EtOH; (c) $Pd(OH)_2$, H_2, MeOH, 5% AcOH; (d) conc. HCl, Δ, 30 min; (e) SO_3·pyridine, 3 Å MS, DMF, 33% (±**3**) (4 steps from **128**).

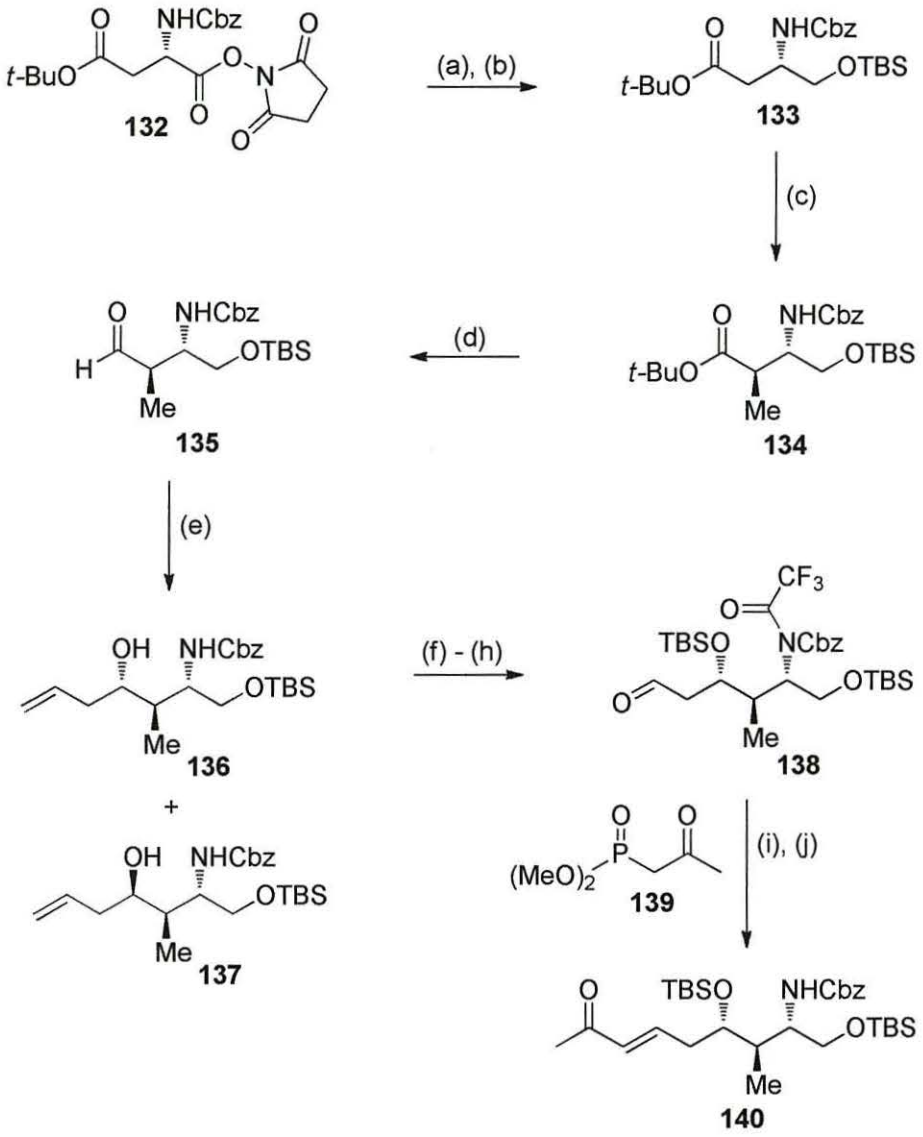
The methodology developed by the Williams group represents the most efficient to date in terms of numbers of reaction steps and overall yields, and uses the least number of protecting group manipulations. It has led to the first enantioselective synthesis of cylindrospermopsin in 19 steps and 0.34-0.57% overall yield and 7-*epi*-cylindrospermopsin in 19 steps and 0.47-0.82% as well as the first racemic synthesis of

the biosynthetic intermediate 7-*deoxy*-cylindrospermopsin in 20 steps and 0.62-1.05% overall yield. The key reactions from these total syntheses are a 1,3-dipolar cycloaddition used to construct the A ring of these metabolites (**115**→**116**) and a Henry reaction of an elaborated nitroalkane with a pyrimidine aldehyde (**122**→**123/15/130**) followed by intramolecular reductive guanylation to form the B-ring.

Model Systems

In addition to the total syntheses reported, several groups have detailed methodology for the preparation of model compounds directed towards the synthesis of the cylindrospermopsin alkaloids.[142-144]

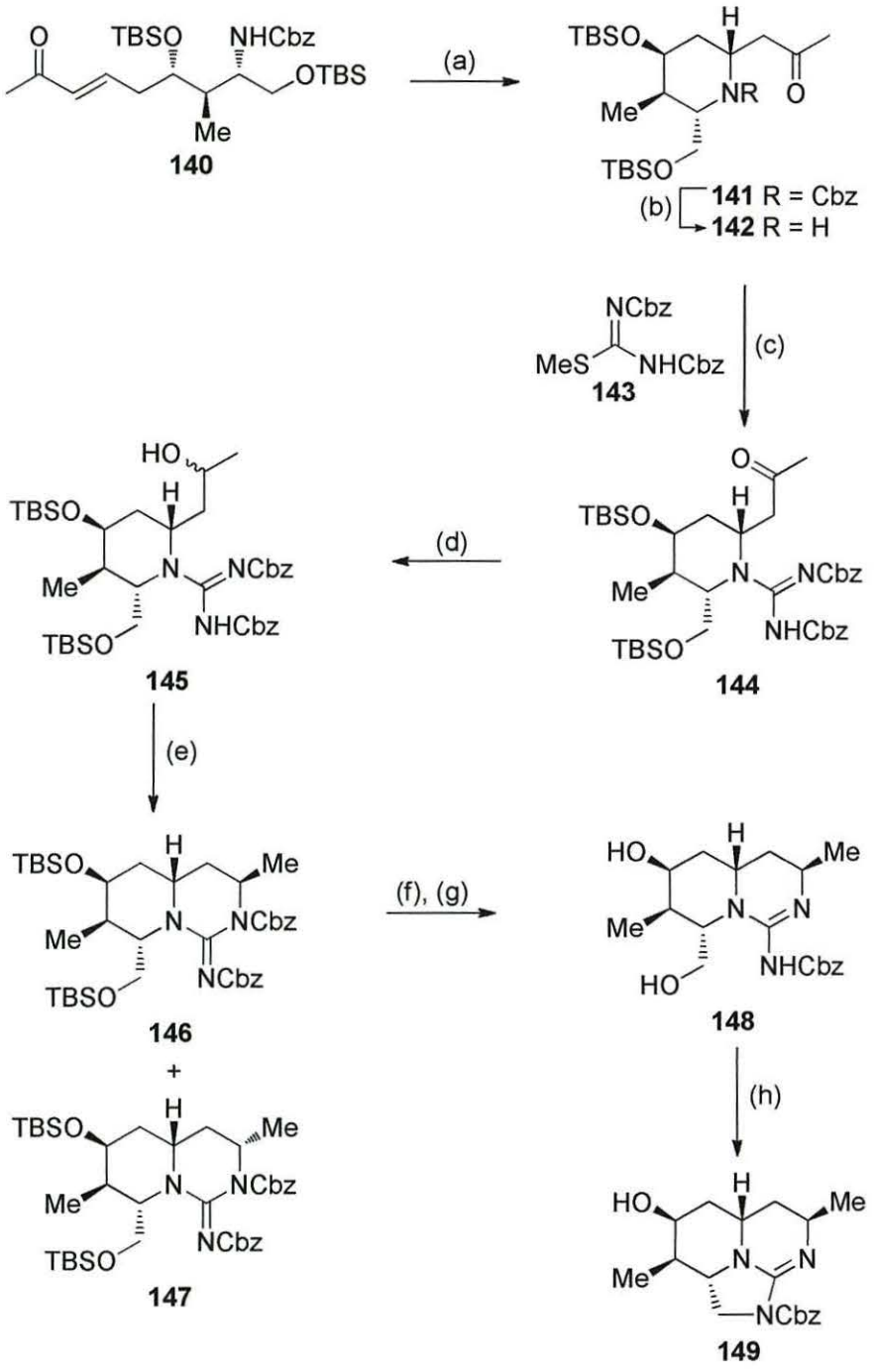
Armstrong and McAlpine have reported the stereoselective synthesis of a tricyclic guanidinium moiety similar to that found in the cylindrospermopsin alkaloids. They employed an intramolecular conjugate addition reaction as a key step in the creation of the piperidine A ring, combined with a Mitsunobu strategy to construct both the B and C rings of the tricycle.[142] Starting with the $NaBH_4$ reduction of commercially available activated aspartic acid derivative **132**, followed by silyl ether protection to give ester **133** in 93% yield. Subsequent addition of methyl iodide to the dianion of **133** at -78 °C gave **134** as the major product. Reduction of the *tert*-butyl ester of **134** with DIBAL gave aldehyde **135** which was allylated using allyl (–)-isopinocamphenylborane giving alcohols **136** and **137** in a 2:1 ratio and 70% yield. This transformation could also be achieved by treatment with allyltributyltin and boron trifluoride etherate in superior yield but with a diminished diastereoselectivity, producing **136** and **137** in a 1.5:1 ratio. Subsequent protection of the alcohol and carbamate groups allowed ozonolysis to proceed smoothly, generating aldehyde **138** in 91% yield. HWE olefination of aldehyde **138** with phosphonate **139** successfully furnished the key cyclisation substrate, enone **140** in 64% yield after workup with methanolic sodium carbonate (Scheme 17).



Scheme 17:- (a) $NaBH_4$, THF, 0 °C; (b) TBSOTf, NEt_3, 2,6-lutidine, 93% (2 steps from **132**); (c) i) LDA, LiCl, THF, -78 °C, ii) MeI, 72%; (d) DIBAL, toluene, -78 °C, 89%; (e) $BF_3 \cdot OEt_2$, allylBu_3Sn, DCM, -78 °C, 51% (**136**), 33% (**137**); (f) TBSOTf, 2,6-lutidine, 100%; (g) TFAA, NEt_3, 90%; (h) O_3, PPh_3, DCM, -78 °C, 91%; (i) LDA, **139**, THF; (j) Na_2CO_3, MeOH, 64%.

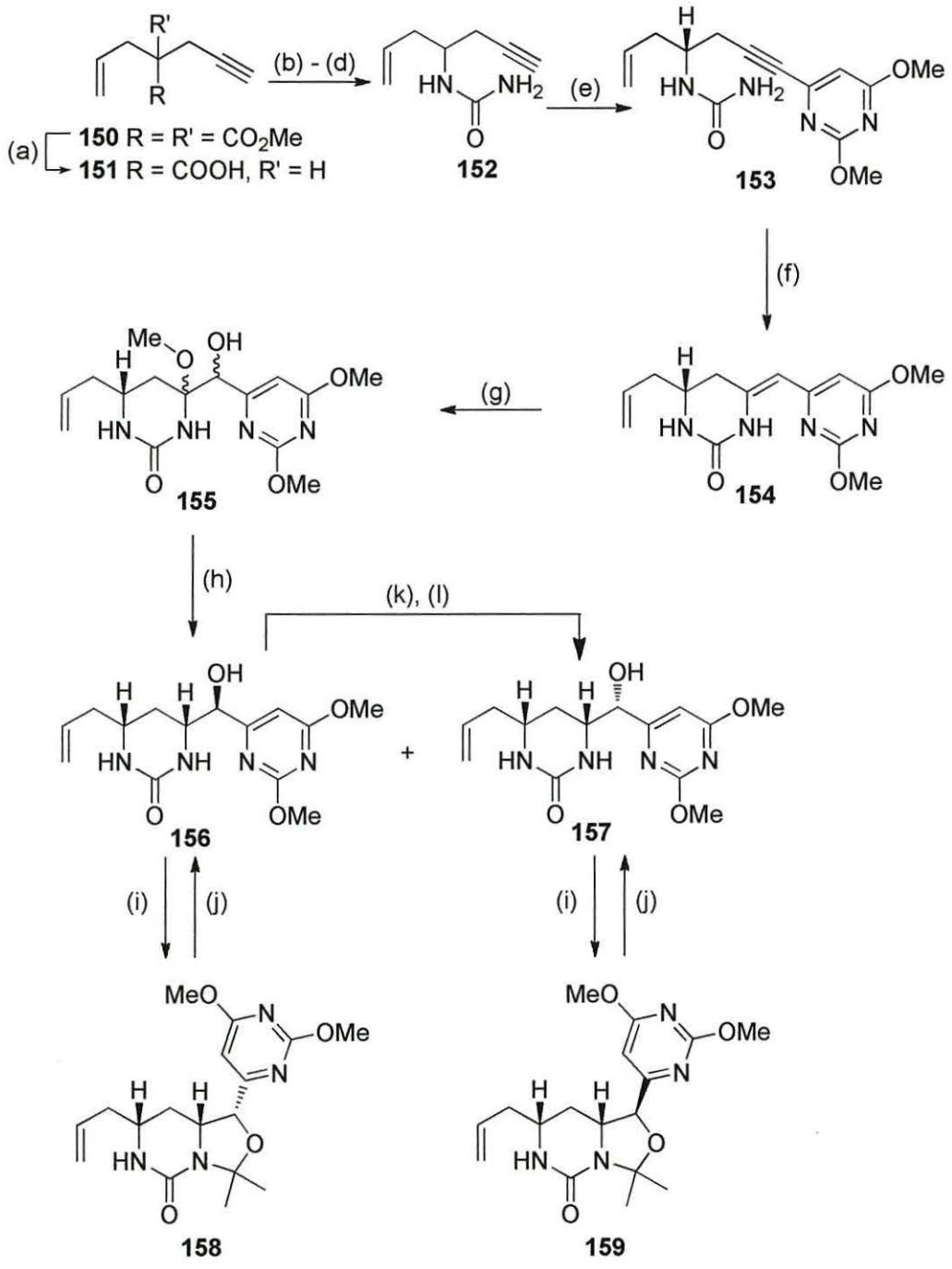
Having prepared enone **140** the key cyclisation reaction was effected by treatment with a catalytic amount of *p*-TsOH in refluxing benzene, giving Cbz protected pyrimidine **141** as a single diastereomer having all four stereocenters of the cylindrospermopsin A ring in place. Hydrogenation of **141** quantitatively generated the corresponding free piperidine **142**, subsequent guanylation of which with methylthioisourea **143** gave the corresponding *bis*-Cbz guanidine **144** in 85% yield. Reduction of the methyl ketone

function of guanidine **144** was conducted under non-diastereocontrolled conditions giving an inseparable 5:1 mixture of alcohols **145**, which upon cyclisation under Mitsunobu conditions gave bicyclic guanidines **146** and **147** in a 5:1 ratio and 75% combined yield. Unfortunately, it was the minor bicycle **147** that was shown to display the desired stereochemistry around the newly formed B ring, however the overall feasibility of the approach was demonstrated using the major product bicyclic guanidine **146**. Selective removal of one Cbz group with sodium hydride in a 1:1 mixture of THF and methanol, followed by treatment with TBAF furnished bicyclic diol **148**. A further Mitsunobu cyclisation using triphenylphosphine and DIAD installed the final C ring giving tricyclic guanidine **149** as the only isolated product in 27% yield (Scheme 18).



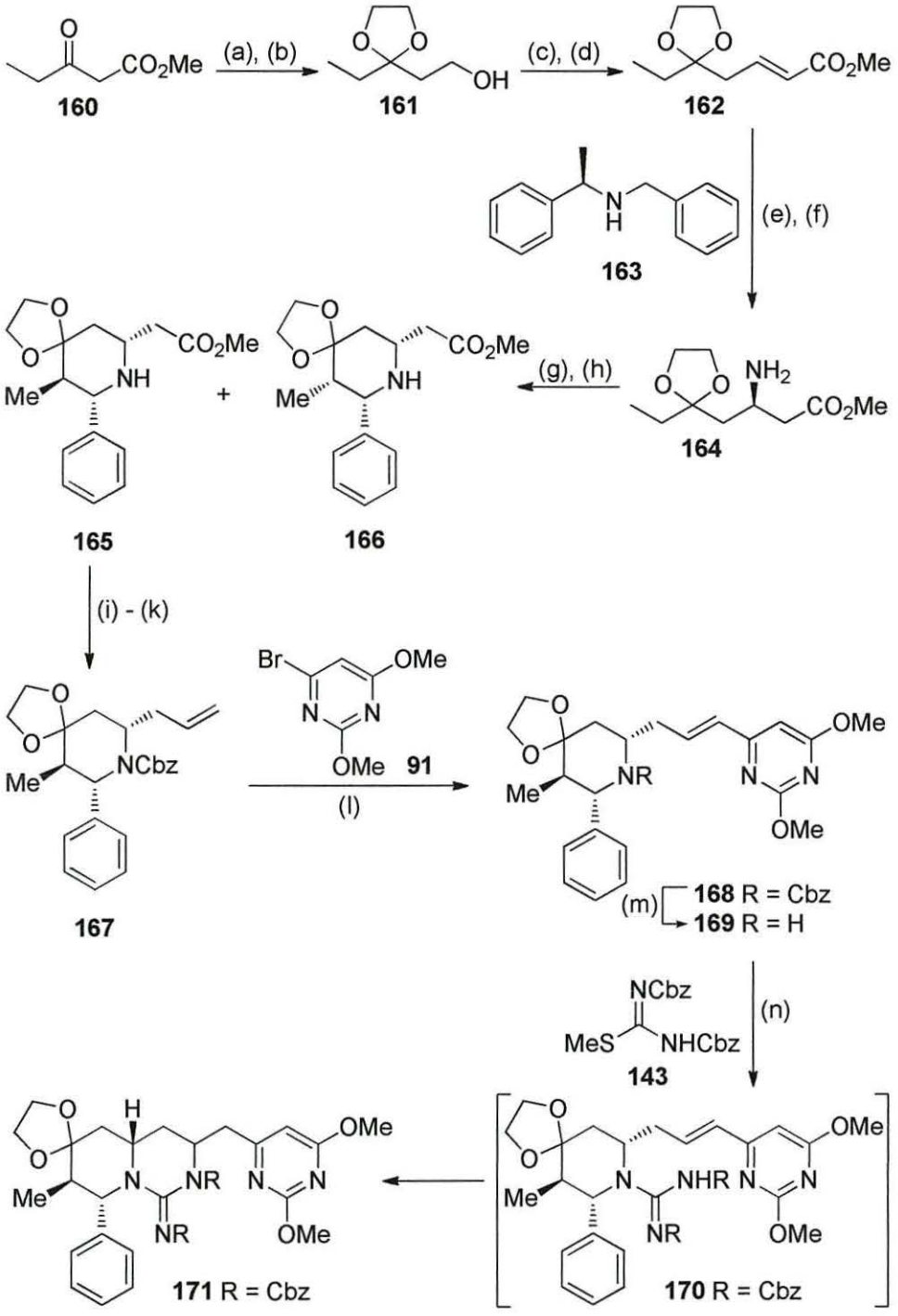
Scheme 18:- (a) *p*-TsOH, benzene, 74%; (b) Pd/C, H_2, 100%; (c) **143**, $HgCl_2$, NEt_3, DMF, 85%; (d) $NaBH_4$, MeOH, 100%; (e) PPh_3, DIAD, 63% (**146**), 12% (**147**); (f) NaH, THF, MeOH, 67%; (g) TBAF, THF, 84%; (h) PPh_3, DIAD, 27%.

A further synthetic approach towards cylindrospermopsin was reported by the Hart research group who developed a methodology for the synthesis of the BD ring system of the toxin, the group employed an intramolecular conjugate addition of a urea to an alkynyl pyrimidine as the key reaction.[143] Saponification of the known diester **150**, followed by acidification and decarboxylation furnished carboxylic acid **151** in 90% yield which was converted to alkynyl urea **152** in high overall yield via a Curtius rearrangement, trapping the intermediate isocyanate with ammonia. Coupling of **152** with 4-bromo-2,6-dimethoxypyrimidine **91** under Sonogashira conditions gave alkynyl pyrimidine **153** in 90% yield. The key step of the sequence was effected by the treatment of **153** with sodium hydride in THF resulting in the clean formation of cyclic urea **154** in very high yield. Treatment of **154** with DMDO in the presence of methanol gave an unstable mixture of *N,O*-acetals **155**, which upon reduction with $NaBH_3CN$ gave an inseparable 78:22 mixture of isomeric alcohols **156** and **157**. Fortunately separation could be achieved by conversion of the mixture of alcohols to their corresponding cyclic *N,O*-acetals **158** and **159**, which were isolated in yields of 58% and 16% respectively. Regeneration of the desired alcohols could then be achieved by acidic hydrolysis giving **156** and **157** in 70% and 60% yields respectively. The major isomer **156** could then be converted to the epimeric alcohol **157** via a Mitsunobu inversion procedure (Scheme 19).



Scheme 19:- (a) i) NaOH, ii) H_3O^+, iii) Δ; (b) $(COCl)_2$; (c) NaN_3, Δ; (d) NH_3, 86%, (3 steps from **151**); (e) $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, **91**, 90%; (f) NaH, THF, 97%; (g) DMDO, MeOH/DCM, acetone; (h) $NaBH_3CN$, MeOH, H_2O, pH 4, 97% (**156** and **157:** 78:22) (2 steps from **154**); (i) $Me_2C(OMe)_2$, CSA, 58% (**158**) 16% (**159**) (3 steps from **154**); (j) HCl, 70% (**156**) 60% (**157**); (k) *p*-NBA, DEAD, PPh_3, THF, Δ; (l) K_2CO_3, MeOH, 91% (2 steps).

The most recent approach towards this family of secondary metabolites has been detailed by Henon and Troin who successfully constructed an ABD ring system comparable to that found in this family of natural products.[144] Key steps in the pairs sequence involve an intramolecular Mannich reaction to form the A ring, followed by addition of guanidine to an activated double bond to form the B ring. Starting from commercially available methyl-3-oxopentanoate **160**, the keto function of which was protected with ethylene glycol followed by $LiAlH_4$ reduction of the methyl ester to afford alcohol **161** in 85% yield. Oxidation of **161** with manganese dioxide gave the corresponding aldehyde, which underwent Wittig olefination to give the unsaturated ester **162** in 56% yield. Diastereoselective conjugate addition of (*R*)-*N*-benzyl-1-phenylethylamine **163** led, after hydrogenation over Pearlman's catalyst to amine **164**, prepared in 6 steps and 26% overall yield from ketone **160**. The key intramolecular Mannich reaction of amine **164** with benzaldehyde under conditions previously developed by the group cleanly afforded a 3:2 mixture of piperidines **165** and **166**, which were isolated in 62% yield and separated chromatographically. The major isomer **165** displayed the correct orientation around the piperidine A ring and was used by the group in the following reactions. Subsequent Cbz protection of the secondary amine functionality of **165**, followed by DIBAL reduction furnished the corresponding aldehyde, which was used without purification in a Wittig olefination with methyl triphenylphosphonium bromide giving piperidine **167** in 55% yield over three steps. Heck cross-coupling of **167** with 4-bromo-2,6-dimethoxypyrimidine **91** gave the desired piperidine **168** in a poor yield of 31%, which could not be improved by modification of the reaction conditions. Removal of the carbamate protecting group was achieved by exposure to TMSI in MeCN, giving the corresponding free piperidine **169** in 67% yield. Treatment of **169** with methylthioisourea **143** in the presence of $HgCl_2$ successfully guanylated the hindered piperidine nitrogen and the resulting guanidine **170** underwent spontaneous cyclisation to give the bicyclic guanidine **171** in 45% yield upon attempted purification on silica gel (Scheme 20).



Scheme 20:- (a) $(CH_2OH)_2$, *p*-TsOH, toluene; (b) $LiAlH_4$, THF, 85% (2 steps from **160**); (c) MnO_2; (d) $Ph_3P{=}CHCO_2Me$, 56% (2 steps from **161**); (e) **163**, BuLi, 0 °C, 72%; (f) $Pd(OH)_2$, H_2 (5 atm), $MeOH/H_2O/AcOH$, 75%; (g) $MgSO_4$, PhCHO, DCM, Δ; (h) *p*-TsOH, toluene, 65 °C, 62% 3:2 (**165**:**166**) (2 steps from **164**); (i) CbzCl, Na_2CO_3; (j) DIBAL; (k) $MePPh_3^+ Br^-$, *n*-BuLi, THF 0 °C, 55% (3 steps from **165**); (l) **91**, $Pd(OAc)_2$, PPh_3, Na_2CO_3, DMF, 31%; (m) TMSI, MeCN, 67%; (n) **143**, $HgCl_2$, NEt_3, DMF, 0 °C, 45%.

Ideality in Synthesis

The cylindrospermopsin alkaloids are a family of structurally unique secondary metabolites that display potent toxicity towards several organ systems and cell lines as well as the potential to act in a carcinogenic capacity. The potent biological activities displayed by these metabolites combined with their widening occurrence and complex functionality has generated a great deal of interest from within the synthetic community, leading to four different groups reporting elegant multi-step total syntheses of all three metabolites.[28]

Judging the efficiency of a chemical synthesis is a difficult process as the objective of a practitioner is not to optimise each reaction step to an industrial level but to explore methodology with the ultimate goal of reaching their target molecule. Consequently the chemical yield, cost and ease in which a certain transformation can be achieved may pay a part in its selection within a total synthesis. Recently the Baran group of the Scripps research institute have addressed this; publishing a simple numerical method to quantify the ideality of a chemical synthesis with the aim of allowing synthetic practitioners the ability to easily make comparisons and pinpoint areas of improvement.[145] The group propose that the percentage "ideality" of a synthesis can be quantified using the following equation:

$$\%\, ideality = \frac{[(\text{No. of construction reactions}) + (\text{No. of strategic redox reactions})]}{(\text{total number of steps})} \times 100$$

Construction reactions (CR) are defined as those which form skeletal bonds (C-C and C-heteroatom) as well as those that directly establish the correct functionality found in the final product such as *strategic redox reactions* (SR). All other reactions fall into the category of a concession step, these are defined as *nonstrategic redox manipulations* (NSR), *functional group interconversions* (FGI) and *protecting group manipulations* (PGM).[145]

Although the concept of percentage ideality does not provide an ultimate measure of a chemical synthesis it does provide a method for comparing the syntheses of the same

target molecule, allowing it to be readily applied to the reported synthesis of cylindrospermopsin, 7-*epi*-cylindrospermopsin and 7-*deoxy*-cylindrospermopsin (Table 2).

Target	Research Group	Steps	Chemical Yield (%)	NSR	PGM	FGI	SR	CR	% Ideality
4	Snider[1] (2000)	20	1.50-2.00	2	8	1	2	7	45%
	Weinreb[1] (2002)	36	0.20-0.25	3	12	8	2	11	36%
	Williams (2006)	19	0.34-0.57	3	5	2	0	9	47%
5	Weinreb[1] (2001)	33	0.26	3	10	8	2	10	36%
	Williams (2004)	19	0.47-0.82	3	5	2	0	9	47%
	White[2] (2002)	25	0.39[2]	3	9	5	2	6	32%
6	Williams[1] (2005)	20	0.62-1.05	3	5	2	1	9	50%

[1]Racemic [2]Based on longest linear sequence.

Table 2:- Percentage ideality of currently published cylindrospermopsin syntheses.

On comparing the results obtained from using Barans methodology it is impressive to note that all the reported synthetic routes score above 30% ideality. The most efficient reported syntheses are those of the Williams group scoring between 47% and 50%, with the highest overall chemical yield being achieved by the Snider group's synthesis of (±)-cylindrospermopsin. Such high levels of synthetic ideality achieved by the Williams group set a very high standard for preparing the metabolites of this structurally complex family of natural products, a feat the group achieved through using the fewest synthetic steps and the least protecting group manipulations.

Aims

Initially it was anticipated that cylindrospermopsin and 7-*epi*-cylindrospermopsin could be separated into two distinct fragments consisting of the uracil D ring and the tricyclic ABC guanidinium core. The tricyclic ring system containing an embedded guanidine group is unique to this family of natural products and will prove to be a challenging synthetic target, it is hoped that this element of the metabolites can be prepared in a stepwise manner mimicking their proposed biosynthesis (Figure 11).

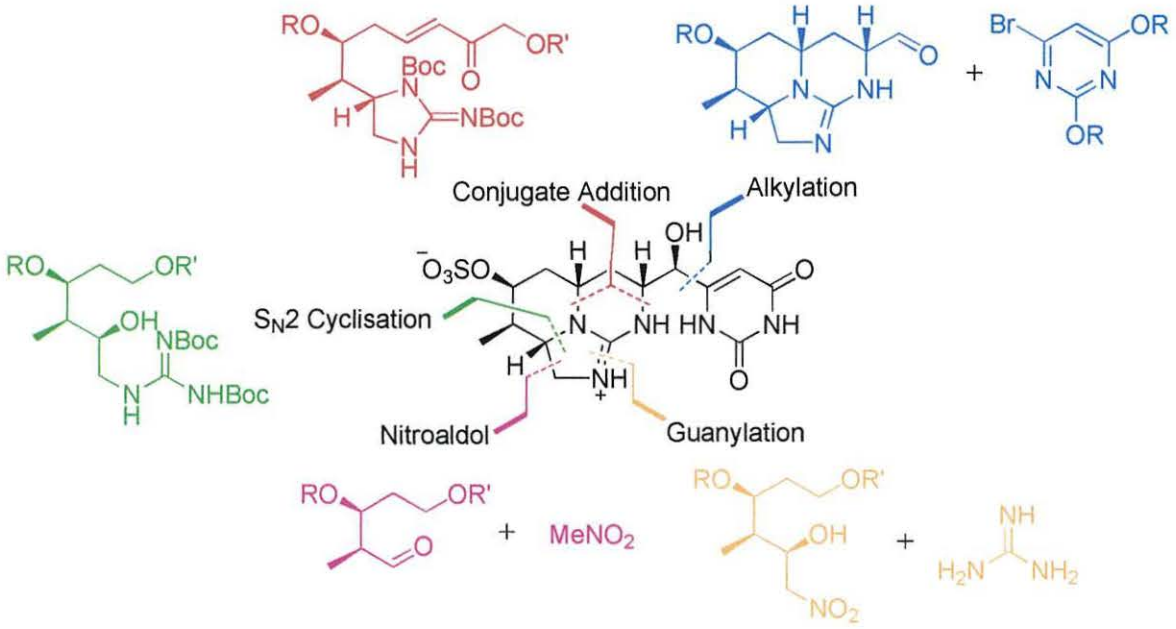
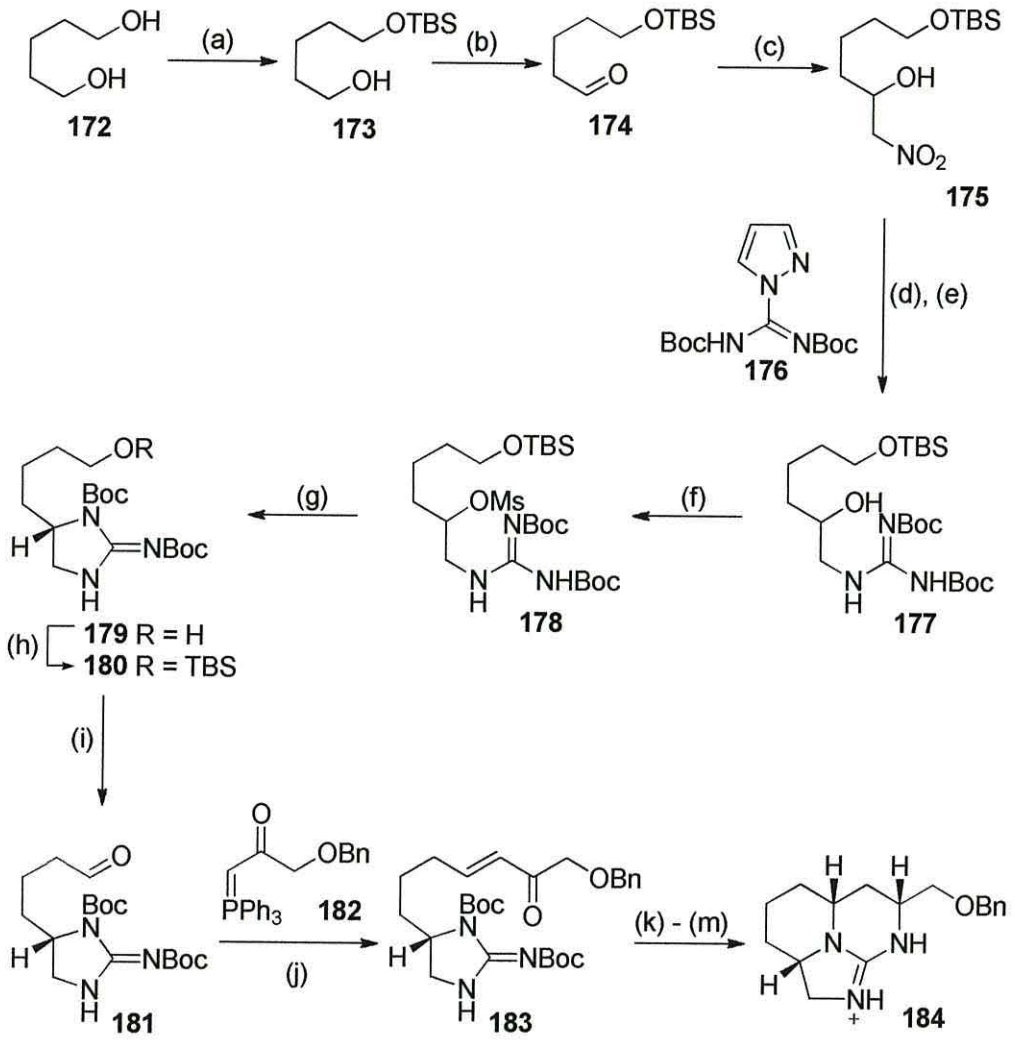


Figure 11:- Retrosynthetic analysis of cylindrospermopsin.

Initial work will concentrate on the preparation of a simplified version of the ABC ring system of the toxins devoid of the methyl and sulphonic acid functionality. Believed to be key to the preparation of such a structural model is the initial stereochemistry of the C ring which is hoped will dictate the relative stereochemical outcome of future ring closures; ultimately leading to an all *syn* relationship as displayed in the target natural products.

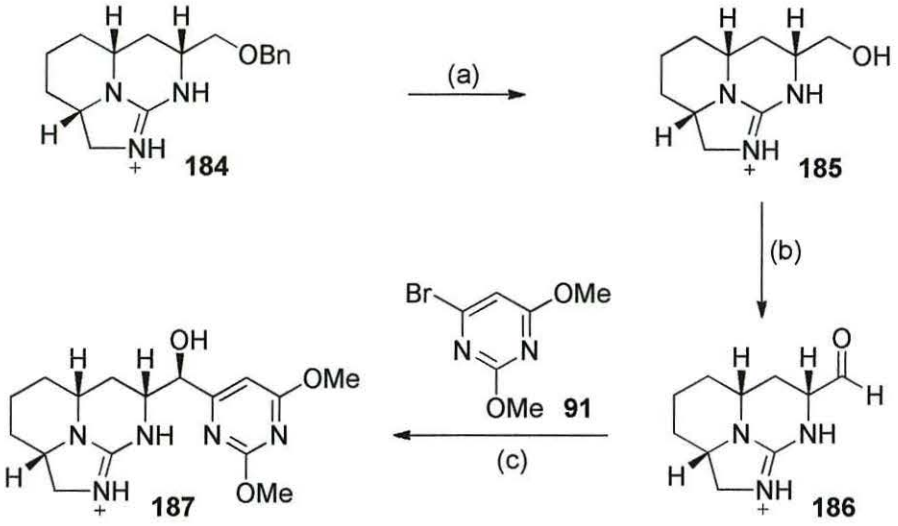
Construction of a model tricyclic system will begin with commercially available 1,5-pentane diol **172** which can be mono-protected as the corresponding silyl ether **173** and the remaining alcohol function oxidised under Swern conditions to give protected aldehyde **174**. A subsequent nitro-aldol (Henry) reaction between **174** and nitromethane will furnish β-hydroxy nitro compound **175**, reduction of the nitro functionality of which to the corresponding primary amine followed by exposure to **176** should give rise to guanidine **177**. At this juncture a good leaving group such as a mesyl group can be introduced at the secondary alcohol position furnishing mesylate **178**, which is hoped will undergo intramolecular cyclisation to afford heterocycle **179** containing the C ring of cylindrospermopsin. The silyl protecting group of **179** can then be removed by treatment with TBAF to give primary alcohol **180** which can oxidised to the corresponding aldehyde **181**. Aldehyde **181** can then be subjected to a Witting olefination with a suitably functionalised phosphorane such as **182** to deliver cyclisation substrate, enone **183**. Removal of the Boc protecting groups of **183** by treatment with TFA should initiate cyclisation ultimately leading to the proposed tricyclic model **184** (Scheme 21).



Scheme 21:- (a) NaH, TBSCl, THF; (b) $(COCl)_2$, DMSO, NEt_3, DCM; (c) $MeNO_2$, DIPEA, DCM; (d) NH_4HCO_2, Pd/C, MeOH; (e) **176**; (f) Ms_2O, pyridine, DMAP, DCM; (g) NEt_3, DCM, Δ; (h) TBAF, THF; (i) $(COCl)_2$, DMSO, NEt_3, DCM; (j) **181**, DCM; (k) TFA, DCM; (l) NH_4OAc, Δ; (m) $NaBH_3CN$, MeOH.

If tricyclic guanidine **184** can be successfully prepared, incorporation of the uracil D ring and bridging hydroxymethylene group will prove crucial in determining the utility of the proposed synthetic pathway. One way this could be achieved in through the unmasking of the protected hydroxyl group of **184** to give primary alcohol **185** which can be oxidised to the corresponding aldehyde **186**, subsequent coupling of **186** with lithiated pyrimidine **91** should allow the requisite functionality to be introduced. Tailoring the reaction conditions employed in the coupling of **186** and **91** to allow for the production of a single diastereoisomer is key to the application of this methodology to the

stereoselective total synthesis of cylindrospermopsin and 7-epi-cylindrospermopsin (Scheme 22).

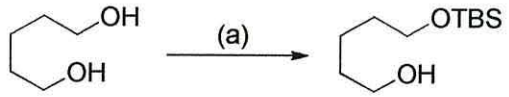


Scheme 22:- (a) H_2, Pd/C, MeOH; (b) $(COCl)_2$, DMSO, NEt_3, DCM; (c) *n*-BuLi, **91**.

If the proposed pathway to a model system representing the cylindrospermopsin alkaloids proves successful it will demonstrate that the chirality present in the initially formed C ring dictates the relative stereochemistry of the A and B rings during their formation and thus mimics the proposed biosynthetic pathway of these metabolites. The methodology should prove versatile enough that the early synthetic precursors can be elaborated to include the requisite functionality of the core tricyclic ring system. A key challenge to the application of this approach to a stereoselective total synthesis would be the development of a highly selective nitro-aldol reaction as the stereochemistry generated at this juncture is key to the latter formation of the tricyclic ring system.

Results and Discussion

The first reaction to be undertaken was the mono-silylation of 1,5-pentanediol, which was performed following the methodology of McDougal and co-workers who described the mono TBS protection of several symmetric diols.[146] Thus alcohol **172** was treated with NaH to generate the corresponding alkoxide which upon exposure to TBSCl furnished silyl ether **173** (Scheme 22).



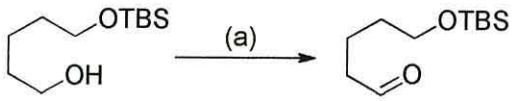
172 **173**

Scheme 22:- (a) i) NaH, THF, rt, 1 h; ii) TBSCl, rt, 1 h, 58-67%.

The reaction proceeded as described in the literature giving the desired TBS ether **173** in isolated yields ranging from 58% to 67%, however the highest yield obtained was considerably lower than that of 87% quoted in the literature.[146] Several repeats of the reaction were carried out on varying scales in order to produce more material for subsequent reactions in the pathway, but the yield could not be improved upon. It is likely that the quality of the sodium hydride used and the hygroscopic nature of the starting material **172** play a key role in the outcome of the reaction.

The successful preparation of the desired compound was confirmed by the spectroscopic data obtained, which were concordant with the literature data.[146] The ^{1}H NMR spectrum clearly showed the incorporation of the desired silyl ether group, evident by the presence of two intense singlets at 0.02 ppm and 0.84 ppm representing the two silicon bound methyl groups and the three *tert*-butyl methyl groups respectively. The ^{13}C NMR spectrum was in agreement, containing signals at -5.6 ppm and 21.7 ppm representing the silicon bound carbon atoms and *tert*-butyl methyl groups, with the quaternary carbon atom of the newly introduced protecting group resonating at 18.0 ppm.

With selectively protected alcohol **173** in hand, generation of the known aldehyde **174** via the oxidation of the remaining primary alcohol functional group was undertaken (Scheme 23).



173 **174**

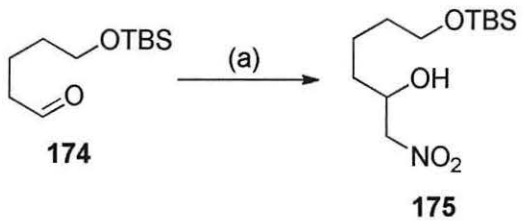
Scheme 23:- (a) i) $(COCl)_2$, DMSO, -78 °C, 20 min; ii) **173**, -78 °C, 20 min; iii) NEt_3, -78 °C, 3 h, 77-89%.

Aldehyde **174** is a known compound and has been regularly reported to be prepared from the PCC oxidation of alcohol **173** as recently as 2007.[147,148] However, due to the carcinogenic nature of hexavalent chromium compounds and the difficulties that can be encountered during the workup of this reaction, it was felt that a safer and more convenient alternative to achieve the desired transformation would be the Swern oxidation which utilises a dimethylchlorosulfonium species generated *in-situ* as an oxidant .[149-152]

The Swern oxidation of alcohol **173** proceeded smoothly and after aqueous workup crude **174** was obtained. Initially it was attempted to purify the reaction material by column chromatography using an ethyl acetate/petroleum ether solvent system on silica gel. However, attempted purification by standard silica gel chromatography led to the formation of a complex mixture of compounds from which **174** could not be isolated. Fortunately, quick filtration of a petrol solution of the crude product through a short plug of silica gel (ca 1cm) eluting with petrol produced the requisite aldehyde **174** in yields ranging from 77-89%.

Confirmation of the formation of **174** was given by the disappearance of the broad OH stretch at 3417 cm^{-1} in the IR spectrum and the appearance of a stretch corresponding to an aliphatic aldehyde at 1728 cm^{-1}. The ^{1}H and ^{13}C NMR spectra also included signals diagnostic of an aldehyde functional group, resonating at 9.73 ppm and 202.4 ppm respectively, and were in agreement with the literature data.[153]

Having successfully prepared aldehyde **174**, preparation of β-hydroxy nitro compound **175** was undertaken by exposing a solution of **174** in DCM to $MeNO_2$ in the presence of a tertiary base (Scheme 25).[154,155]



174 **175**

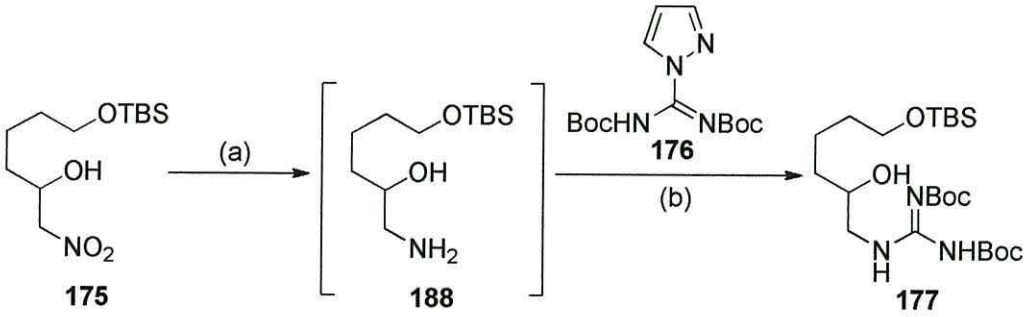
Scheme 25:- (a) $MeNO_2$, DIPEA, DCM, 0 °C to rt, 5 d, 67-81%.

The nitro-aldol reaction between aldehyde **174** and nitromethane proceeded slowly with TLC analysis showing the consumption of aldehyde **174** to take up to five days. However, upon aqueous work up and after column chromatography nitro-alcohol **175** was produced in isolated yields of between 67% and 81%. The preparation of the desired nitro compound was evident by the initial inspection of the IR spectrum which contained a broad OH stretch at 3418 cm^{-1} representing the newly formed alcohol functional group; this was accompanied with the NO_2 asymmetric and symmetric stretches at 1556 cm^{-1} and 1421 cm^{-1} respectively.

Further evidence for the preparation of nitro alcohol **175** was seen in the NMR spectra obtained. The indicative signal was that of the newly introduced methylene group which was observed resonating at 80.6 ppm in the ^{13}C spectrum and at 4.37 ppm and 4.42 ppm in the ^{1}H spectrum, appearing as two double doublets sharing a coupling constant of 12.6 Hz. Inspection of the low resolution CI mass spectrum showed a range of fragments with the highest molecular weight fragments corresponding to the ammonium adduct of the desired compound at 295 and the $[M+H]^+$ ion at 278. The high resolution ESI mass spectrum detected a $[M+H]^+$ ion of 287.1784 closely matching the theoretical mass of 287.1782 and thus confirming the correct molecular formula of nitro alcohol **175**.

The next step in the sequence involved the reduction of the newly introduced nitro functionality to give primary amine **188** which can be exposed to the commercially

available guanylating agent **176** in order to furnish β-hydroxy guanidine **177** (Scheme 26).



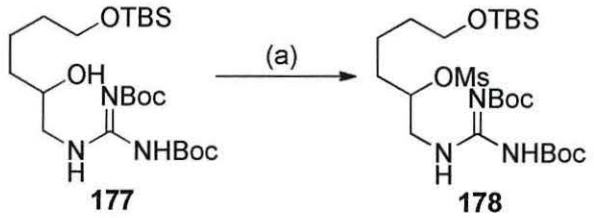
Scheme 26:- (a) $NiCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH, NEt_3, 0 °C to rt, 3 h 15 min; (b) **176**, 48 h, 57-78% (over 2 steps).

Initially the reduction of **175** was investigated using catalytic transfer hydrogenation with ammonium formate and Pd/C. Unfortunately this method of reduction proved unsuccessful returning only unreacted starting material, exchanging the hydrogen source from ammonium formate to gaseous H_2 showed no improvement.[156] This led to the investigation of an alternative reduction method utilising Ni_2B generated *in-situ* from $NiCl_2$ hexahydrate and $NaBH_4$, under these conditions the corresponding primary amine **188** was successfully generated.[157-159] In order to perform the subsequent guanylation step without isolating the intermediary amine **188** sufficient triethylamine was required in order to neutralise residual boric acid form the reduction step. Thus after treatment with triethylamine, exposure of amine **188** to *bis*-Boc guanidine pyrazole **176** gave the desired Boc protected guanidine **177** in yields ranging from 57-78% over 2 steps.[160]

Inspection of ^1H NMR showed the presence of signals associated with the Boc protecting groups of guanidine **177** which appeared as two singlets at 1.47 ppm and 1.52 ppm. The ^{13}C NMR spectrum was slightly more complicated as two signals were present for each different chemical environment of the Boc protecting groups. Signals were evident at 28.0 ppm 28.2 ppm representing the *t*-butyl methyl groups and 79.5 ppm and 83.4 ppm corresponding to the *t*-butyl quaternary carbon atoms. The three most downfield signals of the spectrum appeared at 153.1 ppm 157.4 ppm and 162.9 ppm representing the indicative guanidine quaternary carbon atom and two Boc-carbonyl atoms respectively. The presence of guanidine **177** was further confirmed by mass spectrometry, where the

corresponding $[M+H]^+$ ion was clearly detected and shown to have a mass analogous to the calculated mass for an ion with the same molecular formula.

Having introduced the required *bis*-Boc guanidine functionality, preparation of the corresponding mesylate was undertaken. Thus a solution of guanidine **177** in DCM was treated with mesyl anhydride, pyridine and a catalytic amount of DMAP (Scheme 27).[161]



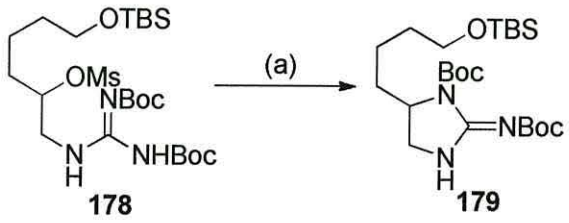
177 **178**

Scheme 27:- (a) Ms_2O, pyridine, DMAP, DCM, 4 d, 84%.

The progress of the reaction was followed by TLC, and upon consumption of the alcohol starting material the reaction was subjected to an aqueous workup, and the crude material purified by column chromatography on silica gel using an ethyl acetate/petroleum ether solvent system. After workup and purification mesylate **178** was obtained in a high yield of 84%. Initial inspection of the IR spectrum of the purified material showed the disappearance of the broad OH stretch at 3300 cm^{-1} and the incorporation of stretches at 1368 cm^{-1} and 1136 cm^{-1} representing the asymmetric and symmetric stretches of the newly introduced mesyl functional group.

Further confirmation that the desired transformation had taken place was observed in the ^1H NMR spectrum where a signal corresponding to the mesylate methyl group was observed as a singlet at 3.30 ppm, this was accompanied by a change in chemical shift for the adjoining methine proton from 3.75 ppm in guanidine **177** to 4.14 ppm in **178**. These observations were mirrored in the ^{13}C NMR spectrum where a new methyl signal was observed at 40.3 ppm which was again accompanied by a change in the chemical shift of the neighbouring methine proton from 71.8 ppm in the starting material to the more upfield resonance of 55.7 ppm. Problems arose when trying to obtain mass spectroscopic data for **178** as the highest molecular weight fragment observed was at 551 Daltons, 18 mass units below that that of the expected $[M+H]^+$ ion of the desired compound

suggesting the loss of a molecule of water. Whilst the mass spectroscopic data cast some doubt on the exact structure of **178**, attempts to induce the key intramolecular cyclisation were undertaken. Thus a solution of mesylate **178** in anhydrous acetonitrile was heated to 80 °C in the presence of excess DIPEA (Scheme 28).[162]



Scheme 28:- (a) DIPEA, CH_3CN, 80 °C.

The progress of the ring closure was monitored by TLC. However, up to 72 hours after the reaction had been initiated only unreacted starting material was detected. Regardless the reaction was quenched with a saturated solution of NH_4Cl and extracted with chloroform. Unfortunately the presence of only unreacted starting material was confirmed by both ^1H and ^{13}C NMR. With no more mesylate in hand more forcing conditions such as performing the transformation or in a sealed vessel or at a higher temperature in a higher boiling solvent could not be immediately investigated. Therefore an alternative cyclisation methodology was investigated alongside the preparation of further mesylate **178**.

Recently the Ōmura research group had reported the total synthesis of three natural products containing a similar structural motif to heterocycle **179**, namely Guadinomine B **189**, Guadinomine C_2 **190** and Guadinomic acid **191** (Figure 11).[162,163]

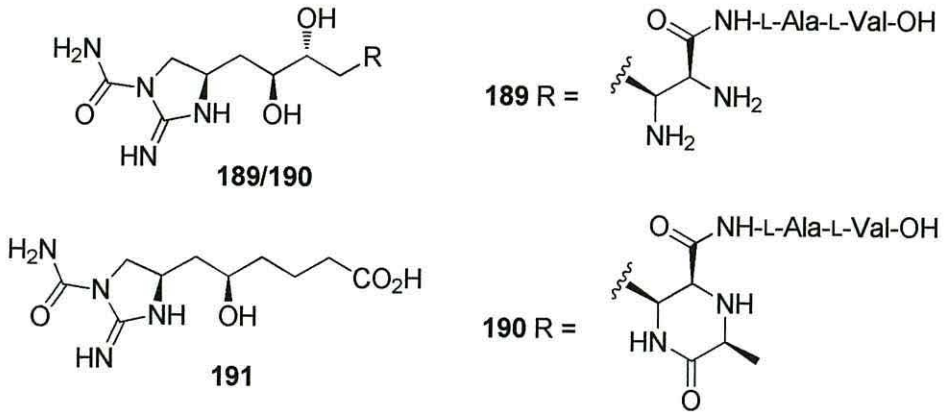
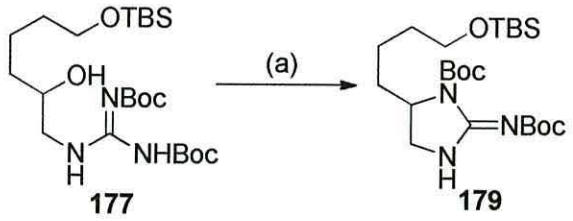


Figure 11:- Recently synthesised natural products structurally similar to guanidine **179**.

Discussion with Dr. T. Hirose a collaborator of Prof. Ōmura led to the investigation of a more efficient methodology that would allow heterocycle **179** to be prepared from the direct cyclisation of β-hydroxy guanidine **177**. Under the newly investigated conditions a DCM solution of guanidine alcohol **177** was treated with I_2, PPh_3 and imidazole (Scheme 29).[163]



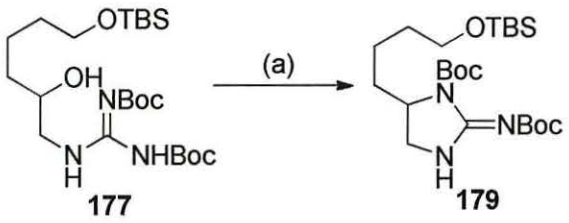
Scheme 29:- (a) PPh_3, I_2, imidazole, DCM, -20 °C, 2 h 30 min, 89-98%.

The reaction proceeded smoothly with total consumption of the starting material being evident via TLC. Once complete, the reaction was subjected to an aqueous workup and the crude material purified by column chromatography, initially giving guanidine heterocycle **179** in a 7% isolated yield.

The spectroscopic data obtained were in agreement with that expected for heterocycle **179**. The ^1H NMR spectrum displayed signals relating to the functionality present, with changes diagnostic of a successful cyclisation being evident as the chemical shift of the methylene group now contained within the 5-membered ring changed from

3.36 ppm (1H) and 3.54 ppm (1H) in the starting material to 3.45 ppm (1H) and 3.82 ppm (1H) in **179**. The chemical shift of the adjacent methine group also changed from 3.75 ppm to 4.12 ppm. Analogous changes were observed to the same functionality in the ^{13}C NMR spectrum were the methylene and methine groups shifted from 47.6 ppm and 71.8 ppm in guanidine alcohol **177** to both resonating at 56.4 ppm in heterocycle **179**. Both the low and high resolution ESI mass spectra obtained contained a $[M+H]^+$ ion of 473 Daltons further confirming the successful preparation of guanidine **179**.

In order to achieve a consistently acceptable yield for the cyclisation of guanidine **177** various reaction conditions were investigated (Table 3). To begin with the reaction was conducted as described by Ōmura; starting at 0 °C and allowing the mixture to stir and warm to room temperature (Entries 1-3). Initially under such conditions guanidine **179** was isolated in a disappointing yield of 7% (Entry 1), however, this could be significantly improved to 23% by reducing the reaction time (Entry 2), whilst reducing the molecular equivalents of reagents used appeared to have a negligible effect (Entry 3). Further improvement was also observed when the reaction temperature was maintained at 0 °C, this, coupled with an even shorter reaction time allowed **179** to be isolated in a more acceptable yield of 45% (Entry 4). The results so far suggested that heterocycle **179** may be decomposing or undergoing unwanted side reactions when the reaction mixture had been allowed to warm to rt, therefore the reaction was performed at a further reduced temperature of -20 °C (Entries 5-6). Here the best results were obtained; with near quantitative yields consistently being attained when the reaction temperature was maintained at -20 °C, although slightly longer reaction times were required in order for the reaction to reach completion (Entry 6).



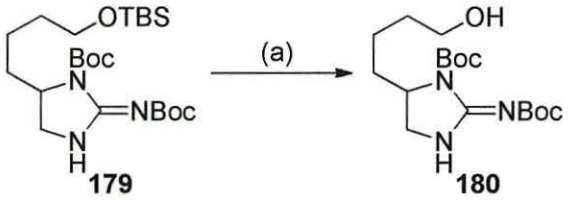
177 179

(a) See Table 3

Entry	I_2 (eqv)	PPh_3 (eqv)	Imidazole (eqv)	Temperature	Reaction time	Isolated yield
1	3.5	4	5	0 °C to rt	6 h	7%
2	3.5	4	5	0 °C to rt	3 h	23%
3	2	3	4	0 °C to rt	3 h	26%
4	2	2.3	3.9	0 °C	1 h	45%
5	2	2.3	3.9	-20 °C to 0° C	1 h	87%
6	2	2.3	3.9	-20 °C	2 h 30 min	89-98%

Table 3:- Reaction conditions employed to optimise the cyclisation of guanidine **177**.

Having optimised the reaction conditions for the formation of guanidine heterocycle **179**, the next step in the pathway involved the removal of the TBS protecting group to allow for the further elaboration of the alkyl side chain. In order to deprotect the primary alcohol functional group a THF solution of guanidine **179** was treated with 1 molar equivalent of TBAF (Scheme 30).[164,165]



179 180

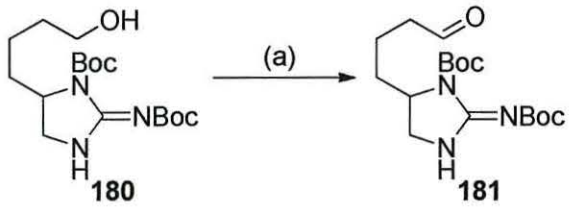
Scheme 30:- (a) TBAF, THF, 0 °C to rt, 24 h, 83-99%.

The reaction was initiated at 0 °C and allowed to stir and warm to rt, after 24 hours TLC analysis indicated the complete consumption of silyl ether **179**. Thus the reaction was quenched with a saturated solution of NH_4Cl, extracted with chloroform and the organic extracts washed with brine. The residue obtained was then purified by column chromatography using an ethyl acetate/petroleum ether solvent system. Fractions eluting

in neat ethyl acetate gave heterocyclic alcohol **180** in very high yields ranging from 83% to 99%.

Identification of the resultant primary alcohol **180** was achieved initially by appearance of a diagnostic broad OH stretch in the IR spectra at 3318 cm^{-1}. Confirmation was achieved by the observation of changes in the ^{1}H NMR spectrum which no longer contained signals representative of a TBS ether functional group. Also noted was an upfield shift in the methylene group adjacent to the newly deprotected alcohol functionality which resonated at 3.50 ppm, compared to 3.59 ppm in the starting material. Key changes were also observed in the ^{13}C NMR spectrum, were the same methylene group again resonated at an upfield frequency of 61.8 ppm compared to 62.7 ppm in the starting material. The signals representing the *t*-butyl methyl groups, quaternary carbon atom and silicon bound methyl groups of a TBS ether were also absent. The low resolution mass spectroscopic data were in agreement with the NMR data, detecting a $[M+H]^{+}$ ion of 358 Daltons representative of alcohol **180**.

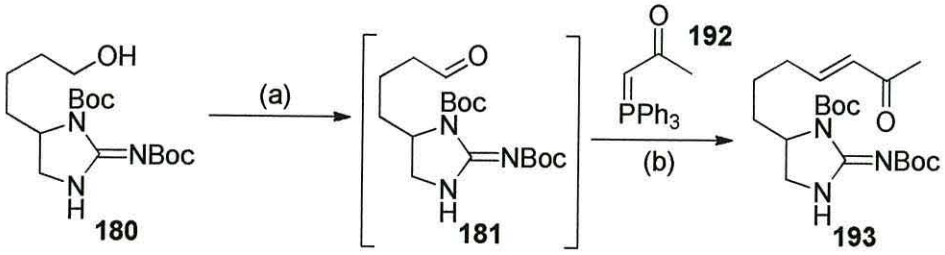
Having accomplished the deprotection of silyl ether **179**, oxidation of the resultant alcohol **180** to the corresponding aldehyde was undertaken. Initial investigations centred on the use of the previously utilised Swern oxidation to achieve this transformation (Scheme 31).[150-152]



Scheme 31:- (a) i) $(COCl)_2$, DMSO, -78 °C, 20 min; ii) **180**, -78 °C, 20 min; iii) NEt_3, -78 °C, 2 h.

The progress of the reaction was monitored by TLC and after 2 hours no more starting material was shown to be present. The reaction was then subjected to an aqueous workup, dried over $MgSO_4$ and evaporated. Unfortunately analysis of the ^{1}H NMR spectrum of the crude material indicated the presence of a complex mixture devoid of

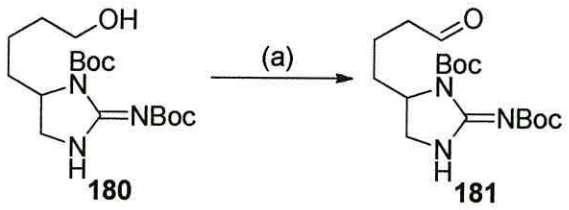
signals diagnostic of an aldehyde functional group. At this stage it was suspected that aldehyde **181** may have formed under the reaction conditions but may be unstable, to test this theory a Swern *in-situ* Wittig reaction was undertaken using the simple readily available phosphorane **192** in the hope of forming enone **193** (Scheme 32).[166]



Scheme 32:- (a) i) $(COCl)_2$, DMSO, -78 °C, 20 min; ii) **180**, -78 °C, 20 min; iii) NEt_3, -78 °C, 2 h; (b) **192**, rt, 24 h.

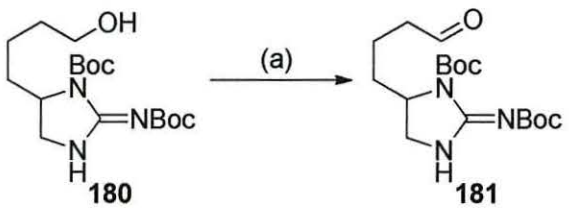
The oxidation phase of the reaction was performed as previously described and upon the complete consumption of alcohol **180** phosphorane **192** was added and the mixture allowed to warm to rt and stir for 24 hours. The reaction was then subjected to an aqueous workup and the crude residue inspected by ^1H NMR. Again the spectrum was indicative of a complex mixture and devoid of signals associated with either the aldehyde functional group of **181** or the enone functionality of guanidine **193**. As a result of the inability to form enone **193**, it was evident that the guanidine alcohol **180** was incompatible with the reaction conditions. The most likely explanation being that HCl liberated from the generation of the key alkoxysulfonium ion intermediate had resulted in the loss of the acid labile Boc protecting groups.[150] Therefore efforts were directed towards finding a procedure capable of oxidising alcohol **180** whilst being compatible with the acid sensitive functionality present within the molecule.

This led to the use of the milder but equally efficient Dess-Martin periodinane as an oxidant in the hope of generating aldehyde **181**. Therefore alcohol **180** was dissolved in DCM and exposed to an excess (3 eqv) of the Dess-Martin reagent (Scheme 33).[162,167]



Scheme 33:- (a) DMP, DCM, rt, 3 h 30 min, 96%.

In the process of finding a consistent method for the oxidation of heterocyclic alcohol **180**, several different reaction conditions were investigated (Table 4). Initially the reaction was conducted under thoroughly anhydrous conditions, however, upon stirring for 48 hours only unreacted starting material was detected (Entry 1). This led to the addition of *t*-BuOH which has been shown to initiate periodinane based reactions, and resulted in aldehyde **181** being formed in 79% yield (Entry 2).[167,168] The addition of H_2O has also been shown to have a beneficial effect on the DMP oxidation, which results in the formation of an acetoxyiodinane oxide which is a more efficient oxidant of alcohols.[169,170] In relation to the transformation under investigation the addition of H_2O further increased the yield of aldehyde **181** to 87% (Entry 3). However the use of reagent grade DCM stored under an air atmosphere gave a very high yield of 96% (Entry 4) probably a result of the small amount of water present within the solvent. Under the conditions described thus far a threefold excess of DMP has been used in order to reduce the reaction time in the hope of preventing unwanted side reaction resulting from the acidic nature of the DMP reagent. Fortunately the reaction could be performed under buffered conditions by the addition of pyridine, thus negating the need for excess DMP, under such conditions aldehyde **181** was obtained high yields ranging from 86% to 96% (Entry 5).[171]



(a) See Table 4

Entry	DMP (eqv)	Solvent	Additive	Time	Yield
1	3	Anhydrous DCM	—	48 h	0%
2	3	Anhydrous DCM	*t*-BuOH (1 eqv)	3 h	79%
3	3	Anhydrous DCM	H_2O (1 eqv)	2 h 30 min	87%
4	3	Reagent grade DCM	—	3 h 30 min	96%
5	1	Reagent grade DCM	Pyridine (3 eqv)	24 h	86-96%

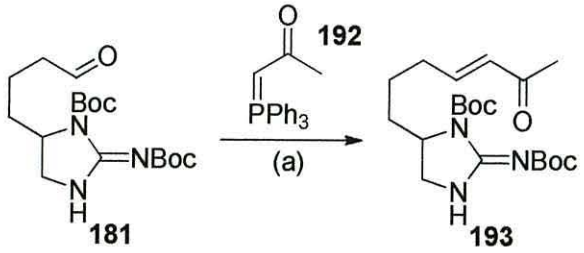
Table 4:- Reaction conditions investigated for the DMP oxidation of guanidine **180**.

Attempts were made to purify aldehyde **181** by column chromatography; however, even the use of NEt_3 washed silica gel led to significant losses in material. Fortunately, when an excess of the DMP reagent was used trituration with Et_2O gave **181** of sufficient purity for use in subsequent steps. Material generated using the buffered pyridine system proved the simplest to purify, requiring only filtration to remove insoluble impurities followed by azeotropic evaporation with toluene to remove excess pyridine. The ease with which the pyridine buffered reaction could be purified and the ability to use much less of the relatively expensive oxidant made this method the method of choice for the oxidation of alcohol **180**.

The initial characterisation of aldehyde **181** was achieved by NMR spectroscopy, where signals indicative of an aldehyde functional group were observed at 9.71 ppm and 201.2 ppm in the ^{1}H and ^{13}C NMR spectra respectively. Confirmation that the functional group interconversion had proceeded successfully was also evident in the IR spectrum which displayed a new stretch representative of an aldehyde at 1710 cm^{-1}, whilst being devoid of the broad OH stretch of the starting material at 3318 cm^{-1}. Inspection of the low resolution ESI mass spectrum showed the presence of a base peak with a mass of 356 Daltons representing the $[M+H]^+$ ion of aldehyde **181**. The high resolution mass spectrum

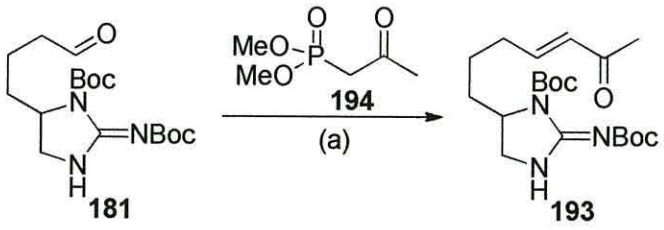
detected a $[M+H]^+$ ion of 356.2186 closely matching the theoretical mass of 356.2180 and thus confirming the correct molecular formula of aldehyde **181**.

Having heterocyclic aldehyde **181** in hand installation of the key enone functionality via a Wittig olefination was investigated. Therefore a DCM solution of aldehyde **181** was cooled to 0 °C and treated with commercially available phosphorane **192** (Scheme 34).[172,173]



Scheme 34:- (a) **192**, DCM, 0 °C to rt, 24 h.

The Wittig reaction between aldehyde **181** and phosphorane **192** proceeded smoothly with TLC analysis indicating that the complete consumption of aldehyde **181** had occurred within 24 hours. Further indication that the reaction had been a success was given in the ^{1}H NMR spectrum of the crude reaction material which displayed signals indicative of the expected enone functional group. Unfortunately problems were encountered during purification as the triphenylphosphine oxide by-product could not be fully removed by either trituration or column chromatography, leading to unacceptable losses in material. However, an analogous HWE olefination using phosphonate **194** circumvented this problem, as the dialkylphosphate salt by-product generated is easily removed by aqueous extraction (Scheme 35).[174,175]

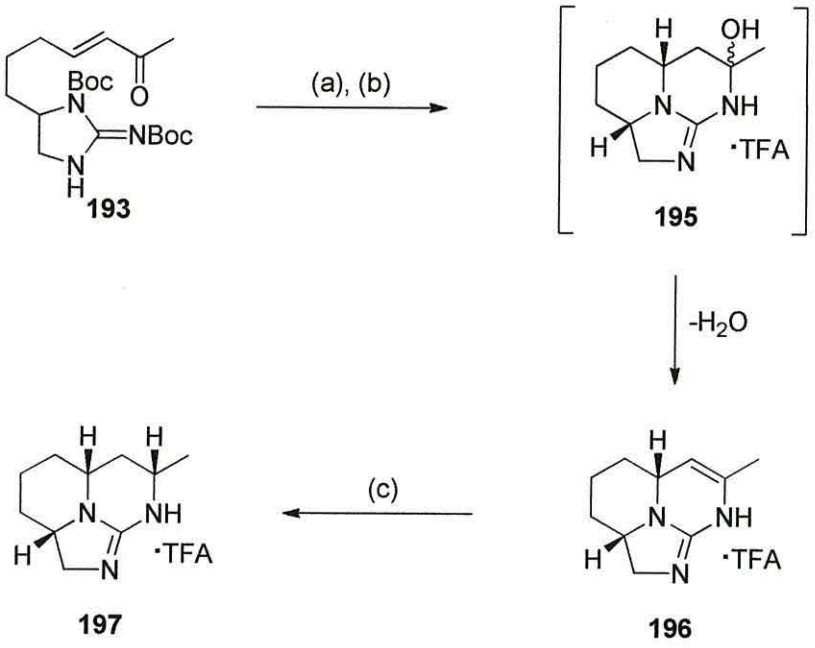


Scheme 35:- (a) LiCl, **194**, DIPEA, MeCN, rt, 48 h, 61-86%.

Initial attempts at the olefination were conducted using LiBr as a cation source for co-ordination with the phosphonate and NEt_3 as a base for deprotonation. However, under these conditions only unreacted starting material was returned. It was reasoned that this failure may be the result of the highly hygroscopic nature of LiBr, which may have hydrated even though the reaction was conducted under anhydrous conditions.[176] This eventuality led to the use of reaction conditions developed by Masamune and Roush who employed the less hygroscopic lithium salt, LiCl as a cation source.[177] Under these conditions enone **193** was consistently generated in yields of 61-86%.

Inspection of the ^1H NMR spectrum showed the presence of signals associated with the newly incorporated enone functionality. Signals representative of the alkene protons appeared as and doublet at 6.07 ppm and a doublet of triplets at 6.74 ppm both sharing the expected *trans* coupling constant of 15.8 Hz. The same methine protons were also observed in the ^{13}C spectrum at 131.7 ppm and 146.9 ppm, and were accompanied by a quaternary carbon atom representing the enone carbonyl group at 198.3 ppm. Both the low and high resolution mass spectra were in agreement with the expected structure of heterocyclic guanidine **193**, with the low resolution spectrum containing a base peak of 396 Daltons representing the $[M+H]^+$ ion of **193** and a heavier fragment at 792 Daltons representing the corresponding dimer.

Having developed a reliable method for the incorporation of the requisite enone functionality, the formation of a tricyclic ring system representative of the cylindrospermopsin alkaloids could be undertaken. Initial investigations utilised guanidine **193** before committing to the preparation of a more complex phosphonate that would allow for the future inclusion of the uracil D ring. Therefore, Boc deprotection of enone **193** was undertaken, in the hope of generating hemiaminal **195** and subsequently tricyclic enamine **196**, which on treatment with $NaBH_3CN$ should furnish saturated guanidine heterocycle **197** (Scheme 36).

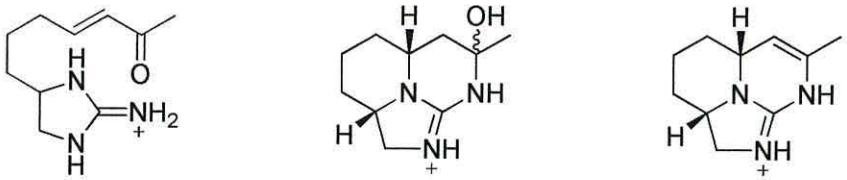


Scheme 36:- (a) TFA, DCM; (b) NEt_3, DCM; (c) $NaBH_3CN$, MeOH.

Initially only a small quantity of guanidine **193** was available. Thus it was decided that removal of the Boc protecting groups would be carried out in an NMR tube and reaction progress monitored by ^1H NMR. After 6 hours of frequently agitating a $CDCl_3$ solution of guanidine **193** containing TFA, ^1H NMR analysis suggested that the deprotection had proceeded successfully as the Boc *t*-butyl groups at 1.49 ppm and 1.50 ppm in **193** were no longer observed. Next the residual TFA was removed by co-evaporation with $CHCl_3$. The resultant material was taken in DCM and stirred in the presence of NEt_3 for 24 hours to remove any remaining TFA and initiate Michael addition to the alkene moiety. It was envisaged that Michael addition may be accompanied by spontaneous nucleophilic attack on the enone carbonyl group, generating hemiaminal **195** and enamine **196**, although removal of water from the reaction medium may be required to push the reaction towards complete enamine formation.[178,179]

Upon stirring in the presence of NEt_3 the solvent was removed from the reaction under reduced pressure and the remaining material concentrated under high vacuum to remove residual NEt_3. An aqueous workup was not undertaken as it was suspected that the resultant guanidine salts may have a high affinity for H_2O. Inspection of the crude material by ^1H NMR indicated that a transformation had taken place and that signals associated with enone **193** were no longer present. Species of interest were observed in

the mass spectrum having molecular weights of 196 Daltons and 178 Daltons which could be representative of hemiaminal **195** and enamine **196** (Figure 12). Unfortunately column chromatography using a methanol/chloroform solvent system was unable to provide a suitably pure sample for further analysis, as the material of interest co-eluted with a triethylammonium 2,2,2-trifluoroacetate salt impurity.



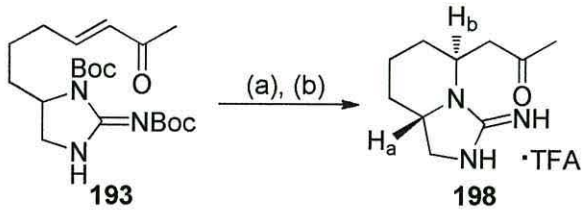
193
$C_{10}H_{18}N_3O^+$
MW: 196

195
$C_{10}H_{18}N_3O^+$
MW: 196

196
$C_{10}H_{16}N_3^+$
MW: 178

Figure 12:- Expected species generated from the cyclisation of enone **193**.

Due to the tentative positive results obtained from the initial cyclisation more enone **193** was prepared and the reaction repeated on a larger scale. The reaction was conducted as previously described with the exception of the deprotection step which was conducted in a 1:1 mixture of DCM:TFA.[180] By using smaller increments when purifying the crude reaction material by gradient elution chromatography it was possible to fully remove the previously problematic NEt_3 salt impurity and consistently isolate a single compound as a viscous oil in yields of between 54% and 70%. Unfortunately the data obtained for this material were consistent with that expected for bicyclic ketone **198** containing the A and C ring of the desired compound (Scheme 37).



193 **198**

Scheme 37:- (a) TFA, DCM (1:1), 24 h; (b) NEt_3, DCM, 24 h, 54-70%.

Evidence for the formation of bicyclic ketone **198** was initially obtained from the IR spectrum which was devoid of the broad OH stretch that would be associated with a

structure such as that of hemiaminal **195**, but contained a carbonyl stretch at 1682 cm^{-1} compared to that of 1605 cm^{-1} in the starting material. The ^{13}C NMR spectrum was further indicative of structure **198** displaying 10 different carbon atom environments containing a carbonyl signal at 206.7 ppm as well as those of two methine protons. The first methine proton H_a resonated at 53.7 ppm with the second H_b appearing at 47.6 ppm, these were accompanied with a signal representative of the methyl group of **198** downfield at 30.9 ppm. The same methyl group was also observed in the ^1H NMR spectrum as a singlet at 2.20 ppm. The two methine protons were also clearly observed in the ^1H NMR spectrum with H_a appearing at a triplet of doublets resonating at 3.85 ppm and H_b appearing as a triplet at 4.39 ppm. The presence of guanidine **198** was further confirmed by the low resolution ESI mass spectrum where the expected $[M+H]^+$ ion was observed at 196 Daltons and the corresponding $[2M+H]^+$ dimer at 391 Daltons. Confirmation that **198** was isolated as the corresponding TFA salt was obtained via negative ion ESI mass spectroscopy were a base peak of 113 Daltons was evident. Fortunately it was possible to slowly crystallise the viscous oil obtained from column chromatography from ethyl acetate to give a suitable crystal of **198**; X-ray crystallographic examination of which indicated that the methine protons attached to C-3 and C-7 displayed an *anti* configuration (Figure 13).

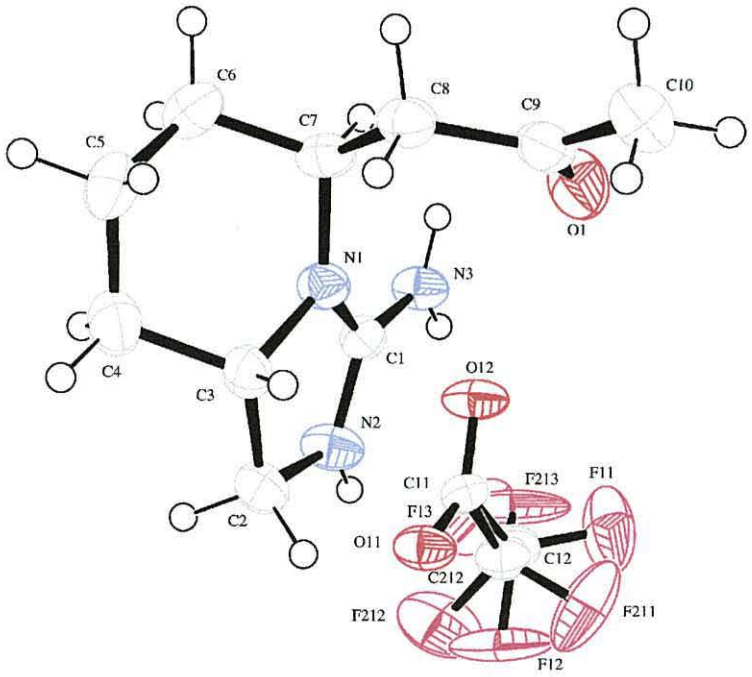
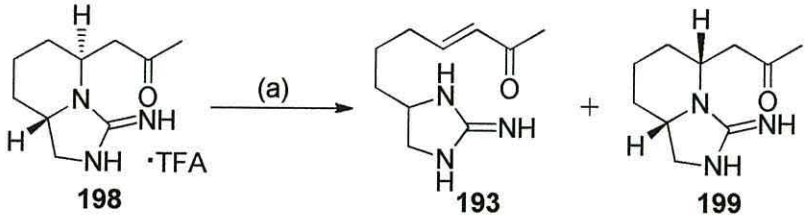


Figure 13:- ORTEP representation of bicyclic guanidine **198** (See Appendix A).

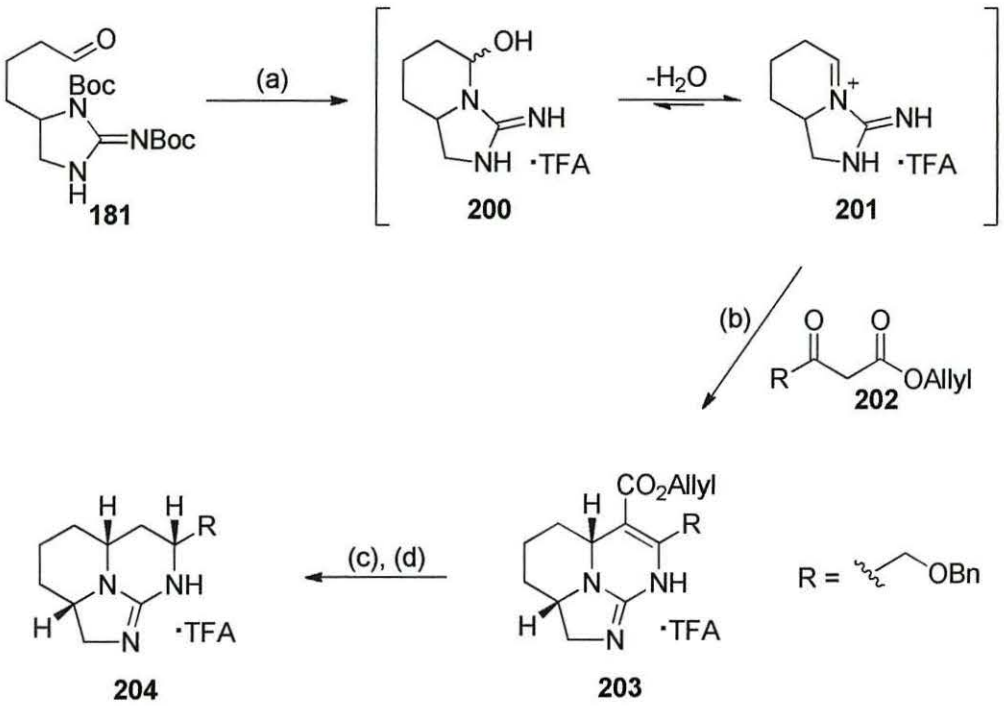
The retro-Michael addition of guanidine to an enone has previously been proposed to occur in the synthesis of a complex natural product,[181] therefore it was attempted to epimerise bicyclic ketone **198** via the reversible addition of the guanidine moiety under basic conditions (Scheme 38).



Scheme 38:- (a) TBD, DMF, 90 °C, 6 h.

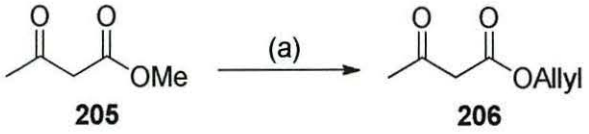
Thus guanidine **198** was dissolved in DMF and exposed to the strong guanidine base triazabicyclodecene (TBD) followed by heating at 90 °C for 6 hours. Upon cooling to rt the solvent was evaporated and the residue dissolved in MeOH and treated with TFA for a further 6 hours, followed by purification via column chromatography. Unfortunately only two fractions were isolated, one representing TBD and the other bicyclic guanidine **198**. Products indicative of retro-Michael addition/equilibration, namely enone **193** and guanidine **199** were not detected.[182]

With attempts to equilibrate the relative stereochemistry of bicycle ketone **198** via a retro-Michael addition proving unsuccessful, it became apparent that the current synthetic approach was no longer suitable for the generation of a tricyclic ring system representative of the cylindrospermopsin alkaloids. This led to a revision of the synthetic strategy, seeking a new methodology capable of meeting the original aim of the project. This came in the form of a tethered Biginelli condensation. It was expected that deprotection of a previous synthetic intermediate, namely aldehyde **181** would yield a mixture of intermediates consisting of hemiaminal **200** and the iminium ion species **201** which could be condensed with a suitable β-keto ester such as **202** to furnish tricyclic guanidine **203**.[183-187] Removal of the unneeded allyl ester and double bond functionality from the resultant dihydropyrimidine ring could then be achieved using known chemistry leading to the saturated tricyclic ring system **204** (Scheme 39).[188-190]



Scheme 39:- (a) TFA/DCM (1:1); (b) **202**, morpholine acetate, Na_2SO_4, CF_3CH_2OH, Δ; (c) $Pd(PPh_3)_4$, pyrrolidine, THF/MeOH; (d) $NaBH_3CN$, AcOH/MeOH.

It was decided that initial investigations regarding the utility of a tethered Biginelli condensation would be undertaken using β-keto ester **206**, as this compound represents one of the simplest allyl substituted 1,3-dicarbonyl compounds. Therefore the preparation of ester **206** was undertaken via the transesterification of methyl acetoacetate **205** with allyl alcohol following the procedure of Gilbert and co-workers (Scheme 40).[191]

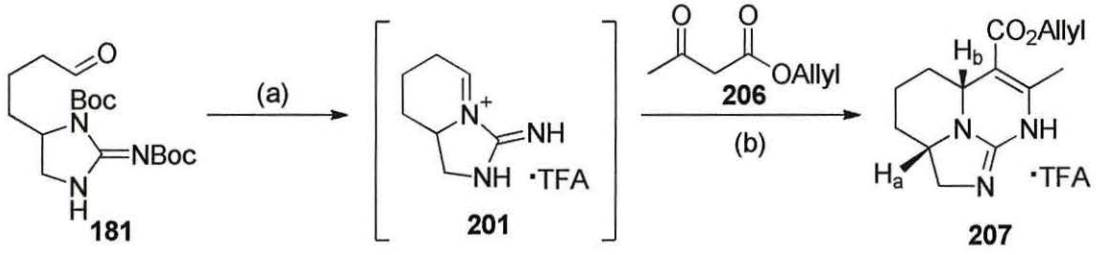


Scheme 40:- (a) allyl alcohol, DMAP, 3 Å MS, toluene, Δ, 36 h, 52-63%.

Reaction progress was monitored by TLC and after 36 hours no more starting material was shown to be present. The reaction was then cooled to rt filtered through a small plug of Celite® and the filtrate washed with a saturated solution of NH_4Cl and brine. Purification of the crude material was achieved by bulb to bulb distillation using a Kugelrohr, giving the desired β-keto ester **206** in yields of 52-63%.

Inspection of the ^{1}H NMR spectrum indicated that the transesterification had proceeded successfully as only one distinct methyl signal was visible in the NMR spectrum resonating at 2.14 ppm. Further evidence indicative of the incorporation of the allyl functionality was evident by the appearance of a doublet at 4.51 ppm representing the allylic methylene group, combined with the alkene methylene and methine protons appearing as multiplets at 5.18 ppm (2H) and 5.79 ppm (1H) respectively. Further agreement was observed in the ^{13}C NMR spectrum which contained 7 different chemical environments with the alkene methylene and methine groups of the allyl ester clearly visible at 118.3 ppm and 131.3 ppm. The data of both spectra were in very close agreement to the literature data.[191]

Having the desired β-keto ester in hand condensation with iminium ion **201** under buffered conditions was investigated. Thus aldehyde **181** was deprotected by treatment with TFA followed by exposure of a trifluoroethanol solution of the resultant un-purified material to allyl ester **206** in the presence of morpholine acetate and Na_2SO_4 (Scheme 41).[183,184]

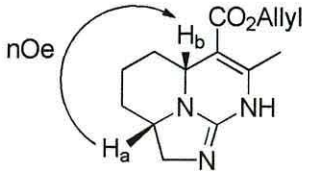


Scheme 41:- (a) TFA/DCM (1:1), 24 h; (b) **206**, morpholine acetate, Na_2SO_4, CF_3CH_2OH, 60 °C, 48 h, 4% (over 2 steps).

Due to the highly polar nature of the guanidinium species generated from the deprotection of aldehyde **181** it was difficult to monitor the progress of the reaction by TLC. However, a new spot became evident and increased in intensity over a period of 48 hours. The reaction mixture was then cooled to rt, filtered and the crude material purified by column chromatography using a methanol/chloroform solvent system which furnished tricyclic guanidine **207** in a low yield of 4%.

Inspection of the material obtained by ^{13}C NMR indicated the presence of 14 distinct carbon atom environments as expected for the desired compound. The most notable signals indicative of a successful transformation included the 2 quaternary carbon atoms of the newly formed enamine double bond which resonated at 101.5 ppm and 146.7 ppm. Further signals of interest were observed at 57.9 ppm and 52.7 ppm which were representative of the methine groups CH_a and CH_b. Signals representative of the allyl ester were also clearly visible, the vinyl methylene and methine groups appeared at 118.2 ppm and 132.2 ppm with the oxygen bound methylene group and carbonyl carbon atom resonating at 65.0 ppm and 165.1 ppm respectively. The allyl ester functionality was also clearly visible in the ^1H NMR displaying comparable chemical shifts to those of ester **206**. Further indication that a successful transformation has occurred was given by the appearance of signals representative of the H_a and H_b protons which were observed as a apparent triplet of triplets with J = 1.9 Hz and 7.0 Hz at 3.91 ppm and a double doublet with J = 2.8 Hz and 11.4 Hz at 4.41 ppm. The $[M+H]^+$ ion of tricyclic guanidine **207** and the corresponding dimer were observed in the low resolution ESI mass spectrum, with confirmation of the correct molecular formula of **207** being obtained through high resolution mass spectroscopy.

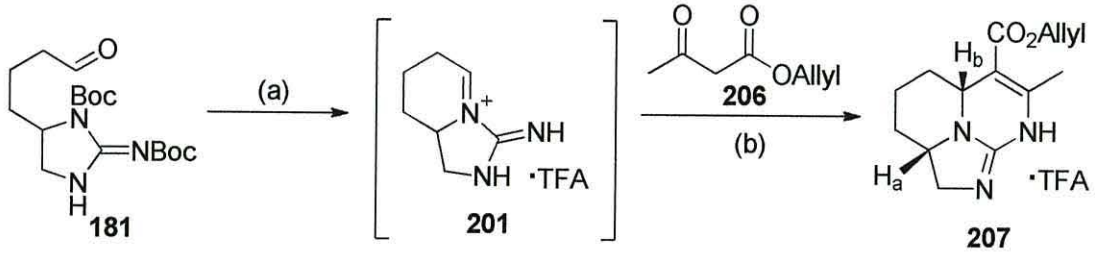
Having satisfactorily characterised the molecular structure of the newly formed tricyclic guanidine **207**, efforts were directed towards determining the relative stereochemistry of the H_a and H_b methine protons. As the product was only isolable as an oil a second crystal structure was not possible. This led to the use of 2D NOESY NMR spectroscopy to detect the presence of any through space nOe effects between the two groups. On performing the experiment a cross-peak between the two protons of interest H_a and H_b was observed in the 2D NOESY spectrum of guanidine **207**, thus confirming that the isolated material displayed the desired *syn* relative stereochemistry analagous to that observed in the cylindrospermopsin alkaloids (Figure 14).



207

Figure 14:- Key nOes observed in the 2D NOESY spectrum of guanidine **207**.

With the successful preparation of tricyclic guanidine **207** which displayed the desired *syn* relationship between the H_a and H_b protons, further investigations were required in order to improve the efficiency of the tethered Biginelli condensation to a level that would be acceptable for use towards a total synthesis. Therefore a variety of conditions was investigated in the hope of improving the overall yield of the reaction (Table 5).



(a) and (b) See Table 5

Reaction conditions[1]						
Entry	**Deprotection**	**β-keto ester**	**Morpholine Acetate**	**Temperature**	**Reaction Time**	**Yield**
1	TFA	3	1	60 °C	48 h	4%
2	TFA	2	1.5	60 °C	72 h	8%
3	TFA	1	1	60 °C	96 h	10%
4	TFA	3	1	60 °C	96 h	12%
5	TFA	5	2.5	70 °C	12 d	21%
6	AcOH	5	2.5	70 °C	12 d	36-43%
7[2]	AcOH	5	2.5	100 °C	6 d	26%
8[2]	AcOH	8	4	100 °C	12 d	37%

[1]All reactions were performed in CF_3CH_2OH in the presence of Na_2SO_4 [2]Reaction performed in a Carius tube.

Table 5:- Reaction conditions investigated for the tethered Biginelli condensation between guanidine **201** and β-keto ester **206**.

Initial investigations were conducted using TFA for the deprotection of aldehyde **181** followed by condensation of the resultant material with varying equivalents of **206**, in the presence of morpholine acetate and Na_2SO_4 at 60 °C (Entries 1-4). An incremental increase in the reaction time from 48 hours to 96 hours allowed for an appreciable increase in the yield from 4% to 10%, with an excess of β-keto ester generating a further 2% increase in the isolated yield (Entries 1-4). One possible explanation for the increased yield observed when increasing the molecular equivalent of **206**, is that the condensation proceeds via the enol tautomer of this species and thus by increasing the amount of **206** present the amount of the corresponding enol is also increaced.[186] From this point onwards an excess of both morpholine acetate and **206** was used for the reaction conditions investigated. Because of the progressive increase in yield observed with increased reaction time the reaction was allowed to proceed over a 12 day period at a slightly higher temperature of 70 °C (Entry 5). Using such a prolonged reaction time had the greatest observed effect on the isolated yield of guanidine **207** which increased to an acceptable 21%.

Further significant improvements to the yield were also observed when all the anionic species involved in the reaction were of the same nature. Under such conditions the TFA used in the initial deprotection step was replaced with AcOH, which allowed for an even greater increase in the isolated yield to between 36% and 43% (Entry 6). Interestingly, it was observed that upon standing the acetate counterion present in guanidine **207** was lost and it was replaced by a carbonate which was confirmed by the ESI negative ion mass spectrum. More forcing conditions were also investigated where the reaction was performed at 100 °C in a sealed vessel, however, no improvement in the isolated yield was observed (Entries 7-8).

Suspected reaction intermediates were observed through TLC analysis of the reaction and during purification. The least polar fractions contained β-keto ester **208** which had arisen from the reaction of the **206** with morpholine acetate and was formed in 13% yield based on the amount of ester **206** used in the reaction. The structure of **208** was determined by NMR and GC/MS which were both concordant with literature data.[192] Additionally no tricyclic products containing morpholine derived esters were observed in any of the reactions run. During purification it was observed that the latter fractions of the

desired compound **207** were contaminated with two slightly more polar compounds. Unfortunately it was not possible to isolate these compounds in pure form, however inspection of the ESI mass spectrum of the mixture indicated the presence of several other species. One of these gave a molecular ion at 280 Daltons which might represent one of the structures **209/210/211** as all these species have the same molecular formula. The NMR spectroscopic data obtained for this material were complex with the ^{13}C NMR spectrum of the mixture giving some indication of the nature of the by-products. The spectrum contained a signal representative of a ketone carbonyl group at 202.1 ppm in conjunction with two distinct allyl ester fragments in a ratio of 72:28 representing a mixture of guanidine **207** and possibly one of the bicyclic intermediates **209** or **210** (Figure 15).

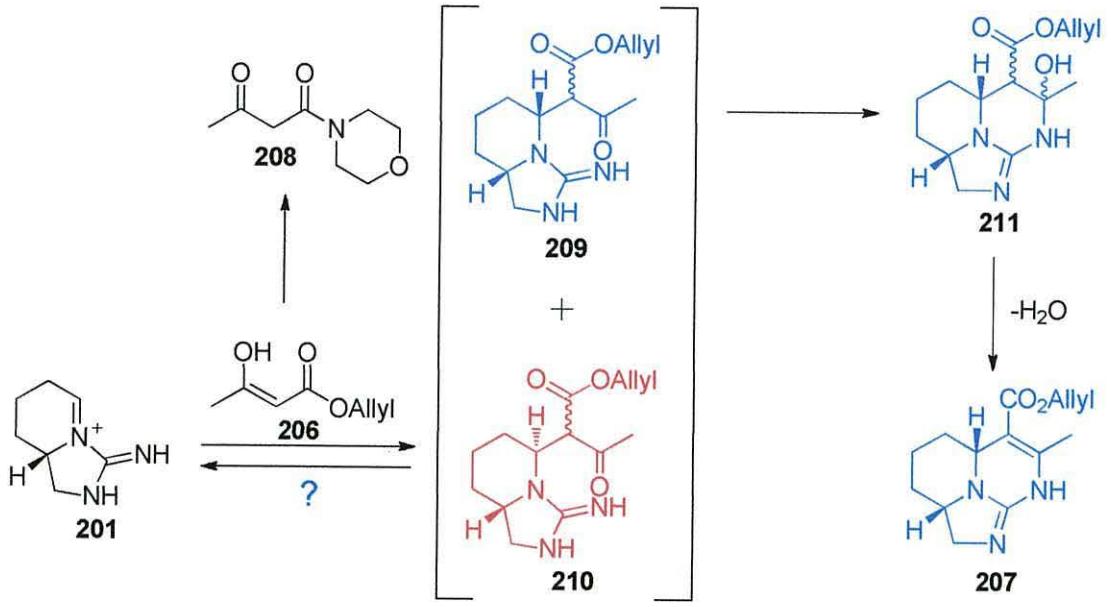
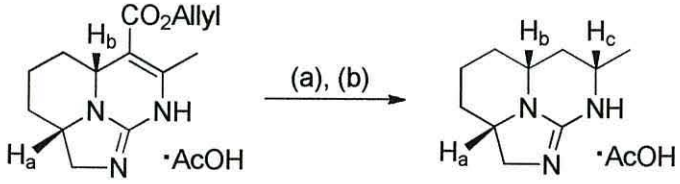


Figure 15:- Mechanistic possibility for the tethered Biginelli condensation between guanidine **201** and β-keto ester **206**.

In an attempt to obtain a clearer understanding of the reaction, these suspected intermediates were resubjected to the original reaction conditions. After heating for 12 days NMR analysis of the material obtained indicated that no further reaction had taken place. This was a little disappointing as this result suggests that the reaction is possibly not under equilibrium control as suggested in figure 15 and would indicate that the by-product observed was guanidine **210** and that the condensation between **201** and **206**

proceeds via an irreversible process. Again possibly more work is needed here to discover if the nature of the catalyst for this reaction has an effect on the yield and reversibility of the addition.

Having established reaction conditions that allow for the reproducible preparation of tricyclic guanidine **207** in appreciable quantities, efforts were directed at the removal of the allyl ester and double bond functionalities. Thus a solution of **207** in a mixture of THF/MeOH was treated with a catalytic amount of $Pd(PPh_3)_4$ and pyrrolidine followed by reduction of the resultant enamine with $NaBH_3CN$ (Scheme 42).[188]



207 **212**

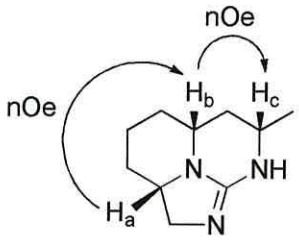
Scheme 42:- (a) $Pd(PPh_3)_4$, pyrrolidine, THF/MeOH, 90 min;
(b) $NaBH_3CN$, AcOH/MeOH, 0 °C to rt, 16 h, 57% (over 2 steps).

The initial cleavage of the allyl ester/decarboxylation step proceeded rapidly with full consumption of the starting material being evident in less than 2 hours. This was followed by reduction of the resultant enamine with $NaBH_3CN$ in AcOH/MeOH over 16 hours. Due to the highly polar nature of the resultant compounds an aqueous workup was not undertaken and the crude material purified by flash column chromatography using a methanol/chloroform solvent system containing 1% AcOH, allowing guanidine **212** to be isolated in a 57% yield.

Inspection of the IR spectrum of the purified material revealed the disappearance of the ester carbonyl and alkene double bond stretches at 1679 cm^{-1}, 1637 $cm^{-1}$and 1605 cm^{-1} respectively, indicating that the desired transformation had proceeded successfully. Confirmation was given by the ^{13}C NMR which was also devoid of the allyl ester and enamine functionality, but did contain three methine signals resonating at 58.2 ppm, 51.6 ppm and 47.0 ppm representing the three methine groups CH_a, CH_b and CH_c respectively. The ^{1}H NMR spectrum was also devoid of the allyl ester functionality previously

observed in guanidine **207**, whilst the three methine protons of guanidine **212** were clearly evident at 3.36 ppm, 3.58 ppm and 3.79 ppm. The ESI mass spectrum gave agreeable results displaying a base beak of 180 Daltons representing the $[M+H]^+$ ion of tricyclic guanidine **212**, with the negative ion spectrum detecting the desired acetate counterion, but with low relative abundance suggesting that exchange of the counterion may have occurred upon standing.

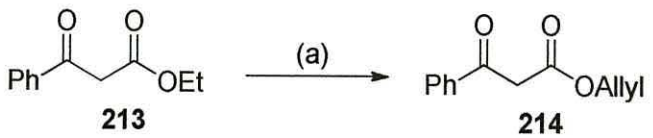
Confident of the successful preparation of guanidine **212**, further investigation was targeted towards establishing the relative stereochemistry of the newly introduced methine proton H_c. This was again achieved by 2D NOESY NMR where, as before a through space nOe effect was observed between the H_a and H_b protons. This was accompanied by an observed nOe between the H_b and H_c protons, confirming that they were all on the same face and therefore the utility of this approach towards the synthesis of the cylindrospermopsin alkaloids (Figure 16).[193]



212

Figure 16:- Key nOes observed in the 2D NOESY spectrum of guanidine **212**.

Before adapting the methodology described thus far towards a stereoselective total synthesis, a tethered Biginelli condensation utilising the phenyl substituted β-keto ester **214** was undertaken in order to ascertain the scope of this key cyclisation reaction. Allyl ester **214** was prepared by treating ester **213** with a large excess (15 eqv) of allyl alcohol in the presence of a catalytic amount of DMAP following the procedure of Kanda and co-workers (Scheme 43).[194]

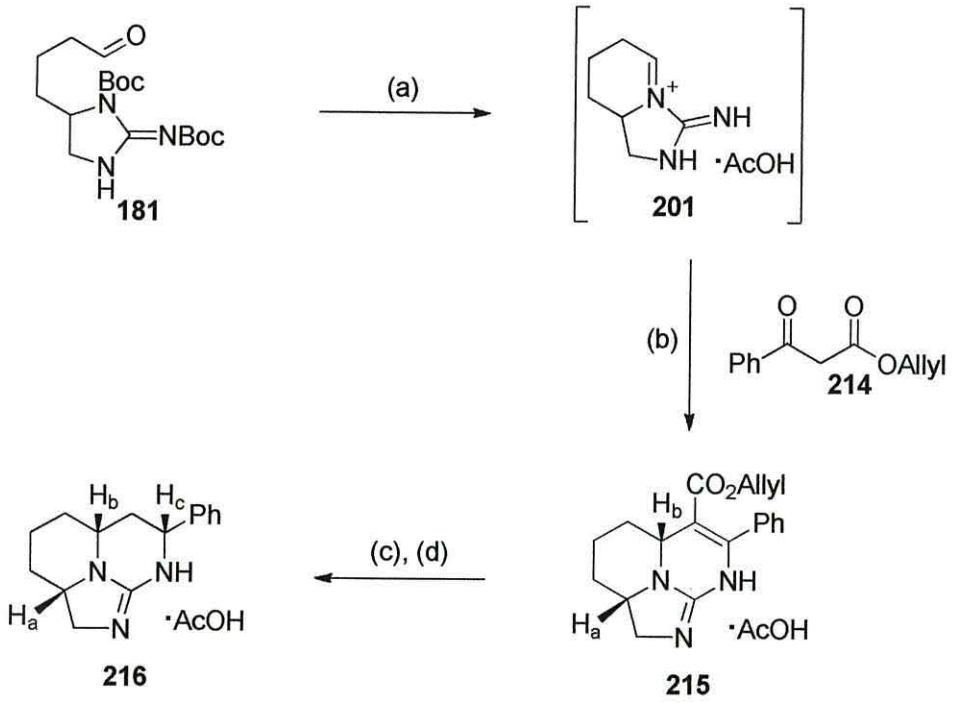


213 **214**

Scheme 43:- (a) allyl alcohol, DMAP, Δ, 5 d, 71%.

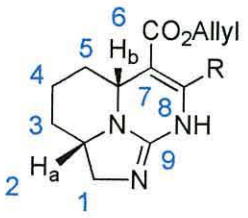
The reaction proceeded as described in the literature and after 5 days TLC analysis showed no more starting material to be present. Therefore the mixture was cooled to rt and subjected to an aqueous workup followed by purification of the resultant material by bulb to bulb distillation giving ester **214** in a 71% yield. Inspection of the NMR spectra obtained indicated the inclusion of the desired allyl ester functionality and displayed a very close agreement with the reported data.[194]

Having β-keto ester **214** in hand condensation with iminium ion **201** and subsequent deallylation/decarboxylation was undertaken using the previously described reaction conditions (Scheme 44).



Scheme 44:- (a) AcOH, 24 h; (b) **214**, morpholine acetate, Na_2SO_4, CF_3CH_2OH, 70 °C, 12 d, 26% (Over 2 steps); (c) $Pd(PPh_3)_4$, pyrrolidine, THF/MeOH, 2 h; (d) $NaBH_3CN$, AcOH/MeOH, 0 °C to rt, 16 h, 53% (over 2 steps).

The initial condensation between guanidine **201** and ester **214** proceeded smoothly and after column chromatography in a methanol/chloroform solvent system containing 1% acetic acid the expected tricyclic guanidine **215** was isolated in a 26% yield. The isolated material gave NMR, IR and mass spectroscopic data both representative of the expected guanidine and concordant with previous findings.



207 R = Me
215 R = Ph

207		**215**	
chemical environment	δ_C (ppm)	chemical environment	δ_C (ppm)
1	48.0	1	48.2
2	57.9	2	58.0
3	29.5	3	29.6
4	21.3	4	21.3
5	31.3	5	31.3
6	52.7	6	53.4
7	101.5	7	103.5
8	146.7	8	144.5
9	154.6	9	153.5

Table 6:- Tabulated ^{13}C NMR spectroscopic data for guanidines **215** and **207**.

In particular a very close similarity was observed between the ^{1}H and ^{13}C NMR spectra of Biginelli adduct **215** and the previously prepared guanidine **207**, the greatest resemblance was observed throughout the backbone of the tricyclic ring system. One of the most evident similarities in the ^{1}H NMR spectra was that of the H_b proton which was observed as a double doublet with coupling constants of 11.4 Hz and 2.8 Hz in guanidine **207** and a double doublet with couplings of 11.1 Hz and 2.7 Hz in **215**, indicating that H_b is in an axial position. Unfortunately a lot of the signals of interest were observed as broad multiplets in **215** making further direct comparisons between the ^{1}H data difficult, however a high level of consistency was observed in the chemical shifts in both systems. The most striking resemblance was observed between the ^{13}C spectra of both intermediates (Table 6/Figure 17), here there was a very close correlation between the chemical shifts of the CH_a and CH_b methine groups of guanidine **207** which resonated at

57.9 ppm and 52.7 ppm and those of guanidine **215** which appeared at 58.0 ppm and 53.4 ppm. The high level of similarity seen between these two systems suggests that that the H_a and H_b protons of tricyclic guanidine **215** display a *syn* relationship as previously seen in the nOe investigations of guanidine **207**.

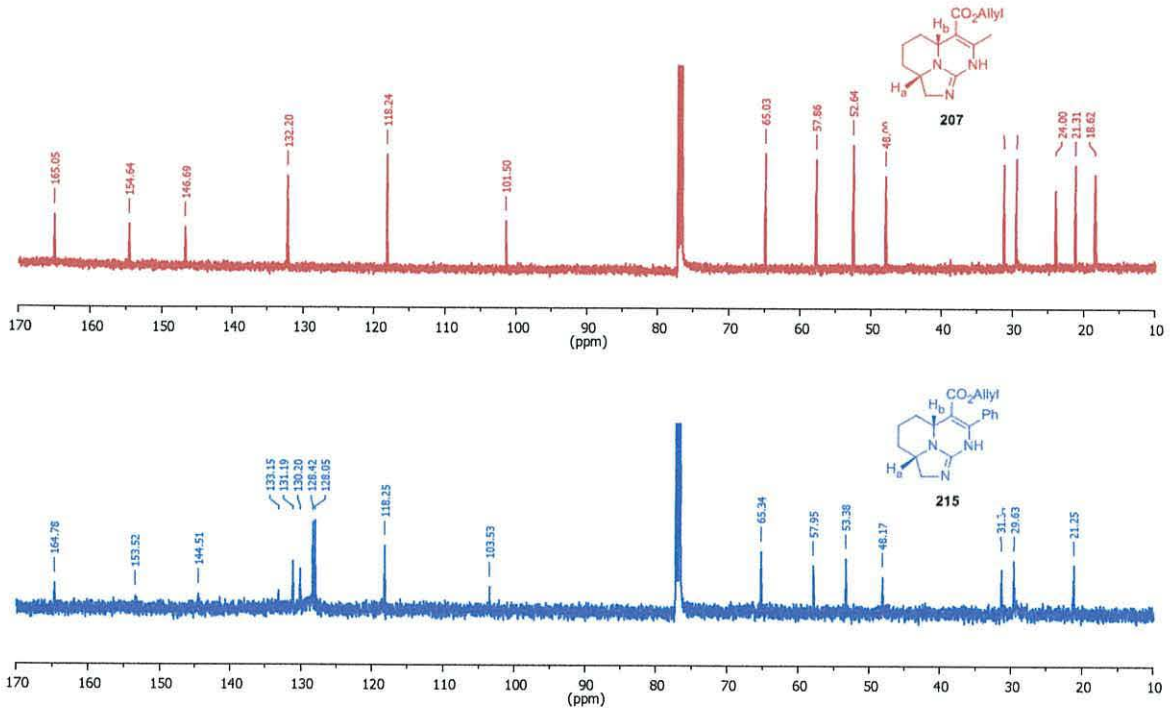
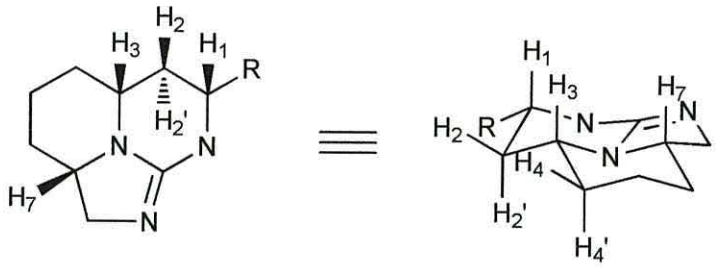


Figure 17:- Visual comparison of the ^{13}C NMR spectra of Biginelli adducts **207** and **215**.

With guanidine **215** in hand, efforts were directed towards the deallylation/decarboxylation which was undertaken by treatment with a catalytic amount of $Pd(PPh_3)_4$ and pyrrolidine, followed by reduction of the resultant enamine with $NaBH_3CN$ under acidic conditions as previously described. The material obtained was then purified by column chromatography which gave the desired tricyclic guanidine **216** in a 53% yield. The spectroscopic data collected for the isolated material were indicative of guanidine **216** with the 3 key methine protons clearly visible in the ^{13}C NMR spectrum at 50.4 ppm, 54.0 ppm and 57.1 ppm, representing CH_b, CH_a and CH_c respectively.

The use of 2D HSQC NMR made it possible to correlate all the proton and carbon signals and therefore assign all the regions in the ^{1}H NMR, allowing comparisons to be made between guanidine **216** and the previously prepared methyl substituted guanidine

212 (Table 7). Initially the orientation of H_1 was investigated. This proton was observed as a double doublet at 4.63 ppm with coupling constants of 11.5 Hz and 3.3 Hz. This combination of a large and a small coupling constant indicates that H_1 displays an axial orientation rather that an equatorial one as two smaller coupling constants would be observed for such a configuration. Next to be determined was the orientation of the other key methine proton H_3, this proton appeared as an apparent triplet of triplets with two large axial-axial coupling constants of 11.0 Hz and 11.3 Hz and two smaller axial-equatorial coupling constants of 3.3 Hz and 4.0 Hz, confirming that it has also adopted an axial configuration. These results, combined with the very close similarities observed in the NMR spectra of Biginelli aducts **207** and **215**, confirm that the saturated tricyclic guanidine **216** also displays the desired all *syn* relationship required for the synthesis of the cylindrospermopsin alkaloids as observed in the previously conducted nOe studies.

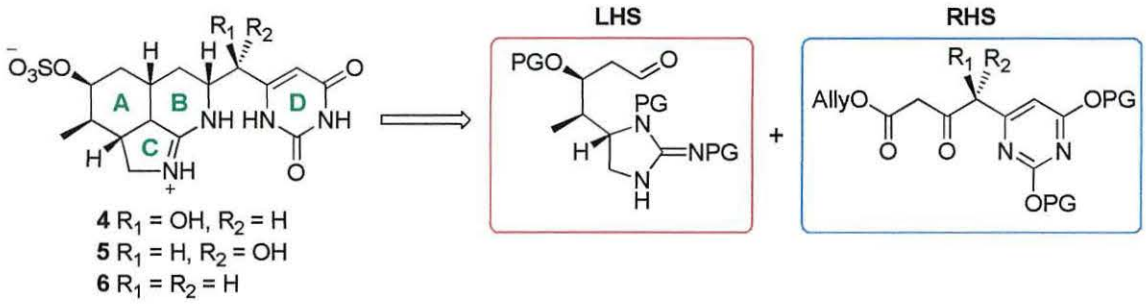


212 R = Me
216 R = Ph

216								
			coupling constants (Hz)					
H	δ (ppm)	multiplicity	H_1	H_2	$H_{2'}$	H_3	H_4	$H_{4'}$
H_1	4.63	dd		3.3	11.5	—	—	—
H_2	2.36	app dt, ddd	3.3		13.5	3.3	—	—
$H_{2'}$	1.80	app dt, ddd	11.5	13.5		11.0	—	—
H_3	3.54	app tt, dddd	—	3.3	11.0		4.0	11.3
H_4	1.26	m	—	—	—	—		—
$H_{4'}$	1.99	m	—	—	—	—	—	
212								
H_1	3.58	m		—	—	—	—	—
H_2	2.18	app dt, ddd	3.6		13.6	3.6	—	—
$H_{2'}$	1.40	app, dt, ddd	11.1	13.6		11.0	—	—
H_3	3.37	m	—	—	—		—	—
H_4	1.30	m	—	—	—	—		—
$H_{4'}$	1.95	m	—	—	—	—	—	

Table 7:- NMR analysis of tricyclic guanidine **216**.

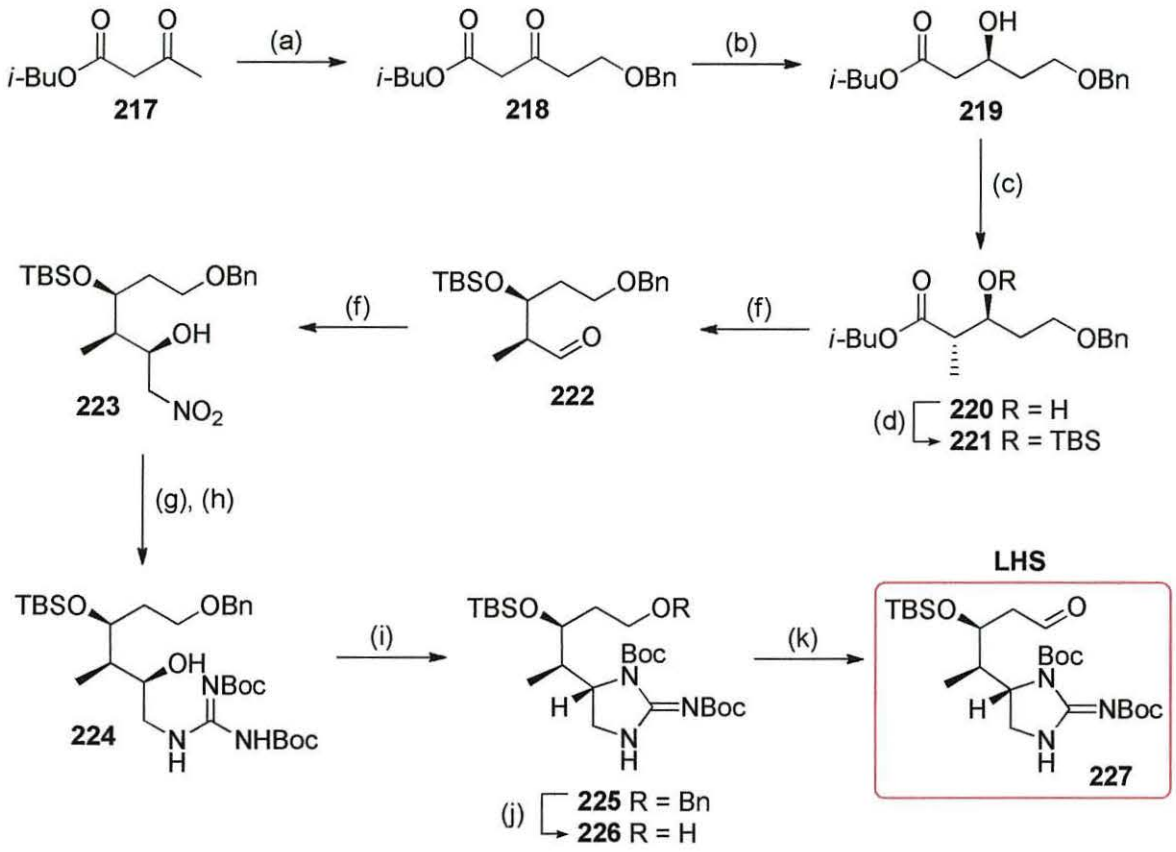
With such positive findings from the tethered Biginelli condensations conducted using previously prepared racemic material, the adaptation of this methodology towards a stereoselective total synthesis of the cylindrospermopsin alkaloids could now be undertaken. Due to the convergent nature of the key tethered Biginelli reaction it was now possible to split the target molecules into two distinct fragments consisting of a suitably functionalised aldehyde the "LHS" and a suitable allyl ester the "RHS" (Figure 18).



4 R_1 = OH, R_2 = H
5 R_1 = H, R_2 = OH
6 R_1 = R_2 = H

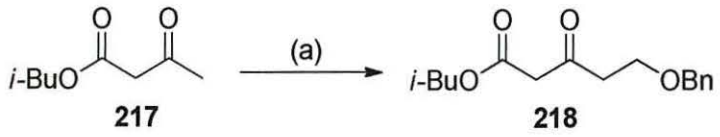
Figure 18:- Retrosynthetic analysis allowing for the preparation of all 3 metabolites.

The current synthetic work will focus on the preparation of the LHS substrate, the starting point for which will be commercially available isobutyl acetoacetate **217**. Alkylation of **217** with benzyl chloromethyl ether should furnish the chain elongated β-keto ester **218** which can undergo selective reduction to give alcohol **219**. Introduction of the A ring methyl substituent could then be achieved by a Fráter-Seebach homologation using LDA and MeI giving rise to alcohol **220**, subsequent protection of which with TBSCl will furnish TBS ether **221**. Low temperature reduction of the ester moiety of **221** with DIBAL should furnish aldehyde **222** which can then be subjected to a stereoselective nitro-aldol reaction to install the key C ring stereocenter **223**. Reduction of the nitro group can then be achieved by treatment with $NiCl_2/NaBH_4$, whilst exposure of the resulting free amine to guanylating agent **176** should yield guanidine **224**. Cyclisation of β-hydroxy guanidine **224** using I_2/PPh_3/imidazole should proceed without issue installing the C ring with the requisite stereochemistry **225**. Removal of the benzyl protecting group from heterocyclic guanidine **225** should furnish primary alcohol **226**, with subsequent DMP oxidation giving rise to the LHS substrate aldehyde **227** (Scheme 45).



Scheme 45:- (a) NaH, *n*-BuLi, BOMCl THF; (b) (*S*-BINAP)$RuCl_2$, H_2, MeOH; (c) LDA, DMPU, MeI, THF, -78 °C; (d) TBSCl, imidazole, DMF; (e) DIBAL, DCM -78 °C; (f) Jacobsen's catalyst, $MeNO_2$, DCM; (g) $NiCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH, NEt_3; (h) **176**; (i) I_2, PPh_3, imidazole, DCM; (j) H_2 Pd/C MeOH; (k) DMP, DCM.

The first reaction from the new synthetic methodology to be undertaken was the alkylation of isobutyl acetoacetate **217**. The conversion was achieved by generation of the corresponding dianion of **217** by successive treatment with NaH and *n*-BuLi, followed by the dropwise addition of benzyl chloromethyl ether (Scheme 46).[195]

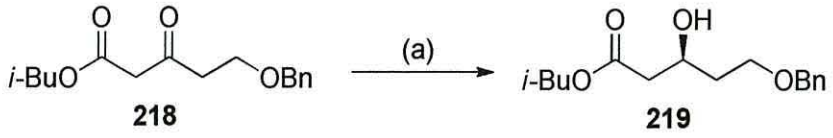


Scheme 46:- (a) i) NaH, 0 °C, 30 min; ii) *n*-BuLi, -25 °C, 30 min; iii) BOMCl, THF, -25 °C to rt, 24 h, 43-58%.

The reaction proceeded as described with the monoanion and dianion being evident from observed colour changes from clear colourless to pale yellow and an eventual deep/dark yellow. After exposure of the dianion solution to BOMCl for 24 hours, the reaction was cooled to 0 °C and the pH adjusted to pH 2-3 by the dropwise addition of ice cold 1 M HCl, diluted with DCM and extracted with EtOAc. Purification of the crude material was achieved by column chromatography on silica gel using an EtOAc/petroleum ether solvent system giving ester **218** in yields of 43-58%.

Inspection of the ^{1}H NMR spectrum indicated the presence of the desired β-keto ester by signals representative of the newly included benzyl group comprising of a singlet at 4.51 ppm representing the benzyloxy methylene group and a multiplet at 7.31 ppm representing 5 aromatic protons. The ^{13}C NMR spectrum corroborated the evidence observed in the ^{1}H NMR spectrum, displaying 13 signals indicative of 13 different chemical environments as expected for ester **218**. Key signals were observed at 19.0 ppm, 27.6 ppm and 71.4 ppm representing the methyl, methine and methylene groups of the isobutyl ester, combined with two quaternary carbon atom signals at 167.1 ppm and 201.3 ppm representing the ester and ketone carbonyl groups respectively. All the spectroscopic data showed close agreement with the reported literature data.[195]

The next objective to be overcome was the enantioselective reduction of β-keto ester **218** to the corresponding β-hydroxy ester (*S*)-**219**. Initially it was anticipated that this transformation could be achieved using the highly enantioselective hydrogenation described by Noyori using a (*S*-BINAP)$RuCl_2$ catalyst.[196,197] However, the same transformation could also be achieved by an aqueous based enzymatic reduction using readily available baker's yeast.[198] Significant investigations into the baker's yeast reduction of the 5-(benzyloxy)-3-oxo-pentanioate moiety in **218** with varying ester groups have been reported in the literature, the results obtained indicate that the isobutyl ester displays the optimum balance between yield and enantioselectivity.[199] Thus β-keto ester **218** was reduced with fermenting baker's yeast following the procedure of Brooks and co-workers (Scheme 47).[199]



218 **219**

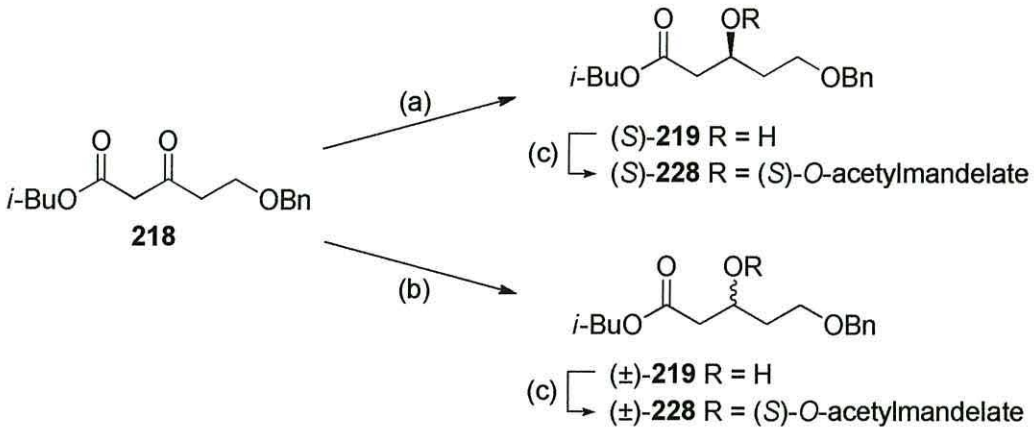
Scheme 47:- (a) i) Sucrose, baker's yeast, H_2O, 35 °C, 30 min;
ii) **218**, 48 h, 43-66%.

Reaction progress was monitored via TLC which indicated the full consumption of the starting material within 48 hours. The reaction mixture was then diluted with DCM and filtered through a pad of Celite®, and the filtrate subjected to an aqueous workup. Purification of the crude material was achieved chromatographically on silica gel using an ethyl acetate/petroleum ether solvent system which gave alcohol **219** in yields ranging from 43% to 66%. Interestingly an almost linear increase in the isolated yield was observed in conjunction with the time in which the yeast had been exposed to the atmosphere. Reports have indicated that the aging of the yeast can have a beneficial effect on the selectivity of some reductions, but no positive effects towards the yield of the reaction have been reported.[200]

Initial confirmation that the reduction had proceeded successfully was given by the IR spectrum which contained a broad OH stretch at 3492 cm^{-1} whilst only containing one carbonyl stretch at 1727 cm^{-1}. Further evidence was visible in the ^1H NMR spectrum were the newly introduced hydroxyl proton was observed as a singlet at 3.31 ppm along with the adjacent methylene group resonating at 4.18 ppm. The ^{13}C NMR spectrum was also indicative of structure **219**, being devoid of the ketone carbonyl signal observed at 201.3 ppm in the starting material, whilst containing the expected downfield methine group at 66.7 ppm. The presence of alcohol **219** was also confirmed by the ESI mass spectrum which displayed a base peak of 281 Daltons matching that expected for the desired product.

The specific rotation of alcohol **219** was measured as +10.9 being comparable to that of opposite stereoisomer, (*R*)-**219** which has a reported optical rotation of -9.6.[201] The enantioselectivity of the reduction was determined by comparing the spectra of the

mandelate derivatives of (*S*)-**228** and (±)-**228**, prepared from a DCC mediated coupling between (*S*)-*O*-acetyl mandelic acid and either (*S*)-**219** or (±)-**219** (Scheme 48).[202]



Scheme 48:- (a) baker's yeast, H_2O, 35 °C, 48 h, 43-66%; (b) $NaBH_4$, MeOH, rt, 3 h, 75%; (c) (*S*)-*O*-acetyl mandelic acid, DCC, DMAP, 0 °C to rt, 48 h, (*S*)-**228** (59%), (±)-**228** (60%).

Inspection of the ^1H NMR spectra of the chiral derivatives (*S*)-**228** and (±)-**228** indicated the presence of a mixture of isomers, as expected. The clearest signals for use in determining the ratio of the isomers present were those of the benzylic methylene group were each proton appeared as a doublet. Comparison of the integrals for this functional group for both isomers present in mandelate ester (*S*)-**228** indicated a 95:05 ratio between the major and minor product, and therefore that the baker's yeast reduction of β-keto ester **218** had furnished alcohol (*S*)-**219** with an enantiomeric excess of 90% (Figure 19).

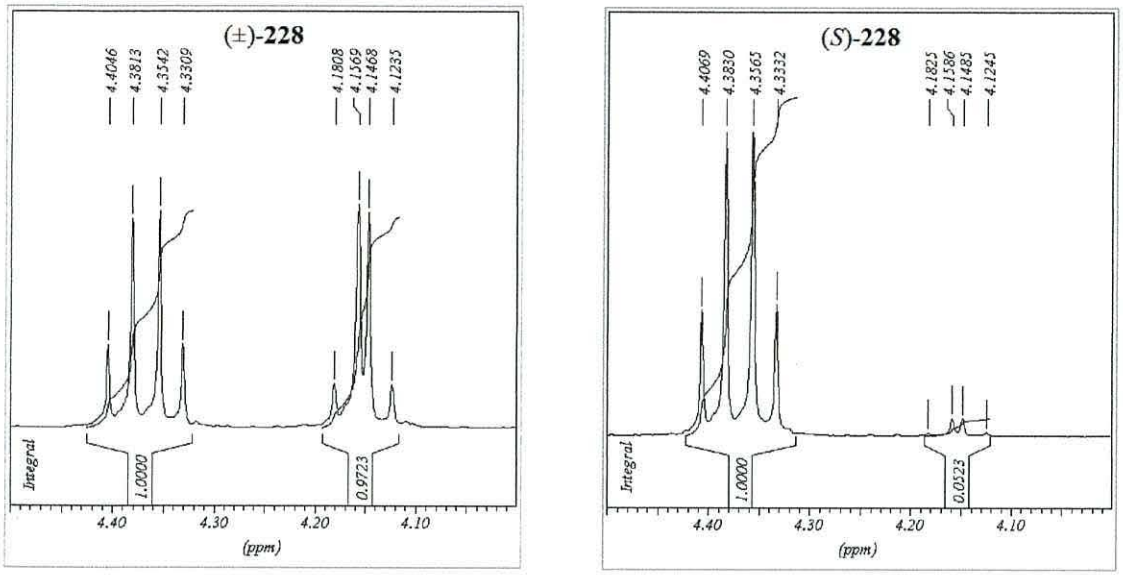
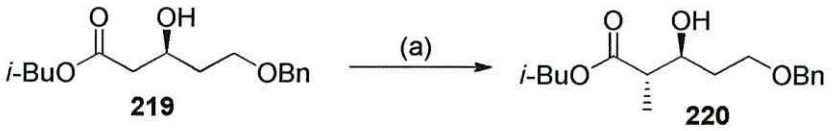


Figure 19:- ^{1}H NMR integrals for the benzylic methylene protons of (±)-**228** and (*S*)-**228**.

Having characterised alcohol (*S*)-**219** and determined the enantioselectivity in which the compound had been prepared, the next step in the pathway involved the inclusion of the A ring methyl group. This was achieved using an anti-selective Fráter-Seebach alkylation which was initially reported by G. Fráter in 1979.[203] Therefore, the dianion of alcohol **219** was prepared by treatment with a slight excess of LDA (2.1 eqv) followed by exposure to a solution of MeI in DMPU (Scheme 49).[203-206]

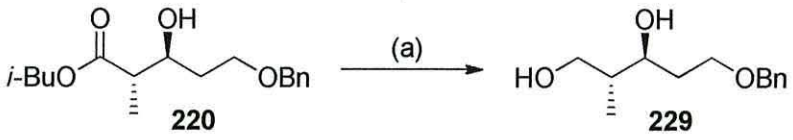


Scheme 49:- (a) i) DIPA, *n*-BuLi, THF, 0 °C, 30 min; ii) **219**, 90 min, -78 °C to -40 °C; iii) MeI, DMPU, -78 °C to rt 5 h 30 min, 70-74%.

Reaction progress was monitored by TLC and 5 hours and 30 minutes after the addition of MeI no more alcohol **219** was detectable. The reaction was then quenched with a saturated solution of NH_4Cl, the pH adjusted to pH 7 by the slow addition of 2 M HCl and the mixture extracted with diethyl ether. Purification was achieved by column chromatography on silica gel using a diethyl ether/petroleum ether solvent system which allowed the desired product, alcohol **220** to be isolated in consistently high yields of between 70% and 74%.

Inclusion of the new methyl group was evident by the appearance of a three hydrogen doublet with J = 7.3 Hz at 1.13 ppm in the ^{1}H NMR spectrum as well as a multiplet resonating at 2.52 ppm representing the adjacent methine group. The same methyl and methine groups were also observed in the ^{13}C NMR by signals resonating at 13.6 ppm and 45.4 ppm respectively. The ^{13}C NMR spectrum also displayed signals representative of 14 different carbon atom chemical environments as expected for the desired alcohol **220**. Further evidence for the preparation of **220** was given by the ESI mass spectrum which contained a base signal of 295 Daltons representing the $[M+H]^{+}$ ion of alcohol **220**. Close monitoring of the progress of the reaction was found to be crucial, as if the reaction was allowed to proceed for a greater length of time material of a slightly higher *Rf* was isolated along with **220** during purification. Inspection of the ^{1}H NMR spectrum of this material indicated that it was the corresponding methyl ether of **220** which was evident by the inclusion of a singlet in the spectrum at 3.31 ppm.

It was possible to determine the diastereomeric excess of the alkylation by reducing alcohol **220** to the corresponding diol **229**. This was achieved by the dropwise addition of DIBAL to a solution of **222** in DCM cooled to 0 °C (Scheme 50).[207]



220 **229**

Scheme 50:- (a) DIBAL, DCM, 0 °C to rt, 24 h, 64%.

Upon consumption of the starting material the reaction was quenched by the slow addition of methanol and diluted with EtOAc. To aid in the removal of the resulting aluminium salts from the reaction mixture, Rochelle Salt was added followed by stirring until 2 layers were evident. After extraction and purification by column chromatography, analysis of the material obtained by ^{1}H NMR spectroscopy indicated the presence of two distinct methyl signals resonating at 0.86 ppm and 0.91 ppm (Figure 20). Comparison of the integrals of the signals of interest indicated an approximate selectivity of 90:10 (*syn*/*anti*) which is slightly lower that of 95:05 which is regularly reported in the literature, a result that is possibly due to the presence of underlying impurities effecting the integrals of the NMR spectrum.[206,208]

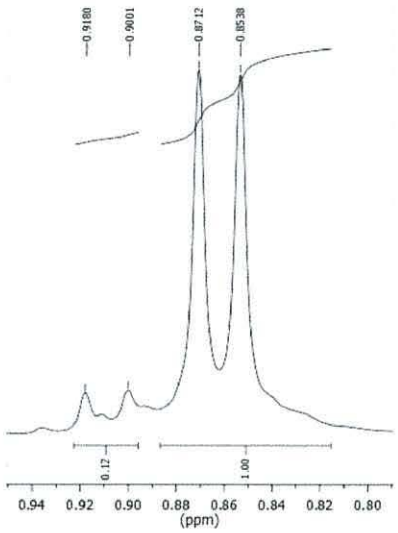
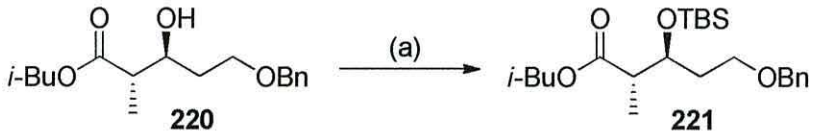


Figure 20:- ^{1}H signals representative of the methyl group of diol **229**.

With compound **220** in hand protection of the secondary alcohol functionality could now be undertaken. This was achieved using the Corey protocol which involves the addition of imidazole and TBSCl to a DMF solution of **220** (Scheme 51).[209]



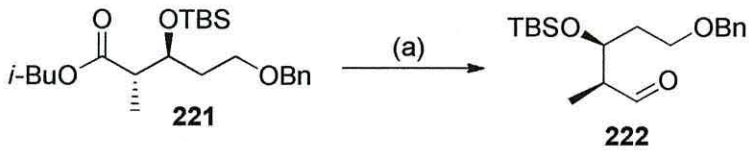
Scheme 51:- (a) TBSCl, imidazole, DMF, 0 °C to rt, 48 h, 70-88%.

The progress of the silylation was followed by TLC, and upon consumption of the alcohol starting material the reaction was quenched with water and the mixture extracted with hexane. Purification of the crude material was achieved by column chromatography using a diethyl ether/petroleum ether solvent system which allowed the desired TBS ether **221** to be isolated in consistently high yields of between 70% and 88%.

Initial indication that the protection of alcohol **220** had been a success was given by the IR spectrum which was devoid of the broad OH stretch indicative of the starting material at 3504 cm^{-1}. Confirmation of the successful incorporation of the TBS group was evident from the ^{1}H NMR spectrum which contained two intense singlets resonating at 0.07 ppm and 0.88 ppm representing the two methyl groups and the *t*-butyl groups from

the silyl protecting group. Signals associated with the newly introduced protecting group were also evident in the ^{13}C NMR spectrum. The silicon methyl groups appeared as two distinct signals resonating at -4.9 ppm -4.7 ppm, whilst the *t*-butyl group gave a quaternary carbon atom resonating at 18.0 ppm and three methyl groups at 25.7 ppm.

Having successfully prepared the silyl ether **221**, reduction of the ester functionality directly to the corresponding aldehyde **222** was undertaken. Thus a solution of ester **221** in toluene was cooled to -78 °C and treated with DIBAL (Scheme 52).[210]



221 **222**

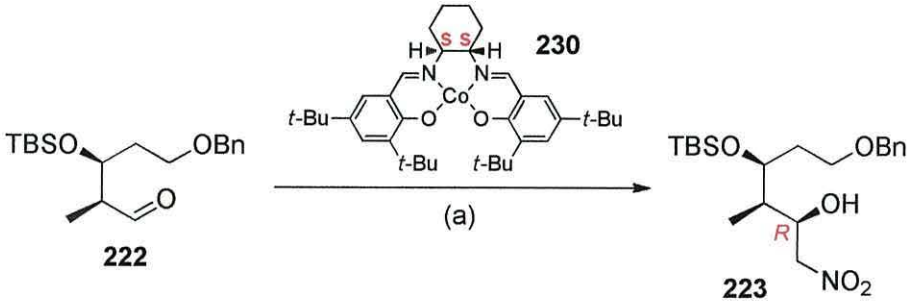
Scheme 52:- (a) DIBAL, toluene, -78 °C, 1 h, 57-66%.

The addition of DIBAL to the cooled reaction mixture was conducted in a dropwise manner and the progress of the reduction followed carefully by TLC in the hope of minimising over-reduction to the corresponding alcohol. After 1 hour ester **221** was no longer detectable by TLC, thus the reaction was quenched at -78 °C by the dropwise addition of methanol, diluted with EtOAc and a saturated solution of Rochelle Salt added to sequester any unwanted aluminium salts. After an aqueous workup the crude material was purified quickly by column chromatography on silica gel which gave the desired aldehyde **222** in a 66% yield.

Inspection of the ^{1}H NMR spectrum indicated that the desired functional group interconversion had proceed successfully as an aldehyde proton signal was clearly visible at 9.73 ppm, whilst signals representative of the *i*-butyl functionality at 0.86 ppm, 1.85 ppm and 3.78 ppm were no longer present. These observations were mirrored in the ^{13}C NMR spectrum which displayed the expected aldehyde carbonyl carbon signal at 204.5 ppm, whilst also being devoid of any of the *i*-butyl signals from the starting material. Further inspection of the ^{13}C NMR spectrum revealed the presence of 15 other carbon environments, as expected for structure **222**. Additional confirmation that the transformation had been a success was evident from the low resolution ESI mass

spectrum which displayed a base peak at 337 Daltons representing the $[M+H]^+$ ion of aldehyde **222**, which was accompanied by the corresponding ammonium adduct at 354 Daltons.

Having avoided the need for an inefficient 2 step reduction/oxidation procedure by generating aldehyde **222** directly from ester **221**, investigations could now be focussed towards a highly selective nitro-aldol reaction between **222** and $MeNO_2$. Several different catalysts have been investigated for their ability to increase the enantioselectivity of the Henry reaction,[211,212] with recent success being achieved by catalysts derived from cobalt(II)-salen complexes.[162,163,213,214] Thus the preparation of (*R*)-nitro alcohol **223** was investigated using the commercially available (*S,S*)-salen-cobalt catalyst **230** as this catalyst has been shown to influence the formation of (*R*)-nitro-alcohols with high selectivities of 78-97% de (Scheme 53).[162]

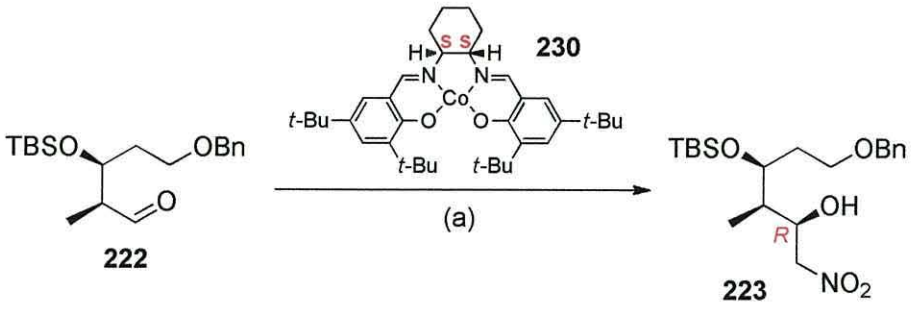


Scheme 53:- (a) **230**, $MeNO_2$, DIPEA, DCM, rt, 7 d, 80%.

In order to determine the effectiveness of catalyst **230**, four initial reactions were conducted, two at room temperature and two at -20 °C. One reaction from each pair was conducted in the presence of **230** and the other without so that the effect of the catalyst on the diastereochemical outcome of the reaction could be clearly compared (Table 8). Reaction progress monitored via TLC and on completion the mixtures were quenched with a saturated solution of NH_4Cl and extracted with DCM. Analysis of the crude material by TLC indicated the presence of two spots of interest having close *Rf* values of 0.28 and 0.30 (20% EtOAc/petrol) suggesting a mixture of diastereomers. Attempts were made during purification by column chromatography to isolate pure samples of each compound, however this proved unsuccessful, so both spots were combined and the mixture characterised.

Indications of a successful transformation were observed first in the IR spectrum which displayed a broad OH peak at 3447 cm^{-1} representative of **223** whilst also being devoid of the aldehyde carbonyl stretch of the starting material at 1728 cm^{-1}. Both the ^{13}C and ^{1}H NMR spectra displayed a complex mixture of signals with two distinct intensities, further confirming the presence of a mixture of diastereoisomers as expected. Signals indicative of the successful preparation of nitro-alcohol **223** were observed in the ^{13}C NMR spectrum at 68.4 ppm and 77.3 ppm representing the newly introduced methine and methylene groups respectively. The same groups were also observed in the ^{1}H NMR spectrum but unfortunately the new methine signal displayed the same chemical shift as the benzylic methylene group, with all three protons appearing as a multiplet at 4.47 ppm. However the signals representative of the methylene group adjacent to the nitro functionality were clearly visible as a double doublet at 4.17 ppm and a multiplet at 4.77 ppm. Conformation of the desired compound was achieved via the high resolution ESI mass spectrum which detected a $[M+H]^+$ ion of 398.2358 Daltons closely matching the theoretical mass of 398.2357 Daltons and thus confirming the correct molecular formula of nitro alcohol **223**.

Having determined the ability of the reactions to successfully generate the desired nitro-alcohol **223**, investigations could be directed towards the effect of the catalyst upon the selectivity of the reaction. Conveniently it was possible to determine the diastereomeric ratio of the compounds produced by ^{1}H NMR (Figure 21). It was found that the most appropriate signal to use was that of the methine proton adjacent to the OTBS group as it was clearly identifiable and free from overlapping signals from other functional groups. Performing the reaction at room temperature in the presence of the catalyst gave the best results, having a diastereomeric ratio of 62:38 (Entry 1). Reactions conducted in the absence of the **230** showed a propensity for the opposite diastereomer (Entries 2 and 4). This effect was greatest when the reaction was performed at a reduced temperature where a dr of 37:63 was observed (Table 8).



(a) See Table 8

Conditions[1]					
Entry	**Catalyst**	**Temperature**	**Time**	**Yield**	**dr[2]**
1	0.1	rt	7 d	80%	62:38
2	——	rt	7 d	61%	45:55
3	0.1	-20 °C	11 d	53%	58:42
4	——	-20 °C	11 d	43%	37:63

[1]All reactions were performed in DCM, using 2.5 eqv of DIPEA as base. Reactions conducted at reduced temperatures were cooled prior to the addition of DIPEA [2]The diastereomeric ratio was determined by ^{1}H NMR.

Table 8:- Reaction conditions investigated for the nitro-aldol reaction between aldehyde **222** and $MeNO_2$.

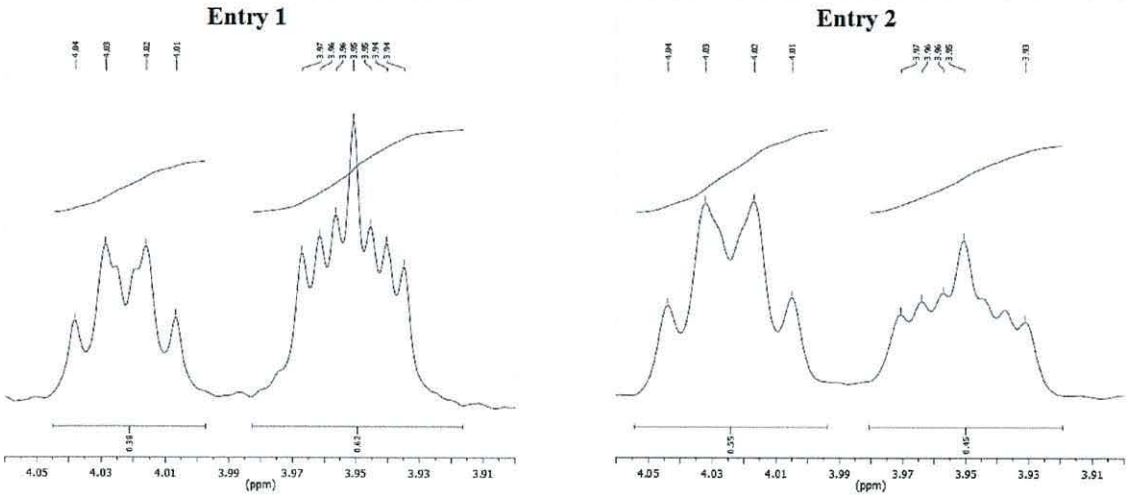
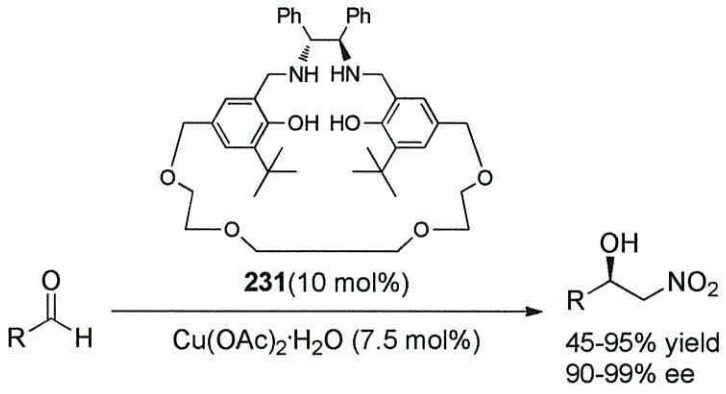


Figure 21:- ^{1}H NMR integrals for the methine group adjacent to the silyl ether in nitro-alcohol **223**.

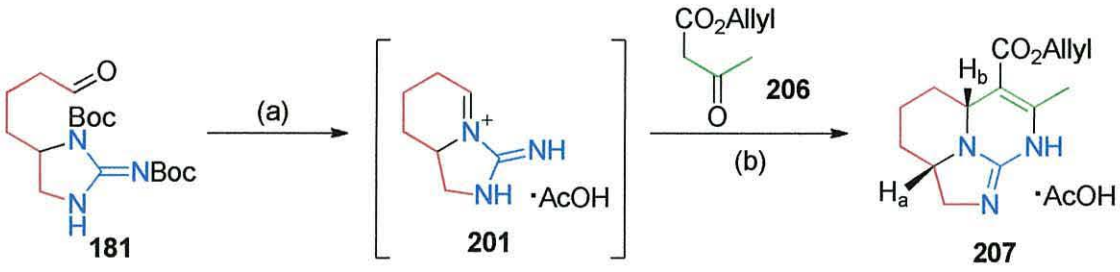
Due to the relativity low selectivities observed it is clear that further investigations are required in order to optimise the reaction conditions to an acceptable level in favour of the formation of the desired diastereomer. These initial results suggest that the reaction selectivity at room temperature and when cooled to -20 °C are largely similar, indeed there is a slightly better selectivity at the higher temperature for the desired (*R*)-alcohol **223** (entries 1 versus 3 and 2 versus 4), however this is a very small bias and is probably statistically insignificant. This observation might imply that it may be more advantageous to conduct future investigations at elevated temperatures in order to maximise selectivity. It may also be beneficial to investigate the ability of other catalysts to affect the desired transformation. Recently the chiral macrocyclic ligand **231** has been used in conjunction with $Cu(OAc)_2 \cdot H_2O$ and has shown promise in catalysing the asymmetric nitro-aldol reaction. A variety of substrates were investigated including aromatic, heteroaromatic, aliphatic, and α,β-unsaturated aldehydes, and the corresponding nitro-aldol adducts isolated in high yields and high enantioselectivities of between 90% and >99% (Scheme 54).[215] Interestingly the combination of macrocyclic ligand **231** and $Cu(OAc)_2 \cdot H_2O$ shows a selectivity towards the formation of the corresponding (*R*)-nitro alcohol making it ideal for use in the preparation of β-hydroxy nitro compound **223**.



Scheme 54:- Chiral macrocyclic ligand for use in the nitro-aldol reaction.

Conclusions and Future Work

During the course of this work a significant contribution has been made towards the synthesis of cylindrospermopsin and related metabolites, namely the construction of a model system representative of the guanidinium core of these natural products. Key to the formation of this model system has been a tethered Biginelli condensation between the iminium species **201**, generated from the deprotection of aldehyde **181** and a β-keto ester such as **206** (Scheme 55).[193]

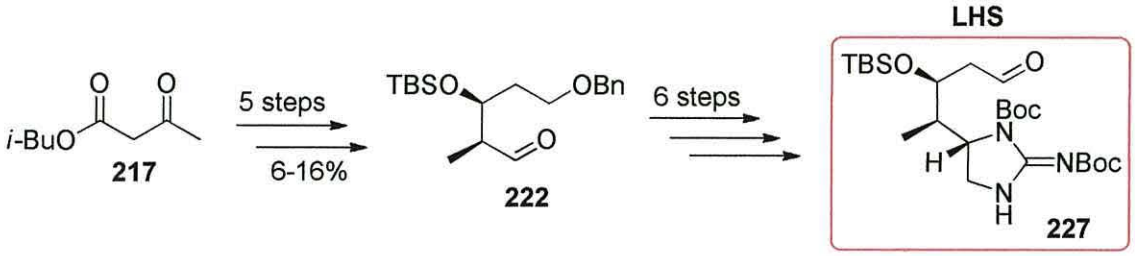


Scheme 55:- a) AcOH, 24 h; (b) **206**, morpholine acetate, Na_2SO_4, CF_3CH_2OH, 70 °C, 12 d, 36-43% (Over 2 steps).

To date reaction conditions have been optimised to allow for the preparation of Biginelli adduct **207** in yields of between 36% and 43%. These encouraging results have been obtained by running the reaction in a morpholine acetate buffered CF_3CH_2OH solution containing Na_2SO_4 as desiccant. However, an area that needs addressing is the extended reaction time that is required in order to generate usable quantities of guanidine **207**. One improvement may be to employ microwave dielectric heating as this has been shown to significantly reduce the reaction time of the conventional multicomponent Biginelli condensation.[216] In addition the use of a different counterion might also increase the yield, for example the use of morpholine benzoate or tosylate however, time was not available for the investigation of this in the study.

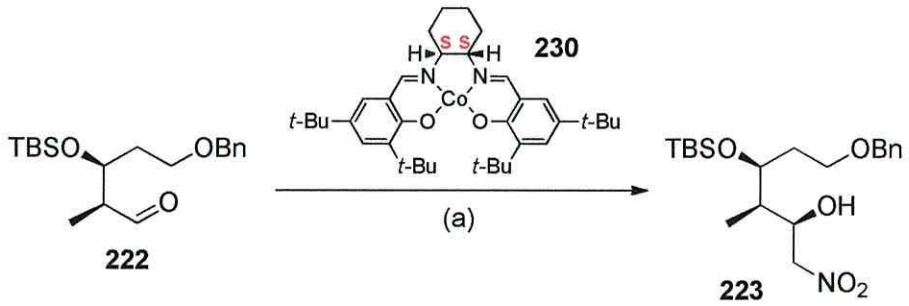
Current efforts have concentrated on the preparation of the LHS precursor, heterocyclic guanidine **227** towards the synthesis of the cylindrospermopsin alkaloids. At

this time aldehyde **222** has been prepared from isobutyl acetoacetate **217** in a stereoselective manner over 5 steps and in 16.5% overall yield (Scheme 56).



Scheme 56:- Current synthetic progress towards the LHS precursor.

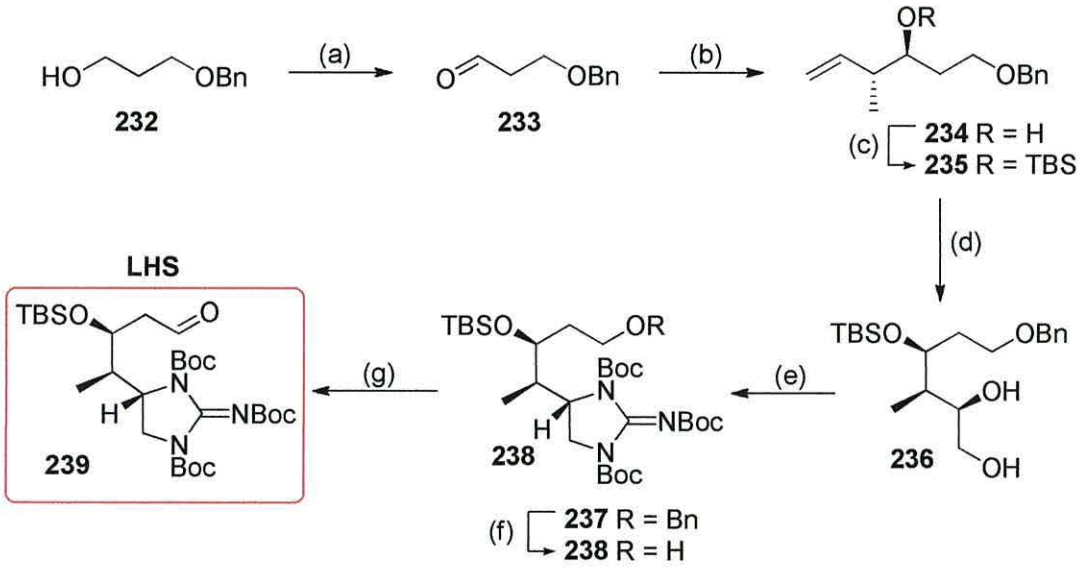
Recent work has culminated with investigations into the stereoselective nitro-aldol reaction between aldehyde **222** and nitromethane. Due to the limited investigations that have been undertaken the requisite nitro-alcohol **223** has only been prepared with a low diastereoselectivity of 24% via the use of the (*S*,*S*)-salen cobalt complex **230** (Scheme 53). However, future investigations will hopefully optimise the molecular equivalents of reagents used and the catalyst loading employed in order to favour the preparation of the required diastereoisomer. If investigations prove successful the desired isomer should be able to be obtained in its enantiomerically pure form for use in future reaction steps using preparative HPLC.



Scheme 53:- (a) **230**, $MeNO_2$, DIPEA, DCM, rt, 7 d, 80%.

However, if it is not possible to obtain a satisfactory level of selectivity from catalyst **230** or other catalysts, an alternative and potentially more efficient pathway to the LHS precursor has been envisaged. This pathway begins with easily prepared 3-benzyloxy-1-propanol **232** which can be oxidised to the corresponding aldehyde **233** under Swern conditions.[150-152,217] The resultant aldehyde can then be subjected to a Brown

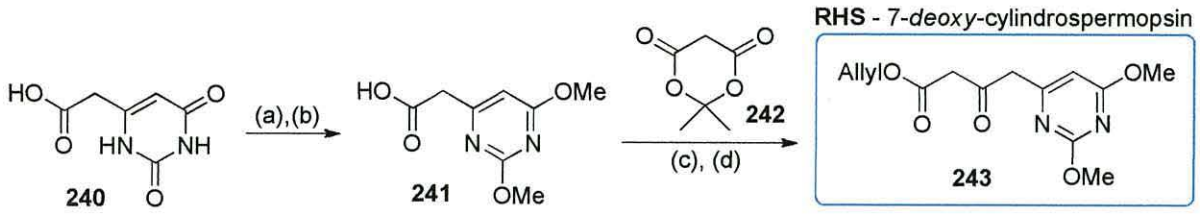
asymmetric crotylation with trans-2-butene and (+)-Ipc_2BOMe which should furnish **234** with high selectivity.[218,219] The newly formed alcohol group can then be protected as the corresponding silyl ether **235**.[209] The alkene functionality of **235** can then be converted to diol **236** under Sharpless *bis*-hydroxylation conditions.[220] Introduction of the guanidine group and subsequent cyclisation can be achieved under Mitsunobu conditions with *tri*-Boc-guanidine to give heterocyclic guanidine **237** containing the C ring of the cylindrospermopsin alkaloids.[221] Subsequent removal of the benzyl protecting group by hydrogenolysis, followed by oxidation of the resulting alcohol **238** with Dess-Martin periodinane should yield the desired aldehyde **239** containing one extra nitrogen terminus protecting group than that of aldehyde **227** (Scheme 57).[167]



Scheme 57:- (a) $(COCl)_2$, DMSO, NEt_3, DCM, -78 °C; (b) (+)-Ipc_2BOMe, (*E*)-2-butene, *t*-BuOK, *n*-BuLi, $BF_3{\cdot}Et_2O$, THF, -78 °C; (c) TBSCl, imidazole, DMF; (d) AD-mix-β, K_2OsO_4, *t*-BuOH/H_2O; (e) PPh_3, DIAD, *N,N',N''-Tri*-Boc-guanidine; (f) H_2, Pd/C, MeOH; (g) DMP, DCM.

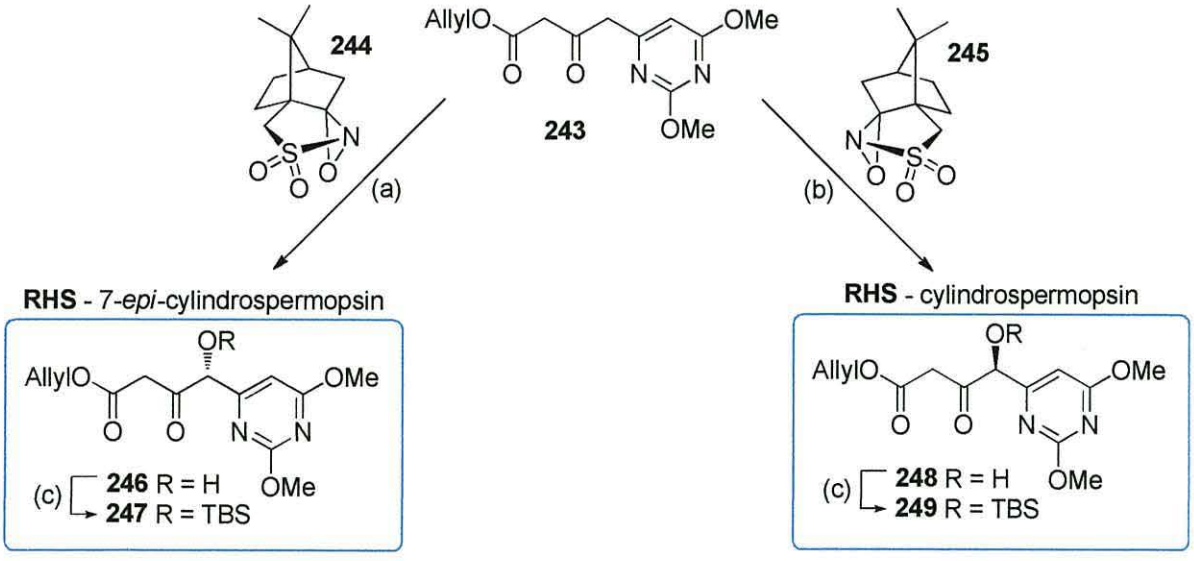
Concomitant to the preparation of the LHS substrate will be the preparation of the relevant RHS precursors for use in the key tethered Biginelli condensation. The simplest precursor will be the one that is required for the synthesis of 7-*deoxy*-cylindrospermopsin, one possible pathway to this sub-unit starts with uracil-4-acetic acid **240**, reaction of which with phosphorus oxychloride followed by treatment with sodium methoxide will yield the corresponding protected pyrimidine **241**.[222,223] A DCC mediated coupling of **241**

with Meldrum's acid **242** followed by treatment with refluxing allyl alcohol should furnish the desired β-ketoester precursor **243** (Scheme 58).[224,225]



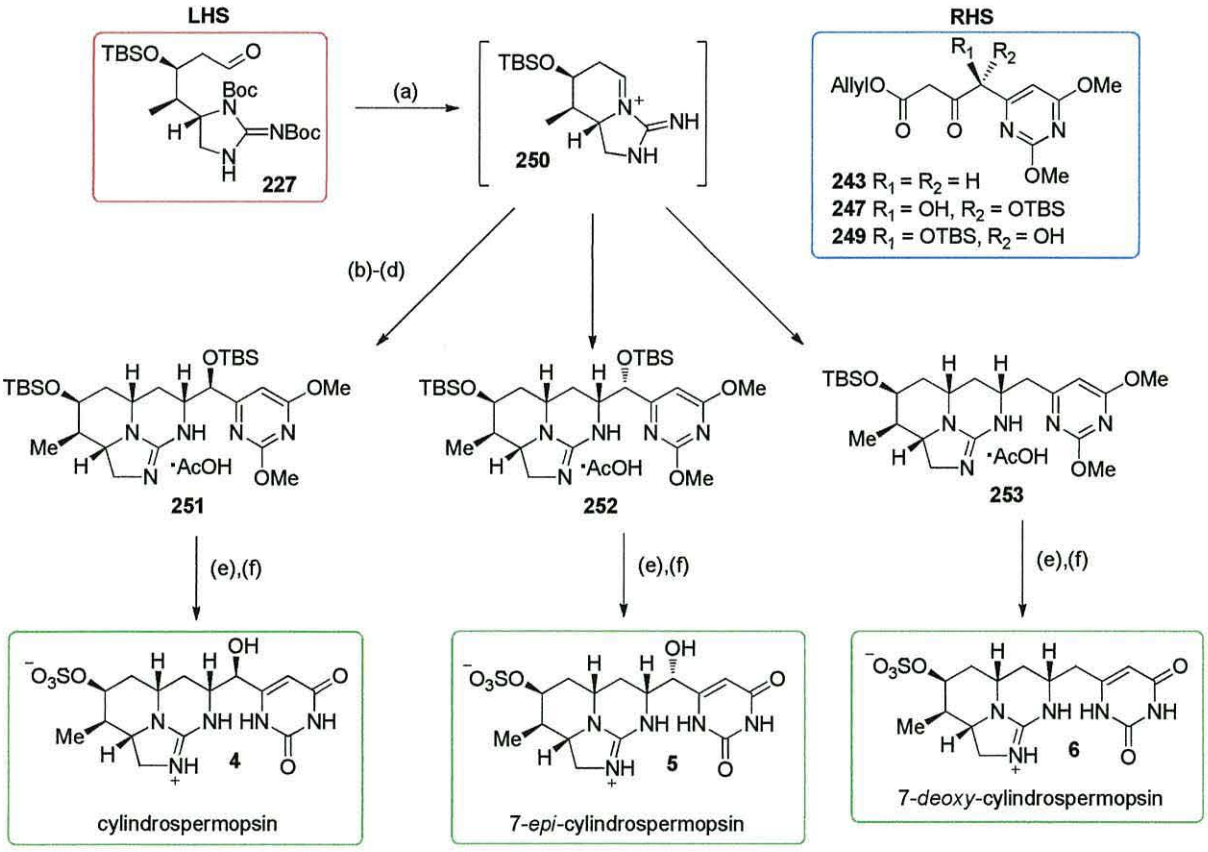
Scheme 58:- (a) $POCl_3$; (b) NaOMe, MeOH; (c) Meldrum's acid, DCC, DMAP; (d) allyl alcohol, Δ.

At this juncture it should be possible to prepare the remaining RHS substrates for the synthesis of cylindrospermopsin and 7-*epi*-cylindrospermopsin from β-ketoester **243**. Treatment of **243** with NaH followed by LDA should generate the corresponding dienolate which can then be oxidised using either Davis oxaziridine **244** or **245** to give alcohols **246** and **248**.[226,227] Finally protection with TBSCl should yield the required condensation substrates **247** and **249** (Scheme 59).



Scheme 59:- (a) NaH then LDA, (1*S*)-(+)-(Camphorsulfonyl)oxaziridine (**244**), -78 °C, THF; (b) NaH then LDA, (1*R*)-(-)-(Camphorsulfonyl)oxaziridine (**245**), -78 °C, THF; (c) TBSCl, imidazole, DMF.

The marrying of the two fragments of interest will be achieved using the previously described tethered Biginelli conditions, beginning with the deprotection of aldehyde **227** followed by condensation of the resulting intermediates with either **243/247/249**.[193] Interestingly, greater yields of tricyclic guanidines may be observed in comparison to the racemic model system previously investigated as the top face of the resulting iminium species **250** may be partially blocked from attack by the methyl and TBSO groups. Subsequently a short three step sequence involving deallylation/decarboxylation and reduction should yield tetracyclic guanidines **251/252/253**. Global deprotection of which by treatment with refluxing HCl followed by sulphonation using known chemistry should generate all three metabolites of the cylindrospermopsin family of natural products (Scheme 60).[130]



Scheme 60:- (a) acetic Acid, 24 h; (b) morpholine acetate, (**243**, **247** or **249**), Na_2SO_4, CF_3CH_2OH, Δ; (c) $Pd(PPh_3)_4$, pyrrolidine, THF/MeOH; (d) $NaBH_3CN$, AcOH/MeOH; (e) HCl, 100 °C; (f) SO_3·DMF, pyridine, DMF.

A key aim when developing the present synthetic approach towards the cylindrospermopsin alkaloids was the efficiency in which the target natural products could be obtained. Therefore, the potential ideality of both of the proposed synthetic pathways (Schemes 54, 55 and 58) were quantified using the methodology of Gaich and Baran, as previously discussed in the context of the reported total syntheses of these metabolites (Table 9).[28, 145]

Target	Research Group	Steps	Chemical Yield (%)	NSR	PGM	FGI	SR	CR	% Ideality
4, 5, 6	Murphy Alkylation	17	___	4	4	0	2	7	53%
	Murphy Crotylation	14	___	3	5	0	1	5	43%
4	Williams (2006)	19	0.34-0.57	3	5	2	0	9	47%
5	Williams (2004)	19	0.47-0.82	3	5	2	0	9	47%
6	Williams[1] (2005)	20	0.62-1.05	3	5	2	1	9	50%

[1]Based on longest linear sequence [2]Racemic

Table 9:- Projected efficiencies of the proposed paths to the cylindrospermopsin alkaloids.

On comparing the projected ideality of the synthetic paths currently under investigation within the Murphy group it is clear that they have the potential to rank among the best published syntheses to date, and are expected to generate the desired metabolites in the least number of steps. Interestingly, the methodology that relies on Brown's asymmetric crotylation as a key step displays is lower % ideality that that of the method relying on a Fráter-Seebach alkylation even though the former should produce the requisite natural products in fewer steps. Due to the high selectivity and reproducibility's achieved with the Brown asymmetric crotylation and Sharpless dihydroxylation reactions it is expected that this pathway is likely to furnish the LHS precursor with much higher overall selectivity that that of the alternative pathway which relies heavily on a stereoselective nitro-aldol reaction. Therefore unless significant progress can be made

with regards to the selectivity in which nitro-alcohol **223** is obtained, the shorter "Murphy crotylation" pathway appears to be the methodology of choice for future investigations within the group.

Experimental

General Procedures

Unless otherwise noted, reactions were magnetically stirred and monitored by TLC. The chromatograms were visualised with either iodine, phosphomolybdic acid, potassium permanganate, 2,4-Dinitrophenylhydrazine or under UV light. All anhydrous reactions were conducted under a static argon atmosphere using oven dried glassware that had previously been cooled under a constant stream of nitrogen.

Materials

Starting materials and reagents were purchased from commercial suppliers and used without further purification unless otherwise noted. All anhydrous solvents used were distilled over either sodium wire and benzophenone (THF/Et_2O) or calcium hydride (DCM/DMPU/DIPA) and used either immediately or stored over molecular sieves prior to use.[228] Anhydrous DMF was prepared by sequential drying of reagent grade DMF with 3 Å molecular sieves.[229] NaH was purchased as a 60 % dispersion in mineral oil and given three successive washes with hexane prior to use. The concentration of *n*-BuLi was determined by titration against DPAA.[230] 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.

Instrumentation

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer with an internal deuterium lock at ambient temperature at 500 MHz and 125 MHz with internal references of δ_H 7.26, δ_C 77.0 and δ_H 3.31 δ_C, 49.00 for $CDCl_3$ and CD_3OD respectively. Melting temperatures were determined using a Gallenkamp MF370 instrument. 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 at the EPSRC National Mass Spectrometry Service Centre based in Swansea. Infrared samples were prepared as thin films in chloroform using sodium chloride plates and the spectra recorded on a Bruker Tensor 37 FT-IR. Specific rotations were recorded on an Optical

Activity Polar 2001 polarimeter, with the temperature, concentration and solvent recorded in each case.

Spectra

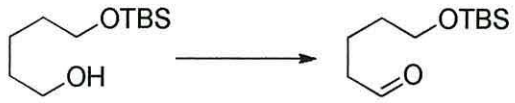
Spectrometric data (NMR, X-Ray and Mass Spec) for all characterised compounds is available for viewing on the attached electronic supplementary data CD.

Preparation of 5-(*tert*-butyl-dimethyl-silanyloxy)-pentan-1-ol (173)

To a stirred suspension of sodium hydride (3.86 g, 100 mmol, 1 eqv) in anhydrous THF (100 mL) was added pentane-1,5-diol (10.52 mL, 100 mmol, 1 eqv), after one hour *tert*-butyldimethylsilyl chloride (15.07 g, 100 mol, 1 eqv) was added and vigorous stirring continued for a further hour. The reaction mixture was then diluted with diethyl ether (600 mL) and washed with potassium carbonate (aq. 10%, 200 mL), brine (200 mL) and the organic layer dried over magnesium sulfate. After purification by flash column chromatography on silica gel using ethyl acetate/petroleum ether (10:90, 40:60), **174** was obtained as a clear oil (14.7 g, 67.1 mmol, 67%). R_f= 0.36 (20% ethyl acetate in petrol); ν_{max} (chloroform)/cm^{-1} 3417 (OH, br), 2932 (CH, w), 2858 (CH, m), 1471 (w), 1389 (w) and 1361 (w); δ_H (500 MHz; $CDCl_3$) -0.01 (s, 6H, *t*-BuSi($\underline{CH_3}_2$), 0.84 (s, 9H, *t*-$\underline{Bu}Si(CH_3)_2$), 1.30-1.36 (m, 2H, CH_2), 1.46-1.55 (m, 4H, 2 x CH_2), 2.74 (s, 1H, OH) and 3.53-3.57 (m, 4H, 2 x CH_2); δ_C (125 MHz; $CDCl_3$) -5.6 (2 x CH_3, *t*-BuSi($\underline{CH_3}_2$), 18.0 (TBSO, C), 21.7 (CH_2), 25.6 (3 x CH_3, *t*-$\underline{Bu}Si(CH_3)_2$), 32.1 (CH_2), 32.2 (CH_2), 62.2 (CH_2, CH_2OTBS) and 62.9 (CH_2, CH_2OH).

Reaction Comments: This reaction was repeated 6 times on scales ranging from 25 to 100 mmol to give alcohol **173** in 58% to 67% yield.

Preparation of 5-(*tert*-butyl-dimethyl-silanyloxy)-pentanal (174)



To a cooled (-78 °C) and stirred solution of oxalyl chloride (3.98 mL, 46.4 mmol, 1.6 eqv) in anhydrous DCM (120 mL) was added anhydrous DMSO (5.96 mL, 84.0 mmol, 2.9 eqv). After 20 minutes a solution of alcohol **173** (6.34 g, 29.0 mmol, 1 eqv) in anhydrous DCM (50 mL) was added to the reaction mixture. After a further 20 minutes triethylamine (24.2 mL, 173.9 mmol, 6 eqv) was also added. After 3 hours TLC analysis indicated the complete consumption of **173** and the reaction was diluted with brine (100 mL) and warmed to rt. After separation the organic layer was washed with brine (2 x 100 mL), HCl (0.25 M, 3 x 100 mL) and water (3 x 100 mL). After drying and evaporation the resulting yellow oil was dissolved in petroleum ether (50 mL) and passed through a plug of silica (ca. 1 cm) eluting with further petroleum ether (200 mL). After evaporation **174** was obtained as a pale yellow oil (5.57 g, 25.7 mmol, 89%) which was used without further purification. R_f = 0.62 (20% ethyl acetate in petrol); ν_{max} (chloroform)/cm^{-1} 2954 (CH, s), 2930 (CH, s), 2857 (CH, m), 1728 (C=O, sh), 1472 (w), 1388 (w) and 1361 (w); δ_H (500 MHz; $CDCl_3$) 0.01 (s, 6H, *t*-BuSi(C<u>H</u>$_3$)$_2$), 0.85 (s, 9H, *t*-BuSi(C<u>H</u>$_3$)$_2$), 1.50-1.54 (m, 2H, C<u>H</u>$_2$CH$_2$OTBS), 1.64-1.70 (m, 2H, C<u>H</u>$_2$CH$_2$CHO), 2.42 (td, 2H, J = 7.4, 1.7 Hz, C<u>H</u>$_2$CHO), 3.59 (t, 2H, J = 6.2 Hz, C<u>H</u>$_2$OTBS) and 9.73 (t, 1H, J = 1.7 Hz, CHO); δ_C (125 MHz; $CDCl_3$) -5.4 (2 x CH_3, *t*-BuSi(C<u>H</u>$_3$)$_2$), 18.2 (TBSO, C), 18.6 (CH_2, C<u>H</u>$_2$CH$_2$OTBS), 25.8 (3 x CH_3, *t*-BuSi(CH$_3$)$_2$), 32.0 (CH_2, C<u>H</u>$_2$CH$_2$CHO), 43.5 (CH_2, C<u>H</u>$_2$CHO), 64.5 (CH_2, C<u>H</u>$_2$OTBS) and 202.4 (C=O, aldehyde).

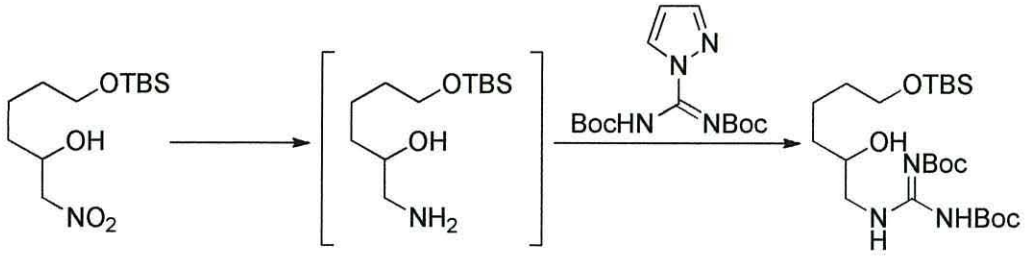
Reaction Comments: This reaction was repeated 4 times on scales ranging from 7 to 67 mmol to give aldehyde **174** in 77% to 89% yield.

Preparation of 6-(*tert*-butyl-dimethyl-silanyloxy)-1-nitro-pentan-2-ol (175)

To a stirred solution of aldehyde **174** (4.93 g, 22.7 mmol, 1 eqv) in DCM (80 mL) at rt was added nitromethane (73.7 mL, 1.36 mol, 60 eqv) and the mixture cooled (0 °C) and diisopropylethylamine (9.90 mL, 56.9 mmol, 2.5 eqv) added. Reaction progress was monitored via TLC and after 5 days the reaction was quenched with an ammonium chloride solution (sat, 200 mL), separated and the aqueous layer extracted with chloroform (3 x 100 mL). The combined organic extracts were washed with brine (100 mL), dried over anhydrous magnesium sulfate and purified by flash column chromatography on silica gel using ethyl acetate/petroleum ether (10:90, 30:70). Fractions eluting in 10:90 ethyl acetate/petroleum ether gave **175** as a pale yellow oil (5.11 g, 18.4 mmol, 81%). R_f = 0.29 (20% ethyl acetate in petrol); ν_{max} (chloroform)/cm^{-1} 3418 (OH, br), 2953 (CH, s), 2931 (CH, s), 2858 (CH, sh), 1556 (NO_2 asymmetric stretch, s), 1471 (m), 1463 (m), 1421 (NO_2 symmetric stretch, sh), 1385 (w) and 1361 (m); δ_H (500 MHz; $CDCl_3$) 0.05 (s, 6H, *t*-BuSi(C<u>H</u>$_3$)$_2$), 0.89 (s, 9H, *t*-<u>Bu</u>Si(CH$_3$)$_2$), 1.41-1.62 (m, 6H, 3 x CH_2), 2.87 (s, 1H, OH), 3.62 (t, 2H, J = 5.9 Hz, <u>C</u>H$_2$OTBS), 4.31-4.34 (m, 1H, <u>CH</u>OH), 4.37 (dd, 1H, J = 12.6, 8.5 Hz, <u>C</u>H$_2$NO$_2$) and 4.42 (dd, 1H, J = 12.6, 3.1 Hz, <u>C</u>H$_2$NO$_2$); δ_C (125 MHz; $CDCl_3$) -5.4 (2 x CH_3, *t*-BuSi(<u>C</u>H$_3$)$_2$), 18.3 (TBSO, C), 21.6 (CH_2, <u>C</u>H$_2$CH$_2$CHOH), 25.9 (3 x CH_3, *t*-<u>Bu</u>Si(CH$_3$)$_2$), 32.1 (CH_2, <u>C</u>H$_2$CH$_2$OTBS), 33.4 (CH_2, CH$_2$<u>C</u>H$_2$CHOH), 62.8 (CH_2, <u>C</u>H$_2$OTBS), 68.6 (CH, <u>CH</u>OH) and 80.6 (CH_2, <u>C</u>H$_2$NO$_2$); LRMS, CI, *m/z* 295 ([M+NH$_3$]$^+$, 3%), 278 ([M+H]$^+$, 9%), 235 (5), 234 (30), 219 (7), 218 (20), 217 (100), 176 (5), 159 (8), 132 (6), 102 (3), 91 (6) and 85 (6); HRMS, ESI, *m/z* $C_{12}H_{28}NO_4Si$, requires 287.1782, found 287.1784 [M+H]$^+$.

Reaction Comments: This reaction was repeated 4 times on scales ranging from 2 to 65 mmol to give nitro-alcohol **175** in 76% to 81% yield.

Preparation of *N,N'-bis*-(*tert*-butyloxycarbonyl)-*N''*-(6-(*tert*-butyldimethylsilyloxy)-2-hydroxyhexyl)-guanidine (177)

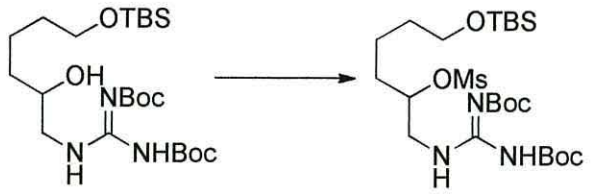


To a vigorously stirred, cooled (0 °C) solution of nickel (II) chloride hexahydrate (8.67 g, 36.5 mmol, 3 eqv) in methanol (50 mL) was added sodium borohydride (4.10 g, 109.4 mmol, 9 eqv) and stirring continued for 30 minutes. To the resultant black suspension was added nitro-alcohol **175** (3.38 g, 12.2 mmol, 1 eqv) in methanol (20 mL), followed by the portion-wise addition of further sodium borohydride (9.23 g, 246.8 mmol, 20 eqv). The reaction mixture was stirred for a further two hours at rt and then filtered through a pad of Celite® (ca. 5 cm) and washed with methanol (3 x 50 mL). Triethylamine (149 mL, 1.07 mol, 88 eqv) was added and after stirring for 45 minutes, the guanylating agent **176** (4.14 g, 13.4 mmol, 1.1 eqv) dissolved in methanol (15 mL) was also added. After 48 hours the reaction was diluted with water (400 mL) and extracted with ethyl acetate (3 x 250mL). The combined organic extracts were dried over magnesium sulfate, evaporated and the resultant reaction material purified by flash column chromatography on silica gel using ethyl acetate/petroleum ether (5:95, 25:75). Fractions eluting in 10:90 ethyl acetate/petroleum ether gave **177** as a clear oil (4.66 g, 9.5 mmol, 78%). R_f = 0.34 (40% ether in petrol); ν_{max} (chloroform)/cm^{-1} 3330 (OH, m), 3291 (NH, sh), 3156 (NH, m), 2982 (CH, sh), 2933 (CH, s), 2859 (CH, sh), 1724 (C=N, sh), 1618 (C=O, s), 1601 (C=O, s), 1577 (NH bend, m), 1472 (w), 1460 (w), 1411 (m), 1394 (m), 1368 (sh) and 1335 (s); δ_H (500 MHz; $CDCl_3$) 0.04 (s, 6H, *t*-BuSi($\underline{CH_3}_2$), 0.88 (s, 9H, *t*-$\underline{Bu}$Si(CH_3)$_2$), 1.35-1.63 (m, 6H, 3 x CH_2), 1.47 (s, 9H, Boc), 1.52 (s, 9H, Boc), 3.33-3.39 (m, 1H, $\underline{CH_2}$NH), 3.54 (ddd, 1H, J = 14.2, 6.3, 2.2 Hz, $\underline{CH_2}$NH), 3.62 (t, 2H, J = 6.3 Hz, $\underline{CH_2}$OTBS), 3.74-3.76 (m, 1H, $\underline{CH}$OH), 4.59 (s, 1H, OH), 8.67 (t, 1H, J = 4.4 Hz, NH) and 11.47 (s, 1H, NH); δ_C (125 MHz; $CDCl_3$) -5.3 (2 x CH_3, *t*-BuSi($\underline{CH_3}_2$), 18.3 (TBSO, C), 21.8 (CH_2, $\underline{CH_2}CH_2CHOH$), 26.0 (3 x CH_3, *t*-$\underline{Bu}$Si(CH_3)$_2$), 28.0 (3 x CH_3, Boc), 28.2 (3 x CH_3, Boc), 32.7 (CH_2, $\underline{CH_2}CH_2OTBS$), 34.9 (CH_2, $\underline{CH_2}CHOH$), 47.6 (CH_2, $\underline{CH_2}NH$), 63.1 (CH_2,

$\underline{CH_2}$OTBS), 71.8 (CH, $\underline{CH}$OH), 79.5 (Boc, C), 83.4 (Boc, C), 153.1 (C=N, guanidine), 157.4 (C=O, Boc) and 162.9 (C=O, Boc); LRMS, CI, *m/z* 491 ($[M+H]^+$, 40%), 434 (4), 390 (3), 373 (4), 370 (1), 320 (2), 275 (3), 274 (9), 273 (14), 248 (6), 232 (11), 231 (44), 217 (100), 204 (11), 175 (4), 174 (5), 160 (15) 159 (21), 145 (20), 132 (16), 104 (18), 92 (18), 60 (30), 58 (49) and 45 (71); HRMS, ESI, *m/z* $C_{23}H_{48}N_3O_6Si$, requires 490.3307, found 490.3312 $[M+H]^+$.

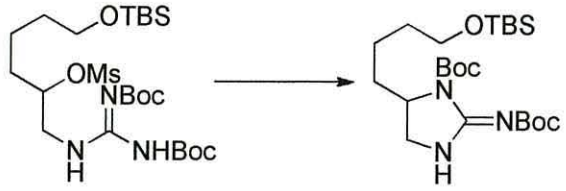
Reaction Comments: This reaction was repeated 10 times on scales ranging from 0.8 to 22 mmol to give guanidine **177** in 76% to 81% yield.

Preparation of *N,N'-bis*-(*tert*-butyloxycarbonyl)-*N''*-(6-(*tert*-butyldimethylsilyloxy)-2-methanesulfonate)-guanidine (178)



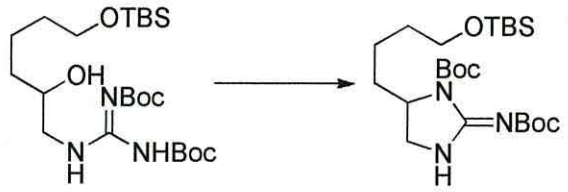
To a stirred solution of **177** (529 mg, 1.08 mmol, 1 eqv) in anhydrous DCM (5 mL) was added anhydrous pyridine (0.87 mL, 10.77 mmol, 10 eqv), methanesulfonic anhydride (375 mg, 2.15 mmol, 2 eqv) and 4-dimethylaminopyridine (26 mg, 0.22 mmol, 0.2 eqv). Reaction progress was monitored via TLC and after 4 days the reaction was quenched with an ammonium chloride solution (sat, 100 mL) and the aqueous layer extracted with chloroform (3 x 100 mL). The combined organic extracts where then washed with brine (100 mL) and dried over magnesium sulfate. After evaporation, purification was achieved by flash column chromatography on silica gel using ethyl acetate/petroleum ether (10:90, 50:50). Fractions eluting in 20:80 ethyl acetate/petroleum ether gave **178** as a waxy oil (520 mg, 0.92 mmol, 84%). R_f = 0.40 (20% ethyl acetate in petrol); ν_{max} (chloroform)/cm^{-1} 3346 (NH, w), 3005 (CH, m), 2978 (CH, m), 2953 (CH, m), 2931 (CH, m), 2853 (CH, m), 1757 (C=N, s), 1703 (C=O, m), 1650 (C=O, m), 1532 (NH bend, w), 1473 (w), 1460 (w), 1368 (SO_2 asymmetric stretch, s), 1314 (sh), 1283 (s), 1251 (sh) and 1163 (SO_2 symmetric stretch, s); δ_H (500 MHz; $CDCl_3$) 0.05 (s, 6H, *t*-BuSi($\underline{CH_3}_2$), 0.81 (s, 9H, *t*-$\underline{Bu}Si(CH_3)_2$), 1.17-1.73 (m, 6H, 3 x CH_2), 1.44 (s, 9H, Boc), 1.45 (s, 9H, Boc), 3.33 (s, 3H, $OSO_2\underline{CH_3}$), 3.52-3.56 (m, 2H, $\underline{CH_2}NH$), 3.66-3.71 (m, 2H, $\underline{CH_2}OTBS$) and 4.12-4.16 (m, 1H, $\underline{CH}OMs$); δ_C (125 MHz; $CDCl_3$) -5.6 (2 x CH_3, *t*-BuSi($\underline{CH_3}_2$), 18.0 (TBSO, C), 20.7 (CH_2, $\underline{CH_2}CH_2CHOMs$), 25.7 (3 x CH_3, *t*-$\underline{Bu}Si(CH_3)_2$), 27.8 (3 x CH_3, Boc), 27.9 (3 x CH_3, Boc), 32.3 (CH_2, $\underline{CH_2}CH_2OTBS$), 33.4 (CH_2, $CH_2\underline{CH_2}CHOMs$), 40.3 ($CH_3$, $OSO_2\underline{CH_3}$), 46.7 (CH_2, $\underline{CH_2}NH$), 55.7 (CH, $\underline{CH}OMs$), 62.3 (CH_2, $\underline{CH_2}OTBS$), 80.0 (Boc, C), 83.6 (Boc, C), 143.9 (C=O, Boc), 149.4 (C=O, Boc) and 156.9 (C=N, guanidine); LRMS, ESI, *m/z* 551 ([M+H-H_2O]$^+$, 6%), 473 (27), 451 (2), 417 (6), 373 (14), 351 (6), 302 (3), 271 (5), 213 (3), 188 (6), 154 (12), 133 (43), 113 (32), 99 (27), 84 (50) and 58 (100).

Attempted preparation of *tert*-butyl 2-((*tert*-butoxycarbonyl)imino)-5-(4-((*tert*-butyldimethyl silyl)oxy)butyl)imidazolidine-1-carboxylate (179)



To a stirred solution of mesylate **178** (352 mg, 0.62 mmol, 1 eqv) in anhydrous acetonitrile (2.5 mL) was added diisopropylethylamine (0.54 mL, 3.10 mmol, 5 eqv) and the mixture heated to 80 °C for 48 hours. Upon cooling to rt the reaction was quenched with an ammonium chloride solution (sat, 10 mL) separated and the aqueous layer extracted with chloroform (3 x 15 mL). The combined organic extracts where washed with brine (20 mL) and dried over anhydrous magnesium sulfate. Purification was not undertaken as only unreacted starting material was recovered (232 mg, 0.41 mmol, 66%).

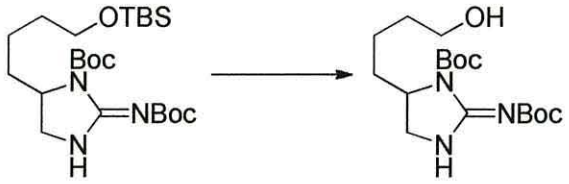
Preparation of *tert*-butyl 2-((*tert*-butoxycarbonyl)imino)-5-(4-((*tert*-butyldimethylsilyl)oxy)butyl)imidazolidine-1-carboxylate (179)



A stirred solution of alcohol **177** (7.79 g, 15.9 mmol, 1 eqv) in DCM (150 mL) was cooled (-20 °C) and triphenylphosphine (9.59 g, 36.6 mmol, 2.3 eqv), imidazole (4.22 g, 62.0 mmol, 3.9 eqv) and iodine (7.48 g, 31.8 mmol, 2 eqv) were added. Reaction progress was monitored by TLC and after stirring for 2 hours 30 minutes the reaction mixture was diluted with chloroform (150 mL), washed with an ammonium chloride solution (sat, 150 mL) and then brine (150 mL). After drying over magnesium sulfate and evaporation, the reaction material was purified by flash column chromatography on silica gel using ethyl acetate/petroleum ether (10:90, 40:60). Fractions eluting in 30:70 ethyl acetate/petroleum ether gave **179** as a pale yellow oil (7.31 g, 15.5 mmol, 98%). R_f = 0.18 (40% ethyl acetate in petrol); ν_{max} (chloroform)/cm^{-1} 3313 (NH, m), 2954 (CH, sh), 2930 (CH, sh), 2858 (CH, sh), 1760 (C=N, sh), 1703 (C=O, s), 1651 (C=O, s), 1604 (NH bend, m), 1532 (m), 1473 (w), 1460 (w), 1438 (w) and 1368 (s); δ_H (500 MHz; $CDCl_3$) 0.02 (s, 6H, *t*-BuSi(C<u>H</u>$_3$)$_2$), 0.87 (s, 9H, *t*-BuSi(CH$_3$)$_2$), 1.30-1.67 (m, 6H, 3 x CH$_2$), 1.47 (s, 9H, Boc), 1.51 (s, 9H, Boc), 3.38-3.52 (m, 1H, C<u>H</u>$_2$NH), 3.59 (t, 2H, J = 6.5 Hz, C<u>H</u>$_2$OTBS), 3.75-3.88 (m, 1H, C<u>H</u>$_2$NH) and 4.08-4.16 (m, 1H, C<u>H</u>CH$_2$NH); δ_C (125 MHz; $CDCl_3$) -5.3 (2 x CH$_3$, *t*-BuSi(C<u>H</u>$_3$)$_2$), 18.3 (TBSO, C), 20.7 (CH$_2$, C<u>H</u>$_2$CH$_2$CH), 25.9 (3 x CH$_3$, *t*-BuSi(CH$_3$)$_2$), 28.1 (3 x CH$_3$, Boc), 28.1 (3 x CH$_3$, Boc), 32.6 (CH$_2$, C<u>H</u>$_2$CH$_2$OTBS), 33.6 (CH$_2$, C<u>H</u>$_2$CHCH$_2$NH), 56.4 (CH, C<u>H</u>CH$_2$NH), 56.4 (CH$_2$, C<u>H</u>$_2$NH), 62.7 (CH$_2$, C<u>H</u>$_2$OTBS) and 82.8 (Boc, C), four quaternary carbon signals were not detected; LRMS, CI, *m/z* 473 ([M+H]$^+$, 100%), 430 (2), 416 (20), 372 (76), 357 (35), 330 (2), 316 (16), 302 (12), 272 (18), 257 (31), 243 (7), 214 (8), 184 (15), 159 (6), 148 (13), 132 (29), 116 (11), 84 (57), 69 (37), 58 (96) and 45 (44); HRMS, ESI, *m/z* $C_{23}H_{46}N_3O_5Si$, requires 472.3201, found 472.3201 [M+H]$^+$.

Reaction Comments: This reaction was repeated 13 times on scales ranging from 0.2 to 21 mmol to give heterocycle **179** in 89% to 98% yield.

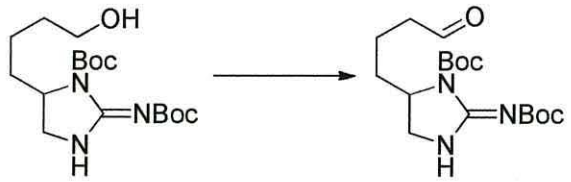
Preparation of 2-*tert*-butoxycarbonylimido-4-(4-hydroxy-butyl)-imidazolidine-1-carboxylic acid *tert*-butyl ester (180)



To a stirred solution of guanidine heterocycle **179** (72.4 mg, 0.154 mmol, 1 eqv) in THF (2 mL) at 0 °C was added a solution of TBAF in THF (1 M, 0.16 mL, 0.16 mmol, 1 eqv). Reaction progress was monitored by TLC and after 24 hours the reaction was quenched with an ammonium chloride solution (sat, 2 mL) and extracted with chloroform (3 x 5 mL). The combined organic extracts were then washed with brine (5 mL) and dried over magnesium sulfate. After evaporation, purification was achieved by flash column chromatography on silica gel using ethyl acetate/petroleum ether (80:20 to 100:0) and ethyl acetate/methanol (90:10). Fractions eluting in ethyl acetate gave **180** as a clear oil (54.4 mg, 0.152 mmol, 99%). R_f = 0.03 (100% ethyl acetate); ν_{max} (chloroform)/cm^{-1} 3318 (OH, br), 2981 (CH, m), 2932 (CH, m), 2865 (CH, m), 1745 (C=N, sh), 1701 (C=O, m), 1649 (C=O, m), 1602 (NH bend, m), 1532 (sh), 1476 (w), 1457 (w) and 1370 (s); δ_H (500 MHz; $CHCl_3$) 1.22-1.40 (m, 2H, CH_2), 1.44 (s, 9H, Boc), 1.45 (s, 9H, Boc), 1.46-1.58 (m, 4H, 2 x CH_2), 3.27-3.35 (m, 1H, $\underline{CH_2}NH$), 3.50 (t, 2H, J = 6.3 Hz, $\underline{CH_2}OH$), 3.66-3.72 (m, 1H, $\underline{CH_2}NH$) and 3.93-4.40 (m, 1H, $\underline{CH}CH_2NH$); δ_C (125 MHz; $CHCl_3$) 20.2 (CH_2, $\underline{CH_2}CH_2CH$), 27.8 (3 x CH_3, Boc), 27.8 (3 x CH_3, Boc), 32.0 (CH_2, $\underline{CH_2}CHCH_2NH$), 33.2 (CH_2, $\underline{CH_2}CH_2OH$), 56.0 (CH, $\underline{CH}CH_2NH$), 56.0 (CH_2, $\underline{CH_2}NH$), 61.8 (CH_2, $\underline{CH_2}OH$), 80.2 (Boc, C) and 82.6 (Boc, C), three quaternary carbon signals were not detected; LRMS, CI, *m/z* 358 ([M+H]$^+$, 55%), 319 (5), 302 (36), 279 (7), 258 (29), 243 (18), 211 (5), 202 (30), 186 (13), 156 (8), 133 (40), 116 (29), 98 (20), 79 (48), 69 (53), 58 (100) and 45 (54); HRMS, ESI, *m/z* $C_{17}H_{31}N_3O_5$, requires 358.2336, found 358.2335 [M+H]$^+$.

Reaction Comments: This reaction was repeated 10 times on scales ranging from 0.1 to 18 mmol to give the requisite alcohol **180** in 83% to 99% yield.

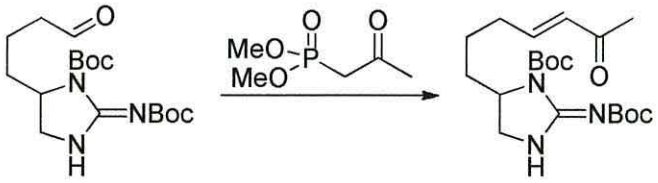
Preparation of *tert*-butyl 2-(*tert*-butoxycarbonylimino)-5-(4-oxobutyl)imidazolidine-1-carboxylate (181)



To a solution of alcohol **180** (260 mg, 0.73 mmol, 1 eqv) in DCM (2 mL) at rt was added pyridine (0.18 mL, 2.19 mmol, 3 eqv) and Dess-Martin periodinane (310 mg, 0.73 mmol, 1 eqv) and the mixture stirred for 24 hours. The reaction was then filtered through a tight plug of cotton wool, diluted with chloroform (20 mL) and washed with water (3 x 5 mL). Residual pyridine was removed by azeotropic evaporation with toluene (3 x 10 mL) to give **181** as a clear oil (249 mg, 0.70 mmol, 96%) which was used immediately without further purification. R_f = 0.30 (1% methanol in ethyl acetate); ν_{max} (chloroform)/cm^{-1} 3336 (NH, br), 2987 (CH, m), 2942 (CH, m), 2882 (CH, m), 1758 (C=N, s), 1710 (C=O, s), 1656 (C=O, m), 1609 (C=O, m), 1536 (NH bend, sh), 1482 (w), 1443 (w), 1374 (s) and 1320 (s); δ_H (500 MHz; $CDCl_3$) 1.41 (s, 9H, Boc), 1.46 (s, 9H, Boc), 1.50-1.62 (m, 4H, 2 x CH_2), 2.44 (td, 2H, J = 6.7, 1.0 Hz, $\underline{CH_2}CHO$), 3.43 (dd, 1H, J = 12.6, 3.2 Hz, $\underline{CH_2}NH$), 3.79 (dd, 1H, J = 12.6, 9.3 Hz, $\underline{CH_2}NH$), 4.05-4.09 (m, 1H, $\underline{CH}CH_2NH$) and 9.71 (s, 1H, CHO); δ_C (125 MHz; $CDCl_3$) 16.6 (CH_2, $\underline{CH_2}CH_2CHO$), 27.9 (3 x CH_3, Boc), 28.0 (3 x CH_3, Boc), 33.0 (CH_2, $\underline{CH_2}CHCH_2NH$), 43.2 (CH_2, $\underline{CH_2}CHO$), 55.9 (CH, $\underline{CH}CH_2NH$), 55.9 (CH_2, $\underline{CH_2}NH$), 80.5 (Boc, C), 83.1 (Boc, C) and 201.2 (C=O, CHO), three quaternary carbon signals were not detected; LRMS, ESI, *m/z* 414 (15), 388 ([M+MeOH+H]$^+$, 100%), 356 ([M+H]$^+$, 15%), 332 (6), 316 (12), 300 (10), 260 (14), 214 (12), 182 (10) and 164 (4); HRMS, ESI, *m/z* $C_{17}H_{30}N_3O_5$, requires 356.2180, found 356.2186 [M+H]$^+$.

Reaction Comments: This reaction was repeated in excess of 20 times on scales ranging from 0.2 to 1.5 mmol to give aldehyde **181** in 86% to 96% yield.

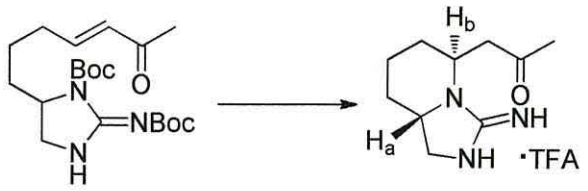
Preparation of *tert*-butyl 2-(*tert*-butoxycarbonylimino)-5-((*E*)-6-oxohept-4-enyl)imidazolidine-1-carboxylate (193)



To a stirred suspension of lithium chloride (28.2 mg, 0.65 mmol, 1.2 eqv) in anhydrous acetonitrile (3 mL) was added phosphonate **194** (75 μL, 0.54 mmol, 1 eqv). After 10 minutes, diisopropylethylamine (0.11 mL, 0.65 mmol, 1.2 eqv) was then added and the mixture stirred for a further 10 minutes. At this point aldehyde **181** (192 mg, 0.54 mmol, 1 eqv) dissolved in acetonitrile (2 mL) was added and the mixture stirred for 48 hours. The reaction was diluted with water (15 mL) and extracted with diethyl ether (3 x 15 mL) and the combined organic extracts washed with brine (10 mL) and dried over magnesium sulfate. After evaporation, compound **193** (183 mg, 0.46 mmol, 86%) was obtained as a pale yellow oil and used without further purification. R_f = 0.22 (100% ethyl acetate); ν_{max} (chloroform)/cm^{-1} 3320 (NH, w), 2980 (CH, m), 2933 (CH, m), 2860 (CH, m), 1764 (C=N, s), 1707 (C=O, s), 1678 (C=O, m), 1650 (C=C, m), 1605 (C=O, m), 1532 (NH bend, sh), 1374 (m) and 1327 (m); δ_H (500 MHz; $CDCl_3$) 1.22-1.69 (m, 6H, 3 x CH_2), 1.49 (s, 9H, Boc), 1.50 (s, 9H, Boc), 2.23 (s, 3H, $\underline{CH_3}$), 3.42-3.52 (m, 1H, $\underline{CH_2}NH$), 3.77-3.87 (m, 1H, $\underline{CH_2}NH$), 4.09-4.18 (m, 1H, $\underline{CH}CH_2NH$), 6.07 (d, 1H, J = 15.8 Hz, CH_2CH=$\underline{CH}$) and 6.74 (dt, 1H, J = 15.8, 7.0 Hz, $CH_2\underline{CH}$=CH); δ_C (125 MHz; $CDCl_3$) 22.8 (CH_2, $\underline{CH_2}CH_2CH$), 27.0 (CH_3), 28.1 (3 x CH_3, Boc), 28.1 (3 x CH_3, Boc), 32.1 (CH_2, $\underline{CH_2}CHCH_2NH$), 33.3 (CH_2, $\underline{CH_2}CH$=CH), 56.1 (CH, $\underline{CH}CH_2NH$), 56.1 (CH_2, $\underline{CH_2}NH$), 80.8 (Boc, C), 83.1 (Boc, C), 131.7 (CH, $CH_2\underline{CH}$=CH), 146.9 (CH, CH_2CH=$\underline{CH}$) and 198.3 (C=O, enone), three quaternary carbon signals were not detected; LRMS, ESI, *m/z* 791 ([2M+H]$^+$, 79%), 762 (7), 708 (3), 692 (6), 593 (7), 448 (12), 430 (17), 412 (49), 396 ([M+H]$^+$, 100%), 374 (2), 356 (13), 340 (27), 296 (10), 279 (19), 256 (6), 240 (23), 212 (3) and 196 (17); HRMS, ESI, *m/z* $C_{20}H_{34}N_3O_5$, requires 396.2493, found 396.2497 [M+H]$^+$.

Reaction Comments: This reaction was repeated 8 times on scales ranging from 0.1 to 1.5 mmol to give enone **193** in 61% to 86% yield.

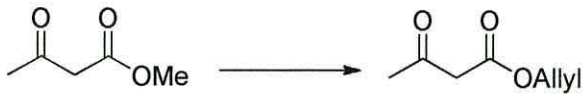
Preparation of (5*S*,8a*S*)-5-(2-oxopropyl)hexahydroimidazo[1,5-*a*]pyridin-3(2*H*)-iminium trifluoroacetate (198)



To a stirred solution of enone **193** (162 mg, 0.41 mmol, 1 eqv) in DCM (1.5 mL) was added trifluoroacetic acid (1.5 mL) and the mixture stirred at rt for 24 hours. After evaporation under reduced pressure, residual trifluoroacetic acid was removed by azeotropic evaporation with chloroform (3 x 15 mL) and the residue dried under high vacuum for 4 hours. The resultant guanidinium salt was dissolved in DCM (4 mL) and triethylamine (70 μL, 0.45 mmol, 1.1 eqv) was added and the mixture stirred at rt for 48 hours. After evaporation, purification by flash column chromatography on silica gel using methanol/chloroform (0:100 to 14:86 in 1% increments) gave **198** (89 mg, 0.28 mmol, 70%) as a viscous oil. An analytical sample was obtained by slow crystallisation from ethyl acetate. *Rf* = 0.08 (10% methanol in chloroform); m.p. 149-153 °C; ν_{max} (chloroform)/cm^{-1} 3400 (NH, m), 2953 (CH, m), 2875 (CH, m), 1714 (C=N, sh), 1682 (C=O, m), 1579 (NH bend, m), 1530 (m), 1482 (w), 1430 (m), 1367 (m) and 1320 (w); δ_H (500 MHz; $CDCl_3$) 1.44 (td, 1H, *J* = 13.6, 3.2 Hz), 1.55-1.59 (m, 1H), 1.63 (d, 1H, *J* = 13.6 Hz), 1.70-1.75 (m, 1H) 1.80 (dt, 1H, *J* = 13.6, 3.2 Hz), 1.92 (dd, 1H, *J* = 12.8, 3.0 Hz), 2.20 (s, 3H, C=O$\underline{CH_3}$), 2.54 (dd, 1H, *J* = 18.3, 3.2 Hz, $\underline{CH_2}$C=O), 3.04 (dd, 1H, *J* = 18.3, 9.3 Hz, $\underline{CH_2}$=O), 3.23 (dd, 1H, *J* = 9.5, 7.3 Hz, $\underline{CH_2}$NH), 3.77 (t, 1H, *J* = 9.5 Hz, $\underline{CH_2}$NH), 3.85 (app td, 1H, *J* = 11.5, 3.6 Hz, $\underline{H_a}$), 4.37-4.41 (m, 1H, $\underline{H_b}$) and 9.75 (s, 1H, NH); δ_C (125 MHz; $CDCl_3$) 18.2 (CH_2, $\underline{CH_2}CH_2CHCH_2NH$), 28.9 ($CH_2$, $\underline{CH_2}CHCH_2NH$), 30.9 (CH_3, C=O$\underline{CH_3}$), 31.0 (CH_2, $\underline{CH_2}CHCH_2C$=O), 44.3 (CH_2, $\underline{CH_2}NH$), 45.4 (CH_2, $\underline{CH_2}$C=O), 47.6 (CH, $\underline{CH_b}$), 53.7 (CH, $\underline{CH_a}$), 157.9 (C=N, guanidine) and 206.7 (C=O, ketone); LRMS, Positive ESI, *m/z* 391 ([2M+H]$^+$, 22%), 196 ([M+H]$^+$, 100%), 138 (5) and 130 (8); LRMS, Negative ESI *m/z* 227 ([2M-H]$^-$, 44%), and 113 ([M-H]$^-$, 100%); HRMS, ESI, *m/z* $C_{10}H_{18}N_3O$, requires 196.1444, found 196.1441 [M+H]$^+$.

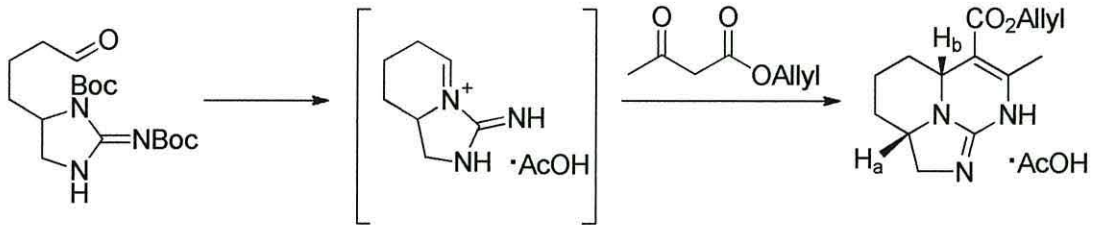
Reaction Comments: This reaction was repeated 6 times on scales ranging from 0.2 to 0.5 mmol to give bicyclic guanidine **198** in 54% to 70% yield.

Preparation of prop-2-enyl-3-oxobutanoate (206)



To a stirred solution of methyl acetoacetate (5.40 mL, 50 mmol, 1 eqv), allyl alcohol (3.40 mL, 50 mmol, 1 eqv) and 4-dimethylaminopyridine (1.22 g, 10 mmol, 0.2 eqv) in toluene (100 mL) was added 3 Å molecular sieves (25 g) and the mixture heated to reflux under an argon atmosphere. Reaction progress was monitored *via* TLC and after 36 hours the mixture was filtered through a plug of Celite® (ca. 2 cm). The filtrate was then washed with an ammonium chloride solution (sat, 3 x 70 mL) and brine (100 mL) and dried over magnesium sulfate. After evaporation, purification by bulb to bulb distillation (Kugelrohr) gave **206** as a clear oil (4.46 g, 31.5 mmol, 63%). R_f = 0.42 (30% ethyl acetate in petrol); ν_{max} (chloroform)/cm^{-1} 3093 (CH, w), 3032 (CH, m), 2953 (CH, w), 1744 (C=O ester, sh), 1718 (C=O ketone, sh), 1655 (C=C, m), 1637 (w), 1452 (w), 1410 (m), 1362 (m) and 1316 (m); δ_H (500 MHz; $CDCl_3$) 2.14 (s, 3H, $\underline{CH_3}C=O$), 3.37 (s, 2H, $\underline{CH_2}C=O$), 4.51 (d, 2H, J = 5.7 Hz, $O\underline{CH_2}CH=CH_2$), 5.12-5.23 (m, 2H, $OCH_2CH=\underline{CH_2}$) and 5.75-5.83 (m, 1H, $OCH_2\underline{CH}=CH_2$); δ_C (125 MHz; $CDCl_3$) 29.8 (CH_3, $\underline{CH_3}C=O$), 49.6 (CH_2, $\underline{CH_2}C=O$), 65.5 (CH_2, $O\underline{CH_2}CH=CH_2$), 118.3 (CH_2, $OCH_2CH=\underline{CH_2}$), 131.3 (CH, $OCH_2\underline{CH}=CH_2$), 166.5 (C=O, ester) and 199.6 (C=O, ketone).

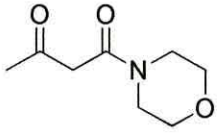
Preparation of (5a*R*,8a*S*)-5-((allyloxy)carbonyl)-4-methyl-3,5a,6,7,8,8a-hexahydro-1*H*-2,2a^1,3-triazaacenaphthylen-2-ium acetate (207)



A solution of aldehyde **181** (144 mg, 0.40 mmol, 1 eqv) in glacial acetic acid (3 mL) was stirred at rt for 24 hours. After evaporation under reduced pressure, residual acetic acid was removed by azeotropic evaporation with chloroform (3 x 15 mL) and the residue dried under high vacuum for 4 hours. The resultant salt was dissolved in trifluoroethanol (1.5 mL) and morpholine acetate (148 mg, 1.01 mmol, 2.5 eqv), **206** (286 mg, 2.01 mmol, 5 eqv) and anhydrous sodium sulfate (1 g) added and the mixture stirred at 70 °C for 12 days. After cooling to rt and evaporation, purification was achieved by flash column chromatography on silica gel using methanol/chloroform (stepwise gradient of 0.5% increments 0:100 to 4:96) containing 1% acetic acid. The fractions eluting in 2:98 methanol/chloroform gave **207** (56 mg, 0.17 mmol, 43%) as a tan oil; on standing or in methanol solution the acetate counterion is slowly converted to the carbonate by atmospheric CO2. R_f = 0.26 (10% methanol in chloroform); ν_{max} (chloroform)/cm^{-1} 3400 (NH, m), 2949 (CH, m), 2932 (CH, m), 2905 (CH, m), 2862 (CH, m), 1696 (C=N, sh), 1679 (C=O, s), 1637 (C=C, s), 1605 (C=C, s), 1449 (w), 1407 (m), 1391 (m) and 1337 (w); δ_H (500 MHz; $CDCl_3$) 1.32-1.40 (m, 1H), 1.51-1.66 (m, 2H), 1.71 (dd, 1H, J = 12.9, 3.2 Hz) 1.87 (dt, 1H, J = 13.3, 2.9 Hz), 1.96 (dd, 1H, J = 13.1, 3.0 Hz) 2.00 (s, 3H, AcOH), 2.36 (s, 3H, CH_3), 3.39 (dd, 1H, J = 10.4, 1.9 Hz, $\underline{CH_2}N$), 3.75 (t, 1H, J = 9.5 Hz, $\underline{CH_2}N$), 3.89-3.93 (m, 1H, $\underline{H_a}$), 4.41 (dd, 1H, J = 2.8, 11.4 Hz, $\underline{H_b}$), 4.63-4.65 (m, 2H, $O\underline{CH_2}CH{=}CH_2$), 5.23-5.32 (m, 2H, $OCH_2CH{=}\underline{CH_2}$) and 5.89-5.97 (m, 1H, $OCH_2\underline{CH}{=}CH_2$); δ_C (125 MHz; $CDCl_3$) 18.6 (CH_3), 21.3 (CH_2, $\underline{CH_2}CH_2CH_a$), 24.0 (CH_3, AcOH), 29.5 (CH_2, $\underline{CH_2}CH_aCH_2N$) 31.3 ($CH_2$, $\underline{CH_2}CH_bCCO_2Allyl$), 48.0 ($CH_2$, $\underline{CH_2}N$), 52.7 (CH, $\underline{CH_b}$), 57.9 (CH, $\underline{CH_a}$), 65.0 (CH_2, $O\underline{CH_2}CH{=}CH_2$), 101.5 ($C{=}\underline{C}CO_2Allyl$), 118.2 ($CH_2$, $OCH_2CH{=}\underline{CH_2}$), 132.2 (CH, $OCH_2\underline{CH}{=}CH_2$), 146.7 ($C{=}\underline{C}CH_3$), 154.6 (C=N, guanidine), 165.1 (C=O, ester) and 179.5 (C=O, AcOH); LRMS, Positive ESI, *m/z* 523

($[2M+H]^+$, 5%), 402 (4), 314 (15), 304 (6), 262 ($[M+H]^+$, 100) and 222 (2); LRMS, Negative ESI *m/z* 165 (6) 113 (20) 77 (100), 75 (62), 60 ($[CO_3]^{2-}$, 37%) and 59 ($[M-H]^-$, 68%); HRMS, ESI, *m/z* $C_{14}H_{20}N_3O_2$, requires 262.1550, found 262.1548 $[M+H]^+$.

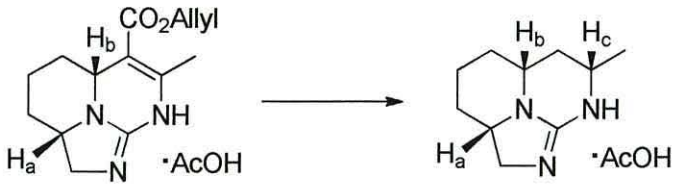
1-morpholinobutane-1,3-dione (208)



Amide **208** (51 mg, 0.30 mmol, 13% (based on **206**)) was isolated from the above reaction as a pale yellow oil. R_f = 0.39 (10% methanol in chloroform); δ_H (500 MHz; $CDCl_3$) 2.24 (s, 3H, CH_3), 3.38 (t, 2H, J = 4.8), 3.53 (s, 2H, C=O$\underline{CH_2}$C=O) 3.58-3.60 (m, 2H) and 3.62-3.65 (m, 4H); δ_C (125 MHz; $CDCl_3$) 30.2 (CH_3), 42.1 (CH_2), 46.7 (CH_2), 49.7 (CH_2), 66.4 (CH_2), 66.6 (CH_2), 165.0 (C=O, amide) and 202.0 (C=O, ketone); LRMS, Positive EI, *m/z* 172 ($[M+H]^+$, 34%), 171 (M^+, 54%), 128 (77), 87 (30), 86 (96), 85 (30), 57 (68), 56 (52) and 43 (100).

Reaction Comments: This reaction was repeated 4 times on a 0.4 mmol scale which gave Biginelli adduct **207** in 36% to 43% yield.

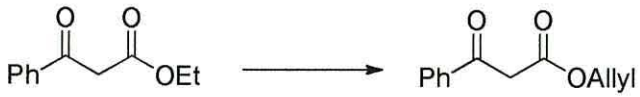
Preparation of (4*S*,5a*R*,8a*S*)-4-methyl-3,4,5,5a,6,7,8,8a-octahydro-1*H*-2,2a^1,3-triazaacenaphthylen-2-ium acetate (212)



To a stirred solution of guanidine **207** (23.5 mg, 0.067 mmol, 1 eqv) in a mixture of anhydrous methanol (1 mL) and anhydrous THF (1 mL) was added tetrakis(triphenylphosphine)-palladium(0) (1.6 mg, 0.0014 mmol, 0.02 eqv) and pyrrolidine (6.5μL, 0.079 mmol, 1.2 eqv). The reaction was stirred at rt and progress monitored by TLC. After 90 minutes the solvent was removed under reduced pressure and the residue dissolved in methanol (0.5 mL) and acetic acid (0.5 mL) and cooled (0 °C), whereupon sodium cyanoborohydride (21.4 mg, 0.340 mmol, 5 eqv) was added and the mixture stirred to rt over 16 hours. After evaporation, the reaction material was purified by flash column chromatography on silica gel using methanol/chloroform (stepwise gradient of 0.5% increments 0:100 to 4:96) containing 1% acetic acid. The fractions eluting in 2:98 methanol/chloroform gave **212** (7.0 mg, 0.034 mmol, 57%) as a pale yellow oil; on standing or in methanol solution the acetate counterion is slowly converted to the carbonate by atmospheric CO_2. R_f = 0.15 (10% methanol in chloroform); ν_{max} (chloroform)/cm^{-1} 3145 (NH, m), 2924 (CH, m), 2853 (CH, m), 1666 (C=N, sh), 1445 (w), 1338 (w) and 1332 (w); δ_H (500 MHz; $CD_3OD/CDCl_3$) 1.16-1.22 (m, 1H), 1.24-1.36 (m, 2H), 1.27 (d, 3H, J = 6.7 Hz, CH_3), 1.40 (ddd, 1H, J = 11.0, 11.1, 13.6 Hz), 1.51-1.54 (m, 1H) 1.92-1.97 (m, 1H), 2.05-2.07 (m, 1H), 2.16 (s, 3H, AcOH), 2.18 (app dt, 1H, J = 13.6, 3.6 Hz), 3.16-3.20 (m, 1H, $\underline{CH_2}N$), 3.34-3.39 (m, 1H, $\underline{H_b}$), 3.56-3.60 (m, 1H, $\underline{H_c}$) and 3.75-3.83 (m, 2H, $\underline{H_a}/\underline{CH_2}N$); δ_C (125 MHz; $CD_3OD/CDCl_3$) 20.7 (CH_2, $\underline{CH_2}CH_2CH_b$), 23.1 (CH_3, AcOH), 30.6 (CH_2, $\underline{CH_2}CH_aCH_2N$) 31.9 ($CH_2$, $\underline{CH_2}CH_bCH_2CH_c$), 37.8 ($CH_2$, $CH_b\underline{CH_2}CH_c$), 47.0 (CH, $\underline{CH_c}$), 48.5 ($\underline{CH_2}N$), 51.6 (CH, $\underline{CH_b}$) and 58.2 (CH, $\underline{CH_a}$), one quaternary carbon signal was not detected; LRMS, Positive ESI, *m/z* 395 (5), 239 (2) and 180 ($[M+H]^+$, 100%); LRMS, Negative ESI *m/z* 113 (7), 77 (100), 75 (7), 60 ($[CO_3]^{2-}$, 30%), 59 ($[M-H]^-$, 12%), 45 (14), 35 (6) and 33 (3); HRMS, ESI, *m/z* $C_{10}H_{18}N_3$, requires 180.1495, found 180.1492 $[M+H]^+$.

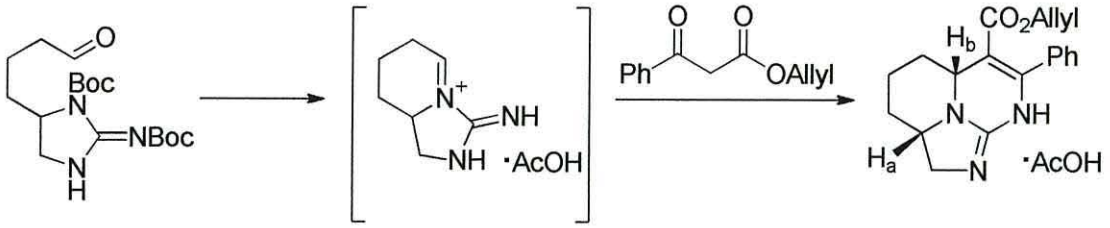
Reaction Comments: This reaction was repeated 2 times on a 0.07 and 0.1 mmol scale to give tricyclic guanidine **212** in 49% to 57% yield.

Preparation of allyl 3-oxo-3-phenylpropanoate (214)



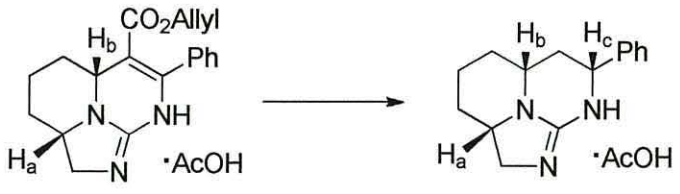
A stirred mixture of ethyl benzoylacetate (10.36 mL, 60 mmol, 1 eqv), allyl alcohol (61.2 mL, 900 mmol, 15 eqv) and 4-dimethylaminopyridine (2.20 g, 18 mmol, 0.3 eqv) was heated to reflux under an argon atmosphere for 5 days. Upon cooling to rt the reaction mixture was dissolved in ethyl acetate (150 mL) and washed with an ammonium chloride solution (sat, 3 x 200 mL) and brine (200 mL) and dried over magnesium sulfate. After evaporation, purification by bulb to bulb distillation (Kugelrohr) gave **214** as a pale yellow oil (8.92 g, 42.6 mmol, 71%). R_f = 0.64 (100% ethyl acetate); ν_{max} (chloroform)/cm^{-1} 3090 (CH, m), 3067 (CH, m), 3032 (CH, m), 2986 (CH, m), 2944 (CH, m), 1743 (C=O ester, s), 1686 (C=O ketone, s), 1644 (C=C, s), 1622 (C=C, s) 1598 (sh), 1497 (sh), 1450 (sh), 1408 (s), 1327 (s) and 1311 (s); δ_H (500 MHz; $CDCl_3$) 4.01 (s, 2H, $\underline{CH_2}C=O$), 4.63-4.65 (m, 2H, $O\underline{CH_2}CH=CH_2$), 5.19-5.31 (m, 2H, $OCH_2CH=\underline{CH_2}$), 5.84-5.92 (m, 1H, $OCH_2\underline{CH}=CH_2$) and 7.37-7.91 (m, 5H, C_6H_5); δ_C (125 MHz; $CDCl_3$) 45.7 (CH_2, $\underline{CH_2}C=O$), 65.8 (CH_2, $O\underline{CH_2}CH=CH_2$), 118.3 (CH_2, $OCH_2CH=\underline{CH_2}$), 128.4 (2 x CH, C_6H_5), 128.7 (2 x CH, C_6H_5), 131.3 (CH, $OCH_2\underline{CH}=CH_2$), 133.7 (CH, C_6H_5), 135.8 (aromatic, C), 167.0 (C=O, ester) and 192.2 (C=O, ketone).

Preparation of (5a*R*,8a*S*)-5-((allyloxy)carbonyl)-4-phenyl-3,5a,6,7,8,8a-hexahydro-1*H*-2,2a^1,3-triazaacenaphthylen-2-ium acetate (215)



A solution of aldehyde **181** (184 mg, 0.52 mmol, 1 eqv) in glacial acetic acid (3 mL) was stirred at rt for 24 hours. After evaporation under reduced pressure, residual acetic acid was removed by azeotropic evaporation with chloroform (3 x 15 mL) and the residue dried under high vacuum for 4 hours. The resultant guanidine salt was dissolved in trifluoroethanol (1.5 mL) and morpholine acetate (190 mg, 1.29 mmol, 2.5 eqv), **214** (528 mg, 2.59 mmol, 5 eqv) and anhydrous sodium sulfate (1 g) added and the mixture stirred at 70 °C for 12 days. After cooling to rt and evaporation, purification was achieved by flash column chromatography on silica gel using methanol/chloroform (stepwise gradient 0:100 to 4:96 in 0.5% increments) containing 1% acetic acid. The fractions eluting in 3:97 methanol/chloroform gave **215** as a tan oil (52 mg, 0.14 mmol, 26%).R_f = 0.24 (10% methanol in chloroform); ν_{max} (chloroform)/cm^{-1} 3338 (NH, m), 2926 (CH, m), 2859 (CH, m), 1693 (C=N, s), 1668 (C=O, s), 1648 (C=C, s), 1606 (C=C, s), 1493 (w), 1448 (m) and 1375 (w); δ_H (500 MHz; $CDCl_3$) 1.53-1.70 (m, 3H), 1.71-1.73 (m,1H), 1.80-1.82 (m, 1H), 2.11-2.13 (m, 1H), 3.48-3.51 (m, 1H, $\underline{CH_2}N$), 3.85-3.92 (m, 1H, $\underline{CH_2}N$), 4.00-4.04 (m, 1H, $\underline{H_a}$), 4.37 (d, 2H, J = 5.4 Hz, $O\underline{CH_2}CH{=}CH_2$), 4.54 (dd, 1H, J = 11.1, 2.7 Hz, $\underline{H_b}$), 4.92-5.04 (m, 2H, $OCH_2CH{=}\underline{CH_2}$), 5.47-5.54 (m, 1H, $OCH_2\underline{CH}{=}CH_2$) and 7.30-7.47 (m, 5H, C_6H_5); δ_C (125 MHz; $CDCl_3$) 21.3 (CH_2, $\underline{CH_2}CH_2CH_a$), 29.6 (CH_2, $\underline{CH_2}CH_aCH_2N$) 31.3 ($CH_2$, $\underline{CH_2}CH_bCCO_2Allyl$), 48.2 ($CH_2$, $\underline{CH_2}N$), 53.4 (CH, $\underline{CH_b}$), 58.0 (CH, $\underline{CH_a}$), 65.3 (CH_2, $O\underline{CH_2}CH{=}CH_2$), 103.5 ($C{=}\underline{C}CO_2Allyl$), 118.3 ($CH_2$, $OCH_2CH{=}\underline{CH_2}$), 128.1 (2 x CH, C_6H_5), 128.4 (2 x CH, C_6H_5), 130.2 (CH, C_6H_5), 131.2 (CH, $OCH_2\underline{CH}{=}CH_2$), 133.2 (aromatic, C), 144.5 ($C{=}\underline{C}Ph$), 153.5 (C=N, guanidine) and 164.8 (C=O, ester); LRMS, Positive ESI, *m/z* 422 ([M+2AcOH]$^+$, 5%), 390 (6), 382 ([M+AcOH]$^+$, 9%), 342 (13), 324 ([M+H]$^+$, 100%), 316 (69), 290 (12) and 276 (8); LRMS, Negative ESI *m/z* 121 (4) 113 (3) 77 (18), 59 ([M-H]$^-$, 100%), 46 (3) and 45 (12); HRMS, ESI, *m/z* $C_{19}H_{22}N_3O_2$, requires 324.1707, found 324.1705 [M+H]$^+$.

Preparation of (4*R*,5a*R*,8a*S*)-4-phenyl-3,4,5,5a,6,7,8,8a-octahydro-1*H*-2,2a^1,3-triazaacenaphthylen-2-iumacetate (216)



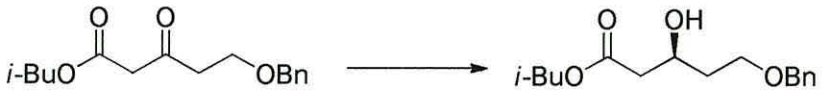
To a stirred solution of guanidine **215** (42.2 mg, 0.11 mmol, 1 eqv) in a mixture of anhydrous methanol (1 mL) and anhydrous THF (1 mL) was added tetrakis(triphenylphosphine)-palladium(0) (2.5 mg, 0.0022 mmol, 0.02 eqv) and pyrrolidine (11 μL, 0.13 mmol, 1.2 eqv). The reaction was stirred at rt and progress monitored by TLC. After 2 hours the solvent was removed under reduced pressure and the residue dissolved in methanol (1 mL) and acetic acid (1 mL) and cooled (0 °C), whereupon sodium cyanoborohydride (34.5 mg, 0.550 mmol, 5 eqv) was added and the mixture stirred to rt over 16 hours. After evaporation, the reaction material was purified by flash column chromatography on silica gel using methanol/chloroform (stepwise gradient of 1% increments 0:100 to 10:90) containing 1% acetic acid. The fractions eluting in 7:93 methanol/chloroform gave **216** as a pale yellow oil (14.9 mg, 0.049 mmol, 53%). R_f = 0.08 (10% methanol in chloroform); ν_{max} (chloroform)/cm^{-1} 3156 (NH, m), 2942 (CH, m), 2928 (CH, m), 2863 (CH, m), 1671 (C=N, sh), 1607 (C=C, m), 1556 (C=C, m), 1455 (w) and 1379 (w); δ_H (500 MHz; CD_3OD) 1.22-1.29 (m, 1H), 1.34-1.45 (m, 1H), 1.58 (m, 1H), 1.80 (app dt, 1H, J = 13.5, 11.5, 11.0 Hz), 1.96-2.02 (m, 2H), 2.00 (s, 3H, AcOH), 2.09-2.12 (m, 1H), 2.36 (app dt, 1H, J = 13.5, 3.3, 3.3 Hz), 3.26-3.31 (m, 1H, $\underline{CH_2}N$), 3.54 (app tt, 1H, J = 11.3, 11.0, 4.0, 3.3 Hz, $\underline{H_c}$), 3.83-3.91 (m, 2H, $\underline{H_a}/\underline{CH_2}N$), 4.63 (dd, 1H, J = 11.5, 3.3 Hz, $\underline{H_c}$) and 7.34-7.43 (m, 5H, C_6H_5); δ_C (125 MHz; $CDCl_3$) 22.2 (CH_2, $\underline{CH_2}CH_2CH_b$), 23.0 (CH_3, AcOH), 29.5 (CH_2, $\underline{CH_2}CH_aCH_2N$), 30.9 ($CH_2$, $\underline{CH_2}CH_bCH_2CH_c$), 38.7 ($CH_2$, $CH_b\underline{CH_2}CH_c$), 48.6 (CH_2, $\underline{CH_2}N$), 50.4 (CH, $\underline{CH_b}$), 54.0 (CH, $\underline{CH_c}$), 57.1 (CH, $\underline{CH_a}$), 126.1 (2 x CH, C_6H_5), 128.5 (CH, C_6H_5), 129.1 (2 x CH, C_6H_5), 139.1 (aromatic, C), 155.9 (C=N, guanidine) and 174.0 (C=O, AcOH); LRMS, Positive ESI, *m/z* 483 ([2M+H]$^+$, 6%), 284 (4) and 242 ([M+H]$^+$, 100%); LRMS, Negative ESI *m/z* 120 (10), 92 (12), 76 (42), 75 (100), 59 ([M-H]$^-$, 18%), 45 (16) and 32 (5); HRMS, ESI, *m/z* $C_{15}H_{20}N_3$, requires 242.1648, found 242.1652 [M+H]$^+$.

Preparation of isobutyl 5-(benzyloxy)-3-oxopentanoate (218)

To a cooled (0 °C) vigorously stirred suspension of sodium hydride (0.86 g, 25.9 mmol, 1.1 eqv) in anhydrous THF (100 mL) was added isobutyl acetoacetate (3.81 mL, 23.5 mmol, 1 eqv), after 30 minutes the reaction was cooled (-25 °C) and *n*-BuLi (11.4 mL, 25.9 mmol, 1 eqv) added and the mixture stirred for a further 30 minutes. Benzyl chloromethyl ether (60% by NMR assay, 6.0 mL, 25.9 mmol, 1.1 eqv) was then added and the reaction stirred to rt over 24 hours. The mixture was then cooled (0 °C) and the pH adjusted to pH 2-3 by the slow addition of ice-cold 1 M HCl, then diluted with DCM (20 mL) and extracted with EtOAc (3 x 100 mL). The organic extracts were then washed with brine (100 mL) and dried over magnesium sulfate. After evaporation, purification was achieved by flash column chromatography on silica gel using diethyl ether/petroleum ether (05:95, 15:85). Fractions eluting in 10:90 diethyl ether/petroleum ether gave **218** as a pale yellow oil (3.81 g, 13.7 mmol, 58%). R_f = 0.44 (20% ethyl acetate in petrol); ν_{max} (chloroform)/cm^{-1} 3091 (CH, w), 3068 (CH, w), 3036 (CH, w), 2962 (CH, m), 2933 (CH, m), 2874 (CH, m), 1742 (C=O ester, s), 1717 (C=O ketone, s), 1650 (w), 1472 (w), 1457 (w), 1368 (m) and 1314 (m); δ_H (500 MHz; $CDCl_3$) 0.92 (d, 6H, J = 7.0 Hz, $(CH_3)_2CHCH_2$), 1.94 (n, 1H, J = 7.0 Hz, $(CH_3)_2CHCH_2$), 2.83 (t, 2H, J = 6.3 Hz, CH_2CH_2OBn), 3.50 (s, 2H, $C{=}OCH_2C{=}O$), 3.75 (t, 2H, J = 6.3 Hz, CH_2CH_2OBn), 3.91 (d, 2H, J = 6.7 Hz, $(CH_3)_2CHCH_2$), 4.51 (s, 2H, OCH_2Ph) and 7.27-7.36 (m, 5H, OCH_2Ph); δ_C (125 MHz; $CDCl_3$) 19.0 (2 x CH_3, $(CH_3)_2CHCH_2$), 27.6 (CH, $(CH_3)_2CHCH_2$), 43.1 (CH_2, $C{=}OCH_2C{=}O$), 49.7 (CH_2, CH_2CH_2OBn), 64.9 (CH_2, CH_2CH_2OBn), 71.4 (CH_2, $(CH_3)_2CHCH_2$), 73.2 (CH_2, OCH_2Ph), 127.7 (2 x CH, C_6H_5), 127.7 (CH, C_6H_5), 128.4 (2 x CH, C_6H_5), 137.9 (aromatic, C), 167.1 (C=O, ester) and 201.3 (C=O, ketone); LRMS, Positive ESI, *m/z* 586 (12), 416 (5), 296 ($[M+NH_4]^+$, 100%) and 171 (3); HRMS, ESI, *m/z*$C_{16}H_{26}O_4N$, requires 296.1856, found 296.1859 $[M+NH_4]^+$.

Reaction Comments: This reaction was repeated 10 times on scales ranging from 8 to 26 mmol to give β-keto ester **218** in 43% to 58% yield.

Preparation of (*S*)-isobutyl 5-(benzyloxy)-3-hydroxypentanoate (219)



To a vigorously stirred solution of sucrose (25 g) in H_2O (250 mL) heated to 35 °C was added dry active baker's yeast (25 g), after 30 minutes β-keto ester **218** (1.96 g, 7.0 mmol) was added and stirring continued for 48 hours. Upon cooling to rt DCM (200 mL) was added and the reaction mixture stirred for a further 2 hours after which time the reaction was filtered through a pad of Celite® (ca. 4 cm) which was washed with further DCM (3 x 30 mL). The phases were then separated and the aqueous phase extracted with DCM (2 x 100 mL), the combined organic extracts were then dried over magnesium sulfate. After evaporation, purification was achieved by flash column chromatography on silica gel using diethyl ether/petroleum ether (05:95, 20:80). Fractions eluting in 10:90 diethyl ether/petroleum ether gave **219** as a clear oil (1.30 g, 4.6 mmol, 66%, er 95:5).[1] R_f = 0.16 (20% ethyl acetate in petrol); $[\alpha]_D^{21}$ +10.9 (c 0.01, $CHCl_3$); ν_{max} (chloroform)/cm^{-1} 3492 (OH, br), 3093 (CH, w), 3066 (CH, w), 3031 (CH, w), 2962 (CH, m), 2874 (CH, m), 1727 (C=O, s), 1498 (w), 1472 (m), 1454 (m), 1380 and 1369 (m); δ_H (500 MHz; $CDCl_3$) 0.87 (d, 6H, J = 7.0 Hz, $(\underline{CH_3})_2CHCH_2$), 1.69-1.79 (m, 2H, $\underline{CH_2}CH_2OBn$), 1.85 (n, 1H, J = 7.0 Hz, $(CH_3)_2\underline{CH}CH_2$), 2.44 (d, 2H, J = 6.3 Hz, $C{=}O\underline{CH_2}CHOH$), 3.31 (s, 1H, $CH\underline{OH}$), 3.55-3.65 (m, 2H, $CH_2\underline{CH_2}OBn$), 3.82 (d, 2H, J = 6.7 Hz, $(CH_3)_2CH\underline{CH_2}$), 4.15-4.20 (m, 1H, $\underline{CH}OH$) 4.45 (s, 2H, $O\underline{CH_2}Ph$) and 7.20-7.29 (m, 5H, $OCH_2\underline{Ph}$); δ_C (125 MHz; $CDCl_3$) 18.9 (2 x CH_3, $(\underline{CH_3})_2CHCH_2$), 27.5 (CH, $(CH_3)_2\underline{CH}CH_2$), 36.0 ($CH_2$, $\underline{CH_2}CH_2OBn$), 42.0 (CH_2, $C{=}O\underline{CH_2}CHOH$), 66.7 (CH, $\underline{CH}OH$), 67.7 (CH_2, $CH_2\underline{CH_2}OBn$), 70.6 (CH_2, $(CH_3)_2CH\underline{CH_2}$), 73.1 ($CH_2$, $O\underline{CH_2}Ph$), 127.5 (2 x CH, C_6H_5), 127.6 (CH, C_6H_5), 128.3 (2 x CH, C_6H_5), 136.0 (aromatic, C) and 172.4 (C=O, ester); LRMS, Positive ESI, *m/z* 583 ($[2M+Na]^+$, 40%), 298 ($[M+NH_4]^+$, 37%) and 281 ($[M+H]^+$, 100%); HRMS, ESI, *m/z* $C_{16}H_{25}O_4$, requires 281.1747, found 281.1746 $[M+H]^+$.

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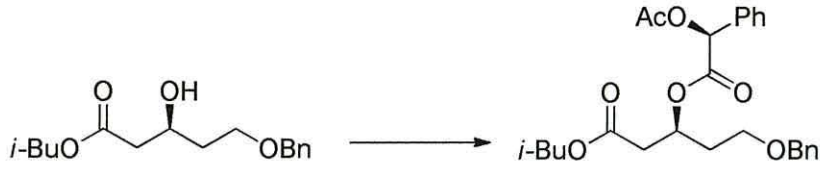
[1]The enantiomeric ratio was determined by preparation of the corresponding (*S*)-*O*-acetylmandalate ester and integrating the ^1H NMR spectrum at δ = 4.22/4.25 and δ = 4.43/4.48 (5:95).

Reaction Comments: This reaction was repeated 12 times on scales ranging from 3 to 7 mmol to give alcohol **219** in 43% to 66% yield.

Preparation of (±)-isobutyl 5-(benzyloxy)-3-hydroxypentanoate ((±)-219)

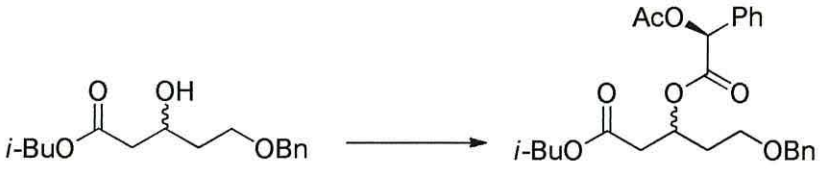
To a stirred solution of β-keto ester **218** (720 mg, 2.59 mmol, 1 eqv) in methanol (15 mL) at ambient temperature was added $NaBH_4$ (98 mg, 2.59 mmol, 1 eqv), portion-wise. Reaction progress was monitored via TLC and after 3 hours the reaction was quenched with H_2O (20 mL) and extracted with diethyl ether (3 x 50 mL). The organic extracts were then washed with brine (50 mL) and dried over magnesium sulfate. After evaporation, purification was achieved by flash column chromatography on silica gel using diethyl ether/petroleum ether (05:95, 20:80). Fractions eluting in 15:85 diethyl ether/petroleum ether gave **(±)-219** as a clear oil (544 mg, 1.94 mmol, 75%). R_f = 0.14 (20% ethyl acetate in petrol); $[\alpha]_D^{21}$ +2.3 (c 0.01, $CHCl_3$); ν_{max} (chloroform)/cm^{-1} 3504 (OH, br), 3018 (CH, w), 2964 (CH, m), 2926 (CH, m), 2868 (CH, m), 2855 (CH, m), 1724 (C=O, s), 1471 (w), 1455 (w), 1379 and 1371 (w); δ_H (500 MHz; $CDCl_3$) 0.87 (d, 6H, J = 7.0 Hz, $(\underline{CH_3})_2CHCH_2$), 1.72-1.76 (m, 2H, $\underline{CH_2}CH_2OBn$), 1.87 (n, 1H, J = 7.0 Hz, $(CH_3)_2\underline{CH}CH_2$), 2.44 (d, 2H, J = 6.3 Hz, $C{=}O\underline{CH_2}CHOH$), 3.36 (d, 1H, J = 6.3 Hz, $CH\underline{OH}$), 3.55-3.65 (m, 2H, $CH_2\underline{CH_2}OBn$), 3.82 (d, 2H, J = 6.6 Hz, $(CH_3)_2CH\underline{CH_2}$), 4.15-4.21 (m, 1H, $\underline{CH}OH$) 4.45 (s, 2H, $O\underline{CH_2}Ph$) and 7.20-7.29 (m, 5H, $OCH_2\underline{Ph}$); δ_C (125 MHz; $CDCl_3$) 18.9 (2 x CH_3, $(\underline{CH_3})_2CHCH_2$), 27.5 (CH, $(CH_3)_2\underline{CH}CH_2$), 36.0 ($CH_2$, $\underline{CH_2}CH_2OBn$), 41.5 (CH_2, $C{=}O\underline{CH_2}CHOH$), 66.7 (CH, $\underline{CH}OH$), 67.7 (CH_2, $CH_2\underline{CH_2}OBn$), 70.6 (CH_2, $(CH_3)_2CH\underline{CH_2}$), 73.1 ($CH_2$, $O\underline{CH_2}Ph$), 127.5 (2 x CH, C_6H_5), 127.6 (CH, C_6H_5), 128.3 (2 x CH, C_6H_5), 138.0 (aromatic, C) and 172.4 (C=O, ester).

Preparation of (*S*)-isobutyl 3-((*S*)-2-acetoxy-2-phenylacetoxy)-5-(benzyloxy) pentanoate ((*S*)-228)



To a stirred solution of **(*S*)-219** (150 mg, 0.54 mmol, 1 eqv) in DCM (10 mL) cooled to 0 °C was added (*S*)-*O*-acetyl mandelic acid (210 mg, 1.08 mmol, 2 eqv), 4-dimethylaminopyridine (13 mg, 0.11 mmol, 0.2 eqv) and *N,N'*-dicyclohexylcarbodiimide (223 mg, 1.08 mmol, 2 eqv) and the reaction stirred to rt over 48 hours. The resultant precipitate was then filtered and washed with hexane (3 x 10 mL) and the organic extracts washed with 1 M HCl (100 mL) and a sodium bicarbonate solution (sat, 100 mL) and dried over magnesium sulfate. After evaporation, purification was achieved by flash column chromatography on silica gel using diethyl ether/petroleum ether (10:90, 30:70). Fractions eluting in 20:80 diethyl ether/petroleum ether gave **(*S*)-228** as a clear oil (145 mg, 0.32 mmol, 59%). R_f = 0.35 (20% ethyl acetate in petrol); $[\alpha]_D^{21}$ +38.1 (c 0.01, $CHCl_3$); ν_{max} (chloroform)/cm^{-1} 3065 (CH, w), 3033 (CH, w), 2962 (CH, m), 2931 (CH, m), 2873 (CH, m), 1752 (C=O, s), 1743 (C=O, s), 1672 (w), 1496 (m), 1470 (m), 1455 (m), and 1398 (m); δ_H (500 MHz; $CDCl_3$) 0.81 (d, 3H, J = 6.6 Hz, $(CH_3)_2CHCH_2$), 0.82 (d, 3H, J = 6.6 Hz, $(CH_3)_2CHCH_2$), 1.70 (n, 1H, J = 6.6 Hz, $(CH_3)_2CHCH_2$), 1.95-2.00 (m, 2H, CH_2CH_2OBn), 2.19 (s, 3H, OAc), 2.56 (d, 2H, J = 6.7 Hz, $C{=}OCH_2CH$), 3.47-3.62 (m, 4H, $(CH_3)_2CHCH_2/CH_2CH_2OBn$), 4.43 (d, 1H, J = 11.7 Hz, OCH_2Ph), 4.48 (d, 1H, J = 12.0 Hz, OCH_2Ph), 5.4-5.49 (m, 1H, $C{=}OCH_2CH$), 5.83 (s, 1H, $CHOAc$) and 7.26-7.46 (m, 10H, 2 x C_6H_5); δ_C (125 MHz; $CDCl_3$) 18.9 (CH_3, $(CH_3)_2CHCH_2$), 19.0 (CH_3, $(CH_3)_2CHCH_2$), 20.6 (CH_3, OAc), 27.4 (CH, $(CH_3)_2CHCH_2$), 34.0 (CH_2, CH_2CH_2OBn), 39.2 (CH_2, $C{=}OCH_2CH$), 66.0 (CH_2, CH_2CH_2OBn), 70.1 (CH, $C{=}OCH_2CH$), 70.6 (CH_2, $(CH_3)_2CHCH_2$), 73.1 (CH_2, OCH_2Ph), 74.6 (CH, $CHOAc$), 127.5 (2 x CH, C_6H_5), 127.6 (2 x CH, C_6H_5), 128.3 (2 x CH, C_6H_5), 128.8 (CH, C_6H_5), 129.1 (CH, C_6H_5), 135.6 (aromatic, C), 138.3 (aromatic, C), 168.0 (C=O, ester), 169.6 (C=O, ester) and 172.3 (C=O, ester).

Preparation of (±)-isobutyl 3-((*S*)-2-acetoxy-2-phenylacetoxy)-5-(benzyloxy) pentanoate ((±)-228)



To a stirred solution of **(±)-219** (262 mg, 0.93 mmol, 1 eqv) in DCM (10 mL) cooled to 0 °C was added (*S*)-*O*-acetyl mandelic acid (363 mg, 1.87 mmol, 2 eqv), 4-dimethylaminopyridine (23 mg, 0.19 mmol, 0.2 eqv) and *N,N'*-dicyclohexylcarbodiimide (385 mg, 1.87 mmol, 2 eqv) and the reaction stirred to rt over 48 hours. The resultant precipitate was then filtered and washed with hexane (3 x 10 mL) and the organic extracts washed with 1 M HCl (100 mL) and a sodium bicarbonate solution (sat, 100 mL) and dried over magnesium sulfate. After evaporation, purification was achieved by flash column chromatography on silica gel using diethyl ether/petroleum ether (10:90, 30:70). Fractions eluting in 20:80 diethyl ether/petroleum ether gave **(±)-228** as a clear oil (256 mg, 0.56 mmol, 60%). R_f = 0.33 (20% ethyl acetate in petrol); $[\alpha]_D^{21}$ +46.7 (c 0.01, $CHCl_3$); ν_{max} (chloroform)/cm^{-1} 3089 (CH, w), 3065 (CH, w), 3033 (CH, w), 2962 (CH, m), 2873 (CH, m), 1751 (C=O, s), 1741 (C=O, s), 1672 (w), 1605 (w), 1587 (w), 1496 (m), 1470 (m), 1455 (m), and 1371 (m); δ_H (500 MHz; $CDCl_3$) 0.81 (d, 3H, J = 6.7 Hz, $(CH_3)_2CHCH_2$), 0.82 (d, 3H, J = 6.6 Hz, $(CH_3)_2CHCH_2$), 0.90 (d, 3H, J = 6.6 Hz, $(CH_3)_2CHCH_2$), 0.90 (d, 3H, J = 6.7 Hz, $(CH_3)_2CHCH_2$), 1.70 (n, 1H, J = 6.6 Hz, $(CH_3)_2CHCH_2$), 1.78-1.91 (m, 3H, $CH_2CH_2OBn/(CH_3)_2CHCH_2$), 1.95-2.00 (m, 2H, CH_2CH_2OBn), 2.17 (s, 3H, OAc), 2.18 (s, 3H, OAc), 2.56 (dd, 2H, J = 6.7, 2.2 Hz, $C{=}OCH_2CH$), 2.63-2.72 (m, 2H, $C{=}OCH_2CH$), 3.10-3.24 (m, 2H, CH_2CH_2OBn), 4.20-4.49 (m, 4H, $(CH_3)_2CHCH_2/CH_2CH_2OBn$), 3.77-3.86 (m, 2H, $(CH_3)_2CHCH_2$), 4.22 (d, 1H, J = 11.7 Hz, OCH_2Ph), 4.25 (d, 1H, J = 12.0 Hz, OCH_2Ph), 4.42 (d, 1H, J = 11.7 Hz, OCH_2Ph), 4.47 (d, 1H, J = 11.7 Hz, OCH_2Ph), 5.40-5.44 (m, 1H, $C{=}OCH_2CH$), 5.47 (p, 1H, J = 6.9 Hz, $C{=}OCH_2CH$), 5.83 (s, 1H, CHOAc), 5.88 (s, 1H, CHOAc) and 7.23-7.49 (m, 10H, 2 x C_6H_5); δ_C (125 MHz; $CDCl_3$) 18.9 (CH_3, $(CH_3)_2CHCH_2$), 18.9 (CH_3, $(CH_3)_2CHCH_2$), 19.0 (CH_3, $(CH_3)_2CHCH_2$), 19.0 (CH_3, $(CH_3)_2CHCH_2$), 20.6 (CH_3, OAc), 27.4 (CH, $(CH_3)_2CHCH_2$), 27.5 (CH, $(CH_3)_2CHCH_2$), 33.7 (CH_2, CH_2CH_2OBn),

40.0 (CH_2, $\underline{CH_2}CH_2OBn$), 39.2 (CH_2, $C{=}O\underline{CH_2}CH$), 39.2 (CH_2, $C{=}O\underline{CH_2}CH$), 65.7 (CH_2, $CH_2\underline{CH_2}OBn$), 66.0 (CH_2, $CH_2\underline{CH_2}OBn$), 69.8 (CH, $C{=}OCH_2\underline{CH}$), 70.0 (CH, $C{=}OCH_2\underline{CH}$), 70.6 (CH_2, $(CH_3)_2CH\underline{CH_2}$), 70.8 ($CH_2$, $(CH_3)_2CH\underline{CH_2}$), 72.9 ($CH_2$, $O\underline{CH_2}Ph$), 73.0 (CH_2, $O\underline{CH_2}Ph$), 74.5 (CH, $\underline{CH}OAc$), 74.6 (CH, $\underline{CH}OAc$), 127.5 (2 x CH, C_6H_5), 127.5 (2 x CH, C_6H_5), 127.6 (2 x CH, C_6H_5), 127.7 (2 x CH, C_6H_5), 128.3 (2 x CH, C_6H_5), 128.6 (CH, C_6H_5), 128.7 (CH, C_6H_5), 129.0 (CH, C_6H_5), 129.1 (CH, C_6H_5), 133.5 (aromatic, C), 133.9 (aromatic, C), 138.1 (aromatic, C), 138.2 (aromatic, C), 168.0 (C=O, ester), 169.5 (C=O, ester), 169.8 (C=O, ester), 170.0 (C=O, ester) and 170.2 (C=O, ester).

Preparation of (2*S*,3*S*)-isobutyl 5-(benzyloxy)-3-hydroxy-2-methylpentanoate (220)

A stirred solution of diisopropylamine (2.92 mL, 20.7 mmol, 2.65 eqv) in anhydrous THF (20 mL) was cooled (0 °C), to which *n*-BuLi (8.19 mL, 18.4 mmol, 2.35 eqv) was added and the reaction stirred for 30 minutes. The resulting solution of LDA was then cooled (-78 °C) and alcohol **219** (2.19 g, 7.8 mmol, 1 eqv) in anhydrous THF (20 mL) added, and the reaction stirred for a further 90 minutes whilst gradually warming to -40 °C. The resultant amber dianion solution was then cooled (-78 °C) and methyl iodide (0.73 mL, 11.7 mmol, 1.5 eqv) in DMPU (5.94 mL) added followed by stirring to rt for a further 5 hours 30 minutes. The reaction was quenched with an ammonium chloride solution (sat, 20 mL) and the pH adjusted to approximately pH 7 by the slow addition of 2 M HCl. The mixture was then extracted with diethyl ether (3 x 100 mL) and the combined organic extracts washed with brine (3 x 100 mL) and dried over magnesium sulfate. After evaporation, purification was achieved by flash column chromatography on silica gel using diethyl ether/petroleum ether (05:95, 20:80). Fractions eluting in 10:90 diethyl ether/petroleum ether gave **220** as a clear oil (1.69 g, 5.7 mmol, 74%, dr 90:10).[2] R_f = 0.27 (20% ethyl acetate in petrol); $[\alpha]_D^{21}$ +22.6 (c 0.01, $CHCl_3$); ν_{max} (chloroform)/cm^{-1} 3504 (OH, br), 3018 (CH, m), 2963 (CH, m), 2942 (CH, m), 2879 (CH, m), 1720 (C=O, s), 1455 (m), 1381 (m) and 1366 (w); δ_H (500 MHz; $CDCl_3$) 0.87 (d, 6H, J = 6.9 Hz, $\underline{(CH_3)_2}CHCH_2$), 1.13 (d, 3H, J = 7.3 Hz, $CH\underline{CH_3}$), 1.64-1.80 (m, 2H, $\underline{CH_2}CH_2OBn$), 1.84-1.92 (m, 1H, $(CH_3)_2\underline{CH}CH_2$), 2.50-2.54 (m, 1H, $\underline{CH}CH_3$), 3.18 (s, 1H, $CH\underline{OH}$), 3.57-3.67 (m, 2H,$CH_2\underline{CH_2}OBn$), 3.82-3.84 (m, 2H, $(CH_3)_2CH\underline{CH_2}$), 3.86-3.90 (m, 1H, $\underline{CH}OH$) 4.45 (s, 2H, $O\underline{CH_2}Ph$) and 7.21-7.29 (m, 5H, $OCH_2\underline{Ph}$); δ_C (125 MHz; $CDCl_3$) 13.6 (CH_3, $CH\underline{CH_3}$), 18.9 (2 x CH_3, $\underline{(CH_3)_2}CHCH_2$), 27.5 (CH, $(CH_3)_2\underline{CH}CH_2$), 33.6 ($CH_2$, $\underline{CH_2}CH_2OBn$), 45.4 (CH, $\underline{CH}CH_3$), 68.1 (CH_2, $CH_2\underline{CH_2}OBn$), 70.4 (CH_2, $(CH_3)_2CH\underline{CH_2}$), 71.8 (CH, $\underline{CH}OH$), 73.1 (CH_2, $O\underline{CH_2}Ph$), 127.5 (2 x CH, C_6H_5), 127.5 (CH, C_6H_5), 128.2 (2 x CH, C_6H_5), 137.9 (aromatic, C) and 175.4 (C=O, ester); LRMS, Positive ESI, *m/z*

[2]The diastereomeric ratio was determined after reduction to the corresponding diol and by integrating the ^1H NMR spectrum at δ = 0.86 and δ = 0.91 (90:10).

611 ($[2M+Na]^+$, 28%), 295 ($[M+H]^+$, 100%) and 221 (5); HRMS, ESI, *m/z* $C_{17}H_{27}O_4$, requires 295.1904, found 295.1907 $[M+H]^+$.

Reaction Comments: This reaction was repeated 5 times on scales ranging from 1.4 to 20 mmol to give **220** in 70% to 74% yield.

Preparation of (2*R*,3*S*)-5-(benzyloxy)-2-methylpentane-1,3-diol (229)

A stirred solution of alcohol **220** (52.3 mg, 0.179 mmol, 1 eqv) in anhydrous DCM (5 mL) was cooled to 0 °C, whereupon a solution of DIBAL in hexanes (1.0 M, 0.72 mL, 0.716 mmol, 4 eqv) was added dropwise. After 24 hours TLC analysis indicated the complete consumption of **220** and the reaction was quenched at 0 °C by the dropwise addition of methanol (4 mL) and the mixture diluted with ethyl acetate (20 mL). Upon warming to rt a solution of Rochelle salt (sat, 10 mL) was added followed by stirring for 1 hour. The reaction mixture was then extracted with ethyl acetate (3 x 15 mL) and the organic extracts dried over magnesium sulfate. After evaporation, purification was achieved by flash column chromatography on silica gel using diethyl ether/petroleum ether (70:30, 100:0). Fractions eluting in 70:30 diethyl ether/petroleum ether gave **229** as a clear oil (26.0 mg, 0.117 mmol, 65%). R_f = 0.24 (100% diethyl ether); $[\alpha]_D^{19}$ -9.4 (c 0.005, $CHCl_3$); ν_{max} (chloroform)/cm^{-1} 3435 (OH, br), 3031 (CH, m), 2956 (CH, m), 2924 (CH, m), 2857 (CH, m), 1607 (w), 1558 (w), 1494 (w) and 1453 (w); δ_H (400 MHz; $CDCl_3$) 0.86 (d, 3H, J = 7.0 Hz, $CH\underline{CH_3}$), 1.74 (ddd, 1H, J = 3.6, 7.2, 14.4 Hz, $\underline{CH}CH_3$), 1.84 (q, 2H, J = 5.8 Hz, $\underline{CH_2}CH_2OBn$), 3.36 (s, 1H, OH), 3.62-3.81 (5H, m, $\underline{CH_2}OH/CH_2\underline{CH_2}OBn/\underline{CH}OH$), 3.87 (s, 1H, OH), 4.53 (s, 2H, $O\underline{CH_2}Ph$) and 7.31-7.38 (m, 5H, $OCH_2\underline{Ph}$); δ_C (100 MHz; $CDCl_3$) 13.9 (CH_3, $CH\underline{CH_3}$), 34.3 (CH_2, $\underline{CH_2}CH_2OBn$), 40.1 (CH, $\underline{CH}CH_3$), 67.8 (CH_2, $CH_2\underline{CH_2}OBn$), 69.6 (CH_2, $\underline{CH_2}OH$), 73.5 (CH_2, $O\underline{CH_2}Ph$), 78.0 (CH, $\underline{CH}OH$), 127.7 (2 x CH, C_6H_5), 127.9 (CH, C_6H_5), 128.5 (2 x CH, C_6H_5) and 137.6 (aromatic, C).

Preparation of (2*S*,3*S*)-isobutyl 5-(benzyloxy)-3-((*tert*-butyldimethylsilyl)oxy)-2-methylpentanoate (221)

To a stirred solution of alcohol **220** (1.51 g, 5.13 mmol, 1 eqv) in anhydrous DMF (20 mL) cooled to 0 °C was added imidazole (2.10 g, 30.8 mmol, 6 eqv) and *tert*-butyldimethylsilyl chloride (2.32 g, 15.4 mmol, 3 eqv), and the reaction stirred to rt over 48 hours. The reaction mixture was then quenched with water (200 mL), extracted with hexane (3 x 100 mL) and dried over magnesium sulfate. After evaporation, purification was achieved by flash column chromatography on silica gel using diethyl ether/petroleum ether (00:100, 10:90). Fractions eluting in 05:95 diethyl ether/petroleum ether gave **221** as a pale yellow oil (1.85 g, 4.5 mmol, 88%). R_f = 0.48 (20% ethyl acetate in petrol); $[\alpha]_D^{21}$ +20.8 (c 0.01, $CHCl_3$); ν_{max} (chloroform)/cm^{-1} 3020 (CH, m), 2958 (CH, s), 2930 (CH, s), 2894 (CH, s), 2858 (CH, s), 1728 (C=O, s), 1471 (m), 1467 (m), 1460 (m), 1380 (m) and 1368 (w); δ_H (500 MHz; $CDCl_3$) -0.01 (s, 6H, *t*-BuSi($\underline{CH_3}_2$), 0.80 (s, 9H, *t*-$\underline{Bu}$Si(CH_3)$_2$), 0.86 (d, 6H, J = 6.6 Hz, ($\underline{CH_3}_2$)$CHCH_2$), 1.07 (d, 3H, J = 7.3 Hz, $CH\underline{CH_3}$), 1.64-1.80 (m, 2H, $\underline{CH_2}CH_2OBn$), 1.85 (n, 1H, J = 6.6 Hz, (CH_3)$_2\underline{CH}CH_2$), 2.59-2.64 (m, 1H, $\underline{CH}CH_3$), 3.49 (t, 2H, J = 6.8 Hz, $CH_2\underline{CH_2}OBn$), 3.74-3.81 (m, 2H, (CH_3)$_2CH\underline{CH_2}$), 4.11-4.14 (m, 1H, $\underline{CH}$OTBS), 4.42 (s, 2H, $O\underline{CH_2}Ph$) and 7.19-7.29 (m, 5H, $OCH_2\underline{Ph}$); δ_C (125 MHz; $CDCl_3$) -4.9 (CH_3, *t*-BuSi($\underline{CH_3}_2$), -4.7 (CH_3, *t*-BuSi($\underline{CH_3}_2$), 11.3 (CH_3, $CH\underline{CH_3}$), 18.0 (OTBS, C), 19.1 (2 x CH_3, ($\underline{CH_3}_2$)$CHCH_2$), 25.7 (3 x CH_3, *t*-$\underline{Bu}$Si(CH_3)$_2$), 27.7 (CH, (CH_3)$_2\underline{CH}CH_2$), 33.1 (CH_2, $\underline{CH_2}CH_2OBn$), 45.8 (CH, $\underline{CH}CH_3$), 66.5 (CH_2, $CH_2\underline{CH_2}OBn$), 70.4 (CH, $\underline{CH}$OTBS), 70.5 (CH_2, (CH_3)$_2CH\underline{CH_2}$), 72.8 (CH_2, $O\underline{CH_2}Ph$), 127.4 (2 x CH, C_6H_5), 127.5 (CH, C_6H_5), 128.3 (2 x CH, C_6H_5), 138.5 (aromatic, C) and 174.4 (C=O, ester); LRMS, Positive ESI, *m/z* 835 ($[2M+NH_4]^+$, 12%), 467 (5), 426 ($[M+NH_4]^+$, 76%) and 409 ($[M+H]^+$, 100%); HRMS, ESI, *m/z* $C_{23}H_{41}O_4Si$, requires 409.2769, found 409.2770 $[M+H]^+$.

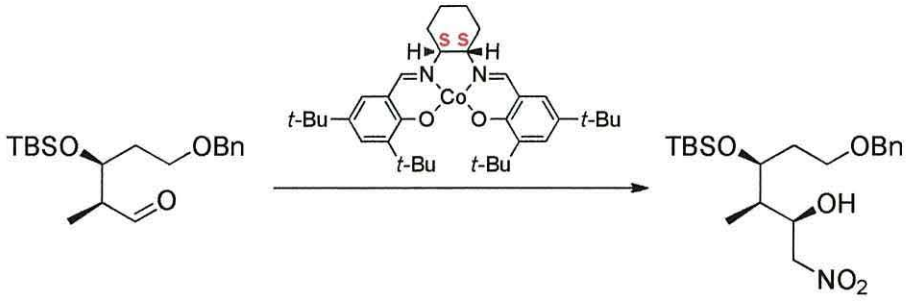
Reaction Comments: This reaction was repeated 3 times on scales ranging from 1 to 14 mmol to give silyl ether **221** in 70% to 88% yield.

Preparation of (2*S*,3*S*)-5-(benzyloxy)-3-((*tert*-butyldimethylsilyl)oxy)-2-methylpentanal (222)

A stirred solution of **221** (213 mg, 0.52 mmol, 1 eqv) in anhydrous toluene (5 mL) was cooled to -78 °C, whereupon a solution of DIBAL in hexanes (1.0 M, 1.04 mL, 1.04 mmol, 2 eqv) was added dropwise. After 1 hour TLC analysis indicated the complete consumption of **221** and the reaction was quenched at -78 °C by the dropwise addition of methanol (5 mL) and the reaction mixture diluted with ethyl acetate (20 mL). Upon warming to rt a solution of Rochelle salt (sat, 10 mL) was added followed by stirring for 30 minutes and the addition of water (20 mL). The reaction mixture was then extracted with diethyl ether (3 x 50 mL) and the organic extracts dried over magnesium sulfate. After evaporation, purification was achieved by flash column chromatography on silica gel using diethyl ether/petroleum ether (00:100, 05:95). Fractions eluting in 03:97 diethyl ether/petroleum ether gave **222** as a clear oil (116 mg, 0.35 mmol, 66%). R_f = 0.66 (20% ethyl acetate in petrol); $[\alpha]_D^{20}$ +26.7 (c 0.01, $CHCl_3$); ν_{max} (chloroform)/cm^{-1} 3089 (CH, w), 3065 (CH, w), 3031 (CH, w), 2956 (CH, s), 2930 (CH, s), 2886 (CH, s), 2857 (CH, s), 1728 (C=O, s), 1496 (w), 1471 (m), 1462 (m), 1455 (m) and 1361 (s); δ_H (500 MHz; $CDCl_3$) 0.07 (s, 6H, *t*-BuSi(C<u>H</u>$_3$)$_2$), 0.88 (s, 9H, *t*-BuSi(CH_3)$_2$), 1.11 (d, 3H, J = 7.0 Hz, CHC<u>H</u>$_3$), 1.76-1.90 (m, 2H, C<u>H</u>$_2$CH_2OBn), 2.51-2.58 (m, 1H, C<u>H</u>CH_3), 3.56 (t, 2H, J = 5.8 Hz, CH$_2$C<u>H</u>$_2$OBn), 4.15-4.19 (m, 1H, C<u>H</u>OTBS) 4.47 (d, 1H, J = 12.0 Hz, OC<u>H</u>$_2$Ph), 4.51 (d, 1H, J = 12.0 Hz, OC<u>H</u>$_2$Ph), 7.27-7.35 (5H, m, OCH$_2$<u>Ph</u>) and 9.73 (d, 1H, J = 2.2 Hz, CHO); δ_C (125 MHz; $CDCl_3$) -4.3 (CH_3, *t*-BuSi(<u>C</u>H$_3$)$_2$), -4.5 (CH_3, *t*-BuSi(<u>C</u>H$_3$)$_2$), 10.0 (CH_3, CH<u>C</u>H$_3$), 18.0 (OTBS, C), 25.7 (3 x CH_3, *t*-BuSi(CH_3)$_2$), 34.7 (CH_2, <u>C</u>H$_2$CH$_2$OBn), 51.6 (CH, <u>C</u>HCH$_3$), 66.3 (CH_2, CH$_2$<u>C</u>H$_2$OBn), 70.5 (CH, <u>C</u>HOTBS), 73.0 (CH_2, O<u>C</u>H$_2$Ph), 127.5 (2 x CH, C_6H_5), 127.6 (CH, C_6H_5), 128.3 (2 x CH, C_6H_5), 138.3 (aromatic, C) and 204.5 (C=O, aldehyde); LRMS, Positive ESI, *m/z* 427 (8), 391 (52), 354 ([M+NH$_4$]$^+$, 32%), 337 ([M+H]$^+$, 100%), 229 (7), 205 (25) and 159 (48); HRMS, ESI, *m/z* $C_{19}H_{33}O_3Si$, requires 327.2193, found 337.2199 [M+H]$^+$.

Reaction Comments: This reaction was repeated 3 times on scales ranging from 0.3 to 2.5 mmol to give aldehyde **222** in 57% to 66% yield.

Preparation of (2*R*,3*R*,4*S*)-6-(benzyloxy)-4-((*tert*-butyldimethylsilyl)oxy)-3-methyl-1-nitrohexan-2-ol (223)



To a stirred solution of aldehyde **222** (100 mg, 0.30 mmol, 1 eqv) in anhydrous DCM (3 mL) at rt was added (*S*,*S*)-salen cobalt complex **230** (18 mg, 0.03 mmol, 0.1 eqv), nitromethane (0.64 mL, 11.84 mmol, 40 eqv) and diisopropylethylamine (0.13 mL, 0.74 mmol, 2.5 eqv). Reaction progress was monitored via TLC and after 7 days the reaction was quenched with an ammonium chloride solution (sat, 10 mL), separated and the aqueous layer extracted with DCM (3 x 5 mL). The combined organic extracts were washed with brine (10 mL) and dried over magnesium sulfate. After evaporation, purification was achieved by flash column chromatography on silica gel using diethyl ether/petroleum ether (02:98, 20:80). Fractions eluting in 05:95 diethyl ether/petroleum ether gave **223** as a pale yellow oil (95 mg, 0.24 mmol, 80%, dr 62:38).[3] R_f = 0.30 (20% ethyl acetate in petrol); ν_{max} (chloroform)/cm^{-1} 3447 (OH, br), 3088 (CH, w), 3065 (CH, w), 3031 (CH, w), 2929 (CH, s), 2955 (CH, s), 2885 (CH, s), 2857 (CH, sh), 1555 (NO_2 asymmetric stretch, s), 1496 (sh), 1471 (sh), 1462 (m), 1455 (NO_2 symmetric stretch, sh), 1379 (w) and 1362 (m); δ_H (500 MHz; $CDCl_3$, major isomer) 0.10 (s, 6H, *t*-BuSi(C<u>H</u>$_3$)$_2$), 0.89 (s, 9H, *t*-BuSi(CH$_3$)$_2$), 1.02 (d, 3H, J = 7.0 Hz, CHC<u>H</u>$_3$), 1.65-1.69 (m, 1H, C<u>H</u>CH$_3$), 1.83-2.06 (m, 2H, C<u>H</u>$_2$CH$_2$OBn), 3.37-3.57 (m, 2H, CH$_2$C<u>H</u>$_2$OBn), 3.81 (s, 1H, OH), 3.94-3.97 (m, 1H, C<u>H</u>OTBS), 4.17 (dd, 1H, J = 12.6, 3.8 Hz, C<u>H</u>$_2$NO$_2$), 4.43-4.51 (m, 3H, C<u>H</u>OH/OC<u>H</u>$_2$Ph), 4.75-4.79 (m, 1H, C<u>H</u>$_2$NO$_2$) and 7.28-7.37 (m, 5H, OCH$_2$<u>Ph</u>); δ_C (125 MHz; $CDCl_3$, major isomer) -4.5 (CH$_3$, *t*-BuSi(C<u>H</u>$_3$)$_2$), -4.8 (CH$_3$, *t*-BuSi(C<u>H</u>$_3$)$_2$),

[3]The diastereomeric ratio was determined by integrating the ^{1}H NMR spectrum at δ= 3.94-3.97 and δ = 4.01-4.04 (62:38).

11.6 (CH_3, $CH\underline{CH_3}$), 17.9 (TBSO, C), 25.7 (3 x CH_3, $\underline{t\text{-Bu}}Si(CH_3)_2$), 34.9 ($CH_2$, $\underline{CH_2}CH_2OBn$), 37.4 (CH, $\underline{CH}CH_3$), 66.3 (CH_2, $CH_2\underline{CH_2}OBn$), 68.4 (CH, $\underline{CH}OH$), 73.0 (CH_2, $O\underline{CH_2}Ph$), 75.8 (CH, $\underline{CH}OTBS$), 77.3 (CH_2, $\underline{CH_2}NO_2$), 127.6 (2 x CH, C_6H_5), 127.7 (CH, C_6H_5), 128.4 (2 x CH, C_6H_5) and 138.0 (aromatic, C); LRMS, CI, *m/z* 849 (8), 817 ($[2M+Na]^+$, 37%), 456 (28), 415 ($[M+NH_4]^+$, 98%) and 398 ($[M+H]^+$, 100%); HRMS, ESI, *m/z* $C_{20}H_{36}NO_5Si$, requires 398.2357, found 398.2358 $[M+H]^+$.

Reaction Comments: The nitro-aldol reaction between aldehyde **222** and $MeNO_2$ was conducted a total of 4 times. This allowed nitro-alcohol **223** to be prepared with selectivities ranging from 62:38 (0.1 eqv catalyst, rt) to 37:63 (0 eqv catalyst, -20 °C) in yields of 43% to 80%. All reactions were conducted on a 0.3 millimolar scale following the general procedure outlined above.

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Appendices

Appendix A - X-Ray Crystallographic Data

University of Southampton · School of Chemistry

EPSRC National Crystallography Service

Table 1. Crystal data and structure refinement.

Identification code	**2009src1065 / DME-176-8-10**	
Empirical formula	$C_{12}H_{18}F_3N_3O_3$	
Formula weight	309.29	
Temperature	120(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	*P*–1	
Unit cell dimensions	$a = 7.9832(6)$ Å	$\alpha = 95.866(3)°$
	$b = 8.8773(7)$ Å	$\beta = 107.215(4)°$
	$c = 12.1929(10)$ Å	$\gamma = 114.500(4)°$
Volume	725.49(10) Å^3	
Z	2	
Density (calculated)	1.416 Mg / m^3	
Absorption coefficient	0.127 mm^{-1}	
F(000)	324	
Crystal	Cut Blade; Colourless	
Crystal size	0.12 x 0.08 x 0.05 mm^3	
θ range for data collection	2.93 – 27.48°	
Index ranges	$-10 \le h \le 9, -11 \le k \le 11, -15 \le l \le 15$	
Reflections collected	12761	
Independent reflections	3301 [$R_{int} = 0.0487$]	
Completeness to $\theta = 27.48°$	99.1 %	
Absorption correction	Semi–empirical from equivalents	
Max. and min. transmission	0.9937 and 0.9849	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3301 / 124 / 222	
Goodness-of-fit on F^2	1.151	
Final *R* indices [$F^2 > 2\sigma(F^2)$]	*R1* = 0.0792, *wR2* = 0.1219	
R indices (all data)	*R1* = 0.1279, *wR2* = 0.1416	
Largest diff. peak and hole	0.319 and –0.254 e Å^{-3}	

Diffractometer: *Nonius KappaCCD* area detector (ϕ scans and ω scans to fill *asymmetric unit* sphere). **Cell determination:** DirAx (Duisenberg, A.J.M.(1992). J. Appl. Cryst. 25, 92-96.) **Data collection:** Collect (Collect: Data collection software, R. Hooft, Nonius B.V., 1998). **Data reduction and cell refinement**: *Denzo* (Z. Otwinowski & W. Minor, *Methods in Enzymology* (1997) Vol. **276**: *Macromolecular Crystallography*, part A, pp. 307–326; C. W. Carter, Jr. & R. M. Sweet, Eds., Academic Press). **Absorption correction**: *SORTAV* (R. H. Blessing, Acta Cryst. A**51** (1995) 33–37; R. H. Blessing, J. Appl. Cryst. **30** (1997) 421–426). **Structure solution**: *SHELXS97* (G. M. Sheldrick, Acta Cryst. (1990) A**46** 467–473). **Structure refinement**: *SHELXL97* (G. M. Sheldrick (1997), University of Göttingen, Germany). **Graphics:** *ORTEP3 for Windows* (L. J. Farrugia, J. Appl. Crystallogr. 1997, 30, 565).

Special details:
All hydrogen atoms were fixed using a standard riding model.
The trifluoroacetate anion was partially disordered over 2 main sites.

Table 2. Atomic coordinates [$\times 10^4$], equivalent isotropic displacement parameters [$Å^2 \times 10^3$] and site occupancy factors. U_{eq} is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Atom	*x*	*y*	*z*	U_{eq}	*S.o.f.*
C1	4244(4)	2482(3)	5322(2)	31(1)	1
C2	6691(4)	3793(4)	4599(3)	40(1)	1
C3	7655(4)	3769(4)	5891(3)	35(1)	1
C4	8778(5)	2735(5)	6039(3)	49(1)	1
C5	9250(5)	2444(5)	7283(3)	53(1)	1
C6	7341(5)	1520(4)	7504(3)	47(1)	1
C7	6139(4)	2501(4)	7370(3)	35(1)	1
C8	7074(4)	4085(4)	8405(3)	36(1)	1
C9	5787(5)	4930(4)	8424(3)	41(1)	1
C10	6677(5)	6527(5)	9402(3)	52(1)	1
N1	5920(3)	2964(3)	6237(2)	32(1)	1
N2	4575(4)	2911(3)	4366(2)	40(1)	1
N3	2431(3)	1646(3)	5322(2)	35(1)	1
O1	4109(3)	4335(3)	7697(2)	56(1)	1
C11	–525(4)	1369(4)	2489(3)	33(1)	1
O11	1134(3)	2274(3)	2477(2)	45(1)	1
O12	–974(3)	638(3)	3245(2)	48(1)	1
C12	–2358(17)	1175(16)	1479(10)	46(1)	0.441(10)
F11	–3441(14)	1720(16)	1852(5)	86(3)	0.441(10)
F12	–1907(10)	1943(14)	672(8)	83(3)	0.441(10)
F13	–3638(18)	–435(12)	938(10)	81(4)	0.441(10)
C212	–2217(14)	1185(13)	1387(8)	46(1)	0.559(10)
F211	–2359(11)	2600(8)	1443(8)	102(3)	0.559(10)
F212	–1949(10)	877(13)	386(5)	91(2)	0.559(10)
F213	–3920(11)	–103(12)	1200(8)	92(3)	0.559(10)

Table 3. Bond lengths [Å] and angles [°].

C1–N3	1.324(3)	C8–C9	1.506(4)
C1–N1	1.327(4)	C8–H8A	0.9900
C1–N2	1.328(4)	C8–H8B	0.9900
C2–N2	1.456(4)	C9–O1	1.214(4)
C2–C3	1.542(4)	C9–C10	1.502(5)
C2–H2A	0.9900	C10–H10A	0.9800
C2–H2B	0.9900	C10–H10B	0.9800
C3–N1	1.481(3)	C10–H10C	0.9800
C3–C4	1.517(4)	N2–H2	0.8800
C3–H3	1.0000	N3–H3A	0.8800
C4–C5	1.528(5)	N3–H3B	0.8800
C4–H4A	0.9900	C11–O12	1.234(3)
C4–H4B	0.9900	C11–O11	1.237(3)
C5–C6	1.523(5)	C11–C212	1.535(9)
C5–H5A	0.9900	C11–C12	1.540(11)
C5–H5B	0.9900	C12–F13	1.311(11)
C6–C7	1.529(4)	C12–F11	1.312(12)
C6–H6A	0.9900	C12–F12	1.305(11)
C6–H6B	0.9900	C212–F213	1.297(9)
C7–N1	1.463(3)	C212–F211	1.305(10)
C7–C8	1.528(4)	C212–F212	1.321(10)
C7–H7	1.0000		
N3–C1–N1	125.6(3)	N1–C7–H7	108.2
N3–C1–N2	122.4(3)	C8–C7–H7	108.2
N1–C1–N2	112.0(2)	C6–C7–H7	108.2
N2–C2–C3	103.3(2)	C9–C8–C7	114.7(2)
N2–C2–H2A	111.1	C9–C8–H8A	108.6
C3–C2–H2A	111.1	C7–C8–H8A	108.6
N2–C2–H2B	111.1	C9–C8–H8B	108.6
C3–C2–H2B	111.1	C7–C8–H8B	108.6
H2A–C2–H2B	109.1	H8A–C8–H8B	107.6
N1–C3–C4	109.7(2)	O1–C9–C10	121.9(3)
N1–C3–C2	102.5(2)	O1–C9–C8	121.7(3)
C4–C3–C2	114.9(3)	C10–C9–C8	116.4(3)
N1–C3–H3	109.8	C9–C10–H10A	109.5
C4–C3–H3	109.8	C9–C10–H10B	109.5
C2–C3–H3	109.8	H10A–C10–H10B	109.5
C3–C4–C5	110.5(3)	C9–C10–H10C	109.5
C3–C4–H4A	109.5	H10A–C10–H10C	109.5
C5–C4–H4A	109.5	H10B–C10–H10C	109.5
C3–C4–H4B	109.5	C1–N1–C7	127.3(2)
C5–C4–H4B	109.5	C1–N1–C3	110.6(2)
H4A–C4–H4B	108.1	C7–N1–C3	120.9(2)
C6–C5–C4	110.1(3)	C1–N2–C2	111.3(2)
C6–C5–H5A	109.6	C1–N2–H2	124.3
C4–C5–H5A	109.6	C2–N2–H2	124.3
C6–C5–H5B	109.6	C1–N3–H3A	120.0
C4–C5–H5B	109.6	C1–N3–H3B	120.0
H5A–C5–H5B	108.2	H3A–N3–H3B	120.0
C5–C6–C7	112.5(3)	O12–C11–O11	129.6(3)
C5–C6–H6A	109.1	O12–C11–C212	117.8(4)
C7–C6–H6A	109.1	O11–C11–C212	112.6(4)
C5–C6–H6B	109.1	O12–C11–C12	112.1(5)
C7–C6–H6B	109.1	O11–C11–C12	118.2(5)
H6A–C6–H6B	107.8	C212–C11–C12	6.7(8)
N1–C7–C8	111.1(2)	F13–C12–F11	101.7(9)
N1–C7–C6	108.4(2)	F13–C12–F12	107.6(11)
C8–C7–C6	112.6(2)	F11–C12–F12	108.4(10)

F13–C12–C11	112.2(10)	F211–C212–F212	105.6(8)
F11–C12–C11	113.4(8)	F213–C212–C11	113.9(8)
F12–C12–C11	112.8(8)	F211–C212–C11	110.7(7)
F213–C212–F211	110.3(9)	F212–C212–C11	113.1(7)
F213–C212–F212	102.8(8)		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters [$Å^2 \times 10^3$]. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + \cdots + 2\,h\,k\,a^*\,b^*\,U^{12}]$.

Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C1	34(2)	27(2)	33(2)	9(1)	14(1)	14(1)
C2	38(2)	42(2)	45(2)	16(2)	23(2)	18(2)
C3	30(2)	33(2)	46(2)	13(1)	20(1)	14(1)
C4	49(2)	53(2)	61(2)	18(2)	29(2)	30(2)
C5	49(2)	65(2)	63(2)	25(2)	22(2)	40(2)
C6	52(2)	43(2)	51(2)	21(2)	17(2)	27(2)
C7	30(2)	37(2)	34(2)	16(1)	11(1)	11(1)
C8	32(2)	45(2)	32(2)	15(1)	12(1)	17(1)
C9	44(2)	51(2)	35(2)	17(2)	21(2)	24(2)
C10	58(2)	58(2)	47(2)	10(2)	26(2)	29(2)
N1	27(1)	36(1)	34(1)	14(1)	13(1)	13(1)
N2	33(1)	43(2)	32(1)	12(1)	11(1)	8(1)
N3	26(1)	39(1)	31(1)	13(1)	8(1)	9(1)
O1	44(1)	80(2)	45(1)	9(1)	12(1)	36(1)
C11	35(2)	29(2)	31(2)	9(1)	10(1)	12(1)
O11	33(1)	50(1)	41(1)	19(1)	12(1)	10(1)
O12	33(1)	58(2)	42(1)	28(1)	11(1)	10(1)
C12	42(2)	50(2)	40(2)	17(2)	12(2)	18(2)
F11	80(5)	132(6)	61(4)	6(4)	7(3)	80(5)
F12	51(3)	94(6)	56(5)	58(4)	–2(3)	–2(4)
F13	80(6)	49(4)	60(5)	–18(3)	–22(4)	22(4)
C212	42(2)	50(2)	40(2)	17(2)	12(2)	18(2)
F211	81(4)	73(4)	120(5)	10(4)	–24(4)	54(3)
F212	71(3)	128(6)	36(2)	11(3)	6(2)	23(4)
F213	31(3)	115(6)	68(4)	65(4)	–3(3)	–15(4)

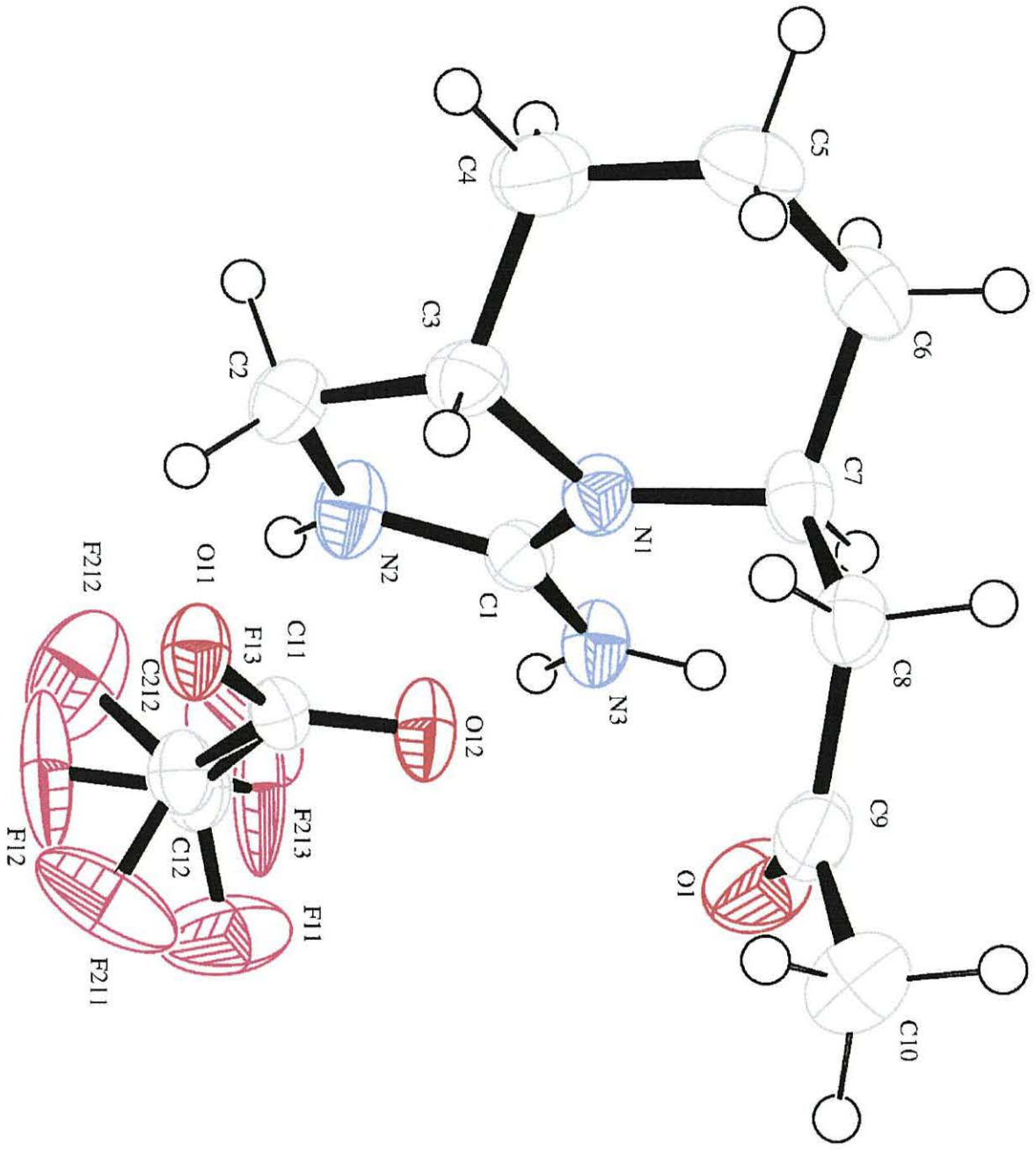
Table 5. Hydrogen coordinates [$\times 10^4$] and isotropic displacement parameters [$Å^2 \times 10^3$].

Atom	*x*	*y*	*z*	U_{eq}	*S.o.f.*
H2A	7117	4982	4522	48	1
H2B	7032	3178	4048	48	1
H3	8549	4969	6398	42	1
H4A	10028	3353	5908	59	1
H4B	7968	1616	5438	59	1
H5A	9961	1751	7363	63	1
H5B	10123	3560	7884	63	1
H6A	7674	1354	8317	56	1
H6B	6518	373	6935	56	1
H7	4788	1718	7343	42	1
H8A	7408	3752	9159	43	1
H8B	8331	4935	8372	43	1
H10A	5684	6918	9363	79	1
H10B	7824	7423	9310	79	1
H10C	7103	6281	10173	79	1
H2	3635	2691	3680	48	1
H3A	2265	1364	5967	42	1
H3B	1392	1372	4676	42	1

Table 6. Hydrogen bonds [Å and °].

D–H···*A*	*d*(*D*–H)	*d*(H···*A*)	*d*(*D*···*A*)	∠(*D*H*A*)
N2–H2···O11	0.88	1.95	2.805(3)	162.4
N3–H3B···O12	0.88	1.96	2.836(3)	177.0
N3–H3A···O12^{i}	0.88	2.14	2.914(3)	147.1

Symmetry transformations used to generate equivalent atoms:
(i) –x,–y,–z+1


C5
C4
C6
C3
C2
C7
N1
N2
C1
C8
N3
O11
F212
C11
F13
O12
C212
C9
F213
C12
F11
O1
F12
F211
C10

Appendix B - Publications

ChemComm

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COMMUNICATION

A biomimetic approach to the cylindrospermopsin alkaloids†

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The tethered Biginelli condensation between hemiaminal 21 and β-keto ester 22 is reported, leading to a model tricyclic core of the cylindrospermopsin alkaloids.

The guanidine containing marine natural product cylindrospermopsin **1** was initially isolated and identified from the cyanobacteria *cylindrospermopsis raciborskii* in 1992[1] after suspicion of its involvement in an outbreak of hepatoenteritis on Palm Island Australia[2] and has since been isolated from several other sources.[3] Cylindrospermopsin displays hepatotoxic,[4] cytotoxic[5] neurotoxic,[6] and carcinogenic effects,[7] which are shared with its equally toxic diastereoisomer 7-epicylindrospermopsin[8] **2** however the deoxygenated analogue 7-deoxycylindrospermopsin **3** is devoid of toxicity[9] (Fig. 1).

Due to the molecules potent biological activity and complex structure it has been the target of four total syntheses[10] and three general synthetic approaches.[11] These methodologies generally rely upon the initial construction of either the A or B rings of the toxin, with the tricyclic core being completed by the installation of the C ring at a later stage.

We were intrigued by the recently proposed biosynthesis of cylindrospermopsin arising from the genetic analysis of the cylindrospermopsin gene cluster from *cylindrospermopsis raciborskii* AWT205.[12] This report suggests that cylindrospermopsin is produced in a stepwise manner from guanidinoacetate **4**, with sequential polyketide extensions and Michael type ring closures to generate in turn the C ring (**5** → **6**), the A ring (**7** → **8**) and the B ring (**9** → **10**) (Scheme 1).

Drawing on this biosynthesis we hoped that by adopting a synthetic strategy in which the C ring system is formed first, the stereochemistry at this ring junction might dictate the subsequent stereochemistry generated in the formation of the A and B rings.[13] We hoped to test this hypothesis on a simple model system and thus began by monosilylating 1,5-pentanediol **11** followed by a Swern oxidation of the remaining alcohol function to furnish aldehyde **12**.[14] A subsequent Henry reaction with nitromethane followed by reduction of the nitro function of **13** and treatment with guanylating agent **14** successfully generated protected guanidine **15**. Mitsunobu type cyclodehydration[15] gave the C-ring heterocycle **16** in 96% yield. Removal of the silyl protecting group with TBAF followed by oxidation with Dess–Martin periodinane generated aldehyde **17**, which underwent a Horner–Wadsworth–Emmons olefination with phosphonate **18** to realise the model substrate enone **19** in 78% overall yield from **16**. We attempted to cyclise the substrate enone **19** under a variety of conditions but were unable to generate any tricyclic material but instead obtained the bicyclic guanidine **20** with an anti-configuration of the two ring protons as confirmed by X-ray crystallography (ESI†). Attempts were made to epimerise this product under both basic and acidic conditions (*via* reversible addition of the guanidine)[16] but this proved unsuccessful (Scheme 2).

A revision of the initial methodology was then proposed which utilised the aldehyde **17**. Removal of the Boc protecting groups from **17** proceeded smoothly upon exposure to acetic acid yielding hemiaminal **21**, this intermediate was then immediately condensed with allyl acetoacetate **22** under the tethered-Biginelli conditions developed by Aron and Overman,[17] successfully generating the tricyclic guanidine **23** in 43% overall yield from **17** (Scheme 2). No evidence of other tricyclic products was apparent however several as yet fully characterised by-products were obtained which might be bicyclic in nature.[18] The relative stereochemistry of **23** was determined by 2D

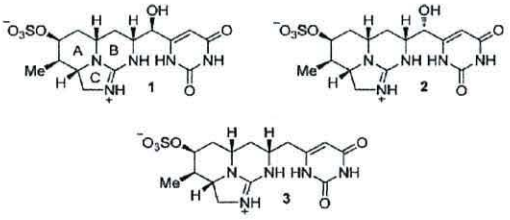
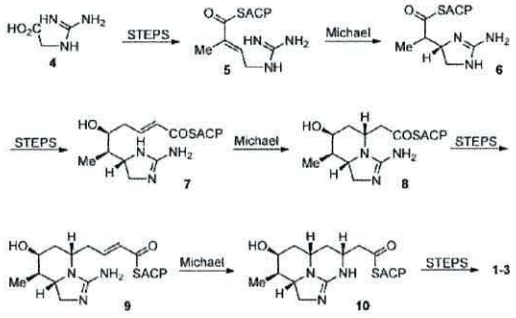


Fig. 1 The cylindrospermopsin alkaloids.



Scheme 1 Proposed biosynthesis of cylindrospermopsin.

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† Electronic supplementary information (ESI) available: Experimental details, crystallographic and spectra data are available. See DOI: 10.1039/c0cc05034b

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The Cylindrospermopsin Alkaloids

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