DISSERTATION

CONCISE ASYMMETRIC SYNTHESES OF THE CYLINDROSPERMOPSIN ALKALOIDS

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY RYAN EDWARD LOOPER ENTITLED THE CONCISE ASYMMETRIC SYNTHESES OF THE CYLINDROSPERMOPSIN ALKALOIDS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

CONCISE ASYMMETRIC SYNTHESES OF THE CYLINDROSPERMOPSIN ALKALOIDS

Presented herein is a concise 18 step asymmetric synthesis of the hepatotoxic cyanobacterial alkaloids cylidrospermopsin, 7-*epi*-cylindrospermopsin, and the purported structure of 7-deoxycylindrospermopsin. Born from a simple amino acid, an intramolecular [1,3]-dipolar cycloaddition of an α-alkoxycarbonyl nitrone and a nitroaldol reaction serve to construct these natural products from a single stereocenter.

The brevity of the synthesis, and the incorporation of the uracil moiety in a late-stage approach, proffers the ability to generate synthetic analogues that have been deployed for biomechanistic evaluation.

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....for...."the turd that can't be polished"

-RMW

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List of Abbreviations

Ac_2O	acetic anhydride
Boc	*tert*-butoxycarbonyl
Boc_2O	di-*tert*-butyldicarbonate
Bn	benzyl
BnBr	benzyl bromide
Bu_2BOTf	dibutylboron trifluoromethanesulfonate
$^{i}BuOCOCl$	isobutylchloroformate
BzCl	benzoyl chloride
Cbz	benzyloxycarbonyl
CbzCl	benzylchloroformate
DEAD	diethyl azodicarboxylate
Dess-Martin Periodinane	triacetoxy *o*-iodoxybenzoic acid
DIAD	diisopropyl azodicarboxylate
DIBAL	diisobutylaluminum hydride
DIC	*N,N*-diisopropylcarbodiimide
DMAP	4-(dimethylamino)-pyridine
DMDO	dimethyldioxirane
DME	dimethoxyethane
DMF	dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1 *H*)-pyrimidinone
DMS	dimethylsulfide
dr	diastereomeric ratio

EDCI	ethyl-dimethylaminopropylcarbodiimide hydrochloride
er	enantiomeric ratio
Et_3N	triethylamine
EtOAc	ethyl acetate
HOAc	acetic acid
HOAt	1-hydroxyazabenzotriazole
HOBt	1-hydroxybenzotriazole
IBX	*o*-iodoxybenzoic acid
Im	imidazole
KHMDS	potassium bis(trimethylsilyl)amide
LDA	lithium *N,N*-diisopropylamide
LHMDS	lithium bis(trimethylsilyl)amide
*m*CPBA	*m*-chloroperbenzoic acid
MeCN	acetonitrile
MOMCl	chloromethyl methyl ether
MsCl	methanesulfonyl chloride
MsOH	methanesulfonic acid
NaHMDS	sodium bis(trimethylsilyl)amide
nOe	nuclear Overhauser effect
NMM	*N*-methylmorpholine
NMO	4-methyl morpholine *N*-oxide
NMP	1-methyl-2-pyrrolidinone
Pd/C	palladium on carbon

$PdCl_2(dppf)\cdot CH_2Cl_2$	dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium dichloromethane solvate
$Pd_2(dba)_3\cdot CHCl_3$	tris(dibenzylideneacetone)dipalladium chloroform solvate
Ph	phenyl
PhH	benzene
PhMe	toluene
PPTS	pyridinium *p*-toluenesulfonate
$^{i}Pr_2NEt$	diisopropylethylamine
TBAF	tetrabutyl ammonium fluoride
TBSCl	*tert*-butyldimethylsilyl chloride
TBSOTf	*tert*-butyl dimethylsilyl trifluoromethansulfonate
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy, free radical
TESOTf	triethylsilyl trifluoromethanesulfonate
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
TfOH	trifluoromethane sulfonic acid
THF	tetrahydrofuran
TIPSCl	triisopropylsilyl chloride
TPAP	tetrapropylammonium perruthenate
TsCl	*p*-toluenesulfonyl chloride
p-TsOH	*p*-toluenesulfonic acid

Chapter 1: Introduction

1.1 Cyanobacterial Toxicity

1.1.1 General Considerations

Like bacteria, cyanobacteria (blue-green algae) are prokaryotic lacking a nucleus or an internal membrane structure.[1] While also unicellular, cyanobacteria often form macroscopic colonies in the environment. They differ from bacteria in their ability to photosynthetically generate food, and are further aided by their ability to fix nitrogen. In fact they get their name from their light harvesting pigment, phycocyanin. These organisms are historically important as their endosymbiosis with eukaryotic cells is thought to have given rise to plants. It is actually a cyanobacterial cell that is considered to be the chloroplast. Cyanobacteria also form symbiotic relationships with filamentous fungi, giving rise to lichens. Commonly found as a symbiont, the secondary metabolites or bioactive natural products generated by these organisms are often incorrectly attributed to the host organism.[2]

Many species of cyanobacteria are beneficial to humans. The filamentous cyanobacterium *Spirulina* has traditionally been harvested as a food source in South America, and is still marketed and sold as a "health-food" supplement.[3] Numerous genera are integral to the nitrogen cycle, fixing atmospheric nitrogen essential for the growth of legumes and rice. However, blooms of toxic cyanobacteria in eutrophic bodies of fresh water pose an immediate world-wide threat to humans who depend on surface water for drinking.[4] Further, wildlife and domesticated animals are frequently affected by both fresh water and marine cyanobacterial blooms. Perhaps the most well recognized outbreak being the "Red-Tide" responsible for paralytic shellfish poisoning or PSP.

Cyanobacteria produce diverse arrays of bioactive natural products. The ecological reasons for the persistence of these metabolites is not fully understood, but is generally attributed to defense.[5] Specifically, toxins are thought to prohibit grazing by zooplankton and marine invertebrates. Those genera commonly associated with toxic metabolites include *Oscillatoria, Aphazinomenon, Anabaena, Nodularia, Lyngbya, Microcystis,* and more recently *Cylindrospermopsis*. Considering the increasing importance of cyanobacterial natural products as drug leads[2] and their implications on human health a brief discussion of these metabolites is warranted.

1.1.2 Neurotoxins

The potent neurotoxin anatoxin-a (**1**) was the first toxin to be isolated and characterized from a freshwater cyanobacterium, *Anabaena flos-aquae* (Figure 1).[6] Simple in structure, **1** is a potent post-synaptic depolarizing neuromuscular blocking agent (LD_{50} = 200 μg/kg), with death occurring minutes after i.p. injection in laboratory mice. Sudden collapse and death is observed in field cases where domestic animals are concerned. Its one-carbon homologue, homoanatoxin-a (**2**) is also neurotoxic (LD_{50} = 250 μg/kg), but isolated from a Scandinavian strain of *Oscillatoria formosa.*[7] The unique cyclic *N*-hydroxygaunidine phosphate ester anatoxin-a(s) (**3**) is also isolated from *A. flos-aquae.*[8]

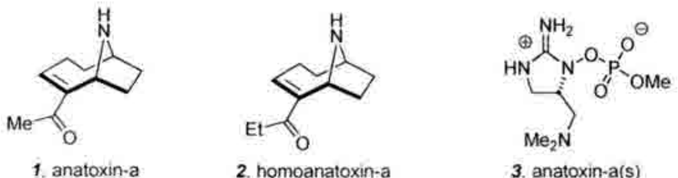


Figure 1. Principal toxins of *Anabaena flos-aquae.*

Acting as a powerful and irreversible peripheral inhibitor of acetylcholinesterase, **3** causes mucoid nasal discharge, tremors, and diarrhea in pigs after ingestion of the cyanobacterium. In laboratory mice **3** is incredibly toxic with an LD_{50} = 20 μg/kg, with death following after a period of 10-30 min.[9]

Perhaps the most famous cyanobacterial toxins are the saxitoxins, the primary agents associated with red tide paralytic shellfish poisoning (Figure 2). Originally isolated from shellfish after poisoning, saxitoxin (**6**) and the *N*-hydroxylated analogue neosaxitoxin (**7**) have subsequently been isolated from *Aphazinomenon flos-aquae*.[10] These substances are among the most toxic substances known with an LD_{50} = 5-10 μg/kg, acting as sodium ion channel blockers. Inhibiting nerve transduction, animals subjected to these toxins die quickly from respiratory arrest.

4 **5** **6**, saxitoxin **7**, neosaxitoxin

Figure 2. The Aphantoxins.

The saxitoxins are held in special regard in the organic community. The structures **4** and **5** were initially assigned for **6** from ^{1}H NMR and chemical degradation studies by Rapoport.[11] However later X-ray crystallographic studies by Clardy on **6**'s *p*-bromobenzoate salt and subsequently Rapoport on its ethyl hemiketal proved the structure as shown.[12,13] The synthesis of saxitoxin's complex architecture was first tackled and elegantly solved by Kishi in 1977.[14] It has since attracted generations of synthetic chemists.

1.1.3 Cytotoxins

Cyanobacteria are beginning to receive recognition as producers of highly cytotoxic metabolites, originally attributed to a host- or macroorganism. Many of these compounds have been found following bioactive fraction screening for microfilament disruption, and in general they inhibit mictrotubule formation.[15] Dolastatin 10 (**8**) was first isolated from the sea hare *Dolabella auricularia* in very low yields (10^{-6} %). It has since been isolated from the marine cyanobacterium *Symploca sp.* (10^{-2} % yield) suggesting that the sea hare ingests the molecule from grazing.[16] This pentapeptide is incredibly cytotoxic, showing zone inhibition >500 units in the Corbett assay against drug insensitive human mammary and colon tumor cell lines. Dolastatin and related analogues are currently being actively developed and have proceeded to Phase II clinical trials as anticancer agents.

8, dolastatin 10

9, aplysiatoxin *X = Br*
10, debromoaplysiatoxin *X = H*

11, cryptophycin

12, dolastatin 19

Figure 3. Cytotoxic metabolites of marine cyanobacteria.

The aplysiatoxins (**9** and **10**) are highly active tumor promoters also originally isolated from the digestive glands of a sea hare *Stylocheilu longicuada*. Moore and co-workers subsequently found that these toxins could be isolated from the marine cyanobacterium *L.*

majuscula, also suggesting ingestion through grazing.[17] These compounds have been shown to be potent protein kinase C activators accounting for their tumor promotion. Further this mode of action produces a severe inflammatory response, recreational swimmers exposed to **9** and **10** suffer from "swimmer's itch".

The depsipeptide cryptophycin (**11**) was isolated by researchers at Merck from *Nostoc* sp. ATCC 53789. This molecule proved to be a potent fungicide, but too cytotoxic to be considered for development. After re-isolation from another *Nostoc sp.*, semisynthetic analogues are proving to be highly efficacious against solid tumor cell lines and show broad spectrum activity against drug-resistant human and murine tumor cells.[18] Eli Lilly has put an analogue, cryptophycin-52, into Phase II clinical trials.[2]

The 14-membered macrocyclic lactone with an appended rhamnopyranoside, dolostatin 19 (**12**), was very recently isolated from *D. auricularia.*[19] Isolated in $8x10^{-8}$ % yield, **12** is also thought to come from a cyanobacterium. This compound displays significant toxicity *in vitro*, active against breast MCF-7 cancer cell lines (GI_{50} = 720 ng/mL) and colon KM20L2 tumors cells (GI_{50} = 760 ng/mL). Unfortunately further biological evaluation will have to await total synthesis, as 600 kg of sea hare yields only 0.5 mg of **12**.

1.1.4 Hepatotoxins

Hepatotoxins are amongst the most commonly encountered cyanobacterial toxins, and have been isolated globally. The peptidal toxins, the microcystins (**13-16**) are cyclic hepta-peptides first isolated from *Microcytis aeruginosa* (Figure 4).[20] These toxins have been implicated in the elevated occurrence of liver cancer in China, where surface water

is relied upon.[21] They are also the only toxins implicated in human fatalities, tragically in the death of sixty people who received microcystin contaminated water at a hemodialysis center in Caru, Brazil.[22] The microcystins all contain the unusual amino acids Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) and Mdha (*N*-methyldehydro-alanine). They are of the general composition: *cyclo*-(D-Ala-**Y**-D-β–MeAsp-**Z**-Adda-D-Glu-Mdha-) where **Y** and **Z** are variable amino acids and these variations denoted in the last two letters of their name. Microcystin-LR (**13**) (where **Y** = leucine and **Z** = arginine) is the most common of the toxins with an LD_{50} = 50 μg/kg. Currently there are over sixty microcystins known with LD_{50}'s ranging from 50 to 800 μg/kg (i.p. injection in mice).[23]

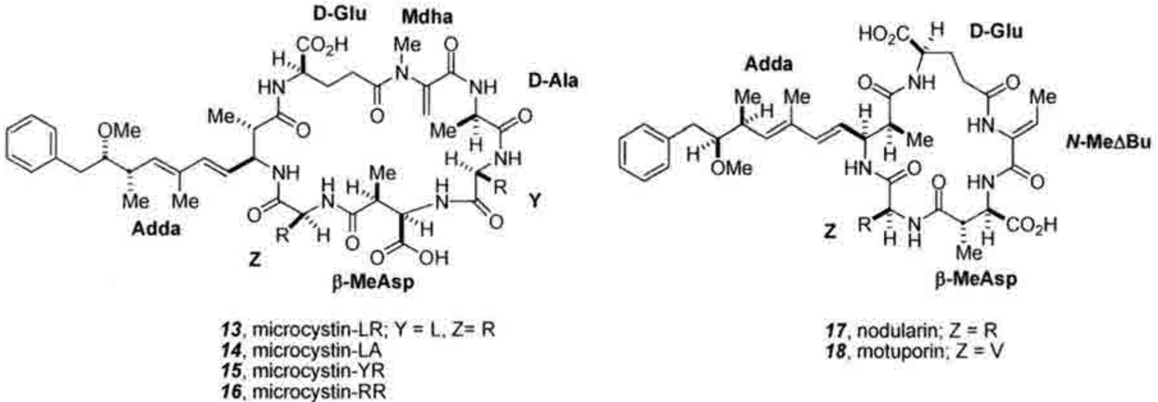


Figure 4. Hepatotoxic peptides.

A number of toxic, cyclic penta-peptides incorporating the Adda residue are also produced by cyanobacteria. Nodularin (**17**) isolated from *Nodularis sp.* has been traced to numerous incidents of animal poisoning. It is equipotent with **13** having an LD_{50} = 50-70 μg/kg (i.p.) in mice.[20] Motuporin (**18**) has been isolated from a marine sponge *Theonella Swinhoei* and also shown to be a potent hepatotoxin.[24] Noting its obvious similarity to **17**, it is also suspected to be of cyanobacterial origin and not a metabolite of the sponge itself.

These peptides have been shown to be highly liver specific due to their active uptake into parenchyma liver cells *via* the multispecific bile acid transport system.[25] More importantly they have been shown to be potent inhibitors of the protein phosphatases PP1 and PP2A.[26] Motuporin (**18**) remains one of the most potent inhibitors of these phosphatases (IC_{50} <1.0 nM). Inhibition of these enzymes is then thought to cause hyperphosphorylation of cytoskeletal proteins leading to hepatocyte swelling (doubling in size) and eventually hemorrhage. Due to the role of threonine and protein phosphatases in cell-signaling, and their implication in tumorgenesis these compounds have been the subject of intense synthetic efforts.[27]

The Cylindrospermopsins, described below, represent the first non-peptidal hepatotoxic alkaloids isolated from cyanobacteria.

1.2 The Cylindrospermopsins

1.2.1 Isolation and Structure Determination

Palm Island, a small tropical island off the northeastern coast of Queensland, Australia, was hit with a serious outbreak of hepatoenteritis in November of 1979. First thought to be a consequence of the consumption of unripe mangoes, 148 people (mostly children) were hospitalized.[28] The observation was later made that the incident occurred a few days after the Solomon Dam, the major water supply for the island, was treated with copper sulfate to control a dense algal bloom in the reservoir. Later epidemiological studies were able to connect the hepatoenteritis outbreak with the water supply.[29]

Subsequent ecological studies of the reservoir were able to show that this body of water suffers frequent cyanobacterial blooms.[30] Two major species were associated with these outbreaks, *Anabaena circinalis* and *Cylindrospermopsis raciborskii*. The first cyanobacterium *A. circinalis* was actually comprised of two varieties, but neither was shown to be toxic in a mouse bioassay.[31] On the otherhand, *C. raciborskii* was shown to be highly toxic. This represented both the first toxic encounter with this cyanobacterium, and also the first time this species had been identified in Australia. Noting the toxicity of this organism it was concluded that the use of copper sulfate to control the bloom must have lysed the cells, releasing a hepatotoxin into the water supply.

Despite the interest in this cyanobacterium, it was not until 1992 that the toxic principles of this *C. raciborskii* were isolated. Bioassay guided fractionation of an extract of freeze dried *C. raciborskii* provided a single compound, cylindrospermopsin (**19**), that displayed hepatotoxic activity indistinguishable from the cyanobacterial extract (Figure 5).[32] This compound was isolated in 0.5% yield from 700 mg of freeze-dried

cyanobacterium, comprising a significant amount of the organism's secondary metabolites. Later studies have shown that cylindrospermopsin content can vary markedly, ranging from undetectable by HPLC-MS to 0.8% of cell dry weight, depending on strain and culture condition.[33]

From positive ion HRFABMS a parent ion of *m/z* 416.1236 (MH^+) was obtained, suggesting the formula $C_{15}H_{21}N_5O_7S$.[32] Further, the UV spectrum of the compound showed a λ_{max} = 262nm (ε 5800) consistent with the presence of a uracil. Detailed analysis of 2D-COSY, HMQC, and HMBC experiments suggested the gross structure depicted in **19**. Biosynthesis of uniformly ^{13}C and ^{15}N (>80%) **19** gave material for ^{13}C-^{13}C COSY experiments that confirmed the contiguous 12-carbon chain.

Figure 5. Cylindrospermopsin's initial structure.

It was noted however, that the ^{13}C signals for C-2 and C-6 (154.8 and 158.1 ppm respectively) in **19** were quite broad. Upon the addition of acid (HCl or HOAc), the signals sharpened and shifted to 153.7 and 156.1 ppm. Moore proposes that this is a result of hydrogen-bonding between N-1 of the uracil and N-18 of the guanidine unit suggesting that the compound exists in both tautomeric forms **19** and **20**. NOE

experiments indicated an interaction between H-8 ↔ H-7 ↔ H5. Assuming the intramolecular co-planar hydrogen bond they proposed that the hydroxyl group on C-7 was axial as shown in figure 5, an assumption we now know to be incorrect (see sec. 1.3.9).

Since its initial isolation from *C. raciborskii*, cylindrospermopsin has been isolated in Thailand and from three other genera of cyanobacteria throughout the world.[34] It has been isolated in Fukui, Japan from *Umezakia natans* in 0.09% yield from this strain.[35] It has also been isolated from *Aphazinomenon ovalisporum* in Israel, and in China from *Rhaphidiopsis curvata*.[36,37]

In 1999, Norris and co-workers reported the isolation of a closely related metabolite to **19**.[38] Initially isolated from *C. raciborskii* it has also been found in *R. curvata* as evidenced by LC-MS.[37] HPLC-MS analysis indicated an MH^+ of $m/z = 400$, suggesting that a single oxygen atom was missing from the structure. *It should be noted here that this isolation paper is not detailed in the physical or spectral properties of the compound; i.e. there is no comprehensive ^{1}H, ^{13}C NMR data or optical rotation reported.*

21, 7-deoxy-cylindrospermopsin ⇌ ***22***

Figure 6. 7-deoxycylindrospermopsin.

The authors make some general comparisons of ^{1}H NMR spectra noting that: 1) The uracil proton at 5.7 ppm (H-5) is missing; 2) there is a peak at 4.57 ppm indicating that

the molecule retains the sulfonated C-12 hydroxyl group; and 3) the peak at 4.43 ppm that corresponds to the methine proton (H-7) is missing. From this they conclude that the structure is deoxygenated at C-7 and is represented by **21** (Figure 6). Further they add "Due to the broadening of peaks compared to cylindrospermopsin, we conclude that this compound is deoxycylindrospermopsin and it exists as the tautomeric forms shown in [**21**] and [**22**]."

Lake Kinneret (The Sea of Galilee), Israel, is the major freshwater source for the country. Following the identification of the cylindrospermopsin producing cyanobacterium *A. ovalisporum* in the resevoir, it became necessary to establish guidelines for acceptable levels in drinking water. In order to do this, large amounts of **19** were required to assess its chronic and acute toxicity. During this isolation a minor metabolite was isolated in 0.01% yield, one tenth of the mass relative to **19**.[39] Detailed NMR studies showed that the compound was 7-epicylindrospermopsin (**23**, Figure 7). NOE experiments observed interactions between H-7 $\leftrightarrow$ H-9_{ax}. Utilizing the same assumption that an intra-molecular hydrogen bond exists; the C-7 hydroxyl group must occupy an equatorial position. From synthesis we now know that this assignment is also reversed (see section 1.3.9).

23, 7-*epi*-cylindrospermopsin

Figure 7. 7-epicylindrospermopsin

1.2.2 Cylindrospermopsin's Biogenesis

Cylindrospermopsin has a unique skeleton. The tetra-substituted guanidine imbedded in its tricyclic core is very rare in natural products. To my knowledge, this substitution pattern for guanidines is only shared by the Batzelladine alkaloids.[40] The rarity of this motif is contrasted by the more common arginine catabolism pathway. Incorporation of the parent mono-substituted guanidine or a single substitution event leads to bis-substituted guanidines, i.e. in saxitoxin (**6**) and anatoxin-a(s) (**3**).

To probe the origin of the guanidine in **19**, Moore and co-workers cultured *C. raciborskii* in the presence of uniformly labeled [^{13}C, ^{15}N]glycine (Figure 8).[41] This experiment showed incorporation into the C-13 methyl group, proving that the methyl group arises from the C1 pool *via S*-adenosyl methionine. Further the intact glycine unit was incorporated into C-14, C-15, and N-16. To probe whether this unusual result was a consequence of a transamidination reaction from arginine to glycine the cyanobacterium was cultured in the presence of [U-^{13}C-^{15}N]arginine.

Figure 8. Proposed reductive cyclization during **19**'s biosynthesis.

However, there was no detectable incorporation of the labeled compound into C-17, N-18, and N-19 of **19**. Asking whether the guanidine unit arose from ureidoglycine (via carbamoyl phosphate), in a fashion analogous to the conversion of ornithine to arginine, the bacterium was fed [4-^{13}C, 3-^{15}N]ureidoacetic acid. Again no incorporation was detected. [4-^{13}C, 3-^{15}N]Guanidinoacetic acid is incorporated into **19**. While it remains unclear how the guanidine arises in **19**, it appears that guanidinoacetic acid is the starter unit for its biosynthesis.

Further studies with [1,2-$^{13}C_2$]acetate showed that its incorporation supports that the carbon chain is polyketide in origin. This then suggested a plausible biogenesis for **19**, starting with a polyketide containing five acetate groups and a guanidinoacetic acid unit attached to an acyl carrier protein (ACP) as in **24**. Minimum energy conformations for such a chain suggest strong intra-molecular hydrogen bond between the hydroxyl group on C-12 and the C-14 carbonyl. Reductive cyclization of the terminal guanidine would then afford **25** which would undergo two subsequent reductive cyclizations to give the tricyclic core in **27**. It is unclear where the uracil comes from, but may well arise from carbamoyl phosphate transfer and cyclization. Sulfonation would then give **19**. The multiple cyclization event is further supported by the fact that a uniformly labeled polyketide intermediate generated from [2-^{13}C,$^{2}H_2$]acetate shows incorporation of ^{1}H's only at the ring juctions in **19** (shown in blue).

There has been putative identification of the genes involved with the production of cylindrospermopsin.[42] Conserved polyketide synthetases (PKS) have been differentiated between *C. raciborskii* strains that produce **19** and those that do not. This has implicated

the *rpoC1* gene in the production of cylindrospermopsin, and may lead to the rapid identification of organisms capable of producing the toxin.

1.2.3 Cylindrospermopsin's Toxicology

Despite two decades' worth of investigation, the exact mode of action for the cylindrospermopsins remains unknown.[43] It is well established that **19** is a potent hepatotoxin with an LD_{50} = 200 μg/kg after 5 days (i.p. injection in mice of purified toxin).[44] Oral administration of the cell free extracts of *C. raciborskii* deliver an LD_{50} value of 4.4 mg/kg. It has subsequently been shown that, in general, oral toxicity is approximately 25 times less than by interperitoneal injection for **19**.[45] In all reports of oral or i.p. investigations of **19**'s toxicity against mice, the liver is the main target organ, although cell extracts of *C. raciborskii* can also affect the heart, thymus, kidneys, and lungs.[46,47] Interestingly 7-epicylindrospermopsin (**23**) is equipotent with **19** in mouse bioassays.[39] 7-deoxycylindsrospermopsin is, however, non-toxic in the same assay.[38] It has also been established that **19** does not inhibit the protein phosphatases (PP1 and PP2A) as do the hepatotoxic microcystins.[32]

Using **19** isolated from *U. natans*, Terau et al. were able to show that one of the first symptoms of poisoning was the detachment of ribosomes from the membrane of the endoplasmic reticulum.[46] Noting this phenotypic similarity to other protein synthesis inhibitors (i.e. cycloheximide), they were able to show that **19** completely inhibited protein synthesis *in vitro* using a globin synthesis assay in a cell free rabbit reticulocyte system. Shortly there-after, it was also shown that in mammalian cell cultures, cell death was accompanied by a marked decrease in glutathione (GSH), a Glu-Cys-Gly tripeptide

essential to the anti-oxidative capabilities of hepatocytes.[48] The mechanism of GSH decrease was investigated and shown to be a result of GSH synthesis inhibition, not through GSH metabolism (i.e. reduction of free-radicals, or toxin conjugation).[49] It remains unclear if this is a result of general protein synthesis inhibition or a direct interaction with γ-glutamylcysteine transferase or glutathione transferase, the enzymes responsible for GSH synthesis. In contrast, it has since been argued that **19** may be a uridine diphosphate (UDP) mimic.[50] In doing so it would interfere with the biosynthesis of ascorbic acid (*via* UDP-glucose) and hamper the anti-oxidative capabilities of hepatocytes. The fall of GSH would thus be a result of interference with GSH reductase.

Further complicating the elucidation of **19**'s mode of action is the involvement of the cytochrome P450 enzyme system. It is noted that the primary toxic effects of **19** are exhibited in the periancinar region of the liver, where most xenobiotic metabolism occurs.[44] Most important is the observation that the addition of cytP450 inhibitors (i.e. α-napthoflavone) provides resistance to the toxicity of **19**.[51]

Besides **19**'s effect on the ribosome, its uracil structure suggests an interaction with DNA or RNA. Indeed the covalent binding of **19**, or its oxidized metabolites, to DNA in poisoned mice has been observed.[44] DNA strand breaks have also been observed, presumably due to the binding of an activated form of **19**.[52] The only current examination of the cytotoxicity of cylindrospermopsin in human cells (lymphoblastoid) showed cytogenetic abnormalities consistent with **19** acting as a spindle poison or causing damage to the centromere/kinetochore machinery.[53] Coupled with the afore-mentioned ribosomal interactions, it remains unclear whether the origin of **19**'s inhibition of protein synthesis is manifested at the transcription or translational level.

1.2.4 Human health impacts and control

Following the discovery that *C. raciborskii* is a toxic cyanobacterium, much has been learned about its ecology.[54] Alarmingly, it has been found in lakes throughout Florida, Minnesota, and Kansas, thought to have colonized these bodies of water within the last thirty years. The fact that **19** has been isolated from at least four genera of cyanobacteria is also alarming. The threat posed to global public health by these molecules in drinking water has prompted the NIH's National Toxicology Program (NTP) and the EPA's Unregulated Contaminant Monitoring Rule (UCMR) to elect **19** for toxicological and environmental evaluation.[55] The European Union has also lauched a major initiative (CYANOTOX) under its Toxin Production in Cyanobacteria (TOPIC) program, aimed at the rapid detection of *C. raciborskii* and its toxins in potential water supplies.[56]

Cylindrospermopsin is a relatively stable compound, unaffected by temperatures above 100 °C. It is also stable to a wide range of pH's (4,7,10) even after 8 weeks.[57] Unlike the microcystins that can be effectively degraded by UV light in ~10 min., **19** has a half-life of 18 h. when exposed to shortwave UV irradiation. This poses major difficulties in the removal of this toxin from water supplies. Fortunately, chlorination appears to sufficiently transform **19** into non-toxic derivatives (see sec. 1.4).[50] However this process has been conducted in the laboratory, with a high concentration of chlorine (1 g/L) over an extended period of time (24 h) and has not been proven as a practical solution to the destruction of **19** in water treatment plants.

1.3 Previous Synthetic Achievements

Containing almost as many hetero-atoms as carbons, embodying a zwitterionic guanidinium-sulfate, a rare tetra-substituted guanidine and a uracil, these highly polar natural products have held the attention of synthetic chemists for over ten years. Following is a chronological discussion of the synthetic approaches toward the synthesis of the cylindrospermopsins.

1.3.1 Weinreb's AC-Model Synthesis

Weinreb's groups was immediately drawn into an attempt to synthesize **19**, and was the first to publish in 1993.[58] Drawing upon their experience with *N*-sulfinyl hetero Diels-Alder chemistry they sought to apply this methodology for the construction of the B-ring and the C7-C8 stereocenters (Scheme 1). Beginning with the amidine **28**, lithiation and treatment with 1-chloro-2,4-hexadiene gave a 4:1 mixture of S_N2 : S_N2' alkylation products.

Scheme 1. a) *t*BuLi, copper (I) pentynoate, 1-chloro-2,4-hexadiene (67%). b) KOH, MeOH, H_2O (94%). c) NaOCN, HCl (48%). d) $SOCl_2$, ImH, −78 °C (67%). e) PhMgBr, THF −78 °C. f) $P(OMe)_3$, MeOH, reflux (65% *2 steps*).

Basic hydrolysis of the amidine and conversion to the ureas with sodium cyanate and HCl gave an inseparable mixture of **29** and **30**. Treatment of this mixture with thionyl chloride

and imidazole gave the *N*-sulfinyl urea which underwent the [4+2] cycloaddition to furnish a single diastereomer of the tricycle **31** in 67% yield. Presumably the transition state depicted is preferred as it minimizes non-bonded interactions between the axial piperidine hydrogen and the internal vinyl hydrogen. The relative stereochemistry of **31** was secured by *X*-ray crystallography. The hydroxyl group at C7 was then cleverly installed by a Mislow-Evans [2,3]-sigmatropic rearrangement of the intermediate phenyl sulfoxide to give the allylic alcohol **32** necessitating the eventual installment of the 6-substituted uracil from this functionality.

1.2.2 Snider's Double Michael Strategy for the AB-ring System.

Snider was the next to publish a model system in 1995 centered on constructing cylindrospermopsin's A-ring through a double Michael addition of ammonia to a dienone (Scheme 2).[59] The requisite dienone **36** on which to perform this reaction was constructed starting with the amination of 1,3-dibromo-2*E*-butane (**33**). This gave the *bis*-TMS protected amine **34** ready for lithiation. Treatment of the lithiated alkene with crotonaldehyde followed by methanolysis of the TMS groups and reprotection of the amine as the *t*-butyl carbamate gave **35** in 44% yield. Oxidation of the resulting *bis*-allylic alcohol with manganese (IV) oxide gave the dienone **36**. The dienone did undergo double Michael addition after treatment with ammonium hydroxide in a sealed tube at 67 °C for 16h, giving the piperidine-4-one **37** in 55% isolated yield. It is reasonable to assume that the stereocontol in this reaction is purely thermodynamically driven, placing all three substituents equatorial as needed for the synthesis of cylindrospermopsin. Further, **37** is accompanied by a minor diastereomer which can be recycled into **37** by re-

subjection to the Michael addition conditions. The stereochemistry of **37** was assumed from the large vicinal coupling constants; $J_{2,3}$ = 10.3 Hz and $J_{5ax,6}$ = 12.0 Hz. This places H_2, H_3, and H_6 in an axial-*anti* relationship. Having constructed the A-ring they turned their attention to the installation of the B-ring guanidine. The carbonyl in **37** was reduced with L-selectride® and the Boc group removed with TFA. Treatment of the amine with *N*-Troc isothioscyanate led selectively to the primary thiourea **38**. The guanidine could then be formed with the aid of mercuric chloride and Et_3N, furnishing a 40% yield of **39** over the two steps. Removal of the Troc group under reductive conditions with zinc in acetic acid afforded the free guanidine **39** in 81% yield. Lastly they established that the sulfate at C12 could be introduced by the action of sulfur trioxide DMF complex, giving **40** in quantitative yield and finishing their model study of the AB-ring system

Scheme 2. a) LHMDS, THF (40-50%). b) *t*BuLi, the *E*-crotonaldehyde. c) MeOH, 24h. d) Boc_2O, NaOH (44%, *3 steps*). e) MnO_2, CH_2Cl_2 (86%). f) NH_4OH, NH_4Cl, MeOH, 67 °C, 16h (55%). g) L-selectride, THF (88%). h) TFA (100%). i) TrocNCS. j) $HgCl_2$, Et_3N, DMF (40% *2 steps*). k) Zn, HOAc (81%). l) DMF·SO_3, DMF (100%).

1.2.3 Weinreb's Synthesis of the A-ring.

Having proved their *N*-sulfinyl urea hetero Diels-Alder strategy would successfully create the C-ring and set the C7-C8 stereocenters, Weinreb's group released their strategy to construct a functionalized A-ring three years after their initial disclosure.[60] They embarked on another Diels-Alder strategy, with a glycinimine providing the piperidine nitrogen (Scheme 3). Thus reaction of the diene **41** with the *N*-Ts imine **42** in the presence of zinc (II) chloride afforded the [4+2] adduct **43** as a single diastereomer.

Scheme 3. a) $ZnCl_2$, PhMe, rt (58%). b) LAH, Et_2O (94%). c) $(COCl)_2$, DMSO, Et_3N (89%). d) MsCl, Et_3N. e) KCN, DMSO (14 %, *2 steps*). f) Ph_3P=CHOMe (5-10%). g) Jones' reagent, Me_2CO (82%). h) $(COCl)_2$, CH_2N_2, Et_2O; Ag_2O, MeOH (22% for **49**, 39% for **50**).

To investigate the necessary carbon homologation, the alcohol **44** was prepared by reduction with LAH and the aldehyde **45** prepared by Swern oxidation of **44**. At this point it is worth discussing some of their follies in this piperidine system. First the aldehyde **45** is very sensitive to base, readily epimerizing to the more stable *trans*-α-amino aldehyde **46**, a problem that we would become acutely familiar with in our synthesis. Secondly, conversion of the hydroxyl-methyl group in **44** to a leaving group affords a system which reacts poorly with nucleophiles, in this case giving the nitrile **47** in only 14% yield. The aldehyde **45** also reacts poorly with phosphorous ylides, giving the homologated enol ether **48** in a crushing 5-10% yield. Lastly, Arndt-Eistert homologation of the acid, prepared by Jones oxidation **45**, gives only a 22% yield of the homologated ester **49**-as a mixture of diastereomers, further illustrating the sensitivity of the α–carbon to undergo epimerization. It should also be noted that the *cis*-substituted piperidine readily closes to the bicycle **50**, a stable entity that we would also encounter.

Noting the difficulty experienced with this hydroxymethyl group, they turned their efforts to incorporating this carbon as a non-labile vinyl group, and directly introducing what would become the C12-alcohol. For this they began with the diene **51** (Scheme 4). Reaction of **51** with the imine **42** catalyzed by $ZnCl_2$ gave a 60% yield, after acidic hydrolysis, of a 22 : 1 mixture of the piperidines **52** and **53**, favoring the *cis*-adduct. Fortunately they found that this mixture could be transformed into a thermodynamically driven 4 : 1 ratio favoring the *trans*-isomer **53** by the action of *p*-TsOH in refluxing benzene. Treatment of **53** with vinyl cuprate effected the 1,4-addition to give a single diastereomer of the vinyl piperidine **54**. Since it is well known that the C2 substituents of acyl piperidines prefers to be axial to minimize $A^{1,3}$-strain, the methyl group also must

adopt an axial configuration leading to the preffered axial attack of the cuprate, giving the 2,6-*cis*-substituted piperidine **54**.

Scheme 4. a) $ZnCl_2$, PhMe. b) 5% HCl (60% *2 steps*). c) $H_2C{=}CHMgBr$, CuI, THF (88%). d) L-selectride®, THF (49%). e) TBSOTf, $i Pr_2NEt$, CH_2Cl_2 (82%). f) LAH, Et_2O (94%). g) BH_3-THF, THF. h) H_2O_2, NaOH (50%, *2 steps*).

Reduction of the carbonyl with L-selectride® gives the correct axial stereochemistry for the secondary alcohol **55** as previously shown by Snider (Scheme 2). Protection of the hydroxyl group as its TBS ether then gave a substrate that could undergo ethoxy-carbonyl reduction giving **56** or a hydroboration sequence to give the homologated alcohol **57**. This sequence avoids the introduction of an epimerization labile center at the α–amino carbon. This signaled the completion of a homologated A-ring synthon, poised for conversion to the diene and subsequent subjection to the *N*-sulfinyl hetero Diels-Alder sequence.

1.2.4 Snider's AC-ring strategy.

After demonstrating that the double Michael addition was competent to construct cylindrospermopsin's A ring, Snider's group focused on the construction of the C ring and the C7-C8 stereocenters. Also three years after their initial report they were able to publish a model system toward this end, based on the intramolecular reductive alkylation of an α–bromo ketone.[61] Utilizing *N*-acyl pyridinium chemistry they began with the addition of an acetylene equivalent to Cbz activated pyridine, giving the terminal alkyne **58** (Scheme 5). Addition of the metallated alkyne to 2,6-dimethoxy pyrimidine (**59**) gave the alkynol which was fully reduced under hydrogenolysis conditions to give **60**. Guanidinylation of the piperidine nitrogen was then effected using the *bis-N*-CBz-protected *S*-Me isothiourea in the presence of mercuric chloride. Oxidation of the benzylic alcohol with Dess-Martin gave the pyrimidyl ketone **61**. Bromination of the ketone was then accomplished with cupric bromide in warm ethyl acetate, giving the unstable diastereomeric mixture of **62**.

Scheme 5. a) CbzCl, THF, $Me_3SiCCMgBr$ (95%). b) K_2CO_3, MeOH (97%). c) EtMgBr then **59** (85%). d) H_2/Pd-C, MeOH (94%). e) *bis-N*-CBz-*S*-Me isothiourea, $HgCl_2$, Et_3N, DMF (74%). f) Dess-Martin periodinane, CH_2Cl_2 (72%). g) $CuBr_2$, EtOAc, 40 °C 15 min.

While the crude mixture of **62** was unstable, they found that immediate treatment of the mixture with hydrogen and catalytic palladium cleaved the Cbz groups, promoting the intramolecular alkylation (Scheme 6). Under these conditions the carbonyl is also reduced providing an 81 : 14 : 4.5 : 0.5 mixture of diastereomers. The major diastereomer **63** was considered to be that corresponding to cylindrospermopsin as the C7 and C8-methine hydrogens show very similar couplings, identical after hydrolysis of the pyrimidine (i.e. **67**).

Note: the stereochemistry depicted in **63-69** is reversed from that reported,[61] reflecting the revision of cylindrospermopsin's C7-stereochemistry, *vide infra*.

Scheme 6. a) H_2/Pd-C, MeOH (81% total yield from **61**). b) conc. HCl reflux, 6h (95% for **67-69**).

The uracil corresponding to 4.5% of the mixture, **69**, was also thought to carry the correct stereochemistry at C8 as H8 displays a large coupling (11.3 Hz) indicating that it is

axially disposed (7-*epi*-cylindrospermopsin exhibits a 6.6 Hz coupling further supporting this assignment, although it was not known at the time). This leaves the other major diastereomer **68** to be epimeric at C8, also suggested by the small equatorial couplings with the C9 methylene group. At this point the stereochemistry of the C8 epimers C7-hydroxyl stereochemistry was not assignable, also a problem that we would meet. Fortunately the major diastereomer from this intramolecular alkylation was that corresponding to **19**. Needing to merge the double Michael synthesis of the AB-ring system, with their new synthesis of the C-ring, they pushed on toward a total synthesis.

1.2.5 Armstrong's Double Displacement Strategy.

Armstrong's group was next to enter the fray, publishing one week after I had begun working on these molecules.[62] In early 2000 they reported an acyclic strategy to construct the three contiguous stereocenters in the A-ring, and a double Mitsunobu strategy to close both the B and C-rings, a strategy that we had also initially proposed to close the B-ring.

They began with the reduction of the aspartic acid derivative **70** (Scheme 7). Protection of the resultant primary alcohol as its TBS ether gave **71** in 93% yield. Using Seebach's amino-ester alkylation protocol, the dianion of **71** could be generated using LDA and alkylated with methyl iodide. Controlled reduction of the *t*-butyl ester gave the aldehyde **72** as a 3:1 mixture of diastereomers favoring the *anti* configuration. Subjection of the aldehyde to Keck allylation conditions afforded the allylic alcohol **73**. The allyl group was transferred in an *anti*-Felkin-Ahn mode, only slightly favoring the *anti-anti* stereo-triad (1.5 : 1), and creating the last of the three contiguous stereocenters in the A-ring. After protection of the secondary alcohol, the carbamate had to be protected as its

trifluoroacetamide derivative to prevent hemi-aminal formation after oxidative cleavage of the terminal alkene. Wittig homologation with the acetone derived phosphonate and release of the trifluoroacetamide under basic conditions gave the enone **75**. This substrate could then be cyclized with *p*-TsOH in refluxing benzene and the Cbz group removed by catalytic hydrogenation to give the free piperidine **76** with all of the A-ring stereocenters intact.

Scheme 7. a) $NaBH_4$, THF. b) TBSOTf, Et_3N, 2,6-lutidine (93% *2 steps*). c) LDA, LiCl then MeI (97%, *3:1 anti:syn*). d) DIBAL-H, PhMe, −78 °C (89%). e) $BF_3 \cdot OEt_2$, allyltributyltin, CH_2Cl_2 (84%, *1.5:1 anti/anti : anti/syn*). f) TBSOTf, 2,6-lutidine (*quant.*). g)TFAA, Et_3N (90%). h) O_3, PPh_3, CH_2Cl_2, −78 °C (91%). i) LDA, $(MeO)_2OPCH_2COCH_3$. j) Na_2CO_3, MeOH (64% *2 steps*). k) *p*-TsOH, PhH (74%). l) H_2/Pd-C (*quant.*).

Installation of the guanidine function was achieved with the Cbz protected isothiourea and mercuric chloride to give **77** (Scheme 8). They were now situated to try the double Mitsunobu displacement strategy to close the remaining B and C-rings. To this end the methyl ketone was reduced with $NaBH_4$ and the resultant secondary alcohol treated with triphenyl phosphine and DIAD. Indeed the Mitsunobu proceeded to close the C-ring, however the bicycles **78** and **79** were produced as a ~5:1 mixture favoring the incorrect

stereochemistry needed for the synthesis of cylindrospermopsin. With little of the needed bicycle **79** produced, they pushed on with **78** to try the second cyclization.

Scheme 8. a) *bis*-*N*-CBz-*S*-Me isothiourea, $HgCl_2$, Et_3N, DMF (85%). b) $NaBH_4$, MeOH (*quant.*). c) PPh_3, DIAD (75%, ~5:1 **78** : **79**), d) NaH, THF, MeOH (67%). e) TBAF, THF (84%). f) PPh_3, DIAD (27%).

In order to achieve the second Mitsunobu, they required a primary carbamate. Fortunately, treatment of **78** with one equivalent of sodium methoxide cleaved only one of the Cbz groups on the guanidine. Fluoride mediated deprotection of the silyl ethers gave the diol **80**. Subjection of **80** to Mitsunobu conditions did indeed effect the second cyclization, closing the B-ring, albeit in 27% yield for **81**. This completed their tricyclic model for cylindrospermopsin.

1.2.6 Weinreb's Uracil Synthesis.

With the C-ring elegantly constructed and a method to fashion a fully elaborated A-ring in place, Weinreb's group needed to devise a synthesis of the uracil. Their [2,3]-sgmatropic rearrangement predicated that the uracil arise from an α,β-unsaturated ester. To study this transformation they devised the model *i*-Pr-substituted ester **82** (Scheme 9).[63] Treatment of **82** with *bis*-*N,O*-trimethylsilyl hydroxylamine in refluxing ethanol

effected both Michael addition and cyclization to give **83**. Acylation of the nitrogen with phenyl chloroformate generated a highly electrophilic intermediate that underwent aminolysis and lactamization upon treatment with ammonium hydroxide, giving the *N*-hydroxy-dihydrouracil **85**. Finally elimination of the hydroxyl function to install the 5,6-unsaturation was found to proceed by tosylation in the presence of DMAP to give the uracil **86**. The only problem left for Weinreb's group to solve was the introduction of the B-ring, and stitching together the pieces needed for the synthesis of cylindrospermopsin.

Scheme 9. a) TMSHNOTMS, EtOH, reflux (68%). b) PhOCOCl, Et_3N, THF. c) NH_4OH, *i*PrOH (65% *2 steps*). d) TsCl, DMAP, $ClCH_2CH_2Cl$, reflux 21h (81%).

1.2.7 Hart's Approach to the C-ring.

Hart's group chose to approach the synthesis of cylindrospermopsin by first addressing the C7-C8-C10 stereo-triad.[64] Starting from the vinyl acetylene **87** a decarboxylation-Curtius sequence afforded the urea **88** (Scheme 10). Sonogashira coupling of the alkyne with the known 2,6-dimethoxy-4-bromopyrimidine (**89**) furnished the alkynyl pyrimidine **90** in 90% yield. Following the precedent of a number of model studies, they found that treatment of the primary urea with NaH in THF effected Michael addition of the anion into the acetylene giving the vinyl pyrimidine **91** in 97% yield. Regio-selective epoxidation of the enamide and subsequent methanolysis gave a

diastereomeric mixture of methylcarbanolamines. Reduction of this mixture with sodium cyanoborohydride gave exclusively the *cis*-cyclic urea, but a 78 : 22 mixture of C7-epimers (**92** and **93**), favoring the wrong stereochemistry for the synthesis of cylindrospermopsin. They did however show that the Martin modified Mitsunobu reaction effected the conversion of **92** to **93**, a reaction later used by Weinreb.

Scheme 10. a) KOH, THF, MeOH. b) 3*N* HCl (90% *2 steps)*. c) $SOCl_2$ (96%). d) NaN_3, Me_2CO, then PhH reflux, then NH_3 (89%). e) Et_3N, CuI, $(Ph_3P)_2PdCl_2$ (90%). f) NaH, THF (97%). g) DMDO, MeOH, CH_2Cl_2, Me_2CO. h) $NaBH_3CN$, MeOH, H_2O, pH = 4 (97% for **64** and **65**). i) DEAD, PPh_3, p-NO_2PhCO_2H, then K_2CO_3, MeOH (91%).

1.2.8 Snider's Racemic Total Synthesis of Cylindrospermopsin.

Drawing primarily from their AC-ring synthesis (sec. 1.2.4) Snider's group was the first to report a racemic total synthesis of cylindrospermopsin in 2000 (Scheme 11).[65] They drew upon Weinreb's addition of organocuprates to a piperidinone to fashion the necessary functionality for the B-ring. Beginning with 3-methylpyridine (**94**) a four step sequence highlighted by nitration and subsequent aromatic substitution with methoxide afforded the oxygenated pyridine derivative **95**. Acylation of the pyridine nitrogen with TrocCl satisfactorily activated the ring for the addition of an alkynyl grignard to give the

piperidinone **96**. Subsequent conjugate addition of vinylcuprate installed the carbon necessary for the B-ring, but as Weinreb observed, with the wrong relative stereochemistry giving **97**. Fortunately acidic-reductive removal of the Troc group quantitatively epimerized the methyl group to the *trans*-isomer, which guided reduction of the carbonyl to set the last stereocenter of the A-ring. Protection of both the amine and alcohol function then afforded the piperidinol **98**. Grignard exchange then generated the alkynylmagnesium species that added to 2,6-dimethoxypyrimidine carboxaldehyde which

Scheme 11. a) H_2O_2, HOAc; b) HNO_3, H_2SO_4; c) K_2CO_3, MeOH; d) 10% Pd/C H_2, 45 psi, 1 week (83% *4 steps*); e) TrocCl, THF; f) TMSCCMgBr, THF (87% *2 steps*); g) $CuBr{\cdot}SMe_2$, $H_2CCHMgBr$, TMSCl (92%); h) Zn, HOAc; i) L-Selectride (90% *2 steps*); j) CbzCl, Na_2CO_3; k) TBSCl, ImH (85% *2 steps*); l) EtMgBr, 2,6-dimethoxypyrimidine carboxaldehyde; m) TBSCl, ImH (73% *2 steps*); n) O_3, DMS, CH_2Cl_2; o)$BnNH_2$, HOAc, PhH; p) $NaCNBH_3$, MeOH (49% *3 steps*); q) 5% Pd/C, H_2, MeOH; r)CNBr, PhH; s) NaH, CbzCl (45% *3 steps*); t) TBAF, THF; u) MnO_2, CH_2Cl_2; v) Ac_2O, pyr. (63% *3 steps*).

after silylation of the resultant secondary alcohol gave **100** in 73% yield. Oxidative cleavage of the alkene followed by reductive amination of the resultant aldehyde with benzylamine afforded the diamine **101** in 49% yield over the three steps. Global reduction of the alkyne, benzyloxy carbonyl, and benzyl amine groups gave the free diamine **102**. In turn the diamine was converted to the guanidine with cyanogen bromide and re-protected as its *bis*-benzyloxycarbonyl derivative **103**. Desilylation with TBAF generated the diol, of which the bezylic function was selectively oxidized with manganese (IV) oxide. Acetylation of the C-12 hydroxyl group then gave a 63% yield of **104** for the three functional group manipulations.

Adhering to their model studies, **104** was treated with cupric bromide in refluxing ethyl acetate which generated a diastereomeric pair of α-bromoketones (Scheme 12). Hydrogenolysis of the benzyloxycarbonyl groups allowed cyclization of the freed guanidine to give an approximately 3:2 mixture of the guanidines **105** and **106**, favoring that needed for the synthesis of cylindrospermopsin.

After considerable experimentation it was found that refluxing **105** in concentrated HCl for 7-12 hours effected the hydrolysis of the dimethoxypyrimidine to give >95% yield of the uracil. Sulfonation was then accomplished by treatment of the diol with sulfur trioxide DMF complex to give cyclindrospermopsin (**19**) in 55-75% yield as a 2:1 mixture with the corresponding *bis*-sulfate. The other diastereomer **106** could also be hydrolyzed to the uracil **107** in 95% yield. Again this represented the first racemic total synthesis of cylindrospermopsin in ~26 linear steps (22 steps from the known compound **95**). Although concise, the α-bromination of **104** is not selective. Further while the

reduction of the resultant guanidinoketone is highly selective to give **105**, the outcome of this reaction failed to independently assign the stereochemistry at C7.

Scheme 12. w) $CuBr_2$, EtOAc, reflux 0.5 h; x) H_2, $Pd(OH)_2$ MeOH (48% for **105**, 28% for **106**); y) conc. HCl, reflux 7h (95%); z) $SO_3 \cdot DMF$, pyr.(55-75%).

1.3.9 Weinreb's Racemic Total Synthesis of 7-epicylindrospermopsin.

Shortly after Snider's completion of the racemic total synthesis of **19**, Weinreb's group published the first racemic total synthesis of 7-epi-cylindrospermopsin (**23**).[66] A synthetic attempt in which all six stereogenic centers are well controlled, they were able to show that the stereochemistry at C-7 initially assigned for **19** was incorrect. They also began using *N*-acylpyridinium chemistry, an extension of their initial Diels-Alder work (Scheme 13). Beginning with 4-methoxypyridine (**106**) acylation with CbzCl followed by the addition of (allyldimethylsilyl)methylmagnesium bromide they were able to generate the vinylogous urethane **107** in 94% yield. *Trans*-selective introduction of the methyl group was accomplished by treatment of the enolate with methyl iodide. Having these two stereocenters in place, allylcuprate stereoselectively added axially to the enone, to generate the remote stereocenter in **108** (see sec. 1.3.3). Again, L-selectride reduction of the ketone cleanly afforded the β-secondary alcohol which after protection as its benzyl ether gave **109**. Tamao oxidation of the silylmethyl group gave the alcohol which could be induced to cyclize on the carbamate to give the oxazolidinone in 88% yield. A hydroboration/oxidation sequence then introduced the primary alcohol in **110** in good yield. Oxidation of the alcohol and Horner-Wadsworth-Emmons homologation gave the requisite *E,E*-diene **111** needed for their hetero Diels-Alder reaction. Reduction of the carbomethoxy function and protection as it *p*MB ether gave **112**.

Scheme 13. a) i. BnOCOCl, THF, -20 °C; ii. CH_2=$CHCH_2Si(Me_2)CH_2MgBr$, Et_2O, -20 ° C; iii. 5% HCl, rt, (94%); b) NaHMDS, MeI, THF, -78 °C, (88%); c) CH_2=CHMgBr, CuI, THF, -78 to -20 °C, (98%); d) L-Selectride, THF, -78 °C, (80%); e) BnBr, NaH, THF; TBAI, reflux, (95%); f) i. KHF_2, $CHCl_3$, TFA; ii. MeOH, $NaHCO_3$, THF, 30% H_2O_2, reflux, (88%); g) Sia_2BH, THF 0 °C; H_2O_2, NaOH, -20 °C-rt, (97%); h) $(COCl)_2$, DMSO, CH_2Cl_2, NEt_3, -55 °C-rt, (84%); i) $(EtO)_2POCH_2CH$=$CHCO_2Me$, LiOH·H_2O, 4 Å MS, THF, reflux, (80%); j) DIBALH, BF_3-Et_2O, CH_2Cl_2, -78 °C, (83%); k) NaH, THF, PMBCl, TBAI, reflux, (96%); l) NaOH, H_2O, EtOH, reflux, (100%); m) NaH, BnBr, TBAI, THF, 0 °C-rt, (65%); n) KOCN, HOAc, pyr, NEt_3, rt, (85%).

Basic hydrolysis of the oxazolidinone followed by selective benzylation of the primary alcohol afforded the free amine **113**. The C1-N unit needed for the construction of the guanidine and the C-ring was then installed as the primary urea by treatment of **113** with potassium isocyanate, which gave **114** in 85% yield.

This generated the substrate needed for their key intramolecular cycloaddition (Scheme 14). Treatment of the primary urea in **114** with thionyl chloride and imidazole smoothly generated the *N*-sulfinyl urea which underwent [4+2] cycloaddition to give a single isomer of the dihydrothiazine oxide **115** in 81% yield. Phenylmagnesium bromide

was then introduced to promote the ring opening / [2,3]-sigmatropic rearrangement to **116**, thereby setting all six streocenters in the natural product.

Scheme 14. o) $SOCl_2$, ImH, CH_2Cl_2, -78 °C-rt, (81%); p) PhMgBr, THF/CH_2Cl_2, -55 °C; $(MeO)_3P$, MeOH, 50 °C, (83%); q) $Me_2C(OMe)_2$, Me_2CO, CSA, reflux, (70%); r) DDQ, H_2O CH_2Cl_2, (82%); s) i. Dess-Martin, CH_2Cl_2; ii. $NaClO_2$, *t*-BuOH, H_2O.; iii. *i*-Pr_2NEt, MeI, DMF, (81%); t) TMSONHTMS, THF, EtOH, (82%); u) PhOCOCl, NEt_3, THF; NH_4OH, *i*-PrOH, (65%); v) Tf_2O, pyr, CH_2Cl_2, (73%); w) Me_3SiCl, MOMCl, *i*-Pr_2NEt, CH_2Cl_2 (80%); x) $Pd(OH)_2$, EtOH, H_2 (71%).

Protection of the C-7 alcohol as its methoxymethyl ether and oxidative removal of the *p*MB group gave **117**, the stereochemistry of which was proven through *X*-ray crystallographic analysis. Alternatively the allylic alcohol could be tied to the urea nitrogen as the acetonide with 2,2-dimethoxypropane. Subsequent removal of the *p*MB group with DDQ and a three step oxidation protocol afforded the methyl ester **118**. The group was then able to implement the methodology developed to install the uracil (sec.

1.2.6). Thus, treatment of **118** with hydroxylamine produced a diastereomeric mixture of β-oxaminoesters **119** which underwent the substitution cyclization sequence to **120** when exposed to phenyl chloroformate and ammonium hydroxide. Elimination of the hydroxyl function affords the *N*-acyl iminium species that tautomerizes to the uracil. Protection of the amide nitrogens, as the *bis*-methoxymethyl uracil derivative, was required in order to activate the urea for guanidine formation. Finally, hydrogenolysis of the benzyl ethers generates the diol **121**, in 71% yield.

It was next required to install the C-ring guanidine. To accomplish this, the C-15 alcohol required conversion to an amine functionality (Scheme 15). They found that treatment of **121** with triphosgene in THF gave an unstable but isolable intermediate thought to be the chloroimidate aminal. Treatment of this intermediate with sodium azide cleanly introduces N-16 as the azide **122**. Hydrolysis of the *N,O*-acetonide then freed the B-ring urea for activation. After a considerable amount of experimentation they found that methyl trifluoromethanesulfonate and 2,6-di-*t*-butyl pyridine gave the *O*-methyl isourea, which suffered attack by N-16 after hydrogenolysis of the azide to give the guanidinium salt **124**. To their surprise, removal of the MOM groups afforded a diol that was not consistent with the one produced by Snider in the transformation of **105** → **19**. Sulfonation of the C-12 hydroxyl group under the conditions applied by Snider afforded 7-epicylindrospermopsin (**23**), not **19**! Since the stereochemistry of the C-7 hydroxyl group had been confirmed by *X*-ray crystallography, the orientation of this group in the natural products was reversed.

Scheme 15. y) i. triphosgene THF, rt; ii. NaN_3, DMF, 65 °C, 86%; z) HCl, THF, H_2O, 85 °C, 72%; aa) MeOTf, 2,6-di-*tert*-butylpyridine, CH_2Cl, -78 °C-rt; bb) 10% Pd/C, EtOH, H_2; cc) 12 N HCl, 95 °C, 43% from **23**; dd) SO_3-DMF complex, DMF, pyr, Na_2SO_4, rt, 70% (+25% bis-sulfate).

To further confirm this unanticipated result, Weinreb's group wanted to produce an intermediate for the synthesis of **19**. In their full account of the synthesis of **23** they were able to show that the C-7 hydroxyl group could be inverted (Scheme 16).[67] Treatment of the protected intermediate **126** with Martin's modified Mitsunobu conditions, and hydrolysis of the *p*-nitrobenzoate gave **127**. Following their synthesis for **23**, they were able to generate the guanidinium diol **129** that matched Snider's intermediate. Knowing that **129** had already been converted to **19**, their assignment was further confirmed.

Scheme 16. a) HCl, H_2O, THF (85%); b) *p*-NBA, Ph_3P, DEAD, PhH; c) K_2CO_3, MeOH (61% *2 steps*); d) $Pd(OH)_2$, EtOH, cyclohexene (95%); e) triphosgene THF, rt; f) NaN_3, DMF, 65 °C, (70% *2 steps*); g) Ac_2O, DMAP, pyr (78%); h) MeOTf, 2,6-di-*tert*-butylpyridine, CH_2Cl, -78 °C-rt; i) 10% Pd/C, EtOH, H_2; j) 12 N HCl, 95 °C (61% *3 steps*).

1.3.10 White's Asymmetric Total Synthesis

Unbeknownst to us at the conception of this project, White's group was also exploring the use of an intra-molecular dipolar cycloaddition strategy.[68] They were able to use the back-end of Weinreb's synthesis to successfully complete the first asymmetric synthesis of **23**, shortly after the revision of **19**'s relative stereochemistry.[69]

They began with the synthesis of the dipolarophile needed to construct the A-ring (Scheme 17). Asymmetric crotylation of the benzyloxyacetaldehyde **130**, using Brown's diisopinylcamphenylmethoxyborane reagent gave the *syn*-homoalyllic alcohol **131** in 45% yield and 94% ee, as determined from its Mosher's ester. This stereocenter was then converted to the *anti*-amino alcohol **132** in a three step sequence. To produce the requisite hydroxylamine for nitrone formation, they chose the indirect oxidation method. Formation of *p*-methoxyphenyl imine and oxidation gave a mixture of oxaziridines **133**.

The free hydroxylamine **134** was then obtained in 60% yield after transamination with hydroxylamine hydrochloride in methanol.

Scheme 17. a) *cis*-2-butene, *t*-BuOK, *n*-BuLi, (+)-MeOB(Ipc)$_2$, Et_2O-THF (45%); b) Ms_2O, pyr, CH_2Cl_2 (100%); c) NaN_3, DMF, 85 °C; d) Ph_3P, THF-H_2O (56% *2 steps*); e) *p*-$MeOC_6H_4CHO$, MeOH, Na_2CO_3, 60 °C; f) *m*-CPBA, CH_2Cl_2, 0 °C → rt; g) $HONH_2 \cdot HCl$, MeOH, 0 °C → rt, 60% from **132.**

The uracil portion of the molecule was constructed from the methionine derived lactone **135** (Scheme 18). Addition of lithiated 4-bromo-2,6-dimethoxy pyrimidine gave a micture of the lactols **136**. Trapping of the primary alcohol as its trityl ether, and reduction of the freed carbonyl with L-selectride afforded a 12 : 1 diastereomeric mixture favoring the *syn*-amino alcohol **137**. Silylation of the secondary alcohol was followed by deprotection of the trityl group and hydrogenolysis of the *N,N*-dibenzyl amine to give the free amino alcohol **138**. The amine was re-protected as its *t*-butoxycarbonyl derivative. Ley oxidation of the primary alcohol then afforded the aldehyde **139** in 91% yield, poised for nitrone formation.

Scheme 18. h) **89**, n-BuLi, $CeCl_3$, Et_2O-THF, -78 °C → rt, (97%); i) Ph_3CCl, Et_3N, DMAP, CH_2Cl_2, Δ, (93%); j) L-Selectride, THF (84%); k) TBSOTf, Et_3N, THF (87%); l) HCO_2H, THF (100%); m) H_2, $Pd(OH)_2/C$, EtOH (81%); n) Boc_2O, Et_3N, CH_2Cl_2 (68%); o) TPAP (cat.), NMO, mol. sieves, CH_2Cl_2 (91%).

Condensation of **134** and **139** in the presence of molecular sieves gave the (*Z*)-nitrone **140** in 60% yield (Scheme 19). Heating **140** in hot toluene effected the intra-molecular [3+2]-dipolar cycloaddition to give a mixture of tricyclic isoxazolidines **141** in a disappointing 2 :1 : 0.5 diastereomeric ratio, presumably due to isomerization of the nitrone at elevated temperatures. Further the major diastereomer possessed the wrong relative stereochemistry at C-12. Reductive cleavage of the *N,O*-bond and acidic removal of the *t*-butoxycarbonyl group gave the diamine **142** in 68% yield from **140**. Treatment of **142** with carbonyl diimidazole gave the more readily handled urea **143**. At this point they needed to correct the stereochemistry of the C-12 alcohol, since all functionalities were suitably protected. Dess-Martin periodinane oxidation of the alcohol and reduction with L-selectride then gave a 15 : 1 diastereomeric mixture favoring the C-12 diastereomer required to complete the synthesis. Finally, hydrogenolysis afforded the diol **144**. This provided a system that was adaptable to Weinreb's chemistry for the installation of the guanidine. Thus treatment of **144** with triphosgene and sodium azide gave **145**. In this case methylation of the urea oxygen was achieved with KHMDS and trimethyloxonium

tetrafluoroborate. Reduction of the azide effected the closure of the C-ring to give **146**. Exposure of **146** to refluxing HCl effected hydrolysis of the pyrimidine and global desilylation. Sulfonation as previously described gave 7-epicylindrospermopsin (**23**), confirming the absolute stereochemistry as shown (7*S*, 8*R*, 10*S*, 12*S*, 13*R*, 14*S*). While representing the first asymmetric total synthesis of a cylindrospermopsin alkaloid in 22 steps, the synthesis suffered multiple protecting group manipulations and the undesirable stereochemical outcome during the intramolecular dipolar cycloaddition.

Confirmed absolute stereochemistry as 7*R*, 8*R*, 10*S*, 12*S*, 13*R*, 14*S*.

Scheme 19. p) MeOH mol. sieves, Δ (60%); q) PhMe, mol. sieves, Δ; r) Zn, NH_4Cl, THF-H_2O; s) HCl, MeOH (68% from **140**); t) Im_2CO, CH_2Cl_2, then K_2CO_3, MeOH (85%); u) Dess-Martin periodinane, CH_2Cl_2; v) L-Selectride, THF; w) H_2/C, $Pd(OH)_2$, EtOH, (55% *3 steps*). x) (i) $(Cl_3CO)_2CO$, THF; (ii) NaN_3, DMF (49%); y) TESOTf, Et_3N, CH_2Cl_2 (99%); z) KHMDS, Me_3OBF_4, CH_2Cl_2; aa) Pd/C, H_2, MeOH; bb) HCl (conc.) Δ, (21% *3 steps*); cc) SO_3·pyr, DMF (63%).

1.4 Biological Studies of Synthetic Analogues

From the large body of synthetic work that exists, toxicological evaluation of synthetic intermediates has given us a structure-activity framework. Runnegar and co-workers have had the opportunity to test synthetic analogues from both the Weinreb and Snider laboratories.[70] Compounds were tested for *in vitro* protein synthesis inhibition in the rabbit reticulocyte lysate system. Natural and racemic cylindrospermopsin are potent inhibitors with IC_{50} values in the nanomolar range (Figure 9). Racemic 7-epicylindrospermopsin (**rac-23**) was equipotent with an IC_{50} = 0.48 μM, again showing that the stereochemistry at C-7 has little effect on these toxins activities. Interestingly the synthetic diol **129** was as potent as the sulfated natural product, indicating that the sulfate is not required for activity. In cultured hepatocytes it was further shown that **19** and **129** had IC_{50}'s of 1.28 and 0.76 μM respectively, further indicating that the sulfate is not required for solubility or cellular uptake. The same is true for the 7-epicylindrospermopsin diol **147**.

19, IC_{50} = 0.21 μM
rac-19, IC_{50} = 0.57 μM

129, IC_{50} = 0.20μM

rac-23, IC_{50} = 0.48 μM

147, IC_{50} = 1.65 μM

Figure 9. Evaluation of *in vitro* protein synthesis inhibition.

Several synthetic analogues were shown to be non-toxic by the *in vitro* and hepatocyte protein synthesis inhibition assays (Figure 10). The AB-model **40** was had no effect at concentrations up to 800 μM. The AC-model showed lowered activity with IC_{50}'s of ~800 μM for *in vitro* protein synthesis and ~250 μM in hepatocyte cultures. The hydroxymethyluracil analogue **148** proved non-toxic at concentrations up to 2000 μM. These results suggest that neither the guanidine, nor the uracil alone are critical for the toxicity of **19** and **23**. It does however suggest that the *tetra*-substituted guanidine is necessary for bioactivity.

Figure 10. Non-toxic synthetic analogues.

From their studies on the chlorination of cylindrospermopsin, Carmeli and co-workers have isolated two oxidation products of **19** (Scheme 20). Treatment of **19** with an aqueous solution of chlorine provided 5-chlorocylindrospermopsin (**149**) and a product lacking the uracil fragment, named cylindrospermic acid (**150**). Both of these analogues showed LD_{50}'s > 10,000 μg/kg after i.p. injection in mice. This further illustrates the necessity of the intact uracil ring, and suggests specific binding of the uracil as substitution destroys its activity.

Scheme 20. Chlorination / oxidation of cylindrospermopsin.

1.5 Conclusion and Goals

Although there has been a tremendous amount of research directed toward the cylindrospermopsin alkaloids in the last twenty years, there is much to be learned. Despite biochemical and structure-activity studies the mode of action of these compounds is still unknown. It thus remains a major research objective in this area to identify **19**'s molecular targets (enzymes) and to elucidate a putative structure of a cytP450 metabolite.

The research described herein aims to achieve a concise asymmetric synthesis of the structurally unique cylindrospermopsin alkaloids. In doing so we may further the development of structure-activity relationships, thereby advancing a biomechanistic hypothesis. Synthesis may also afford competitive inhibitors of this toxin, aiding biochemical study and practical alleviations of intoxication. Total synthetic efforts will also be applied to the genesis of compounds that may aid in; 1) the isolation of enzyme targets and 2) the development of immunological assays (ELISA) for the rapid detection of these toxins in domestic water supplies.

Chapter 2: Initial Synthetic Research

2.1 Retrosynthetic Analysis of Cylindrospermopsin

When contemplating the total synthesis of **19** several factors warranted consideration. The polarity of uracils requires some form of protection, the alkylated pyrimidines allows easy manipulation that can be freed by hydrolysis. From Snider's synthesis we know that the reduction of the C7 ketone is stereoselective.[65] This suggests a strategy that allows the C7 stereochemistry to follow the installation of the C8 stereocenter. We initially thought that an intramolecular alkylation reaction on **156** should set the C8 center (Scheme 21). This intermediate would in turn be generated from the guanidinylation of the protected uracil fragment **155** and an A-ring piperidine like **154**. This guanidinylation event further permitted flexibility in the alkylation step. The B-ring could be closed first through a mitsunobu event to give **156**, or the C-ring could be closed by the alkylation event with subsequent B-ring formation.

crotylglycine

151

152

153

154

1,3- Dipolar Cylcoaddition

Intramolecular alkylation

19

Guanidinylation

155

156

Scheme 21. Initial retrosynthetic analysis.

At the conception of this project the absolute stereochemistry of this family of natural products was not known. To potentially access both antipodes of the natural product it was decided that a simple amino acid starter unit would be beneficial. In particular we decided that crotyl glycine (**151**) would be optimal, as both enantiomers would be readily available from Williams' oxazinone template. We then had to trace the synthesis of **154** to this amino acid.

Recognizing the 1,4-amino alcohol relationship in **154** it was thought that the three contiguous stereocenters in the A-ring might be set in a single intramolecular cycloddition reaction from the nitrone **152**. The intramolecular 1,3-dipolar cycloaddition (1,3-DC) of nitrones has become a powerful tool to access architecturally complex heterocycles.[71] Yet the use of functionalized nitrone precursors, in particular α-alkoxycarbonylnitrones, has found little application in the synthesis of natural products.[72] This is in part due to poor stereoselectivity arising from nitrone *E*/*Z* isomerization, which is probably responsible for the poor selectivity seen by White's group. The development of geometrically (*E*/*Z*) constrained nitrones, from chiral 1,4-oxazin-2-ones (oxazinone N-oxides), obviates this problem, but has remained unexplored in an intramolecuolar fashion.[73]

Further there are two disconnections that can be made for an intramolecular 1,3-DC reaction for the synthesis of **153**. The disconnection chosen by White's group enters the cycloaddition with two stereocenters already set. We rationalized that the intramolecular nature of the cycloaddition might be subject to stricter stereocontrol. Entering the cycloaddition with a single stereocenter would allow the construction of the three

contiguous stereocenters without the need for prior acyclic stereocontrol. This then predicates the construction of **152** from the amino acid **151**.

2.2 Crotyl glycine synthesis

In order to access the nitrone **152** for the key 1,3-DC the crotyl glycinol derivative **163** was targeted (Scheme 22). Initial attempts to generate this structure began with the esterification of *N*-Cbz-Ser (**157**) with thionyl chloride in methanol to give *N*-Cbz-*O*-Me-Ser (**158**) in 97% yield. This "chiral pool" approach was pursued with the desire to produce the large quantities of **152** needed for preliminary synthetic investigations. Protection of the hydroxyl group as the acetonide was best effected by the use of 2,2-dimethoxypropane and borontrifluoride etherate giving **159** in 98% yield. Reduction of the methyl ester was problematic. Aluminum based reducing agents gave large amounts of *N*-methyl acetonide while lithium or sodium borohydrides showed little to no reactivity. The free alcohol **160** could be generated in a moderate 47% yield using calcium borohydride.

Scheme 22. Serine derived attempts at crotyl glycinol.

Formation of the iodide **161** by the conventional triphenyl phosphine/iodine system also proceeded in a disappointing 37% yield. Attempts to alkylate the iodomethylene group were completely unsuccessful. Propynyl lithium, propynyl magnesium bromide, and both alkynyl anion equivalents in tandem with cuprous iodide failed to produce any of the alkyne **162**. Steric hindrance in these protected serine systems may well hamper both the iodination and alkylation reactions.[74]

The alternative route to the glycinol **163** was through the protected acid **166** (Scheme 23). Chemistry established in our group for the synthesis of allylglycine was adapted for the synthesis of this homologue.[75] Initially it was found that commercially available (*E*)-crotyl chloride could be used as the electrophile in the enolate alkylation of Williams' glycine template (**(+)-164**) to give the crotylated lactone **(+)-165** in 64% yield.[76] Lithium in ammonia ably removed the auxiliary to afford (*R*)-*t*-Boc-crotylglycine (**(*R*)-166**) in 57% yield.

Scheme 23. Crotylglycine synthesis from the oxazinone template.

After optimization of this sequence it was found that (*E*)-crotyl iodide, prepared *in situ* from crotyl alcohol, was a more competent electophile in the alkylation giving **(+)-165** in 92% yield after recrystallization from EtOH/H_2O.[77] It was also found that the addition of a proton source aided reduction of the auxiliary. Addition of EtOH during the Birch

reduction gave (*R*)-crotyl glycine ((***R*)-166**) in an improved 68-87% yield, the higher yield reflecting reaction scales <1 mmol.

This template directed synthesis of **(*R*)-166** afforded the amino acid in high optical purity. The lactone adduct **(+)-165** was judged to be greater than 99 : 1 er by HPLC after recrystallization. Interestingly separation of the enantiomers was concomitant with rotamer separation.[78] The optical purity of **(*R*)-166** could only be measured by HPLC after conversion to the free amino acids by treatment with aqueous HCl.

As permitted by the lactone, we now had synthetic access to both enantiomers of the amino acids, affording access to both antipodes of **19** if necessary. It became apparent however, that the use of the lactone **164** would not be amenable to the production of multi-gram quantities of **166** needed for initial synthetic investigations. Recognizing the unsaturation pattern in **166** it seemed plausible that a Claisen- rearrangement may also afford the amino acid. Diimide mediated coupling of (±)-3-buten-2-ol (**rac-167**) with *N*-*t*-Boc-Gly gave the glycine ester **rac-168** in excellent yield (Scheme 24). Treatment of **168** with >2 equiv. of base generated the enolate dianion that smoothly underwent [3,3]-sigmatropic rearrangement to give racemic **166** in essentially quantitative yield. This route was readily scalable to generate ~10-20g of **166**, enabling a racemic platform from which to launch our synthetic forays.

N-t-Boc-Gly; DIC, DMAP; CH_2Cl_2, 0 °C
2.2 eq. NaHMDS; THF, 0 °C-rt; ~*quant.*

rac-167 / ***(R)-(-)-167*** → ***rac-168***, 94% / ***(R)-(+)-168***, 96% → ***rac-166*** / ***(R)-(-)-166***

Scheme 24. Claisen rearrangement for crotyl glycine construction.

Kazmaier has extensively developed the enolate-Claisen rearrangement on a number of amino acid skeletons, varied in ester (alkene) and *N*-acyl-components.[79] This method has been further highlighted in the diastereoselective rearrangement of extended peptides.[80] He has also successfully developed an asymmetric variant utilizing quinine chelated magnesium enolates.[81] Despite these developments, the rearrangement has not been studied on substrates bearing chirality in the alkene partner. It is interesting to note that in this rearrangement it is the enolate geometry that controls the optical identity of the resultant acid. Noting this we hoped that both antipodes of **166** might be available from ***(R)*-168** (Scheme 25). We envisioned chelation, perhaps by a divalent metal atom, giving rise to the *Z*-enolate that would undergo rearrangement to **(*R*)-166**. Conversely a charge separated *E*-enolate should give **(*S*)-166**. This was also attractive as only the *R* enantiomer of **167** is commercially available.

As seen in Table 1 only the chelated Z-enolate pathway has been operational in this rearrangement. Treatment of **(*R*)-168** with NaHMDS in THF gave the acid in 92:8 er. Smaller couterions, i.e. lithium gave lowered er (86:14). Thinking that this erosion was due solely to the dianion leaking into the *E*-enolate, KHMDS was used as a base in conjugation with 18-crown-6 in an attempt to generate a naked enolate that may not experience any chelation. Indeed it appears that this increases the amount of *E*-enolate in the rearrangement, however only decreasing the enantioselectivity for **(*R*)-168** and unable to quantitatively switch the selectivity. Surprisingly the addition of aluminum and zinc also eroded the enantioselectivity. Using the corresponding *N*-carbobenzyloxy analogue of **(*R*)-168** in conjugation with NaHMDS an er of 96:4 was obtained. This demonstrated that the stereochemistry of the ester is almost exclusively translated in the rearrangement

as the alyllic alcohol **(*R*)-167** is available only in 98.5 : 1.5 er. Drawing from similar rearrangements on α-oxymethylene esters, attempts were made to selectively generate the *E* or *Z* enolates using di-butylboron triflate with triethylamine or diisopropylethylamine. Unfortunately the carbamate protecting groups were not stable to these conditions and decomposition ensued.

Scheme 25. The stereodivergent enolate-Claisen hypothesis.

Reagents	Conditions	e.r. (*R* : *S*)[a]
NaHMDS	THF, -78°C-rt	92 : 8
LiHMDS	THF, -78°C-rt	86 : 14
KHMDS, 18-crown-6	PhMe, -78°C-rt	77 : 23
NaHMDS, Et_2AlCl	THF, -78°C-rt	84 : 16
NaHMDS, $ZnCl_2$	THF, -78°C-rt	90 : 10
NaHMDS (*N-Cbz*)	THF, -78°C-rt	96 : 4
Bu_2BOTf, Et_3N (*N-Cbz*)	CH_2Cl_2, 4 °C	Decomp.
Bu_2BOTf, iPr_2NEt (*N-Cbz*)	CH_2Cl_2, 4 °C	Decomp.

a. Determined by HPLC on free AA after HCl hydrolysis

Table 1. Enolate-Claisen rearrangement selectivited.

Unfortunately the rearrangement did not occur below ~0 °C so temperature could not manipulate the stereochemical translation of the rearrangement. At this point the Claisen rearrangement was not further pursued as the lactone chemistry had been optimized for the synthesis of crotylglycine and provided a reproducible method for the synthesis of

***(R)*-166** in high optical purity. *It should be noted here that synthetic manipulations were proven on racemic material.*

2.3 Morpholine Synthesis

With the parent acid **166** in hand, we continued toward the construction of the morpholinone **172** (Scheme 26). The acid could be reduced with lithium aluminum hydride to give the protected amino alcohol **169** in 62-83% yield. This reduction was sometimes problematic, with **169** often accompanied by 10-15% of the free amino alcohol **163** and 5-10% of the corresponding aldehyde. Attempts to deprotect **169** with traditional protic acids (TFA, HCl), TMSI, and TMSCl/phenol led predominantly to the urethane **170**. The hydroxyl group was subsequently protected as the bromo acetate **171**. Removal of the *t*-Boc group with TFA and neutralization of the resulting salt did not produce **172**. 5-*exo*-trig cyclization predominated giving solely *O* to *N*-acyl transfer and the amide **173**.

Scheme 26. Morpholinone tribulations.

The reactivity of the hydroxyl group was circumvented by the use of boron trifluoride, which smoothly removed the carbamate giving the free amino alcohol **163** (Scheme 27).[82] The primary alcohol was noted to react with the *t*-butyl cation, affording up to 20% of the *t*-butylether. Addition of 1,3-dimethoxybenzene sufficed as a cation scavenger to produce **163** as the sole product. The lactone portion of **172** was constructed by the use of α–bromophenyl acetate, thus *in situ* treatment of **163** with this reagent gave a 63% yield of **172**.[83]

Scheme 27. Preparation of the morpholinone.

While this first procedure afforded **172** in acceptable yield, it was desired that the amino alcohol precursor to be isolable and storable, not contaminated with excess 1,3-dimethoxybenzene or its alkylated derivatives. This was essential as **172** was unstable, readily dimerizing to the diketopiperazine (**175**, see Scheme 28). Two routes were executed to cleanly produce the amino alcohol in reasonable yield and scale. The first of these converted **166** to the amino acid methyl ester hydrochloride upon treatment with *in situ* generated HCl in methanol. This salt could be treated with lithium aluminum hydride

to give the free amino alcohol **163** in 65% yield after Kugelhror distillation. The second route utilized the mixed carbonic anhydride reduction of the acid followed by removal of the *t*-butoxycarbonyl group with HCl in methanol. This gave the amine salt **174** as a stable solid. The free amino alcohol performs better in the alkylation cyclization sequence to afford the morpholinone. It is unclear whether this is due to the hygroscopic nature of the salt or increased consumption of **172** to its diketopiperazine in the presence of excess base. The salt **174** can be free-based by extraction into chloroform and used as **163**. The alkylation reaction to form **172** remains the bottleneck in this synthesis. Highly optimized reaction conditions using freshly distilled **163** can routinely give the morpholinone in 60-70% yields but the reaction has not been made scaleable and is most productively run on only a ~5 mmol scale.

As noted before the morpholinone is prone to dimerization and must swiftly be oxidized to the nitrone **152**. This transformation was first accomplished using Davis' oxaziridine (**176**) (Scheme 28, Table 2).[84] Treatment of **172** with two equivalents of **176** gives the nitrone in 75% yield. Benzenesulfonamide, produced after the oxidant is spent, proved difficult to remove from the nitrone upon scale up. Thus the oxidation was attempted using the saccharin derived oxaziridines **177** and **178**. Unfortunately these oxidants do not react at room temperature, and upon refluxing a solution of the morpholinone the diketopiperazine **175** is formed. Both peracetic acid and oxone return low yields of **152**, due to lactone hydrolysis in the aqueous reaction environments. Fortunately it was found that purified *m*-CPBA could be used effectively as an oxidant.[85] Reagent grade *m*-CPBA is not suitable as it contains ~ 30% water, again causing lactone hydrolysis during the oxidation event. Oxidant by-products are water soluble and easily

removed by basic extraction. After realizing that alkene oxidation was not effected at low temperatures, it was found that inverse addition of a solution of **172** into an excess of *m*-CPBA gave a satisfactory 84% yield of the nitrone.

Scheme 28. Morpholinone oxidation.

Reagents	Conditions	Results
176	THF, 0°C	**152** (75 %)
177	THF, 0°C-reflux	**175**
178	THF, 0°C-reflux	**175**
$MeCO_3H$ (aq.)	THF	**152** (47 %)
Oxone	Me_2CO, H_2O pH = 7	**152** (8 %)
m-CPBA	CH_2Cl_2, Na_2HPO_4, -78°C	**152** (72 %)
m-CPBA	CH_2Cl_2, Na_2HPO_4, -78°C *Inverse addition*	**152** (84 %)

Table 2. Oxidation summary.

2.4 Intramolecular Dipolar Cycloaddition

The oxazinone-N-oxide **152** was surprisingly stable, not prone to dimerization nor spontaneous cyclization. Pleasingly, exposure of **152** to elevated temperatures cleanly effected the 1,3-dipolar cycloaddition reaction to give the tricyclic isoxazolidine **153** in 78-84 % isolated yield (Scheme 29). As expected from Oppolzer's work, and subsequent developments of intramolecular N-alkenylnitrone cycloadditions, the nitrone added to the alkene predominantly through the chair like *exo*-transition state $A^{\ddagger}$ to give **153**.[72,86]

Scheme 29. The intamolecular 1,3-Dipolar cycloaddition.

Although the reaction produced **153** as a single diastereomer it was accompanied, as a 10:1 mixture with the regioisomeric isoxazolidine **179**, presumed to arise from transition state $\boldsymbol{B}^{\ddagger}$. Although, at present this minor product is inseparable from **153**, fractional crystallization affords a 2:1 mixture of **153** : **179**. The structure of **179** is supported by a quartet at 3.77 ppm (1H, J = 6.3 Hz) in the ^{1}HNMR spectrum for the alkoxy-methine proton and its nOe effect on its neighboring pseudo-equatorial methylene proton. The gross skeletal structure of **153** was confirmed by HSQC and COSY NMR experiments. The relative stereochemistry of **153** was ultimately determined by single crystal X-ray diffraction (Figure 11).

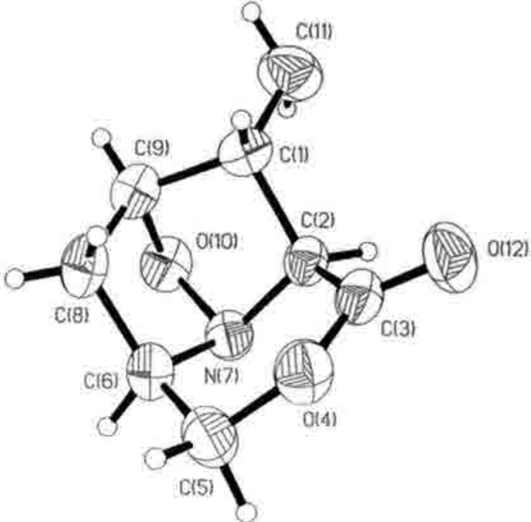


Figure 11. TEP diagram of the tricyclic isoxazolidine **153**.

The regiochemistry of the cycloaddition could be improved with the use of $Sc(OTf)_3$ in dichloromethane. The reaction took ~3 days to reach completion but afforded **153** in an improved 12:1 selectivity. Other Lanthanide triflates, La and Yb were examined, however their triflate salts are available only as the hydrates and the nitrone suffered hydrolysis prior to cycloaddition. Boron trifluoride, even at -78 °C, proved too strong of a Lewis acid leading to substrate decomposition.

Due to the extended reaction times with scandium, the thermal reaction is routinely performed adhering to practical considerations. Initially the thermal cycloaddition was conducted in a sealed tube in toluene at ~200 °C. However, when scaling the reaction it was found that nitrone dimerization and intermolecular cycloaddition competed at increased concentrations. The reaction is best performed at concentrations near 0.01 M. Capacity limitations with sealed tubes led us to utilize refluxing PhMe (~110 °C) as the reaction medium. These conditions afford variable ratios, ranging from 7:1 to 9:1, of **153**:**179**. The reason for this is unclear, but it is noted that the cycloaddtion is not thermally reversible as subjection of a 7:1 mixture to sealed tube conditions does not affect the product ratio.

The exo-mode of cycloaddition appears to dominate in these intramolecular cycloadditions, as also seen with the allyl derivative prepared in an analogous fashion to **153** (Scheme 30). Allyl alcohol (**180**) can be esterified with *N-t*Boc-Gly in 98% yield. Enolate-Claisen rearrangement and mixed carbonic anhydride reduction cleanly gives racemic allylglycinol (**181**). Acidic removal of the Boc group and annulation with phenyl bromoacetate gives the morpholinone that can be oxidized to the nitrone **182** in 83% yield. Thermal cycloaddition induced in this system produces **183**, apparently as a single

regioisomer. The relative stereochemistry was again proven by single crystal X-ray analysis.

180

1. *N*-*t*Boc-Gly, DIC
DMAP, CH_2Cl_2 (98%)
2. NaHMDS, THF (92%)
3. *i*BuOCOCl, Et_3N
$NaBH_4$, THF, H_2O (69%)

181

1. AcCl, MeOH (84%)
2. $PhOCOCH_2Br$
iPr_2NEt, MeCN (63%)
3. *m*-CPBA, Na_2HPO_4 (83%)

182

PhMe, 200°C
sealed tube

183

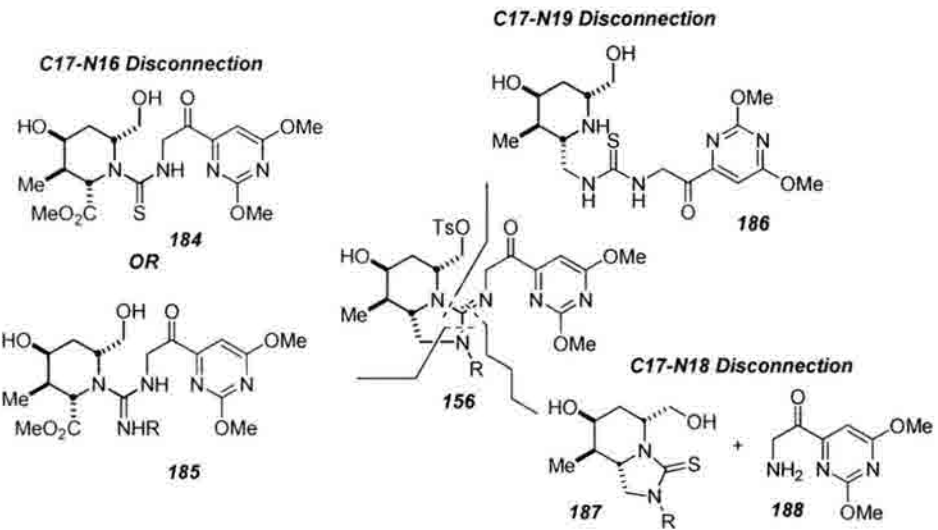
183 X-ray

Scheme 30. Allyl glycine derived isoxazolidine.

At this junction, we had found a reliable method to construct the A-ring of cylindrospermopsin. A single synthetic manipulation, using an intra-molecular dipolar cycloaddition served to set the three contiguous stereocenters of the piperidine.

2.5 Guanidine installation attempts

Our initial synthetic strategy revolved around an intramolecular alkylation event to install the C8 stereocenter from an intermediate like **156**. This predicated that the guanidine be installed early. At the time it seemed fortuitous that the synthesis of **156** would be flexible with three probable disconnections for its construction (Scheme 31). Disconnection of the C17-N16 bond allows guanidine construction from the thiourea **184** or the guanidine **185**. Scission of the C17-N19 bond allows an intramolecular guanidinylation event from the thiourea **186**. An intermolecular guanidinylation of the intact AB-ring synthon **187** and the amino ketone **188** could also be envisioned.



Scheme 31. Guanidine disconnections.

2.5.1 C17-N16 Disconnection

In order to explore the first guanidinylation strategy the lactone in the isoxazolidine **153** required hydrolysis. Reduction would produce a diol, in which it was not envisioned to be able to differentiate the two hydroxyl groups. Unfortunately attempts to hydrolyze the lactone in **153** were unsuccessful (Scheme 32). Treatment of the tricyclic

isoxazolidine with lithium hydroxide did not afford the acid **189**. Methanol at elevated temperatures was also unsuccessful at lactone opening unable to produce the methyl ester **190**. It was reasoned that both the lack of enthalpic gain in the reaction and the proximity of the freed hydroxymethyl greatly favored the return of **153**. It then became apparent that the *N,O*-bond had to be cleaved first. In doing so the intermediate **191** would be generated. This intermediate was predicted to undergo facile ring opening, as doing so would allow all the three carbon based piperidine substituents to assume an equatorial orientation. Indeed hydrogenolysis of the *N,O*-bond in methanol produced the methyl ester **192** in 98% yield. Responding to Armstrong's and Hart's disclosures of their synthetic strategies and Snider's total synthesis, we published the synthesis of this functionalized A-ring precursor.[87]

189 R = H
190 R = Me
LiOH, H_2O, THF
or
MeOH, PhMe, 200 °C
153
H_2, Pd/C
MeOH
98%
191
192

Scheme 32. *N,O*-bond cleavage prevails.

Having now synthesized a piperidine suitable for guanidinylation in a C17-N16 fashion, work focused on the construction of the pyrimidine half of **19**. The *S*-Me thiourea **155** was to be constructed from the α-haloaryl ketone **196** (Scheme 33). Dehydrative halogenation of barbituric acid (**193**) gave the tri-bromopyrimidine **194** in 60 % yield.[88] Nucleophilic substitution with two equivalents of sodium methoxide gave

the dimethoxypyrimidine **195** in 82% yield.[89] Concomitantly the *N*-Cbz thiourea **198** was prepared from the disulfate salt of *S*-Me thiourea (**197**). Subsequent attempts to alkylate the thiourea nitrogen with α-bromomethyl acetate under a variety of conditions were unsuccessful. *It should be noted here that the following discussions on guanidine formations may often utilize glycine equivalents instead of the pyrimidylketone. It was also envisioned that a metallated form of **195** could be added to a glycine derived synthon to afford the analogous system.*

Scheme 33. Pyrimidine preparation.

Undeterred because of Snider's total synthesis in which a guanidine displaces an α-haloarylketone the synthesis of **196** was pursued. Lithium halogen exchange with *t*-butyl lithium smoothly generates the lithiated arene. Previous reports mention the instability of this anion and the need for low temperatures.[88] Thus quenching of the lithioarene at –80 °C with a variety of electrophiles (AcCl, EtOCOCN, $MeOCOCH_3$, and $BrCOCH_2Br$) proved unsuccessful. It was found that the lithioarene added smoothly to acetaldehyde to give the alcohol **200** in high yield (Scheme 34). Swern oxidation then affords the ketone

201 in 75% yield. Unfortunately all attempts to halogenate the methyl group, thus producing **196**, met with failure. Enolate trapping with I_2, Br_2, or NBS afforded complex reaction mixtures, presumably due to competing halogenation of the pyrimidine ring. Utilizing reaction conditions identical to Snider's, i.e. cupric bromide in refluxing ethyl acetate, also failed to return any of the desired α-bromoketone. The alternative then became to install the ketone in a "pre-aminated" fashion. Although we knew that the lithioarene was precocious, the Weinreb amide of *N*-Tr-glycine (**204**) was prepared. Addition of the lithiated pyrimidine gave minimal amounts of **205** and further complications in the deprotection of this compound hailed this a dying route.

Scheme 34. Attempted synthesis of **196** or directly aminated partners.

Experiencing difficulty with the construction of the pyrimidine synthon to be joined to **192** it became necessary to ensure the ability of the piperidine nitrogen to be guanidinylated. Treatment of **192** with benzyl isothiocyanate does not produce the thiourea **206** (Scheme 35). More importantly **192** does not participate in guanidine formation with the *bis*-*N*-*t*Boc-*S*-Me thiourea to produce **207** even in the presence of silver or mercury salts. Further it had also been noted that the piperidine nitrogen cannot be protected as its Boc derivative. It is thought that the two neighboring equatorial

substituents on the piperidine ring sterically encumber the nitrogen and prohibit functionalization.

Scheme 35. Attempted piperidine functionalization.

It was eventually found that highly activated imidazole thioureas could acylate this hindered nitrogen (Scheme 36). Treatment of glycine's benzyl ester **208** with 1,1′-thiocarbonyldiimidazole (TCDI) in acetonitrile cleanly gives **209,** that is surprisingly stable to chromatography. Refluxing a solution of **192** in methanol with this activated thiourea indeed acylates the nitrogen to give **210**. However, cyclization follows to deliver the thioimidazolidinone **211** as the sole product. Attempts to halt this cyclization by removal of the thiourea proton failed. The *N*-benzyl thiourea **213** could be prepared in an analogous manner, however, this reagent failed to react with the piperidine, again attributed to sterics. The carbamate protected thioimidazoles could not be accessed. These would be very electophilic alternatives that may overcome steric encumbrance. Attempts to add the lithium or sodium salts of *N*-Boc-Gly-*O*-Bn or *t*-butylcarbamate to TCDI failed.

Facing the difficulties of functionalizing the piperidine nitrogen, it was decided to install N18 first. Functionalization of this primary or secondary amine would then allow the piperidine nitrogen to react intramolecularly, a much more favorable process.

Scheme 36 labels: BnO / NH2·HCl (208) → TCDI, Et3N, MeCN, 92% → 209; 192 → 209, MeOH, reflux → [210] → 211; 212 (NHBn·HCl) → TCDI, Et3N, MeCN → 213; 192 → 213, MeOH, reflux ✗ → 214

Scheme 36. Thioimidazole acylations.

2.5.2 C17-N19 Disconnection

Following the success of the *N-O* bond cleavage/lactone opening strategy which gave the methyl ester **192** it was thought that hydrogenation in the presence of ammonia would give the amide **216**, thus incorporating N16 (Scheme 37). Conducting the reaction in methanolic ammonia produced solely the methyl ester **192.** In an attempt to increase the relative nucleophilicity of ammonia the reaction was carried out in ethanol. This gave a clean reaction yielding a 3:2 ratio of the desired amide **216** to the ethyl ester **215**. Extending this trend isopropanolic ammonia was utilized as the solvent giving **216** as the sole product by ^{1}H NMR. It was simultaneously found that the *NO*-bond could be cleaved in THF to give **191**, thus eliminating this competing ring opening. Treatment of this

fragile intermediate with ammonia in a sealed tube or benzylamine cleanly gave amides **216** or **217**.

Scheme 37. Synthesis of the N18 amides.

Unfortunately, attempts to reduce amides **216** or **217** to their corresponding amines under a variety of hydridic conditions led to complex reaction mixtures. The analysis of reduction products was further complicated by difficult purification of the resulting highly polar diamino-diols. This led us to adjust the oxidation state of the carbonyl carbon prior to introduction of the amino functionality (Scheme 38). Reduction of the lactone in **153** with diisobutylaluminum hydride gave the lactol **218** in 87% yield, inconsequentially as a ~2 : 1 mixture of anomers. *N-O* bond cleavage and reductive amination of the lactol under hydrogenolysis conditions in ammonium hydroxide/methanol gave the diamine **219**. Thiocarbonyl transfer from TCDI, to avoid handling the diamine, gave the thiourea **220** in 76% yield from lactol. This strategy could also be applied with benzyl amine to give the protected amine **221**. This too could be captured as its thiourea **222** in 80% yield after treatment with TCDI. While it was noted that the primary carboxamide could not be reduced by metal hydrides, it was

subsequently found that the benzylamide could after knowing the characteristics of **221**. A nice reaction sequence whereby the isoxazolidine could be ring opened with benzyl amine was developed. The amide **223** was highly unstable. Purification on silica gel returned the tricycle **153**. Fortunately it was found that refluxing **223** in a suspension of lithium aluminum hydride in THF for 64 h gave the *NO* and amide reduced diamine **221** in 51-63% yield. These sequences afforded flexible synthetic access to A ring synthons with N16 installed.

Scheme 38. Synthesis of the diamines.

With the diamines in hand the N16-N19 thioureas needed installation. Treatment of the diamine **221** with the xanthate derived from Gly-*O*-Me in refluxing DMF gave both the thiourea **224** and the guanidine **225** as evidenced by HRMS (Scheme 39). However, **225** was produced only in trace amounts by ^{1}HNMR, and was not able to be purified. Due to the harsh reaction conditions and the low reactivity of the secondary amine toward the

xanthate, **221** was treated with the imidazole thiourea **209**. This smoothly coupled to the diamine to give the thiourea **226** in 74 % yield as a ~3.5:1 mixture with the regioisomeric thiourea coupled to the piperidine nitrogen, which were easily separable by chromatography. Unfortunately exposure of **226** to mercury or silver salts failed to generate **225**. The more active *S*-methyl thiouronium salt **227** could be generated by treatment of the thiourea with trimethyloxoniuim tetrafluoroborate. Exposure of **227** to several combinations of bases and solvents surprisingly failed to produce **225**.

Scheme 39. N18 functionalized thioureas.

Lacking two urea protons, it was suspected that the *N*-benzyl thiourea might hamper activation, not allowing the formation of the desulferized diimide that would readily undergo guinidinylation. To evaluate this possibility the primary diamine **221** was treated with the imidazole **228** (Scheme 40). After interception of the thiourea, the molecule surprisingly undergoes guanidinylation. However, it further lactonizes to give the apparent guanidine lactone **229**. This assignment is supported by mass, and ^{1}HNMR in which the methylene protons next to the carbonyl become a distinct AB quartet, and the IR shows stretching at 1740 cm^{-1} and 1520 cm^{-1} indicating that both a lactone and a

guanidine are present. Unfortunately this molecule was highly unstable, refuting purification. Attempts to tame its reactivity by protecting the guanidine as its carbamate also met with failure.

Scheme 40. Primary diamine acylation.

Scheme 40. Primary diamine acylation.

Although **229** proved not synthetically useful, it was exciting to discover a successful mode of guanidine closure. The experiment above indicated that the glycine ester would not prove itself a productive enolate synthon, due to the subsequent lactonization. It thus became *imperative* to generate the pyrimidyl aminoketone that could be coupled with TCDI to give **234** (Scheme 41). Noting that the 1,2-amino alcohol functionality could be generated from a nitroaldol reaction, we needed to synthesize the known pyrimidine aldehyde **59**. However, attempts to recreate Sniders synthesis of **59** gave material in inadequate yield.

The aldehyde **59** was instead synthesized from the bromopyrimidine **195**. Negishi cross-coupling of the pyrimidine with vinylzinc chloride afforded a 92% yield of vinyl pyrimidine **230**. Ozonolysis and reductive work-up gave an 86% yield of aldehyde **59**. The condensation of nitromethane and **59** in the presence of sodium methoxide gave exlusively the nitrostyrene. Elimination could be prevented by the use of catalytic triethylamine to give an 87% yield of the nitronol **231**. Reduction of the nitro group with hydrogen in the presence of palladium and TFA gave the amino alcohol **232** in quantitative yield. Following reports that amino alcohol salts could be directly oxidized,

232 was treated with IBX in DMSO to give the presumed amino ketone **233**.[90] This compound was not incredibly stable and could not be manipulated. Treatment with TCDI and base did not afford **234**. Instead it appears that this compound expediently undergoes dimerization to the corresponding pyrazine. Further, the Boc protected amino alcohol derived from **232** could be oxidized to the ketone. Removal of the Boc group gave mixtures appearing to suffer the same fate of dimerization.

Scheme 41. Pyrimidine synthesis

Thwarted again attempting to synthesize a pyrimidyl ketone, it was hoped that the oxazolidine-thione **236** might serve its purpose as a thiourea transfer agent (Scheme 42). Reduction of the nitronol **231** without TFA gave the free amino alcohol **235**. TCDI then introduced the thione unit to afford **236**. It was hoped that the primary amine of **221** would suffice to intercept the thiocarbonyl, after which guanidine formation would provide the driving force for the production of **237**. Attempts to couple **236** and **231** were

not successful. Addition of mercury salts failed to promote the guanidinylation. *S*-Methylation of **236** also failed to yield an intermediate reactive enough for coupling.

Scheme 41. Attempted oxazolidine-thione coupling

Unable to synthesize a pyrimidine synthon with an activated thiourea, needed for coupling to the diamines, the focus turned to the C17-N18 disconnection. Having both the thioureas **220** and **222** in hand, it appeared a logical disconnection to incorporate the aminoalcohol **235**.

2.5.3 C17-N18 Disconnection

The thioureas **220** and **222**, previously prepared as easily handled diamine synthons now found a specific application. However, they didn't prove very useful (Scheme 42). Attempts to couple the aminoalcohol **235** with the unsubstituted thiourea **220** with the aid of various mercury or silver salts returned complex mixtures of products. The benzyl substituted thiourea **222** also failed to produce **238**. It was noticed that the use of mercuric trifluoroacetate actually returned the urea **239** as subsequently proven by an independent synthesis (see sec. 3.3). The more interesting result came from mercuric chloride induced coupling. In this reaction, the thiourea was also hydrolyzed. However this hydrolysis was accompanied by substitution at the hydroxy methyl group. This

pointed to a unique mode of reactivity for these systems. It is thought that Lewis-acid complexation of the thiourea allows attack by the hydroxyl group to give an intermediate like **240**. With a thiophilic Lewis-acid like mercury, nucleophilic attack occurs at the methylene carbon to collapse the tetrahedral intermediate, expelling mercuric sulfide, and generating the urea **241**. This may also be the reaction mode observed in the production of **239** with attendant hydrolysis of the trifluoroacetate ester. Weeks after this discovery, Weinreb's total synthesis appeared in which they observed an orthogonal mode of reactivity in the installation of the B-ring (see Scheme 15), validating this mechanism.

Scheme 42. Attempted AB-ring couplings.

Having established the interference of the hydroxymethyl group in this reaction the diol was protected as its di-*t*-butylsilyl ether **242** (Scheme 43). This protected derivative also failed to couple with **235**. Attempts to generate the *S*-Me thiouronium salt and

subsequent substitution with glycine esters also failed. Surprisingly treatment of **242** with oxalyl chloride in warm toluene to generate the chloroimidazolium salt **244**, proceeded to undergo this intramolecular oxygen transfer leading to the chloro-urea **245**.

Scheme 43. Protected thiourea couplings.

2.6 Conclusions

These initial synthetic investigations provided a reliable method for producing the three contiguous stereocenters in cylindrospermopsin's A-ring through an intra-molecular dipolar cycloaddition, in up to 12:1 selectivity for the stereotriad. Further, methods were also developed to install N16 needed for the synthesis of the B-ring. The progress of the synthesis was marred by our inability to synthesize a pyrimidyl aminoketone needed to execute the intramolecular enolate alkylation. The few guanidine species that had been observed proved unstable or very difficult to purify and work with. This led us to contemplate a late stage installation of the guanidine.

Chapter 3. Synthesis of the Cylindrospermopsin Alkaloids

3.1 Biomechanistic hypothesis

We became interested in the recent reports that cylindrospermopsin may alkylate DNA thus causing single strand breaks.[52,53] This coupled with the long standing observation that **19** is oxidatively activated *in vivo* suggested a plausible mode of action leading to the genotoxicity of these compounds. [48,49] Both *N*-hydroxylation and C-α-hydroxylation by putative cytP450 enzymes is well known.[91] Further the intermediacy of the cytP450 system may account for the redundancy of the C7-stereocenter, as both cylindrospermopsin and 7-epicylindrospermopsin are equipotent.

A number of oxidation pathways can be conceived for this activation of **19**. It is interesting to note that these five potential oxidation events are degenerate, leading to only two activated intermediates (Scheme 44). Through extensive tautomerization these may even lead to a single metabolic intermediate. The first pathway explores oxidation of the B-ring. Hydroxylation of N16 would lead to the *N*-hydroxyguanidine **246**, while C15 oxidation would give the carbinol **247**. Both of these intermediates could undergo expulsion of the hydroxyl group to give the intermediate guanidinimine **248**. This is an intriguing hypothesis as Snider's AC-ring model **67** is not toxic, suggesting the necessity of an intact B ring, or C15. The second pathway addresses oxidation in the northern region. N8-hydroxylation would generate the *N*-hydroxyguanidine **249**. Oxidation of C8 would produce the carbinol **250**, while oxidation at C7 would provide the masked ketone **251**. Again, expulsion of the hydroxyl group followed by tautomerization could funnel all of these intermediates to the enolguanidine **252**. This oxidation pathway may explain the the stereochemical redundancy at C7.

Scheme 44. Degenerative oxidation pathways.

The presence of the uracil strongly suggests a motif for DNA association, either through interchelation or hydrogen bonding complimentarity. Upon "binding" to DNA, both of these intermediates are then thought to be capable of producing electrophilic intermediates (Scheme 45). If protonated, the gunidinimine **248** would generate the intermediate **248′**. Alkylation, probably by the exocyclic amine of guanine (N2), would create a lesion leading to strand breaks. Correspondingly, protonation or hydrogen bonding to the uracil carbonyl may generate the imine **252′**, also capable of being alkylated.

Scheme 45 structure labels: 253, 248′, 254, 252′; DNA regconition stacking; DNA recognition H-bonding

Scheme 45. Potential DNA alkylation events.

3.2 Nitroaldol disconnections

Driven by this hypothesis, we decided to manipulate our synthetic route to probe these possible mechanisms. Having already constructed the AB-ring system the N16-C15 oxidation pathway would not be immediately addressed. When constructing the C-ring and the C7-C8 stereocenters, several means to test the C7-C8-N18 oxidation pathways were devised. These centered around a nitrolaldol disconnection whereby nitromethane became the ultimate source for N18 (Scheme 46).

From the biomechanistic hypothesis it follows that if ***252*** *was an intermediate, the C8-diastereomers of cylindrospermopsin should be equally toxic.* To test this, we desired the ability to synthesize all four diastereomers of the contiguous C7-C8 stereocenters. Of course two of these are natural products, **19** and **23**. The catalytic asymmetric addition of nitromethane to aldehydes is well developed. Following this work, it was thought that both enantiomers of **256** could be derived from the pyrimidine aldehyde **59** utilizing catalysts developed by Shibasaki, Trost, or Evans.[92,93,94] This stereocenter could then persuade the C8 stereochemistry after reductive nitromethylenation to the ureidoaldehyde

255. Seebach has shown that *O*-silyl lithium nitronates can be protonated at low temperatures to give high selectivity (>95:5) for the *anti*-isomer.[95] Thus we should be able to synthesize the 7*R*, 8*R* isomer, needed for the synthesis of **19,** from **256** or the 7*S*, 8*S* isomer from its enantiomer. It was also thought that direct condensation of the homologated nitroalkane **258** with the aldehyde **59** should give the pair of *syn*-diastereomers. This would give the 7*S*, 8*R* diastereomer needed for the synthesis of **23** and the 7*R*, 8*S* diastereomer.[96] This was also an attractive strategy as reductive nitromethylenation of **255** with the nitroalkane **257** would synthesize 7-deoxycylindrospermopsin (**21**). With the nitronols in hand it was then hoped that reduction of the nitro group would effect the intramolecular guanidinylation on a urea like **259**. If this reduction could be controlled, the *N*-hydroxyguanidine **249** would be fashioned, allowing us to test its biological intermediacy.

Scheme 46. The nitroaldol disconnection.

3.3 α-Ureidoaldehyde synthesis

One of the difficulties associated with these disconnections is the need for the α-ureidoaldehyde **255**, which is highly sensitive to epimerization (as seen by Weinreb). We initially attempted to synthesize the aldehyde of the thiourea **222** (Scheme 47). Treatment of **222** under typical Swern oxidation conditions interestingly returned the chloromethyl urea **241**, presumably through the activated intermediate **260**. Further, treatment of **222** with TEMPO and iodosylbenzene diacetate gave the acetate substituted urea **262**, after addition of the thiourea to the oxammonium salt (**261**).

Scheme 47. Thiourea oxidations.

We then became interested in the ability to add productive nucleophiles to the methylene carbon. Unfortunately several combinations of the nitronate anion (Li, K, or Na salt) with mercuric salts or scandium failed to directly produce **258**. The use of

mercuric cyanide even failed to introduce the cyano group. Treatment of **241** with KCN in DMSO in conjunction with NaI gave very little of the nitrile, <10% conversion. These experiments told us that the sulfur was too nucleophillic and must be changed to the urea. They also hinted that the aldehyde would be the only productive electrophile at this position, as nucleophilic substitution is too sterically demanding.

To this end, treatment of **221** with *bis-p*-nitrophenylcarbonate, functioned well to give the urea **239** in 75% yield (Scheme 48). Again attempts to directly oxidize the hydroxymethyl group in **239** proved fruitless. In fact, traditional Swern and Dess-Martin periodinane oxidations showed selectivity for the secondary hydroxyl group, giving the ketone. To prevent this, the diol **239** could be protected as its di-*t*-butyldimethylsilyl derivative. Treatment of this intermediate with TBAF selectively deprotected the primary hydroxyl group affording **264** in 64% yield. Unfortunately treatment of **264** with Dess-Martin reagent, or IBX failed to oxidize the hydroxymethyl group. Swern and Ley oxidations also failed to transform **264** to the aldehyde. The intermediate **264** was difficult to handle, as silyl migration occurred rapidly even at -20 °C to give a synthetically useless mixture of mono, di, and unprotected ureas.

Scheme 48. Formation of the urea.

Noting the recent advances in nitroxyl-mediated oxidation chemistry, and its adaptation to selective oxidations, an effort was launched to oxidize the hydroxymethyl

group of **239** in the presence of the free secondary alcohol.[97] Thus treatment of **239** with catalytic TEMPO and a variety of secondary oxidants was investigated (Table 3). Sodium hypochlorite functioned in a biphasic system to effect the oxidation, however the pH required to sustain the reoxidation (through the generation of hypochlorous acid) also epimerized the sensitive ureidoaldehyde **255**.[98] Reoxidation with *m*CPBA failed to give any material.[99] Noting the very dramatic effects of halide counterions in the oxidation, NCS was used in combination with both TBACl and TBAB in a biphasic system.[100] Again the basic nature of these conditions caused significant levels of epimerization. Success was beginning to be realized with iodobenzene diacetate as the reoxidant in non-aqueous envrionments.[101] While only providing **255** in about 10% conversion, there was no sign of the epimer or oxidation of the secondary hydroxyl group. From this, attempts were made to elaborate these conditions into a synthetically useful reaction.

Reagents	**Conditions**	**Results**[a]
NaOCl	CH_2Cl_2, H_2O, pH = 7.8	~20% conv. + epimer
m-CPBA	CH_2Cl_2, -78° C	Decomp.
NCS, Bu_4NCl	CH_2Cl_2, H_2O, pH = 8.6	~30% conv. + epimer
NCS, Bu_4NBr	CH_2Cl_2, H_2O, pH = 8.6	~30% conv. + epimer
$PhI(OAc)_2$,	CH_2Cl_2, rt	~10% conv.

Table 3. Initial TEMPO oxidations. [a] determined by ^{1}HNMR

This oxidation was capricious to say the least and required prolonged reaction times to reach acceptable conversion levels. The oxidation would sometimes proceed in dichloromethane and not in acetonitrile, but often in acetonitrile and not

dichloromethane. Further, some oxidation would proceed to ~60% in 2 days, but sometimes ~20%. The reasons for this random behavior were not at all clear.

One empirical observation was that an oxidation proceeded faster when concentrated. At first this was actually thought to be related to the low nucleophilicity of the hydroxymethyl group due to an intramolecular hydrogen bond to the urea oxygen. Thus concentration should effect the nature of the inter-intramolecular hydrogen bonding, potentially increasing the oxidation rate. Crude experiments showed that in fact the percent conversion is independent of urea concentration. Reactions run at 5, 7, and 11mM all provided the aldehyde in ~63% conversion after 53h.

This lack of dependence on concentration also indicated that alcohol addition to the oxidant was not rate-limiting. To further test this, the dependence on catalyst loading was examined. Not surprisingly there was no dependence on catalyst concentration either, with reaction conducted in the presence of 20, 40, and 60 mol% TEMPO all showing ~20% conversion after 2h.

This independence of catalyst loading has further implications. When considering the mechanism of TEMPO mediated oxidations (Scheme 49) it is important to note that the nitroxyl radical itself is not the oxidant.[102] It is thought that **265** disproportionates into the hydroxylamine **266** and the oxoammonium salt **267**.[103] It is then **267** that is the active oxidant. Nucleophillic addition of the alcohol to this intermediate is thought to precede Cope-elimination to generate the alcohol and the hydroxyl amine. Iodobenzene diacetate then oxidizes the hydroxylamine back to the nitroxyl radical to complete the catalytic cycle. This then suggests that either reoxidation of the hydroxylamine or the disproportionation of the nitroxyl radical are responsible for the sluggishness of the

reaction. It was further shown that the reaction rate is not dependent on the concentration of iodobenzene diacetate. Thus the disproportionation step must be rate limiting. The addition of acid should promote this disproportionation, and indeed the addition of 1 mol% methanesulfonic acid greatly accelerates the reaction rate leading to 81% conversion in 15 h.[104] Further $MeSO_3H$ was found to be superior to $TsOH{\cdot}H_2O$, AcOH, or Amberlyst-15® in promoting this oxidation. Although this is the logic that led us to develop these optimum oxidation conditions, the role of methanesulfonic acid is still debatable. It could be generating a more reactive re-oxidant by addition to iodobenzene diacetate. It may also protonate the urea carbonyl disrupting the intramoleular hydrogen bond, thus rendering the primary alcohol more nucleophilic.

Scheme 49. TEMPO oxidation mechanism.

Regardless of these debates, optimum conditions were now found to selectively oxidize the primary alcohol of **239** (Scheme 50). The slightly acidic nature of the reaction allows the oxidation to proceed without attendant epimerization, as proven by nOe experiments. It should be additionally noted that chloroform is a better solvent for this reaction. However, commercially available chloroform is stabilized with 1-2% EtOH. Distillation from a mixture of $CaSO_4$ and $MgSO_4$ can remove the EtOH, however

complete exclusion varied from batch to batch. Consequently the reaction is performed in deuterated chloroform that is manufactured without stabilizers. The reaction can be run extremely concentrated, 0.5-1 M, requiring minimal solvent.

Scheme 50. Optimized oxidation conditions.

3.4 Initial nitroaldol approaches

3.4.1 Nitroethyl pyrimidines *via* Swern elimination

Although we had achieved a synthesis of the pyrimidine aldehyde **59** (Scheme 41), the availability of the dimethoxy bromopyrimidine **195** was limited. Requiring three equivalents of expensive phosphourous oxybromide for its synthesis, we desired a more economic synthesis of **59**. This was achieved from orotic acid (Scheme 51). Polychlorination with phosphorous oxychloride gave the acid chloride in 58% yield. Reduction gave the hydroxymethylpyrimidine **269**.

Scheme 51. Synthesis of the protected nitronol.

Nucleophilic displacement of the chlorides with methanol and Swern oxidation gave **59** in good overall yield and was scaleable up to ~ 5g. As before, nitromethane could be added to the aldehyde in the presence of triethylamine to afford **231**. The alcohol could be protected as its *t*-butyldimethylsilyl ether to give racemic **256** that would be evaluated in the nitroaldol before optically enriched material was synthesized.

We also envisaged the nitronol **231** being fashioned into the saturated nitroalkane **257** (Scheme 52). Although not discussed previously, while attempting to make the pyrimidyl aminoketones (Scheme 41), Swern oxidation of the nitronol **231** gave exclusive elimination to afford the nitrostyrene **271** in 93% yield. The nitroalkane **257** could then be obtained after conjugate reduction with sodium borohydride in ethanol.

Scheme 52. Swern mediated nitronol elimination.

Nitroalkenes are incredibly valuable intermediates in organic chemistry; unfortunately their synthesis often necessitates harsh reaction conditions.[105] Usually strong bases such as sodium methoxide, or strong acylating reagents like trifluoroacetic anhydride are

required. Strong mineral acids (i.e. conc. HCl) are productive for the dehydration of β-aryl-nitroalcohols but not alkyl nitronols. Although some milder conditions have been developed for the direct addition elimination with nitromethane (ammonium acetate, MeCN, 200 °C), the dehydration of nitroalcohols in the presence of sensitive functionalities can be difficult.[106]

The potential mechanism of the reaction is also interesting. Presumably an activated intermediate like **272** is formed in the reaction. This intermediate can then suffer two fates. First the sulfenium ylide **273** could be formed after the addition of triethylamine (*path **a***). This would then intramolecularly deprotonate the more acidic nitromethylene protons to eliminate DMSO. Alternatively triethylamine may intermolecularly deprotonate the nitromethylene group and β-eliminate DMSO (*path **b***). Conducting the reaction with DMSO-d_6, we would expect to see an equivalent of D_3CSOCD_2H produced if pathway ***a*** was operative. When the reaction is run in $CDCl_3$ with DMSO-d_6 no additional DMSO-d_5 is observed above the residual 1% by ^{1}HNMR, thus supporting pathway ***b***.

This Swern-type elimination occurs under mildly basic conditions at -78 °C and may find further application. It is also one of the cleanest reactions that I have ever run! As shown in the summary Table 4 the reaction works well on β-aryl-nitroalcohols. β-Nitrostyrene (**276**) can be produced in 97% yield as a single geometric isomer under the same conditions. Alkyl nitronols are a little lower yielding, for example the nitroalcohol derived from cyclohexanone **277** generates **278** in 81% yield. Unsymmetrical β-aryl-nitroalcohols may yield *E/Z* mixtures. In the case of the nitroalcohol derived from phenyl

acetaldehyde, **279**, elimination proceeds in 71% yield and delivers a 1.5:1 mixture of *E:Z* isomers.

Substrate	*Product*	*Yield*
231	**271**	93%
275	**276**	97%
277	**278**	81%
279	**280**	71% 1.5 : 1 *E*:*Z*

Table 4. Swern elimination of β–nitroalcohols.

Having discovered this high yielding transformation we now had facile synthetic access to both of the nitroethyl pyrimidines needed to couple to the aldehyde **255**. Unfortunately conditions could not be found to effect these transformations (Scheme 53). Using triethylamine as the base failed to effect the coupling of either **270** or **257** with the aldehyde. Tetramethylguanidine also failed to couple the two halves but did force epimerization of the aldehyde. The lithium enolates of either **270** or **257**, generated by the addition of *n*-BuLi does not effect the coupling either. These anions appear to be highly unstable undergoing decomposition. Upon addition of the alkyllithium reagent a bright red-orange colour is established, perhaps indicating the presence of a charge-transfer complex that leads to unproductive reactivity.

Scheme 53. Attempted coupling of the nitroethylpyrimidines.

3.4.2 AB-ring nitroalkane nitroaldols

With the failure of this nitroaldol to occur, only noting epimerization, it was deemed advantageous to remove the acidic protons alpha to the aldehyde. To do this the coupling polarities must be reversed as suggested for the *syn*-nitroaldol strategy (Scheme 54). To this end **255** was reacted with an excess of the lithium salt of nitromethane to provide a 74% yield of the nitronol **283**, inconsequentially as a 1.4:1 mixture of diastereomers. Elimination of the alcohol in **283** proved to be non trivial. Unfortunately the Swern elimination could not be applied to this system because of the free secondary alcohol. Treatment with strong acid i.e. refluxing H_2SO_4 or HCl in MeOH failed to dehydrate the nitronol. TFAA in pyridine gave the nitroalkene **284** but a lot of decomposition accompanied the reaction. The use of 2,6-lutidine as the base gave satisfactory mass recovery, but as an inseparable mixture of nitroalkene and trifluoroacetate esters. Longer reaction time to encourage elimination of the nitronol trifluoroacetate spawned decomposition. Fortunately this crude mixture could be reduced to an easily isolable **258** in ~ 50% over the two steps.

Scheme 54. Synthesis of the AB-ring nitroalkane.

Reagents	Conditions	Results
Et_3N	$CDCl_3$	NR
*n*BuLi	THF, -78° C - rt	NR
KO*t*Bu	THF, rt	decomp
KF, 18-crown-6	MeCN, rt	***258*** and ***286***
NaOMe	MeOH, rt	***258*** and ***286***

Table 5. AB-ring nitroalkane nitroaldols reaction.

Conditions could not be found to effect the addition of **258** to the pyrimidine aldehyde **59** (Table 5). The lithium enolate failed to react as did the presumed triethylammonium enolate. Most interestingly the use of potassium fluoride or sodium methoxide led to the return of **258** but concomitant with aldehyde reduction (**286**). The mechanism of this reduction is uncertain. Comparison to the intermediate in the synthesis of **59** affirms the structure of this product. Although one may suggest a base catalyzed disproportionation event, treatment of **59** independently with sodium methoxide in nitromethane does not reduce the aldehyde. Further the acid, the presumed oxidative couple has not been isolated from the reactions.

To rule out interference of the secondary hydroxyl group in the nitroaldol reaction it was slated for protection. The use of TFAA to dehydrate the nitroalcohol **283** generates a

trifluoroacetate ester of the alcohol that is subsequently cleaved under reducing conditions. Replacing acetic anhydride as the acylating agent gave the acetate ester **287** in 80% yield, essentially protecting the alcohol for "free" (Scheme 55). Treatment of **287** with lithium ethoxide and **59** in DMSO-d_6 gratuitously afforded nitroaldol adducts (**288a-d**). Although formed as a 3:2:1:0.5 mixture of diastereomers, it was a starting point! The diastereomeric ratios can easily be determined by integration of the pyrimidine *ipso*-proton singlets. Although the diastereomers are not completely separable by normal phase chromatography, they can be isolated in pairs on silica gel. The relative stereochemistry of **288a-d** was not determined, products were confirmed by LC-MS in which all four diastereomers are separable and provided equivalent nominal masses.

283 → (Ac_2O, DMAP, CH_2Cl_2 then $NaBH_4$, EtOH, 80%) → 287 → (59, conditions) → 288a-d (diastereomers a:b:c:d)

Scheme 55. Synthesis of the ureaacetate and initial nitoraldol studies

Base	Conditions	Yield (%)	dr [a]
LiOEt	DMF,rt	26	*0.6: 0.9: 1:1*
LiO*i*-Pr	DMF, rt	67	*1:1:1:1*
KO^{t}Bu	THF, rt	93	*0.7:0.8:0.8:1*
KO*t*-Bu/DMSO	THF, -78 °C	63	*0.7:0.8:0.8:1*
KOt-Bu/DMSO	PhMe, -78 °C	69	*0.9:0.9:0.7:1*
LiOEt, $MgBr_2$	THF	NR	-
Et_3N, $MgSO_4$	THF	NR	-

Table 6. Benzylurea nitroaldol reaction. *a)* determined by ^{1}HNMR.

With these initial results in hand, a number of bases were screened (Table 6). As seen the diastereoselectivities remain poor. However, the potassium nitroenolate does give

good chemical yields (up to 93%) of the nitroalcohols **288a-d**. Attempts to generate the magnesium nitroenolate by transmetallation failed to give any coupled product. *As a general note*: it appears that Lewis-acids as coactivators in this nitroaldol shut down the reaction.

Before optimization of the nitroaldol reaction, it was pertinent to ensure the efficacy of the intramolecular reductive guanidinylation. Although amines are not sufficiently nucleophilic to intercept ureas, there are examples of hyroxylamines doing so. It was hoped that the transient hydroxylamine in the reduction could effect the guanidinylation. Unfortunately reduction of **288a-d** failed to give the guanidines **105**, **106**, **289**, or **290** (Scheme 56). Avoiding the complications from the diastereomeric mixture of **288a-d**, the urea **287** was reduced to **291**.

Scheme 56. Attempted intramolecular guanidinylation.

Again guanidinylation did not occur. Forcing conditions on this amine, PhMe, 200°C or refluxing TFA, also failed to promote the intramolecular cylization. Attempts to activate the urea were also fruitless. Treatment of **288a-d** with trimethyloxonium tetrafluroborate and reduction also failed to produce the guanidines. It was further shown that the pyrimidine nitronol **231** suffers competitive methylation of the pyrimidine nitrogens (**293**) when exposed to Meerwein's salt.

These experiments indicated that the benzylurea **287** would not be a productive nitroaldol partner in the synthesis of the cylindrospermopsins. The benzyl group could not be removed without destruction of the nitro group. It thus became necessary to introduce a protecting group on the urea that would be removable in the presence of the nitro group. This group must also be removed to accomplish activation of the urea, so that the intramolecular reductive guanidinylation after the nitroaldol could occur.

3.5 The productive nitroaldol approach

From these observations and/or concerns it was decided to pursue a route utilizing a urea protecting group, orthogonal to the nitro group. The *p*-methoxybenzyl (PMB) group was chosen as it may easily be entered into the synthesis as the parent *p*-methoxybenzyl amine and can be oxidatively removed.

Treatment of the tricyclic isoxazolidine **153** with *p*-methoxybenzylamine cleanly opened the lactone to give **294** (Scheme 57). Reduction of this more electron rich amide was unsuccessful using similar conditions for the benzyl series. The lactol could however be reductively aminated under hydrogenolysis to give the diamine. Treatment of this diamine *in situ* with *bis*-*p*-nitrophenylcarbonate then affords the urea **296**. Employing the

oxidation conditions discovered for the benzylurea, the aldehyde **297** can be synthesized in 75% yield. Addition of the lithium salt of nitromethane proceeds in 84% yield. The acetylation-elimination-reduction sequence then affords the homologated nitroalkane **298**. Initially, oxidative cleavage of the PMB group was difficult. Cerric ammonium nitrate returned the expected *p*-methoxybenzaldehyde in all cases, but the free urea could not be isolated. Eventually it was found that refluxing TFA cleanly removed the PMB group to give an 80% yield of **299**.[107]

Scheme 57. Synthesis of the PMB urea.

Surprisingly **299** does not participate in the nitroaldol reaction under conditions used for the construction of **288a-d**. The reason for this remains unclear, but it should be noted that in both attempts to couple **299** or **258** which contain acidic protons (relative to the resultant alkoxide developed after addition of the nitroenolate to the aldehyde) the nitroaldol reaction does not proceed cleanly. Reduction of **299** to the amine also fails to undergo the reductive guanidinylation reaction. It then became necessary to activate the

urea, as its *O*-Me-isourea to promote this closure (Scheme 58). Attempts to alkylate this urea with Weinreb's conditions, using methyl trifluoromethanesulfonate gave predominantly *N*-methylation of the urea. It was eventually found that Meerwein's salt in dichloromethane smoothly promoted the alkylation to give the isourea **300** in 77% yield. Hydrogenolysis of the nitroalkane pleasingly underwent the intramolecular reductive guanidinylation. If the reaction was halted after 0.5h the *N*-hydroxy guanidine **301** could be isolated. Extended reaction times gave the guanidine **302** in 45% yield as its formate salt after chromatography.

AcO Me N N H O NO2 299 — Me_3OBF_4, K_2CO_3, CH_2Cl_2, 77% → 300 (OMe, NO2) — $Pd(OH)_2$, H_2, MeOH; 0.5 h → [301 (N-OH, $HCO_2^{\ominus}$)] — 12 h, 45% → 302 (NH, $HCO_2^{\ominus}$)

Scheme 58. The first intramolecular reductive gunaidinylation.

Despite our newfound ability to construct the guanidine, **300** did not undergo clean addition to the aldehyde **59**. Simultaneously we discovered that the isourea was not stable. Standing in $CDCl_3$ returned the urea **299** even within a couple of hours. The mode of decomposition was initially assumed to be hydrolysis by nucleophilic attack at C17 (Scheme 59, pathway ***a***).[108] Attempts to generate the *S*-Ph thiourea by displacement with thiopheol led exclusively to the urea **299**, illustrating that it was actually displacement of the methyl group (pathway ***b***) that led to this formal hydrolysis. The most convenient way to circumvent this problem was to increase the steric bulk around the carbon-oxygen

bond, most easily accomplished with triethyloxonium tetrafluoroborate. Treatment of **299** with triethyloxonium tetrafluoroborate gave the isourea **303** in 78% yield after optimization, most essential of which was the use of cesium carbonate as the base. This isourea appeared to be stable enough for subsequent synthetic manipulations. It is stable to acid as reduction of **303** in the presence of acetic acid affords the tricyclic guanidine **302** in an improved 96% yield.

Scheme 59. Urea hydrolysis.

With the isourea constructed and directly poised for the reductive guanidinylation event, after addition to the pyrimidine aldehyde, studies of the nitroaldol were conducted (Scheme 60). In this series it was difficult to discern the diastereoselectivity of the nitroaldol after the addition as the pyrimidine singlets overlapped. It was instead decided to directly carry out reductive guanidinylation and determine the diastereo-selectivity from the methine doublets under the secondary alcohol (Table 7). The spectral characteristics of the two diastereomers **105** and **106** are known from Snider's synthesis (Figure 12). The assignment of **290** was made from the larger 7 Hz coupling constant similar to **23**.

Scheme 60. Nitroaldol-reductive guanidinylation sequence.

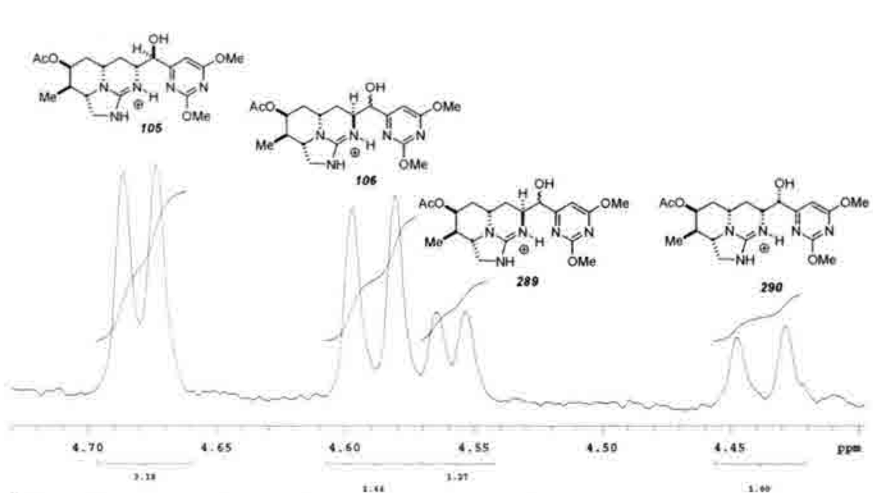


Figure 12. ¹HNMR comparison of the diastereomeric methine doublets.

Entry	Reagents	Conditions	dr(*105:106:289:290*)[a]
1	TBAF[b], $MgBr_2 \cdot OEt_2$	THF, -15 °C	NR
2	TBAF, $Sc(OTf)_3$	THF, -15 °C	NR
3	Alumina (neutral)	CH_2Cl_2, rt, 24h	9.2 : 7.2 : 9.9 : 10
4	Alumina (neutral)	THF, rt, 24h	10 : 4.9 : 5.4 : 9.0
5	TBAF, TESOTf, Et_3N	THF, -78 → -15 °C	8.0 : 10 : 5.4 : 5.6
6	0.5eq TBAF	THF, -40 °C	10 : 8.4 : 3.9 : 3.1
7	0.5eq TBAF	THF, -15 °C	10 : 5.2 : 6.6 : 9.2
8	0.5eq TBAF	THF, rt	10 : 5.1 : 7.0 : 9.8
9	2.0 eq TBAF	THF, -15 °C	0 : 0 : 8 : 10
10	1.5 eq TBAF	THF, -15 °C	0 : 0 : 8.4 : 10
11	1.0 eq TBAF	THF, -15 °C	5 : 2.4 : 7.1 : 10
12	$Ti(OiPr)_4$	CH_2Cl_2	NR

Table 7. Nitroaldol-reductive guanidinylation selectivities. *a*) see Figure 12. *b*) 1M THF

It was found imperative that the nitro-Aldol reaction be quenched with AcOH and reduced. As seen with 2 eq. TBAF (entry 9) reduction gives a 1:0.8 mixture favoring the diastereomer required for the synthesis of 7-epi-cylindrospermopsin. If the nitroaldol products are purified without an acid quench, a ~1:1:1:1 mixture of diastereomers is formed, indicating that the reaction is indeed highly reversible. Thus, all reactions were quenched with 20% AcOH in THF and immediately subjected to reductive guanidinylation. This reversibility upon isolation is undoubtedly responsible for the equimolar mixtures obtained in Table 6.

Again the addition of homogenous Lewis-acids as co-activators impedes the reaction (entries 1,2,12). Surprisingly neutral alumina in dichloromethane affords equimolar amounts of the four diastereomers, where the reaction in THF favors the two diastereomers corresponding to **19** and **23**.

Undoubtedly fluoride ion, acting as the base, has been the most productive reagent for these reactions. Attempts to thwart the reversibility of the nitroaldol by trapping the nitroalcohol as its silyl ether (entry 5) proved futile, leading to a diastereomeric mixture only slightly favoring **105** and **106**.[109,110] The most interesting takehome message from these experiments is the stereochemical dependence on the amount of TBAF in the reaction. Using sub-stoichiometric amouts of TBAF (entries 6-8) at several temperatures favors the construction of **105**. One equivalent of TBAF (entry 11) begins to afford mixtures favoring the production of **289** and **290**. The use of 1.5-2 equivalents then exclusively produces these two diastereomers. These results are contrary to previous findings that 0.5 equivalents provides optimum selectivity.[111] Since the relative stereochemistry of **106** and **289**, in regard to the C7-hydroxyl group remains unknown,

precise stereochemical arguments for the outcomes of these reactions cannot be presented.

Controlling the stereochemistry of this nitroaldol is not a trivial problem. One has to control the enantioface of the aldehyde to which addition occurs. This problem has been solved in the enantioselective addition of nitromethane to aldehydes using the Lewis-acid catalysts like **304** and **305** (Figure 13).[93,94] The real problem lies in substitution of the nitroalkane. Now one is faced with epimerization of the acidic nitro center (pK_a ~8-10), and further the addition products have slightly more acidic α-protons than the parent nitroalkane. The quaternary ammonium cinchonidine catalyst **306** developed by Corey has been able to effect the diastereo-controlled addition of nitromethane to chiral aldehydes, with dr's up to 17:1. The diastereomeric control issue, without substrate influence, has only been successfully addressed by Moruoka who has utilized the elaborate designer quaternary ammonium bifluoride catalyst **307** in conjunction with silylnitronates. Using this system, dr's up to 9:1 have been realized.

Figure 13. Catalysts for the asymmetric nitroaldol reaction.

Attempts to utilize these catalysts have yet to produce favorable results. The lanthanum *tris*-BINOL catalyst **304** produces ~1:1:1:1 mixtures when used in the addition of **287** to **59**, but remains the only Lewis-acid based catalyst to generate nitroaldol products. The copper acetate *bis*-oxazoline **305** in the addition of **302** to **59** caused decomposition. Treatment of **302** with **306**, in combination with potassium fluoride to generate the chrial ammonium fluoride *in situ* also produces ~1:1:1:1 mixtures in less than 10% conversion. Noting that the fluorides are the most productive catalysts in this transformation, current efforts are focused on the direct isolation of **306** as its fluoride salt and its subsequent application.

Another issue complicating this reaction is the extreme electrophilicity of the pyrimidine aldehyde **59**. As seen in Figure 14, **59** immediately undergoes hemiacetal formation in methanol.

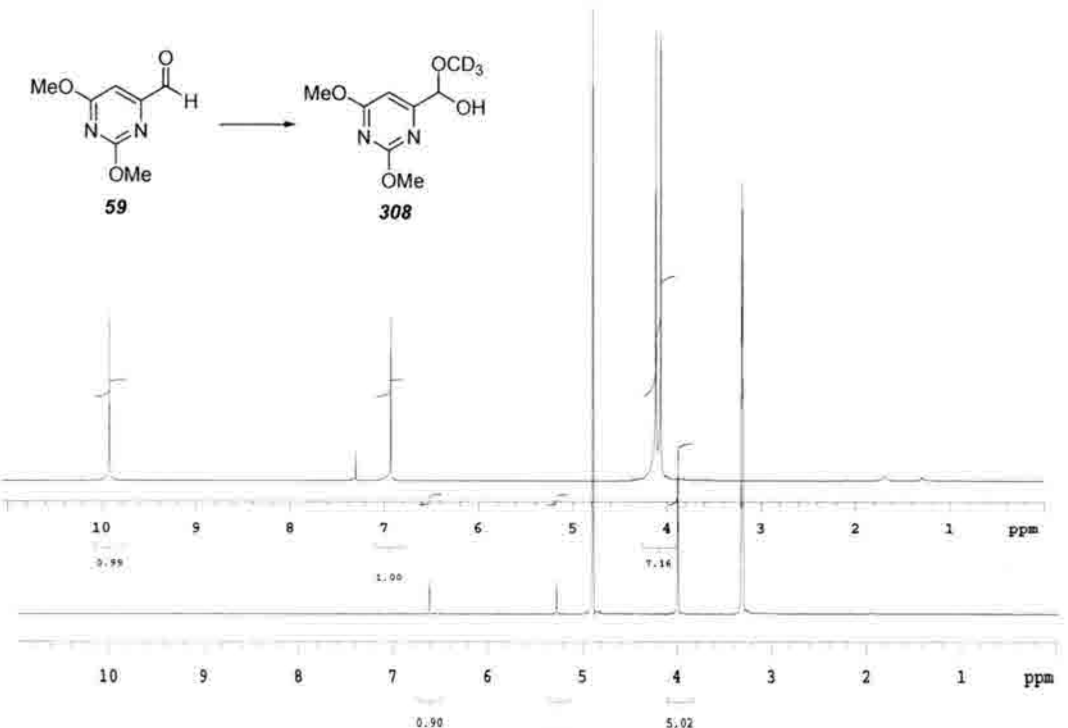


Figure 14. The immediate conversion of **59** to its hemiacetal.

As noted in Table 7, most nitroaldols performed under basic conditions are complete within 15 minutes. This reactivity is probably responsible for the inadequacy of Lewis-acids in the reaction. Interestingly however, the aldehyde is stable in ethanol for up to 12 h. Further this reactivity suggested a possible strategy to inhibit the reversibility of the nitroaldol reaction (Scheme 61).[112] That is treatment of the acetals **309a/b** with a Lewis-acid should generate the oxocarbenium ions **310a/b**. After addion of the nitro-enolate **311a/b** would be produced. Lacking a hydroxyl proton, these protected substrated would not be subject to the retro-nitroaldol reaction. Unfortunately attempts to generate the acetals have been unsuccessful. The hemiacetals are easily intercepted; however, forcing conditions to effect the final substitution have led to decomposition, or partial pyrimidine substitution in the case of **309b**.

MeO, N, N, OMe, O, H
59
MeO, N, N, OMe, OR, OR
309a R = Me
309b R = Bn
LA
MeO, N, N, OMe, ⊕O-R, H
310a/b
MeO, N, N, OMe, O-R, NO_2
311a/b

Scheme 61. Oxocarbenium hypothesis.[112]

Noting that in the reactions with TBAF, a *tetra*-butylammonium counterion is coupled with the nitroenolate, it was hoped that the size of the aldehyde might influence the diastereoselectiviy. Although distant from the reacting aldehyde, the dibenzyloxypyrimidine aldehyde was synthesized (Scheme 62). Treatment of **194** with

the lithium salt of benzylalcohol gave the dibenzyloxy bromopyrimidine **312** in 80% yield. This pyrimidine was then productively added to DMF after lithiation, giving the aldehyde **313** in acceptable yields. Treatment of **302** with **313** and two equivalents of TBAF effected the nitroaldol reaction. Hydrogenolysis cleanly induced the reductive guanidinylation and cleaved the benzylpyrimidine, to directly release the uracil. However **314** and **315** were still produced as a ~1:1 mixture.

Scheme 62. Benzyloxypyrimidine nitroaldol.

Hanessian has shown that the addition of substituted nitroalkanes to aldehydes bearing a stereogenic center produce predominantly the *anti-anti* stereotriad.[113] It was then anticipated that an optically pure dihydrouracil aldehyde like **317** should give rise to the guanidine **316** carrying the correct relative stereochemistry for **19** (Scheme 63). Oxidation could then install the unsaturation. To this end aspartic acid **318** was transformed into its mono-methylester **319**. Condensation with benzyloxycarbonyl isocyanate gave the urea **322** in ~40% yield.[114] However this substrate failed to cyclize to give the dihydrouracil **323** under basic or acidic conditions, falling prey only to ester or imide hydrolysis. Concerned that the free acid was a problem, it was reduced to give the

alcohol **324**, which also failed to cyclize. The *p*-methoxybenzyl ureas corresponding to **322-324** were synthesized from its isocyanate, but also refused to cyclize.

Scheme 63. Attempted synthesis of the dihydouracil.

Another manifold to selectively generate the diastereomer for **19** was envisaged from the nitroketone **329** (Scheme 64). Assuming that the NO bond of the nitro group is reduced first, the reductive guanidinylation should occur. From Snider's synthesis, the resultant ketone should be stereoselectively reduced to give the *anti*-diastereomer required for **19**. Acylimidazoles have been shown to be excellent coupling partners for the synthesis of nitroketones.[115] Treatment of the acid chloride **326** with two equivalents generates the acylimidazole **327**, isolated in low yield. This substrate, however, failed to react with either the tetrabutylammonium or potassium enolates of **302**. The *S*-Ph

thioester **328** could also be fashioned from the aldehyde **59** but again could not be coupled to **302**.

Scheme 64. Attempted synthesis of the nitroketone.

Scheme 64. Attempted synthesis of the nitroketone.

3.6 Total synthesis of 7-epi-Cylindrospermopsin

Although this nitroaldol reaction could not yet be controlled to produce the C7-C8 diastereomers individually, they can all be obtained and we pushed on toward the total synthesis. As noted previously treatment of the isourea **302** with two equivalents of TBAF and reductive guanidinylation affords a 1:0.8 mixture of **290** : **289** (Scheme 65). At this point the pyrimidines are inseparable. Using identical conditions reported by Snider, the pyrimidines were refluxed in HCl for 12 hours. After purification by HPLC a 32% yield of the uracil **330** was obtained, accompanied by 29% of the C8-diasteromer **331**. The uracil **330** spectroscopically matched that from Weinreb's synthesis, confirming its identity as the congener to **23**. The uracil **331** is indeed different than the C8-diastereomer (**107**) produce by Snider. Interesting to note is that these guanidines are isolated as their trifluoroacetate salts after HPLC with 0.1% TFA in the eluent. We and Weinreb's group were unaware of this until our synthesis of 7-deoxycylindrospermopsin (*vide infra*).

Conditions used for sulfonation of the C12 hydroxyl group reported by the other groups were capricious.[116] The use of sulfurtrioxide-pyridine complex in DMF with 3Å molecular sieves reproducibly gave **23** in 59% yield.[117] This was accompanied by the bis-sulfate **332**, as a ~2:1 mixture but easily separated by HPLC. Synthetic **23** had spectroscopic properties identical to those reported. The optical rotation also agreed well: $[\alpha]^{25}{}_{D} = -12.5°$ (c 0.04, H_2O); lit $[\alpha]_D{}^{24} = -20.5°$ (c 0.04, H_2O).[39]

As shown in Scheme 62 the dibenzyloxy pyrimidine **313** can also be used to directly release the uracil. Unfortunately conditions have not been found to directly cleave the acetate during the reductive guanidinylation. These uracil acetates do however circumvent the need for the lengthy acid reflux required for pyrimidine hydrolysis. Exposure of **314/315** to refluxing HCl for only 30 min. effects the cleavage giving **330** and **331** in similar yields after HPLC purification.

1. **59**, 2.0 eq TBAF THF, -15 °C, 15 min. 2. H_2 / $Pd(OH)_2$ MeOH, 5% HOAc

302 → **290** + **289**

conc. HCl reflux, 12 h

1. **313**, TBAF 2. H_2 / $Pd(OH)_2$ MeOH, 5% HOAc 3. conc. HCl reflux, 0.5 h ~1:1

330, 32% $F_3CCO_2^{\ominus}$ + **331**, 29% $F_3CCO_2^{\ominus}$

10 eq. SO_3·pyr DMF, 3Å MS

23, *7-epi-cylindrospermopsin* (59%) + **332** ~2:1

Scheme 65. The total synthesis of 7-epi-cylindrospermopsin.

3.7 Total synthesis of cylindrospermopsin.

The total synthesis of cylindrospermopsin is straightforward from a mixture of the four diastereomers (Scheme 66). Treatment of the isourea **302** with the bis-benzyloxypyrimidine aldehyde **313** and 1.0 eq. TBAF and subsequent reductive guanidinylation affords a 0.9 : 0.9 : 1 : 0.5 mixture of **105** : **106** : **289** : **290**. Fortunately; however, **333** is obtained in 20% yield after acetate hydrolysis and HPLC purification. Sulfonation, again with sulfur trioxide-pyridine complex, gives cylindrospermopsin (**19**) in 60% yield.

1. 1.0 eq.TBAF, *313* THF, -15 °C, 30 min.
2. H_2 / $Pd(OH)_2$ MeOH, 5% HOAc
3. conc. HCl reflux
(0.9 : 0.9 : 1 : 0.5 dr)

333 20% $F_3CCO_2^{\ominus}$

SO_3 pyr
DMF, 3Å MS
60% (6 : 1 *bis-sulfate)*

19 cylindrospermopsin

Scheme 66. Total synthesis of cylindrospermopsin.

Interestingly synthetic cylindrospermopsin carrying the 7*R*, 8*R*, 10*S*, 12*S*, 13*R*, 14*S* configuration exhibits an $[\alpha]^{25}_{D} = +8.0^{\circ}$ (c 0.05, H_2O). The natural material first isolated from *C. raciborskii* displays an opposite rotation; $[\alpha]^{25}_{D} = -30.1^{\circ}$ (c 0.1, H_2O).[32] From *A. ovalisporum* however, the optical rotation is consistent with synthetic **19;** $[\alpha]^{25}_{D} = +12.5^{\circ}$ (c 0.6, H_2O).[39] It would seem unlikely that the two metabolites would carry opposite absolute configurations as the polyketide synthetases involved in their biogenesis are highly conserved.[42] To reconcile these differences in optical rotation,

Circular Dichroism (CD) spectra were obtained on an Aviv model 202 spectrometer in water of natural **19** obtained from *C. raciborskii.* and compared to that of synthetic **19** at ~ 44 μg / mL (Figure 15). Natural cylindrospermopsin displayed a Cotton effect at 264 nm ($\Delta\varepsilon = -6.949$) and 228 nm ($\Delta\varepsilon = -4.243$). Synthetic cylindrospermopsin showed identical Cotton effects at 264 nm ($\Delta\varepsilon = -8.797$) and 229 nm ($\Delta\varepsilon = -4.432$).

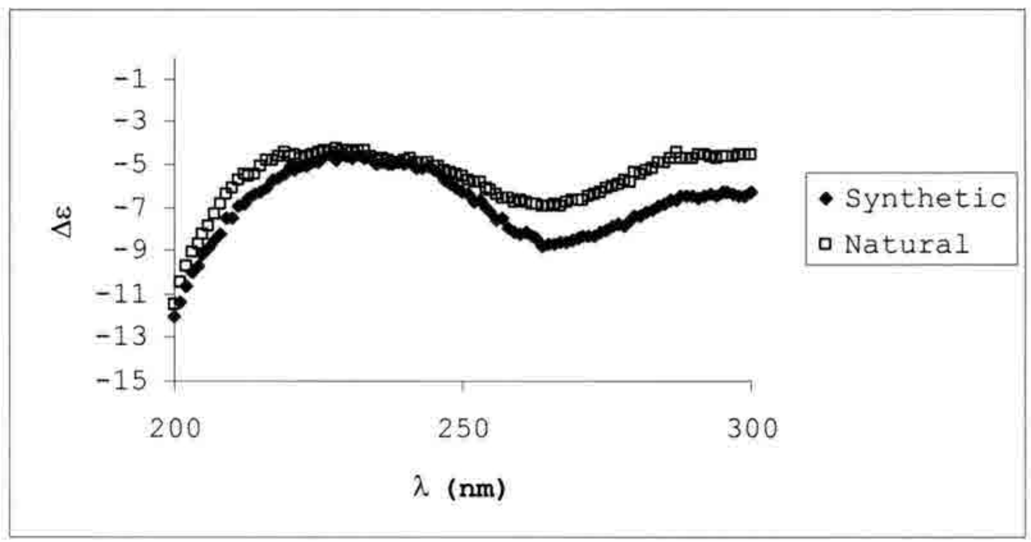


Figure 15. CD spectra of natural and synthetic cylindrospermopsin

Although it is unclear what has caused the opposite optical rotation for **19** isolated from *C. raciborskii* it is now clear that cylindrospermopsin does indeed carry the 7*R*, 8*R*, 10*S*, 12*S*, 13*R*, 14*S* configuration from both organisms (*C. raciborskii* and *A. ovalisporum*).

3.8 Synthesis of 7-deoxycylindrospermopsin.

A nice reaction sequence to couple the nitroalkane and the aldehyde, directly generating the nitroalkene has recently been developed (Scheme 67). Treatment of the isourea with the aldehyde **313** with acetic anhydride in the presence of cesium fluoride affords the nitroalkene **334** in 65% yield. Although fluoride promoted coupling and subsequent acetic anhydride mediated eliminations of nitroalcohols are known, they generally require two distinct steps and require a molar excess of the nitroalkane partner.[118] This sequence generates **334** in a single operation with only one equivalent of both the aldehyde and the nitroalkane, making this protocol amenable to complex molecule synthesis. The nitoalkene is thought to carry the *E* geometry around the tri-substituted double bond. NOE studies show that irradiation of the vinyl proton only enhances the pyrimidine proton. Following the precedent set in Hart's model system it was hoped that reduction of the nitro group would intercept the ene-guanidine **335**. Stereoselective reduction of the alkene should then ensue to afford **336**. Subjection of **334** to the reductive guanidinylation conditions returns a complex mixture, containing products arising from hydrolysis of the intermediate enamine prior to ring closure.

Scheme 67. One-pot condensation/elimination sequence.

To circumvent this hydrolysis and obtain **336** for comparison during this one-step reduction, it was decided to undertake the synthesis in a step-wise manner (Scheme 68). To this end **334** was subjected to conjugate reduction and then the reductive guanidinylation procedure giving a 1:1 mixture of the two uracils **337** and **338**. Again the acetate could be cleaved by brief heating in HCl to give **336** and **337**. At this juncture the C8 stereochemistry was unknown and the mixture was sulfonated to give a 33% yield of each diastereomer **340** and **341** after HPLC purification. Experiments to achieve the one-step, stereoselective reduction are ongoing.

Scheme 68. Synthesis of 7-deoxycylindrospermopsin.

The relative stereochemistry of **336** and **339** was eventually delineated by X-ray crystallography after the diols were separated prior to sulfonation (Figure 16). Interestingly the ^{1}HNMR spectrum of **340** does not agree with spectral comments for

natural **21**. Most pertinently the uracil proton in **340** is definitely evident, contrary to the isolation paper and supported our suspicion of the equilibrium invoked by the authors.

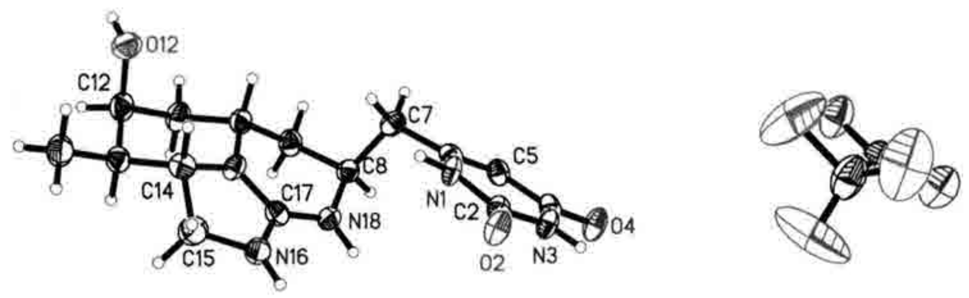
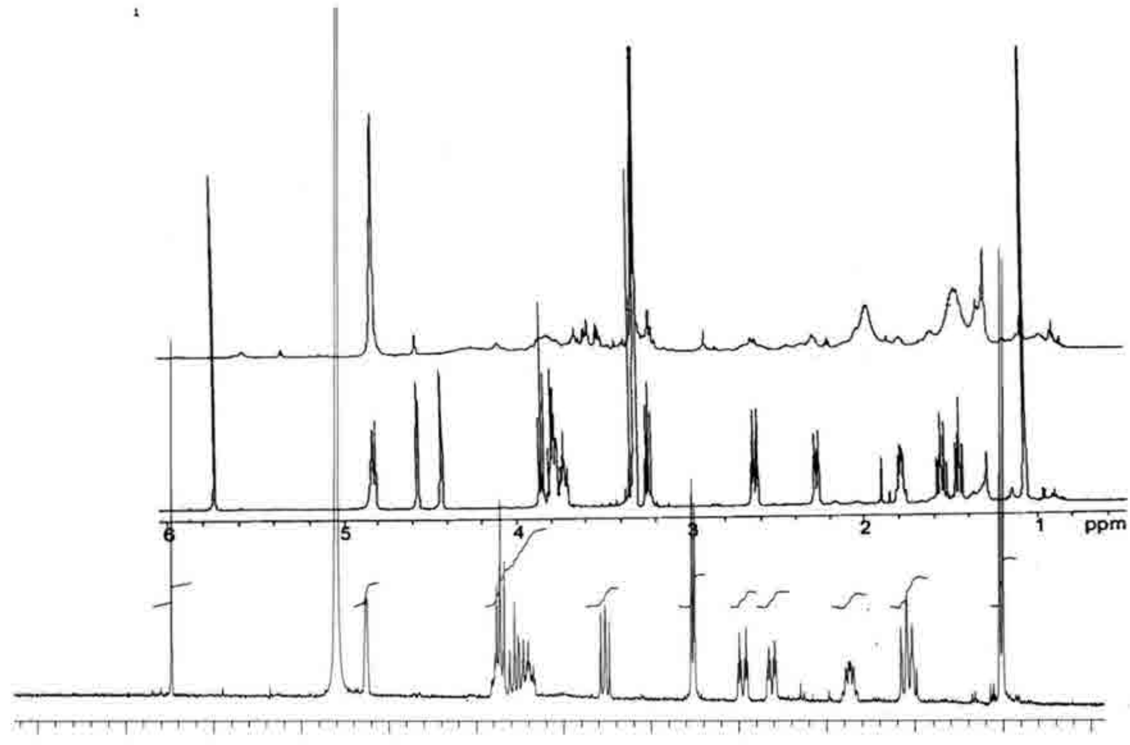


Figure 16. TEP diagram of **339**.



Fiure 17. ^{1}HNMR Comparison ***a*** (top)) **21**, (CD_3OD, 500MHz); ***b***) **19**, (CD_3OD, 500MHz); ***c*** (bottom)) **340** (D_2O, 400 MHz).

After communication was established with Dr. Glen Shaw at Queensland's National Research Center for Environmental Toxicology, we were able to obtain the actual ^{1}HNMR spectra on which the assignment of **21**'s structure was made (compared to **19**). As shown in Figure 17, trace **a**, it is unclear how they were able to publish a structure based on this spectroscopy. We didn't realize that their statement: "Due to the broadening of peaks compared to cylindrospermopsin, we conclude that this compound is 7-deoxycylindrospermopsin and it exists as the tautomeric forms shown in [**21**] and [**22**]," actually meant that they had a mixture of compounds! Perhaps more disturbing is the individual ^{1}HNMR spectrum provided by the authors (Figure 18). This is clearly a mixture of compounds. The toxicology data presented for synthetic **340** (see Chapter 4) also contradicts the structural assignment of **21**.[119] It is interesting to note that **340** or **336** might be biosynthetic intermediates in the genesis of **19** and **23**. We are currently providing Dr. Shaw's laboratory with a sample of **340**, for co-HPLC analysis to probe the natural occurrence of **340** and its relation to **21**.

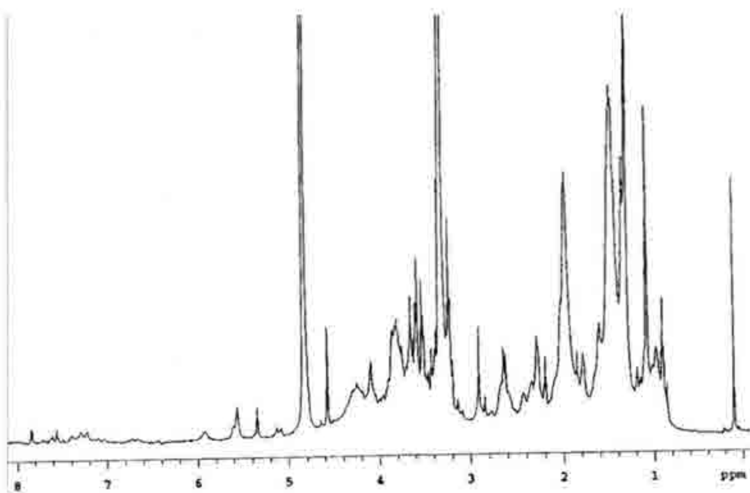


Figure 18. 7-deoxycylindrospermopsin in CD_3OD.

3.9 Synthetic summary and conclusions

Presented below is a summary of the synthetic efforts and achievements described in this dissertation (Scheme 69). The synthesis presented herein represents the shortest successful approach to this family of natural products. Cylindrospermopsin and 7-epicylindrospermopsin can be obtained in only eighteen synthetic manipulations from the commercially available oxazinone template **164**. The putative structure of 7-deoxycylindrospermopsin has been synthesized in nineteen steps. These densely functionalized and highly polar natural products have been synthesized without the introduction of a *single* protecting group, solely for that purpose!

The synthesis is highlighted by an intramolecular dipolar cycloaddition that serves to construct the three contiguous stereocenters of the A-ring in up to 12:1 selectivity. The selective acid mediated oxidation of the urea diol efficiently delivers the sensitive α-ureido aldehyde. Homologation to the nitroalkane provides a versatile intermediate that is used to construct all three of these natural products. A kinetically controlled nitroaldol reaction affords both 7-epicylindrospermopsin and its C8-diastereomer to be deployed for biomechanistic evaluation. Under thermodynamic control, this nitroaldol reaction serves to produce cylindrospermopsin and its C8-diastereomer. A one pot nitromethylenation reaction was developed for the union of the nitroalkane and the pyrimidine aldehyde, leading to the synthesis of 7-deoxycylindrospermosin.

Scheme 69. Synthesis of the cylindrospermopsin alkaloids. a) KHMDS, *E*-crotyl iodide, THF, -78°C (92%, 99%ee); b) Li, NH_3, THF, EtOH (68-87%); c) AcCl, MeOH, 0°C-rt; d) $LiAlH_4$, THF (65% *2* steps); e) $BrCH_2CO_2Ph$, iPr_2NEt, MeCN (63-80%); f) *m*CPBA, Na_2HPO_4, CH_2Cl_2, -78°C (84%); g) PhMe, 200°C *sealed tube*, (78%). h) DIBAL-H, CH_2Cl_2, -78°C (87%); i) $pMBNH_2$, H_2/Pd/C, EtOAc then $(p\text{-}O_2NPhO)_2CO$, MeCN, (81%); j) TEMPO, $PhI(OAc)_2$, 1 mol% MsOH, $CDCl_3$, (75%); k) $MeNO_2$, *n*-BuLi, THF, rt (84%); l) Ac_2O, DMAP, CH_2Cl_2 then $NaBH_4$, EtOH (67%); m) TFA (neat), reflux (80%); n) Et_3OBF_4, Cs_2CO_3, CH_2Cl_2 (78%). o) **59**, 2.0 eq. TBAF, -15 °C, THF; p) $Pd(OH)_2$, H_2, 5% AcOH/MeOH (**337** (82% comb, *2 steps*); q) conc. HCl, reflux 12 h (**330** 32 % *3 steps*); r) SO_3·pyr, DMF, 3Å MS (**23** (59 %); **19** (60 %); **340** (33% *2 steps*). s) **313,** 1.0 eq. TBAF (20 %). t) conc. HCl, reflux 0.5 h (**333**, 20 % *3 steps*) u) **313**, CsF, Ac_2O, MeCN (65%); v) $NaBH_4$, EtOH, rt; v)

Chapter 4. Biological Implications and Contributions

4.1 Inhibition of Protein Synthesis by Synthetic Analogues

To assess our biomechanistic hypothesis we began collaborating with Maria Runnegar's laboratory at the University of Southern California's Keck School of Medicine. The toxicity of our synthetic intermediates was measured by their ability to inhibit protein synthesis both *in vitro* and *in vivo*. The results presented below are preliminary; more thorough evaluations considering statistical variation, to obtain accurate IC_{50} values, await completion.

As *in vivo* evaluations require rat sacrifice and hepatocyte culture, all compounds were first screened for protein synthesis inhibition *in vitro*. This was done using a rabbit reticulocyte lysate system, measuring the incorporation of [^{35}S]-methionine into luciferase protein (see sec. 5.3). Following the conventions of the biological community, cylindrospermopsin will be abbreviated as CY. It should be noted that the identity of the guanidine salt is inconsequential. Control experiments with formic acid show no effect on protein synthesis. Synthetic 7-epi-CY from Weinreb's group (as its TFA salt) and natural 7-epi-CY have identical inhibition profiles, showing that TFA does not inhibit protein synthesis.[70]

These initial *in vitro* results are presented as relative potencies (RP) (Figure 19). They are expressed relative (RP = 1) to synthetic racemic CY diol **rac-333** and 7-epi-CY diol **rac-330** both of which displayed approximate IC_{50}'s ~200 nM, for protein synthesis inhibition, agreeing well with previous results.[70] The ABC-ring systems **rac-301**, **rac-302**, and **rac-342** (prepared by HCl hydrolysis of **302**, see sec 5.2) reinforce the observation that the uracil is crucial for toxicity. The acetate group has an effect, when

comparing **rac-301** and **rac-342**, an interesting observation since the sulfate on CY has no effect. Although **rac-301** is essentially non-toxic the N-hydroxyguanidine **rac-301** is toxic in a dose dependent manner (results not shown). At present unclear, guanidine hydroxylation may have further biological implications. Interestingly the *bis*-sulfate **332** has an RP =15. This may indicate that the sulfates are efficiently hydrolyzed or that steric encumbrances at C7 are tolerable.

Figure 19. Relative potencies for *in vitro* protein synthesis inhibition.

The C8 diastereomers **331** and **rac-107** are toxic in a dose dependent manner, but remain ~two orders of magnitude less potent that the C7 diastereomers. This contradicts the possibility of an identical metabolic intermediate resulting from stereochemical

scrambling at C8. The most exciting result of these initial experiments is that our synthetic 7-deoxy-CY (**340**) is almost equipotent with CY. This further confirms the incorrectly assigned structure of **21**. As noted in Chapter 1, when tested in a mouse bioassay at concentrations equal to CY, **21** showed no toxicity. Again the C8-diastereomer **341** was ~100 fold less potent.

To ensure that these results were competent, the inhibition of protein synthesis was measured *in vivo*. Cultured rat hepatocytes were incubated with the test compounds in the presence of [^{35}S]-methionine, and the radioactivity of the protein precipitate compared to control. As seen in Figure 20, **340** remains a potent inhibitor of protein synthesis. When treated with 10 μM **340**, protein synthesis is completely inhibited (8% of control) and is comparable to cells treated with 1.5 μM natural CY (**19**) and 3.3 μM synthetic **23**.

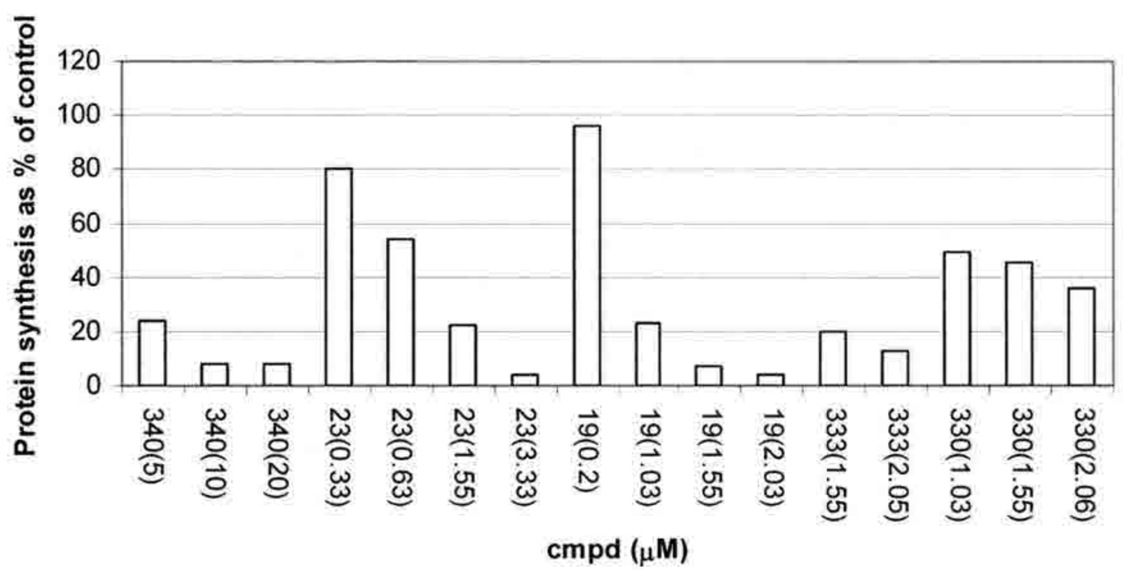


Figure 20. Inhibition of protein synthesis by the C7-diastereomers.

Again the C8 diastereomers proved to be less potent (Figure 21). While **331** inhibits protein synthesis, concentrations 100 fold greater than natural CY are required. 8-epi-deoxy-CY also inhibits protein synthesis *in vivo* although in these experiments it is ~2 fold less toxic that **331**, in contrast to ~equipotent in the *in vitro* assay.

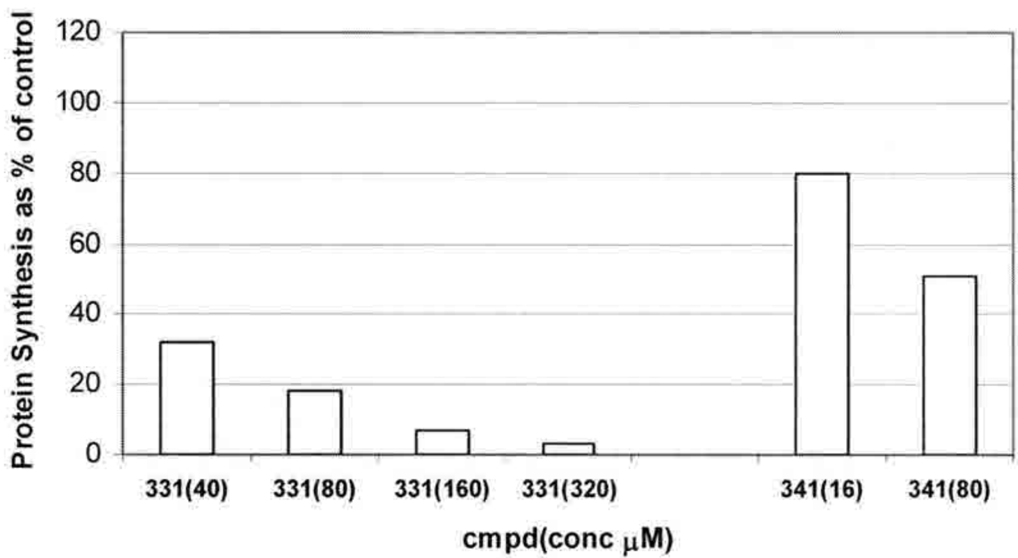


Figure 21. Inhibition of protein synthesis by the C8 diastereomers.

The effect of these synthetic intermediates on the depletion of cellular glutathione (GSH) was also measured (Figure 22, see sec. 5.3). Indeed 7-deoxy-CY shows inhibits the synthesis of glutathione, 40% at 10 μM compared to control. The C8-diastereomer **331** is again approximately 100 fold less active than natural CY. Synthetic 7-epi-CY (**23**) also displays typical inhibition relative to natural CY.

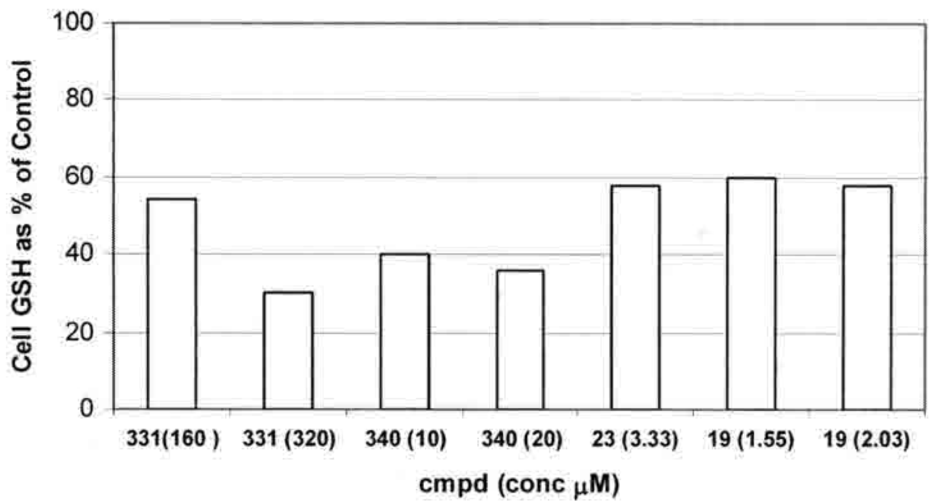


Figure 22. Inhibition glutathione biosynthesis *in vivo*.

The results described above should be interpreted cautiously as concentrations are approximate (see sec. 5.3). However, there are several outstanding "take-home" messages. The first is that C7-oxygenation is not required for toxicity, contrary to the community's beliefs from the mythical structure of **21**! Secondly the C8-stereocehemistry is important for toxicity and a common metabolic intermediate is not intercepted. This contradicts our hypothesis that argues C8 or C7 oxidation as a plausible metabolic outcome. Thirdly blocking the C12 hydroxyl (as its acetate) group does have a measurable effect on the toxicity of the weakly inhibiting ABC-ring systems. This will be further probed by submission of the acetylated uracils **314** and **315** for testing. Lastly, N18 oxidation may still be a plausible oxidation event.

I feel that these considerations narrow a biomechanistic hypothesis for this interesting class of natural products. First it may now be assumed that the northern fringe is "spinach" not contributing to any direct molecular recognition interactions. Considering the planarity of the ABC-ring system as noted in the *X*-ray structure of **341**, the southern

fringe is the "leading edge" of these compounds. Following this it may be suggested that these compounds interchelate rRNA or tRNA aided by potential hydrogen bond pairing to adenine. Their molecular association is potentially stabilized by hydrogen bonding to the secondary alcohol or guanidine. This event may be reversible or irreversible, depending on the intervention of an incipient electrophillic intermediate generated by *cyt*P450 oxidation. Interruption of translation or transloaction in polypeptide elongation then leads to the dramatic inhibition of protein synthesis. The decrease in cellular GSH, preceeding cell death, is then a result of the decreased translation of γ-glutamylcysteine transferase or glutathione transferase needed for the biosynthesis of GSH.

Figure 23. Narrowed biomechanistic hypothesis for the cylindrospermopsins.

Although this hypothesis generates more questions than answers, I think it proffers a new vision to devise experiments and advance our understanding of CY's toxicity.

4.2 Antimicrobial activity

Noting the profound effect of these compounds on eukaryotic protein synthesis, it is desired to evaluate them for their potential ability to inhibit bacterial protein synthesis or glutathione biosynthesis. Promoting a biomechanistic hypothesis reminiscent of the viomycins, tuberactinomycins, and capreomycins it was of interest to probe the cylindrospermopsins for MDR-tuberculosis activity in collaboration with Prof. M. McNeil's laboratory in CSU's Department of Microbiology. Unfortunately cylindrospermopsin diol (**333**) does not show any activity against *M. tuberculosis* H37Ra even at concentrations up to 200 μM.

4.3 Enzyme conjugable cylindrospermopsins

Despite the large amount of research accomplished to efficiently detect these toxins in water supplies, methods have evolved primarily around the use of physical chemical methods; LC, LC-MS post extraction and purification. Concerned that these toxins do not only affect water supplies monitored by such sophisticated equipment, a cheap and reliable field method to detect these toxins is desired. Enzyme linked immunosorbent assays (ELISA's) have fulfilled this requirement for the microcystins and saxitoxins.[120] Conjugation of these toxins to enzymes, usually to Keyhole Limpet hemocyanin, elicits antibodies that can detect these toxins. The saxitoxins generate an incredibly specific response, detectible at pg mL^{-1} concentrations. It is thus highly desirable to develop such an immunoassay for the cylindrospermopsins. The ultimate goal of which is the production of "litmus" paper for their detection. Further, antibodies can also be adapted for immunoaffinity chromatography for the rapid isolation of the natural product.

With the total chemical synthesis in hand we have both the ability and responsibility to apply our new-found knowledge to address this ecologically significant problem. We have begun collaborating with Dr. Titan Fan at Beacon Analytical Biosciences to develop such an assay. The problem with natural CY is its resistance to functionalization. Again, to elicit an immunological response, these compounds must be rendered conjugable to a host enzyme. The microcystins and saxitoxins both contain chemical handles to directly conjugate these toxins; CY does not. The C7-alcohol is not very reactive, as noted synthetically having reacted only partially with sulfur trioxide. The C12 alcohol is sulfated and thus unreactive.

Scheme 70. Attempted C12-hydroxyl functionalization.

Synthetically we have access to the C7, C12 diol suggesting the ability to functionalize the C12 alcohol (Scheme 70). A high yielding synthesis of **344** was developed, unfortunately attempted coupling of this acid to the 7-epi-CY diol **330** failed. Functionalization of these advanced intermediates remains problematic as they are soluble only in water, methanol, and DMF. Fortunately the conciseness of our total

synthesis might allow the introduction of a linkable function early in the synthesis. It is hoped that this succinate linker may be introduced at the nitronol stage (Scheme 71).

Scheme 71. Early linker installation.

We are currently excited at the prospect of using the new nitroalkene derivative **334** for functionalization (Scheme 72). Acting as a Michael acceptor it is hoped that hydroxy-acids can be added producing **348** in essentially a one-pot process. This intermediate may then enter into enzyme conjugation giving **349** and be evaluated for its immunological response.

Scheme 72. New linker strategy.

Perhaps more exiting is the prospect of adding in an ethanolamine derivative. Again in a one pot strategy the amine **350** might be synthesized. This intermediate may provide an orthogonal approach to enzyme conjugation for immunoassay/immunoaffinity chromatography development. More exciting is the prospect of coupling **350** to biotin, generating **351**. In doing so, we may gain insight into CY's specific molecular target through streptavidin affinity chromatography. It should be stressed that these proposed one-pot reactions generate biologically useful compounds in a "handleable" fashion as the precursors are soluble in organic solvents, and only a single HPLC purification would be required.

4.4 Conclusions

Concise asymmetric total syntheses of the cylindrospermopsin alkaloids have been achieved. Focusing not only on the synthetic achievements, I believe that our synthesis of these natural products is productive enough to provide valuable tools to probe the mechanism by which these compounds manifest their toxicity. Advancing our understanding of the structural requirements needed for toxicity, we have gained a new vision for experimental direction.

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Chapter 5: Experimental Section

5.1 General Considerations

Unless otherwise noted, materials were obtained from commercial sources and used without purification. All reactions requiring anhydrous conditions were performed under a positive pressure of argon using flame-dried glassware. Dichloromethane, diisopropylamine, triethylamine, and *N,N*-diisopropylethylamine were distilled from CaH_2 immediately prior to use. Tetrahydrofuran, diethylether, toluene, and dimethylformamide were degassed with argon and passed through a solvent purification system (J.C. Meyer of Glass Contour) containing either alumina or molecular sieves. Flash chromatography was performed on Merk silica gel Kieselgel 60 (230-400 mesh) from EM science with the indicated solvent. Alkyllithium reagents were standardized in THF with diphenylacetic acid as the acid and indicator.[1]

^{1}HNMR spectra were recorded on Varian 300, 400, or 500 MHz spectrometers as indicated. The chemical shifts (δ) of proton resonances are reported relative to $CHCl_3$, DMSO-d_5, HOD, or HD_2COD using the following format: chemical shift [multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, app = apparent), coupling constant(s) (J in Hz), integral].[2,3] ^{13}CNMR spectra were recorded at 75, 100, or 125 MHz. The chemical shifts of carbon resonances are reported relative to the deuterated solvent peak, except those in D_2O which are referenced to methanol.[2]

Infrared spectra were recorded on a Nicolet Avatar 320-FT IR spectrometer. All absorptions are reported in cm^{-1} relative to polystyrene (1601 cm^{-1}). Spectra that were

recorded 'neat' refer to a thin film of pure liquid on NaCl plates. Spectra were also recorded as films deposited from $CDCl_3$ (dep. $CDCl_3$) or CH_2Cl_2 (dep. CH_2Cl_2) solutions on NaCl plates followed by solvent evaporation. Peaks reported in the IR spectrum are described using the following conventions: w = weak, m = medium, s = strong, vs = very strong, sh = shoulder, and br = broad.

Mass spectra were obtained at the Colorado State University CIF on a Fisons VG Autospec. Optical rotations were obtained with a 2 mL, 1 dm cell on a Rudolf Research Autopol III polarimeter operating at 589 nm. $CHCl_3$ was distilled from $CaCl_2$ for optical rotations where indicated. HPLC data was obtained on a Waters 600 HPLC system Interfaced with Varian Dynamax Integration software using the indicated column and eluent conditions. Melting points are uncorrected.

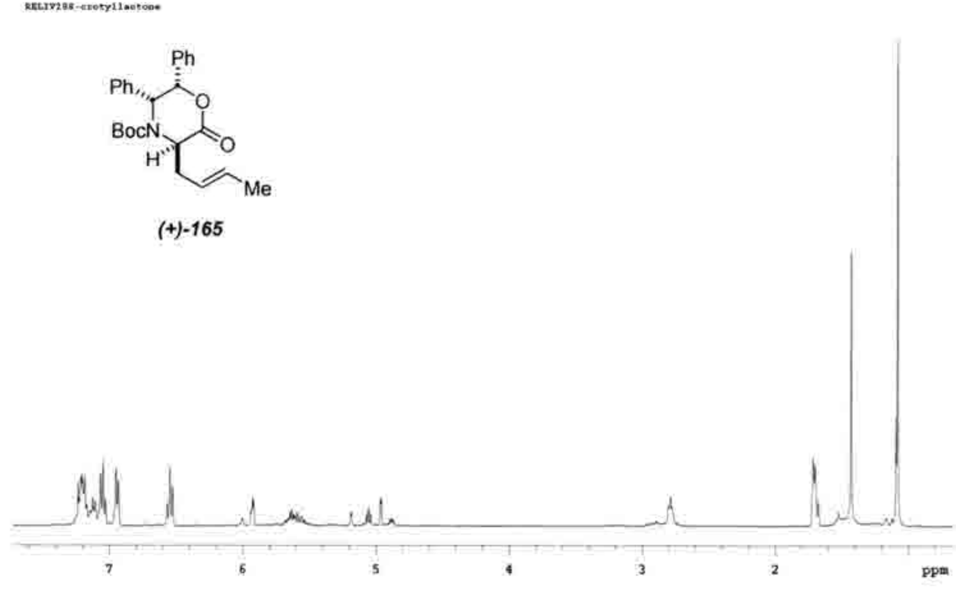
5.2 Experimental procedures

3-(R)-But-2-enyl-2-oxo-5-(R), 6-(S)-diphenyl-morpholine-4-carboxylic acid tert-butyl ester (165).

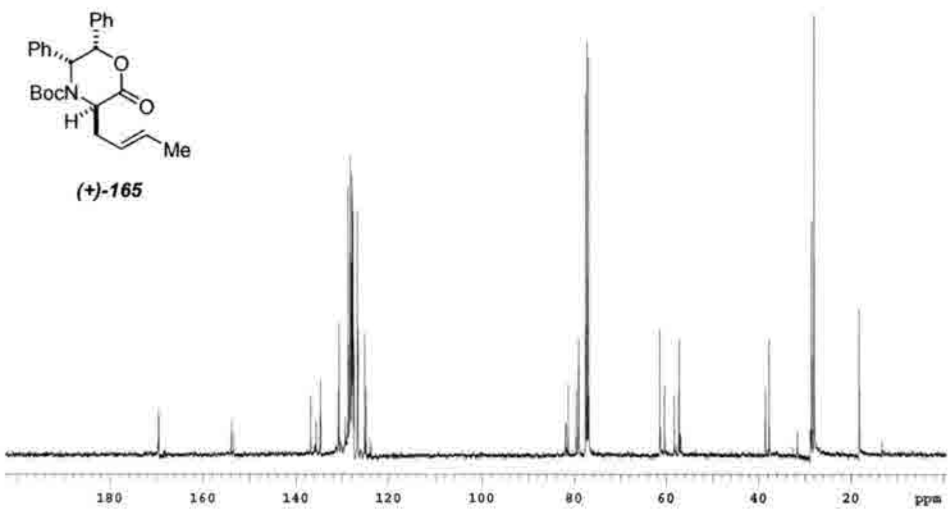
(+)-164 *(+)-165*

To a solution of NaI (6.00g, 40.0 mmol) in MeCN (30 mL) under an argon atmosphere was added TMSCl (5.08 mL, 40.0 mmol) dropwise over 10 min. H_2O (0.36 mL, 20.0 mmol) was then added followed by crotyl alcohol (3.40 mL, 40.0 mmol). After 30 min the reaction was diluted with H_2O (100 mL) and extracted 3 x 50 mL hexanes. The combined organics were washed with sat. $Na_2S_2O_3$, brine, and dried ($MgSO_4$). The organics were then concentrated under aspirator pressure to ~1/4 volume. To this solution, under an argon atmosphere, was added the oxazinone **164** (5.66 g, 16.0 mmol) and THF (100 mL). The mixture was cooled to -78°C and a 0.5 M solution of KHMDS in PhMe (32.0 mL, 16.0 mmol) was added dropwise over 10 min. After 0.5 h the reaction was quenched with sat. NH_4Cl and diluted with Et_2O. The organics were washed with sat. $Na_2S_2O_3$, brine, and dried (Na_2SO_4). Concentration afforded a white solid which was recrystallized from EtOH / H_2O. The while solid was dried at 60°C to constant mass giving the crotyloxazinone (5.97 g, 92%, m.p. 138-141°C). $[\alpha]^{25}_{D} = +13.2°$ (*c* 1.00, $CHCl_3$). Optical purity was determined by HPLC, Chiracel OD-H column eluting with 97:3 hexanes: *i*PrOH at 1 mL/min; (* indicates minor rotamer): 3(*S*), 5(*S*), 6(*R*) t_R = 5.78* min, 6.26 min; 3(*R*), 5(*R*), 6(*S*) t_R = 7.66* min, 9.35min.[4] ^{1}HNMR ($CDCl_3$, 400 MHz, 273 K): (mixture of rotamers, * indicates minor rotamer where discernable) δ 7.28-7.10 (m, 6H), 7.05 (t, *J* = 7 Hz, 2H), 6.94 (d, *J* = 7 Hz, 2H), 6.55 (t, *J*, = 8 Hz, 2 H), 6.00*

(br d, J = 2 Hz, 1H), 5.92 (br d, J = 3 Hz, 1H), 5.7-5.5 (m, 2H), 5.19* (d, J = 2 Hz, 1H), 5.05 (app t, J = 7 Hz, 1H), 4.96 (d, J = 3 Hz, 1H), 4.88* (dd, J = 6, 8 Hz, 1H), 2.80 (br t, J = 6 Hz, 2H), 1.70 (overlapping d, J = 5 Hz, 3H), 1.43* (s, 9H), 1.08 (s, 9H). ^{13}CNMR ($CDCl_3$, 100 MHz, 273 K): (major rotamer) δ 169.52, 153.87, 136.80, 134.68, 130.66, 128.71, 128.26, 127.88, 127.75, 127.67, 126.71, 125.16, 81.31, 79.07, 61.45, 57.21, 37.74, 28.02, 18.22. IR (Dep. $CDCl_3$): 2975(w), 1752, 1700 (both s), 1388, 1166, 700 (all m). HRMS (FAB+): Calc. for $C_{25}H_{29}NO_4$ (*m/z*) 407.2097; found (*m/z*) 407.2094.



^{1}HNMR, $CDCl_3$, 400 MHz; filename: RELIV285-400



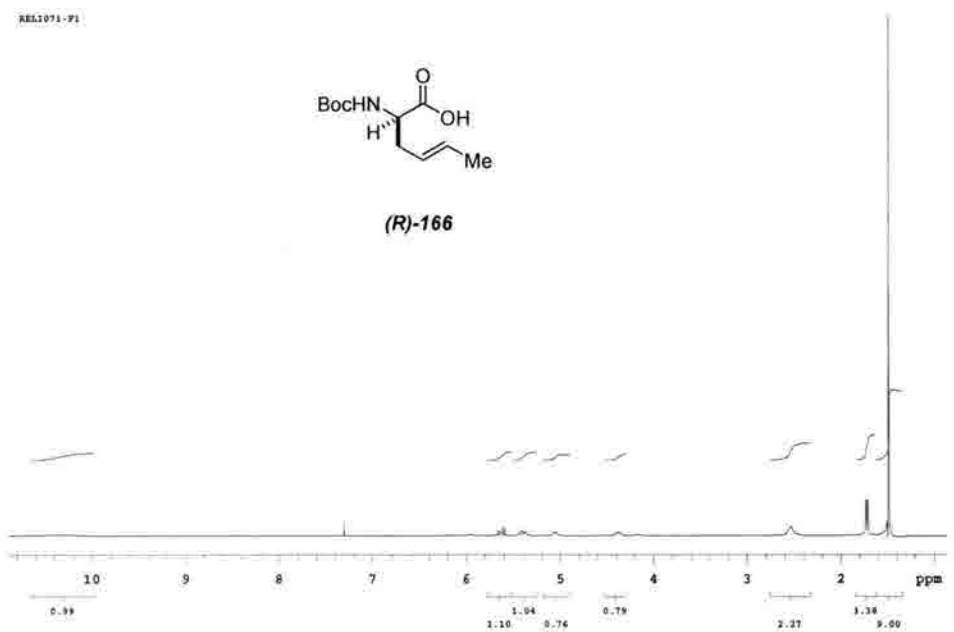
^{13}CNMR, $CDCl_3$, 100 MHz; filename: RELIV288-C13-400

2-(R)-tert-Butoxycarbonylamino-hex-4-(E)-enoic acid ((R)-166).

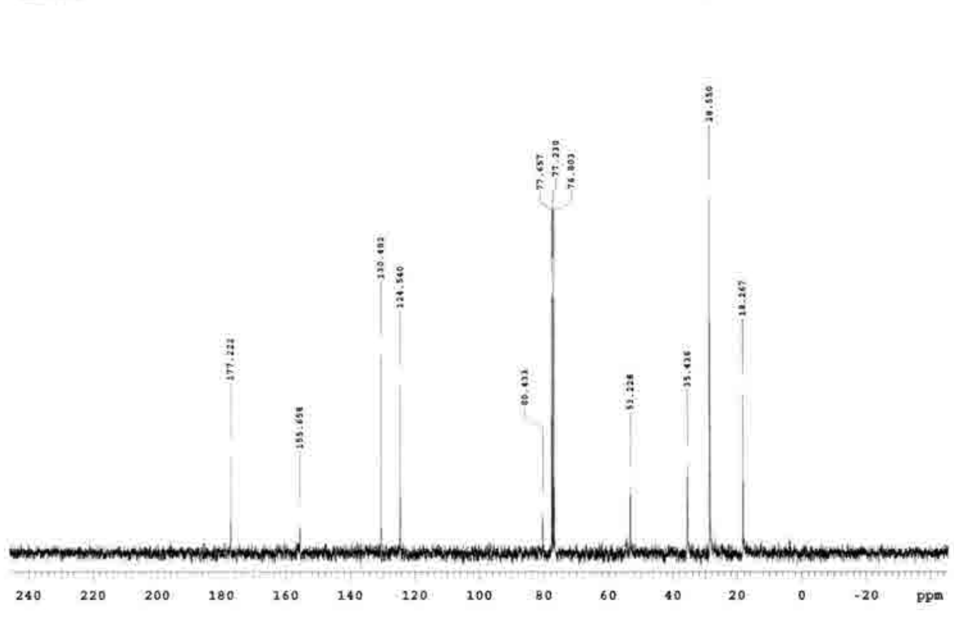
(+)-165 → (R)-166

A flame dried flask fitted with a CO_2 condenser was charged with flattened lithium metal (660 mg, 95.7 mmol) under argon. Ammonia (50 mL) was condensed into the flask at -78°C and the blue slurry stirred for 15min. A solution of the oxazinone **165** (3.00 g, 7.36 mmol) in THF (10 mL) and EtOH (1.29 mL, 22.08 mmol) was added dropwise over 5 min. The cooling bath was removed and the mixture allowed to reflux at -33°C for 0.5 h. The reaction was quenched by the careful addition of NH_4Cl and the ammonia allowed to evaporate. The resulting residue was taken up in sat. $NaHCO_3$ (100 mL) and extracted 2 x Et_2O (50 mL). The aqueous layer was acidified to pH~2 with $NaHSO_4$ and extracted 3 x CH_2Cl_2 (50 mL). The combined organics were washed with brine and dried (Na_2SO_4). Concentration gave the acid as a light yellow oil (1.12 g, 67%) which was used without further purification. *Note*: smaller reaction scale (~1 mmol) resulted in increased ~80 % yields. $[\alpha]^{25}_D = -4.30°$ (*c* 1.0, $CHCl_3$). Optical purity can be determined by HPLC on the free amino acid after hydrolysis with conc. aq. HCl, Crownpak CR column eluting with aqueous $HClO_4$ (pH = 1) at 0.8 mL/min : 2(*R*) t_R = 3.95 min.; 2(*S*) t_R = 5.71 min. ^{1}HNMR ($CDCl_3$, 300MHz): δ 10.25 (br s, 1H), 5.60 (dq, *J* = 15.0, 6.3 Hz, 1H), 5.40-5.24 (m, 1H), 5.00 (d, *J* = 7.7 Hz, 1H), 4.34 (br m, 1H), 2.58-2.40 (m, 2H), 1.66 (dd, *J* = 6.3, 0.9 Hz, 3H), 1.44 (s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.22, 155.66, 130.48, 124.54, 80.45, 52.23, 35.44, 28.55, 18.27. IR (Dep. $CDCl_3$): 3330 (m, br); 2978 (m); 1716 (s, br); 1508

(m); 1165 (s). HRMS (FAB+): Calc. for $C_{11}H_{20}NO_4$ [M+H]: *(m/z)* 230.1392; Found *(m/z)* 230.1393.



^{1}HNMR, $CDCl_3$, 400 MHz; filename: RELI071-F1



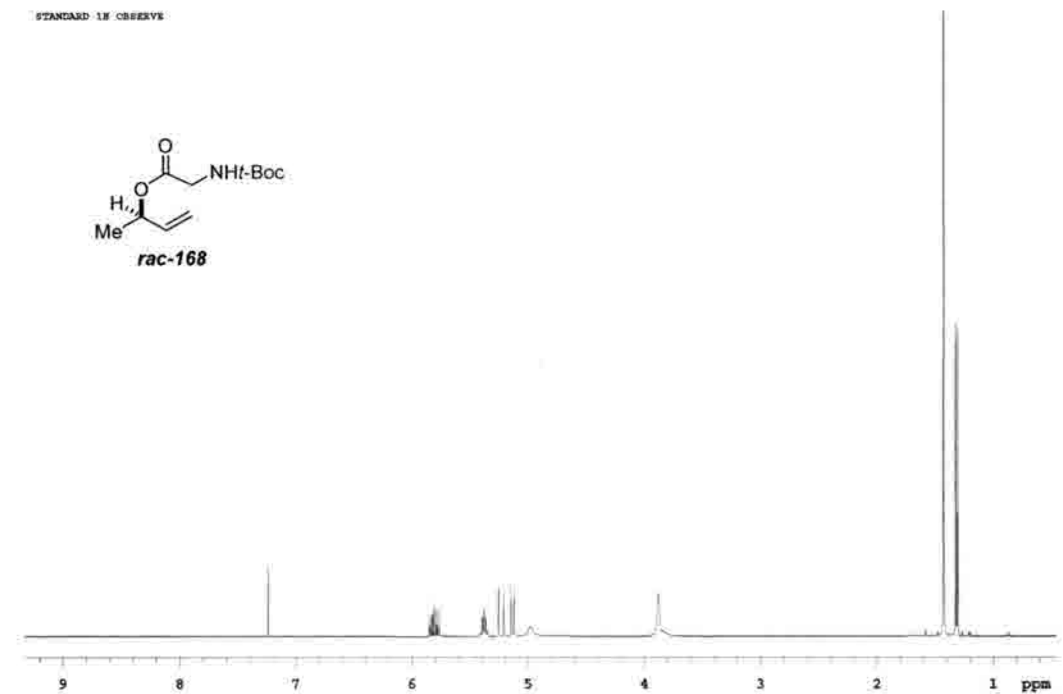
^{13}C NMR, $CDCl_3$, 100 MHz; filename: RELI072-C13

tert-Butoxycarbonylamino-acetic acid 1-methyl-allyl ester (168).

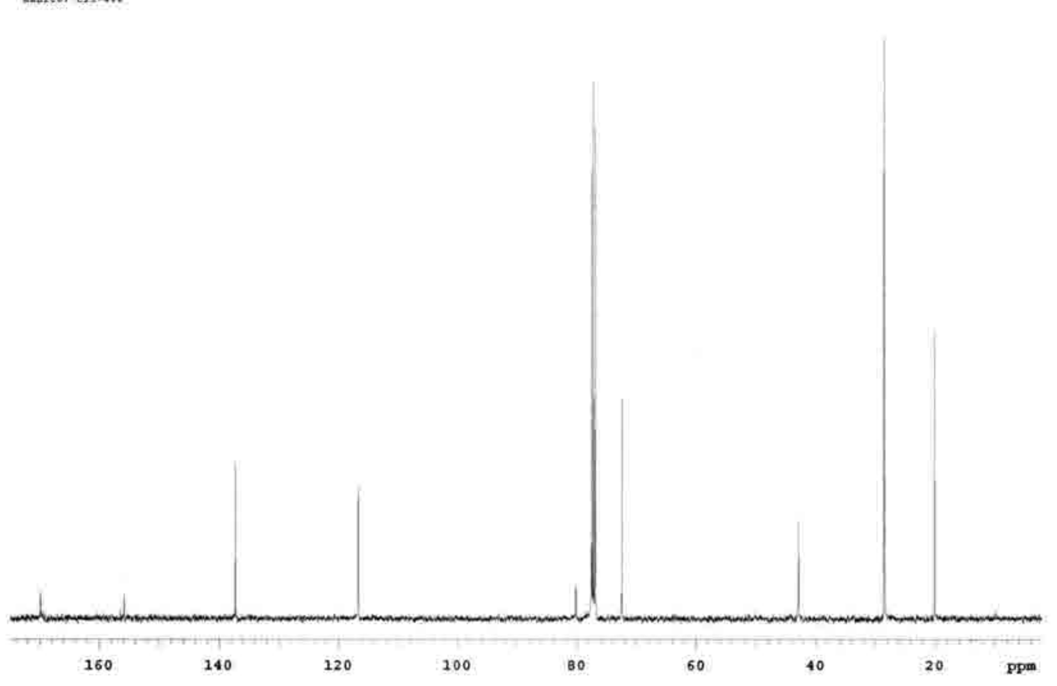
rac-167
(R)-(-)-167

rac-168
(R)-(+)-168

To a solution of 3-buten-2-ol (**167**, 2.00 g, 27.7 mmol), 4-dimethylamino pyridine (10mol%, 346 mg, 2.77 mmol), and *N*-tert-butoxycarbonyl glycine (5.35 g, 30.5 mmol) in CH_2Cl_2 (50 mL) was added diisopropylcarbodiimide (4.78 mL, 30.5 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The mixture was stirred for 2h and filtered through Celite with CH_2Cl_2 (100 mL). The combined organics were washed with 10% HCl, sat. $NaHCO_3$, brine, and dried (Na_2SO_4). The concentrated organics were purified by flash chromatography (6:1 Hex: EtOAc) to give the ester **168** as a colourless oil (6.12 g, 96%). If the ester was derived from (*R*)-(-)-3-buten-2-ol $[\alpha]^{25}_{D} = +17.9°$ (*c* 1.50, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz): δ 5.83 (ddd, *J* = 17.3, 10.5, 6.6 Hz, 1H), 5.40 (qd (app quintet), *J* = 6.6, 6.6 Hz, 1H), 5.25 (dd, *J* = 17.2, 1.2 Hz, 1H), 5.15 (dd, *J* = , 10.5, 1.2 Hz, 1H), 5.00 (br s, 1H), 3.90 (app d, *J* = 3.9 Hz, 2H), 1.45 (s, 9H), 1.33 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.69, 155.84, 137.19, 116.52, 80.07, 72.39, 42.82, 28.53, 20.12. IR (Dep. $CDCl_3$): 3381 (m); 2980 (m); 1751 (s, sh); 1719 (s); 1520 (m); 1368 (m); 1168 (s).HRMS (FAB+): Calc. For $C_{11}H_{20}NO_4$ [M+H]: (*m/z*) 230.1393; Found (*m/z*) 230.1392.



^{1}HNMR, $CDCl_3$, 400 MHz; filename: RELI067-400



^{13}CNMR, $CDCl_3$, 100 MHz; filename RELI067-C13-400

rac-2-tert-Butoxycarbonylamino-hex-4-(E)-enoic acid (166).

To a solution of ester **168** (2.72 g, 11.9 mmol) in THF (30 mL) under an Ar atmosphere was added a 1M solution of Sodium bis(trimethylsilyl)amide in THF (2.2 eq., 26.1 mL, 26.1 mmol) at 0°C. The mixture was allowed to warm to r.t.. After 2h the reaction was quenched with sat. NH_4Cl (5 mL) and brought to pH = 2 by the addition of 10% HCl. The mixture was extracted with Et_2O (3 x 50 mL), the combined organics were washed with brine and dried (Na_2SO_4). Concentration gave **166** as a light yellow oil (2.69g, 99%). All spectral characteristics agreed with **(*R*)-166.**

2-(E)-aminohex-4-en-1-ol- hydrocloride (174)

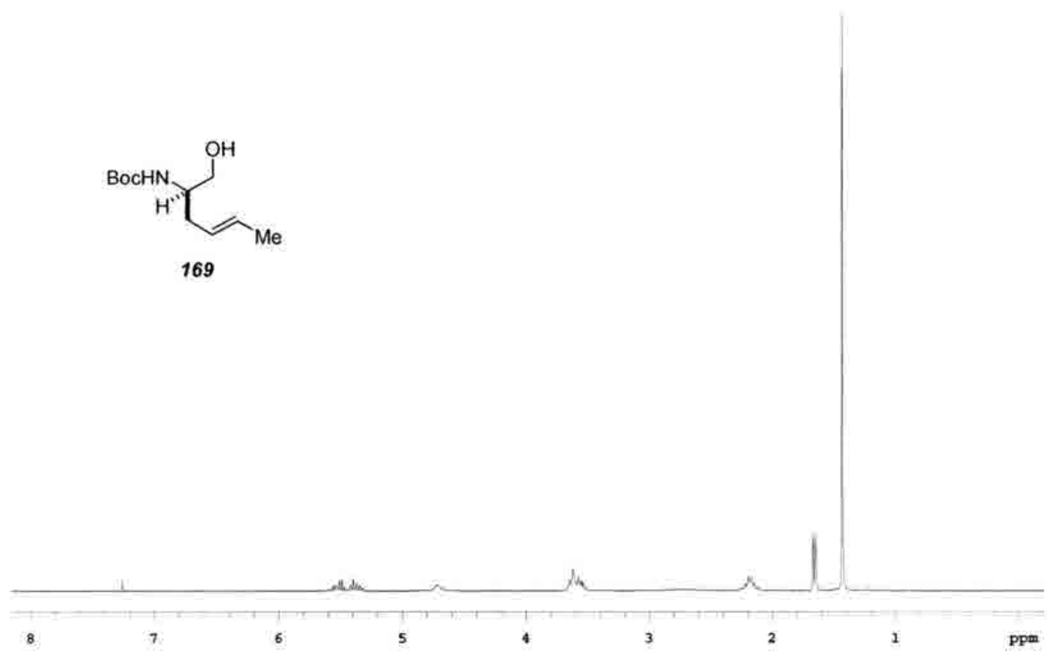
166 169 174

To a solution of the acid **166** (13.5g, 58.9 mmol) was added Et_3N (9.09 mL, 64.7 mmol) in THF (100 mL) and the mixture cooled to 0°C. Ethyl chloroformate (6.16 mL, 64.7 mmol) was then added dropwise over 10 min. After stirring for 20 min the mixture was filtered to remove the Et_3NHCl. This solution was added dropwise to a slurry of $NaBH_4$ (3.34 g, 88.4 mmol) in H_2O (70 mL) over 0.5 h, after the addition another potion of $NaBH_4$ (1.0 g, 26.4 mmol) was added. The mixture was stirred for 3h at rt and quenched by the addition of AcOH. After concentration the mixture was partitioned between Et_2O and H_2O. The organics were further washed 1 x 10% HCl, 1 x sat. $NaHCO_3$, 1 x brine and dried (Na_2SO_4). *[an analytical sample was purified on silica gel eluting with 1:1*

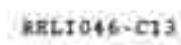
hexanes:EtOAc] Concentration gave a light yellow oil that was added to a mixture of AcCl (6.31 mL, 88.4 mmol) in MeOH (100 mL) that had been stirred for 15 min. The combined solutions were stirred at rt for 8h and concentrated. CH_2Cl_2 (100 mL) was added and the mixture allowed to stand at 0°C until crystallization occurred. The solid was filtered off and washed with Et_2O to give the amine salt **174** as a white solid (4.82 g). The mother liquor was concentrated and diluted with CH_2Cl_2 to afford an additional crop (531 mg, 5.35 g combined, 60%).

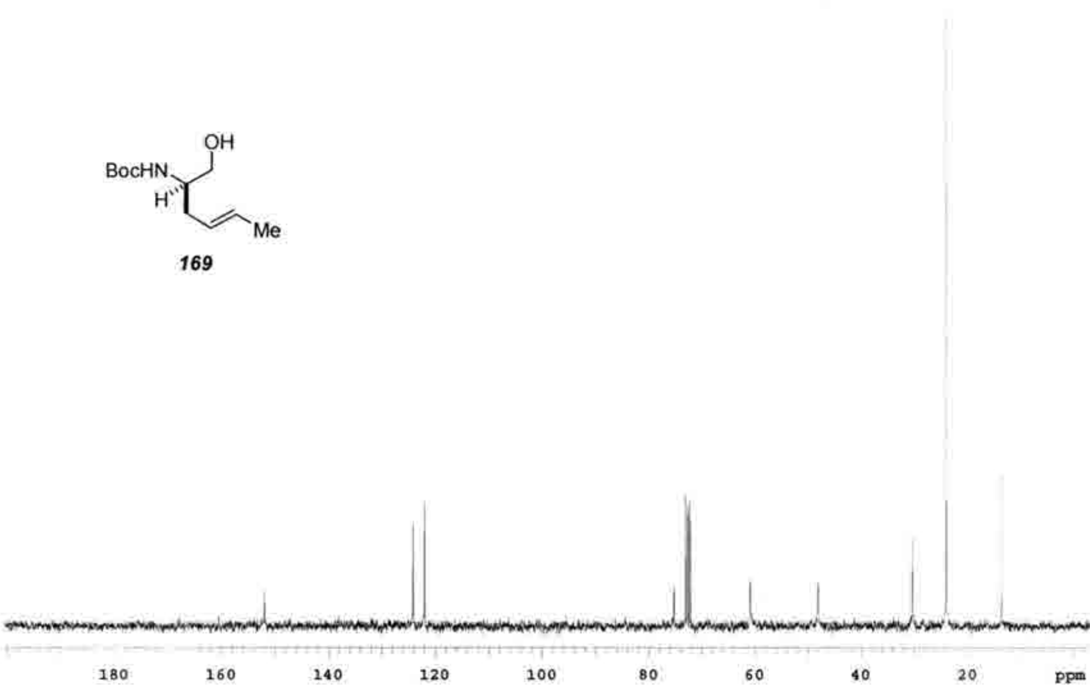
169: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.57 (dq, *J* = 15.6, 6.3 Hz, 1H), 5.42 (dt, *J* = 15.6, 7.1 Hz, 1H), 3.66 (app t, *J* = 9 Hz, 2H), 3.60 (burried m, 1H), 2.27 (ddd, *J* = 13.2, 7.2, 1.2 Hz, 1H), 2.17 (ddd, *J* = 13.2, 6.9, 1.2 Hz, 1H), 1.71 (d, *J* = 6.3 Hz, 3H), 1.48 (s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 151.94, 124.12, 121.98, 75.17, 60.815, 48.06, 30.32, 23.95, 13.58. IR (Dep. $CDCl_3$): 3350 (br, s), 1694 (s), 1520, 1366 (both m), 1172 (s), 1056, 967 (both m). HRMS (FAB+): Calc. For $C_{11}H_{21}NO_3$ [M+H]: 216.1600; Found 216.1599.

174 : ^{1}H NMR (D_2O, 300 MHz): δ 5.73 (dq, *J* = 15, 6.3 Hz, 1H), 5.45 (ddd, *J* = 15, 7.5, 7.5 Hz, 1H), 3.84 (dd, *J* = 12.6, 4.2 Hz, 1H), 3.64 (dd, *J* = 12.6, 7.2 Hz, 1H), 3.38 (dddd, *J* = 7.8, 7.2, 6.6, 4.2 Hz, 1H), 2.40 (ddd, *J* = 15, 7.5, 6.6 Hz, 1H), 2.30 (ddd, *J* = 15, 7.8, 7.5 Hz, 1H), 1.70 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (D_2O, 75 MHz): δ 132.12, 124.22, 61.44, 53.41, 32.65, 17.90.

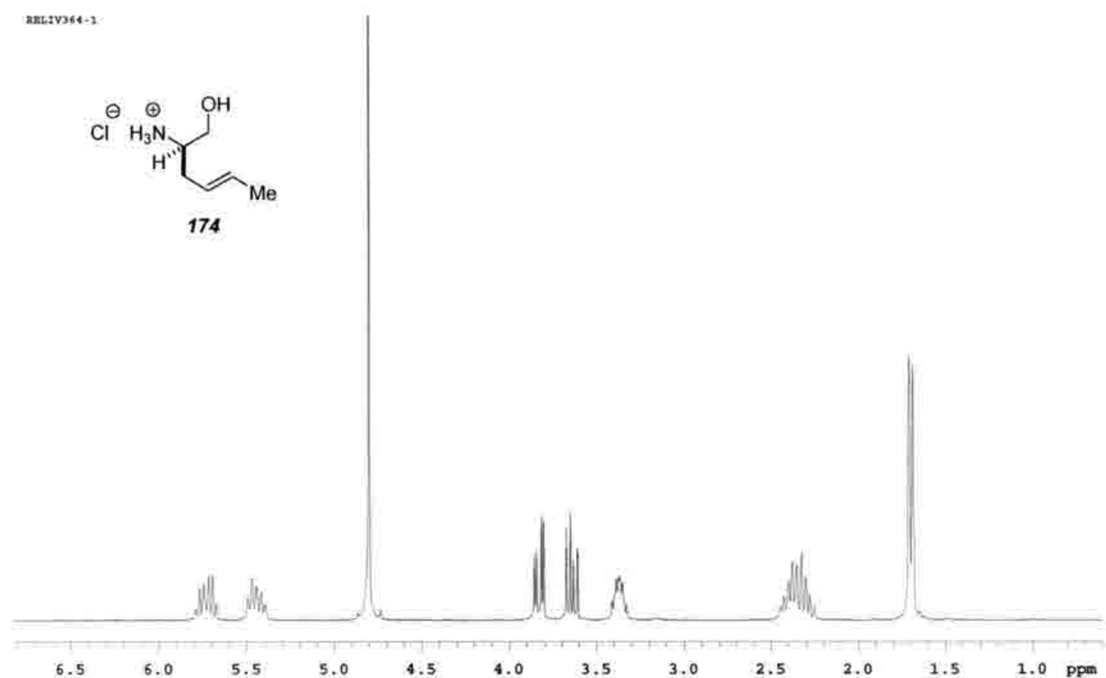


^{1}HNMR, $CDCl_3$, 300 MHz ; filename : RELI054-F2

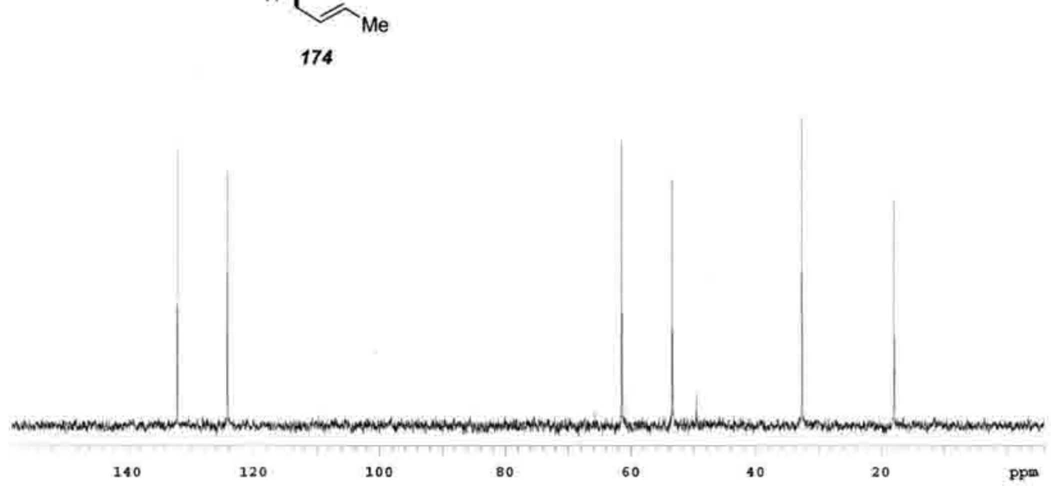




^{13}CNMR, $CDCl_3$, 75 MHz ; filename : RELI054-C13



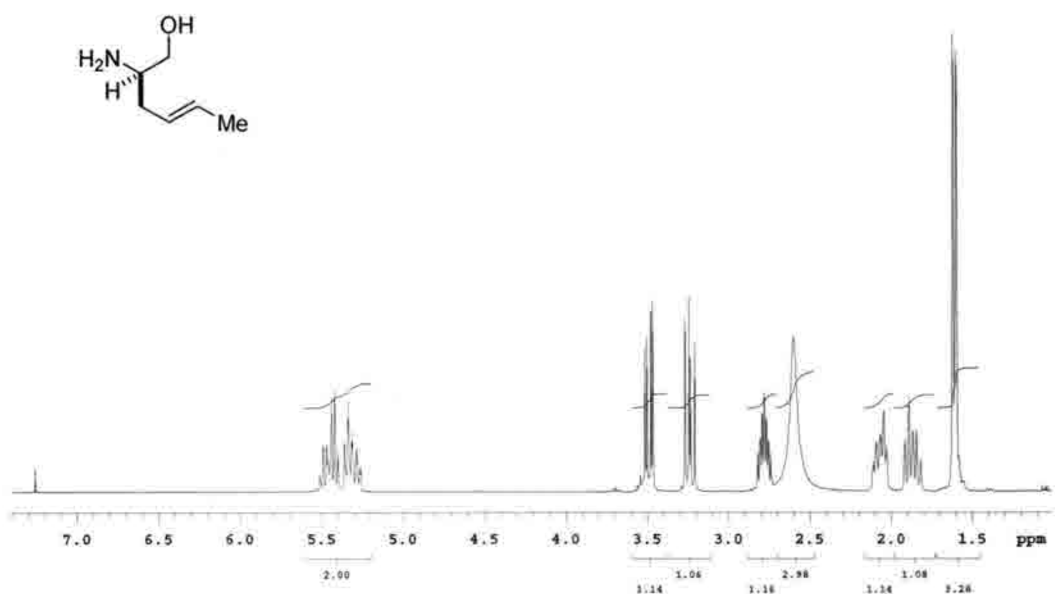
^{1}HNMR, D_2O, 300 MHz ; filename : RELIV364-1



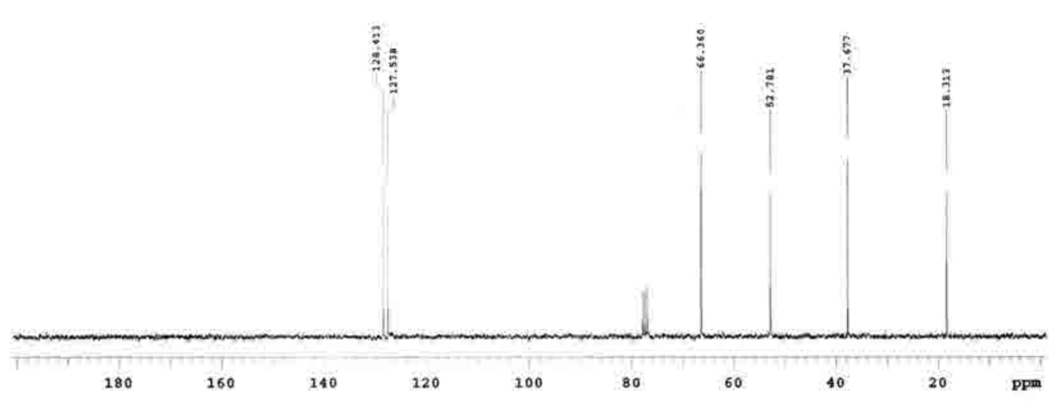
^{13}CNMR, D_2O, 75 MHz ; filename : RELIV364-C13

2-(R,E)-aminohex-4-en-1-ol.

Acetyl chloride (1.39 mL, 19.5 mmol) was added dropwise to MeOH (40 mL) at 0 °C and the solution stirred for 15 min. A solution of the acid (1.49g, 6.49 mmol) in MeOH (3 mL) was added and the mixture allowed to reach rt and stirred an additional 12h. The mixture was concentrated *in vacuo* and further concentrated after the addition of Et_2O (2 x 20 mL) and PhMe (1 x 50 mL). The crude solid was slurried in THF (50 mL) and $LiAlH_4$ (500 mg, 13.2 mmol) added in portions over 0.5 h at 0 °C. After stirring at rt for an additional 3 h the reaction was quenched by the sequential addition of H_2O (0.5 mL), 15% NaOH (0.5 mL), and H_2O (1.5 mL). The mixture was filtered through celite with THF and concentrated. The crude oil was purified by Kugelhror distillation, collecting material between 80-100 °C (0.5 mm Hg) to give the amino alcohol as a clear oil (487 mg, 65%). $[\alpha]^{22}_D = -14.3°$ (*c* 1.00, $CHCl_3$). ^{1}HNMR ($CDCl_3$, 300MHz): δ 5.45 (dq, *J* = 15, 6 Hz, 1H), 5.31 (dddq, *J* = 15, 6, 6, 1.5 Hz, 1H), 3.59 (dd, *J* = 11, 4 Hz, 1H), 3.24 (dd, *J* = 11, 8 Hz, 1H), 2.78 (dddd, *J* = 8, 6, 6, 4 Hz, 1H), 2.60 (br s, 3H), 2.06 (ddd, *J* = 13, 6, 6 Hz, 1H), 1.86 (ddd, *J* = 13, 6, 6 Hz, 1H), 1.61 (dd, *J* = 6, 1.5 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 128.27, 127.39, 66.22, 52.64, 37.54, 18.17. IR (Dep. $CDCl_3$): 3335 (s), 1573, 1435, 1051, 968 (all m). HRMS (FAB+): Calc. for $C_6H_{13}NO$ [M+H]: *(m/z)* 116.1075; Found *(m/z)* 116.1080.



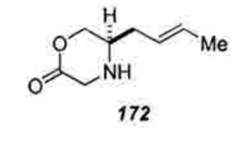
^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELIIaminoalcohol

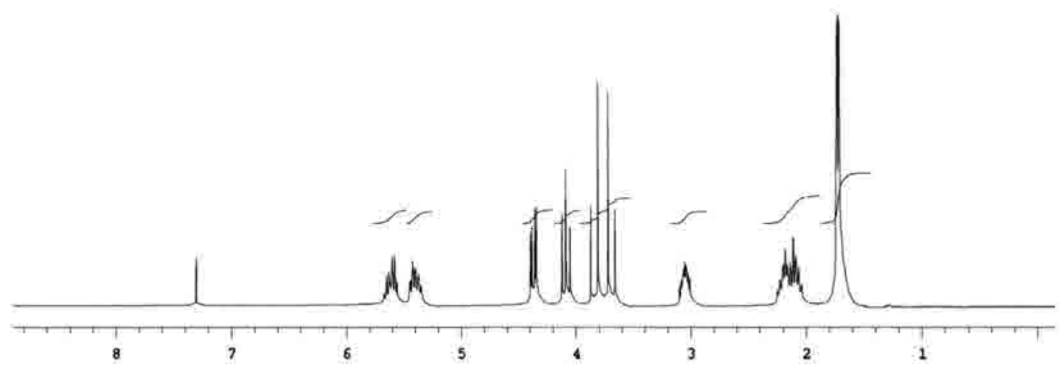


^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELIIaminoalcohol-C13-1

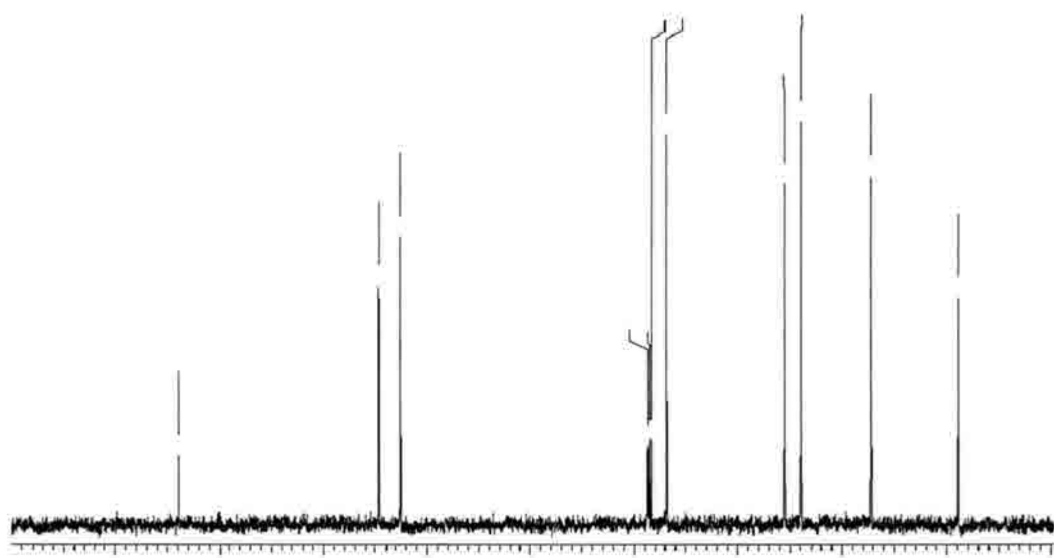
5(R)-But-2-enyl-morpholin-2-one (172).

A solution of the amino alcohol **153** (395 mg, 3.43 mmol) and $i\mathrm{Pr_2NEt}$ (745 mg, 3.46 mmol, 1.01 eq) in MeCN (40 mL) was added drop-wise over 1h to a solution of bromophenyl acetate in MeCN (131 mL, final conc. to be 0.02 M). The mixture was stirred for an additional 4h and concentrated. Purification on silica with a Na_2CO_3 pre-pad eluting with 5% *i*PrOH / EtOAc gave the morpholinone **172** as a colourless oil (335 mg, 63%). $[\alpha]^{22}_{D} = -49.6°$ (*c* 1.00, $CHCl_3$). ^{1}HNMR (CD_3OD, 300 MHz): δ 5.58 (dq, *J* = 15.0, 6.3 Hz, 1H), 5.43 (ddd, *J* = 15.0, 6.6, 1.5 Hz, 1H), 4.38 (dd, *J* = 10.9, 3.7 Hz, 1H), 4.07 (dd, *J* = 10.9, 10.9 Hz, 1H), 3.62 (ABq, dd, *J* = 18.1, 18.1 Hz, 2H), 3.04 (m, 1H), 2.14 (dd, *J* = 6.6, 6.6 Hz, 2H), 1.68 (dd, *J* = 6.3, 1.2 Hz, 3H). ^{13}C NMR (CD_3OD, 75 MHz): δ 170.83, 130.12, 126.99, 75.02, 52.19, 48.24, 35.61, 18.28. IR (Dep. CD_3OD): 3400 (br s), 2964 (s), 1636, 1404 (both m), 1063 (vs). HRMS (FAB+): Calc. for $C_8H_{14}NO_2$ [M+H]: 156.1025; Found 156.1025.





^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELII357-1

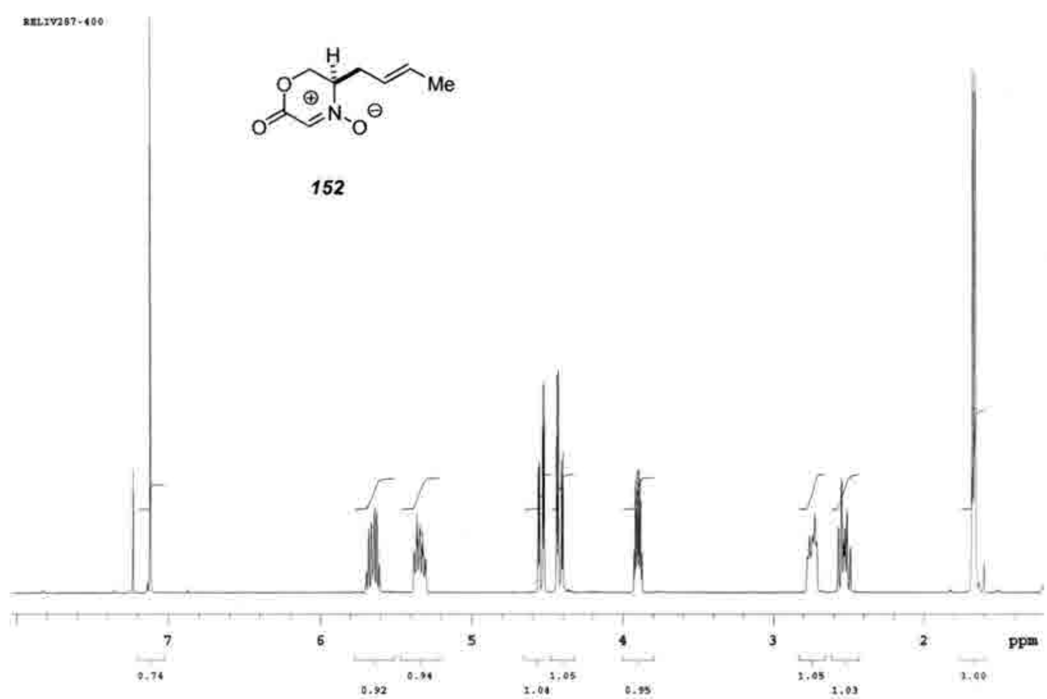


^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELII357-C13

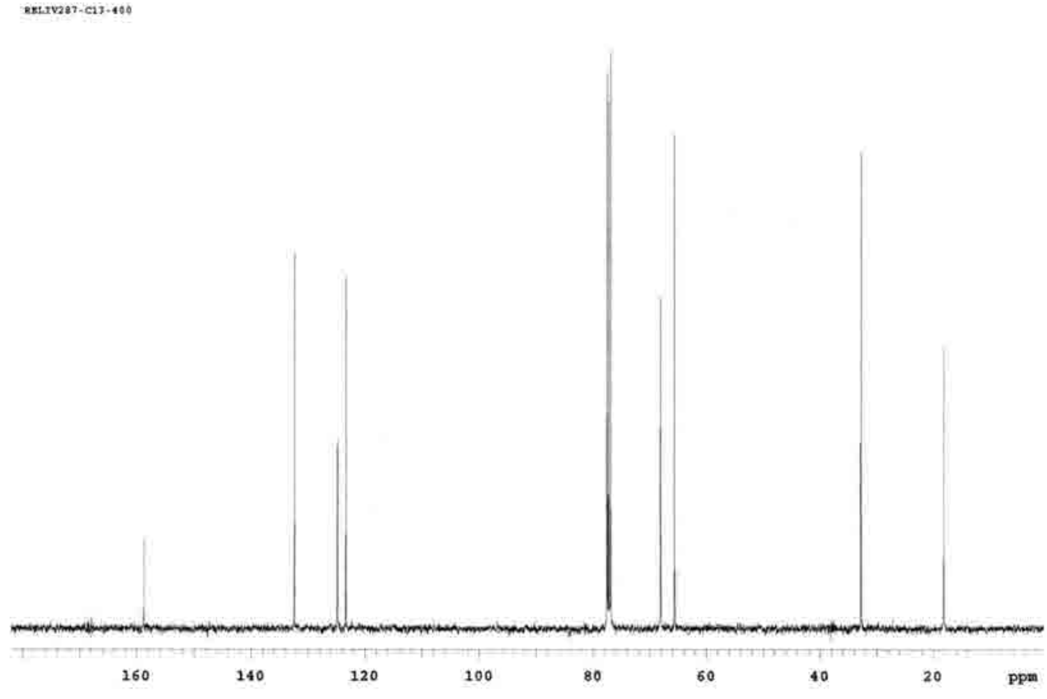
5-(R)-But-2-enyl-4-oxy-5,6-dihydro-[1,4]oxazin-2-one (152).

172 → **152**

A solution of the oxazinone **172** (260 mg, 1.67 mmol) in CH_2Cl_2 (1 mL) was added dropwise over 5 min to a solution of purified *m*CPBA (636 mg, 3.69 mmol) and Na_2HPO_4 (1.18g) in CH_2Cl_2 at −78° C. The reaction was allowed to proceed for 0.5h and quenched with sat. $Na_2S_2O_3$. The mixture was partitioned between H_2O and Et_2O and the organics further washed with 9% Na_2CO_3, brine, and dried (Na_2SO_4). The crude oil was purified on silica eluting with 1:1 hexanes : EtOAc to afford the nitrone as a colourless oil (236 mg, 84%). $[\alpha]^{25}_{D} = +4.00°$ (*c* 4.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.14 (s, 1H), 5.66 (dq, *J* = 15.0, 6.5 Hz, 1H), 5.46-5.30 (m, 1H), 4.58 (dd, *J* = 12.3, 3.9 Hz, 1H), 4.43 (dd, *J* = 12.3, 3.9 Hz, 1H), 3.92 (dddd, *J* = 9.3, 3.9, 3.9, 3.9 Hz, 1H), 2.82-2.70 (m, 1H), 2.61-2.49 (m, 1H), 1.69 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 158.19, 132.27, 124.74, 123.25, 68.08, 65.62, 32.81, 18.27. IR (Dep. $CDCl_3$): 1715, 1556 (both s), 1209 (m), 1061, 968 (both w). HRMS (FAB+): Calc. For $C_8H_{12}NO_3$ [M+H]: 170.0818; Found 170.0817.



^{1}HNMR, $CDCl_3$, 400 MHz; filename: RELIV287-400

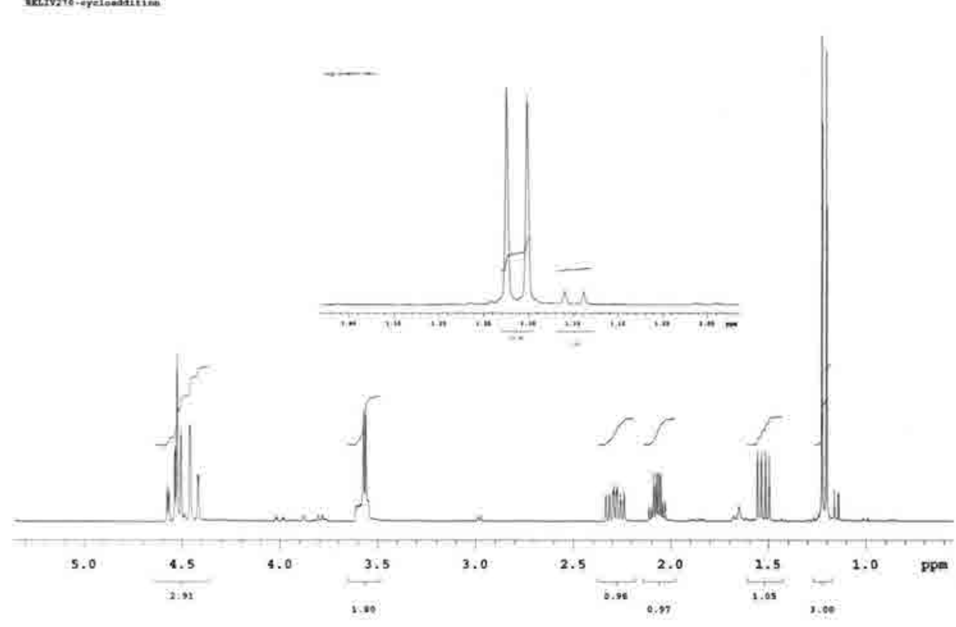


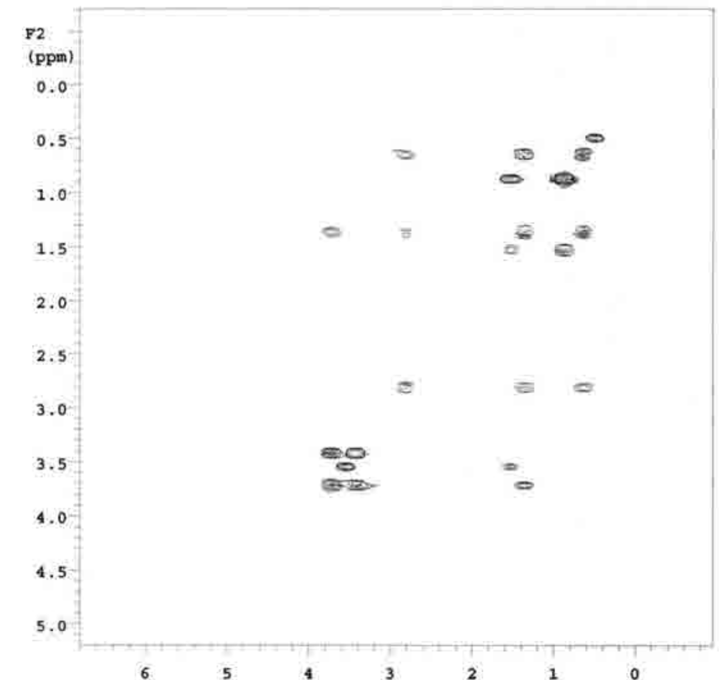
^{13}CNMR, $CDCl_3$, 100 MHz; filename: RELIV287-C13-400

2-(S)-Methyl-5-(S),9-(R)-dioxa-8-(S)-aza-tricyclo[5.2.1.0^{3,8}]decan-4-one (153).

(1)

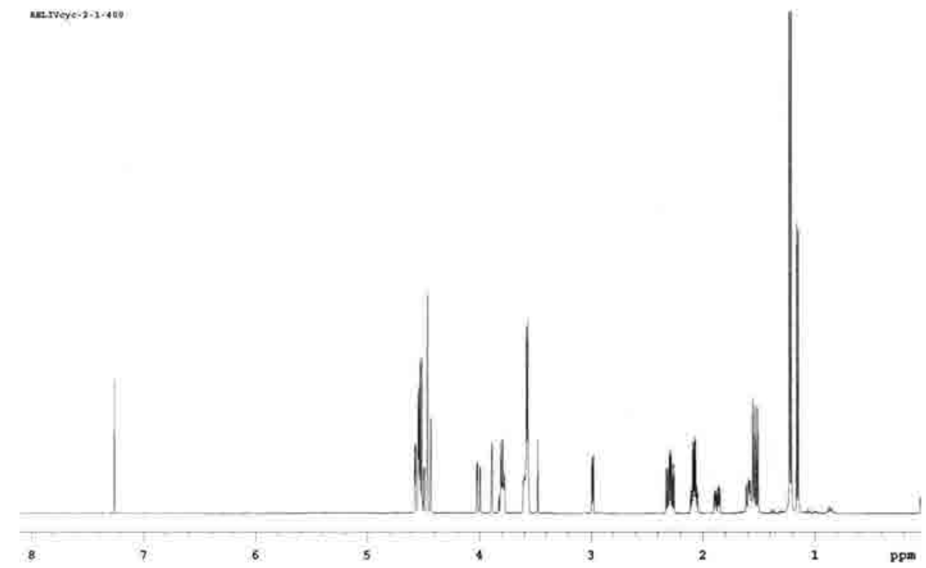
The nitrone **152** (60 mg, 0.35 mmol) was dissolved in dry toluene (7 mL) to be 0.05 M. This solution was heated in a sealed tube at 200 °C (sand bath temperature) for 2.5 h. The mixture was then cooled and the solvent removed *in vacuo*. The crude organics were purified on silica eluting with 1:1 hexanes: EtOAc to afford the tricyclic isoxazolidine **153** (47 mg, 78%) as a colourless oil which solidified upon standing. An analytical sample was recrystallized from pet. ether / CH_2Cl_2 (m.p. 78-80 °C). $[\alpha]^{25}_D = +3.6°$ (*c* 0.52, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.56 (dd, *J* = 12.3, 2.7 Hz, 1H), 4.53 (d, *J* = 6.9 Hz, 1H), 4.45 (dd, *J* = 12.3, 1.2 Hz, 1H), 3.58 (burried m, 1H), 3.58 (d, *J* = 3.6 Hz, 1H), 2.30 (ddd, *J* = 11.7, 10.8, 5.4 Hz, 1H), 2.08 (qd, *J* = 6.9, 3.7, 1H), 1.56 (dd, *J* = 12.0, 6.0 Hz, 1H), 1.22 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.92, 85.14, 70.37, 65.12, 57.70, 51.71, 34.69, 19.70. IR (Dep. $CDCl_3$): 2966 (w), 1746 (vs), 14548, 1404 (both w), 1227 (m), 1117 (w), 988 (m). HRMS (FAB+): Calc. for $C_8H_{12}NO_3$ [M+H]: 170.0817; Found 170.0812.



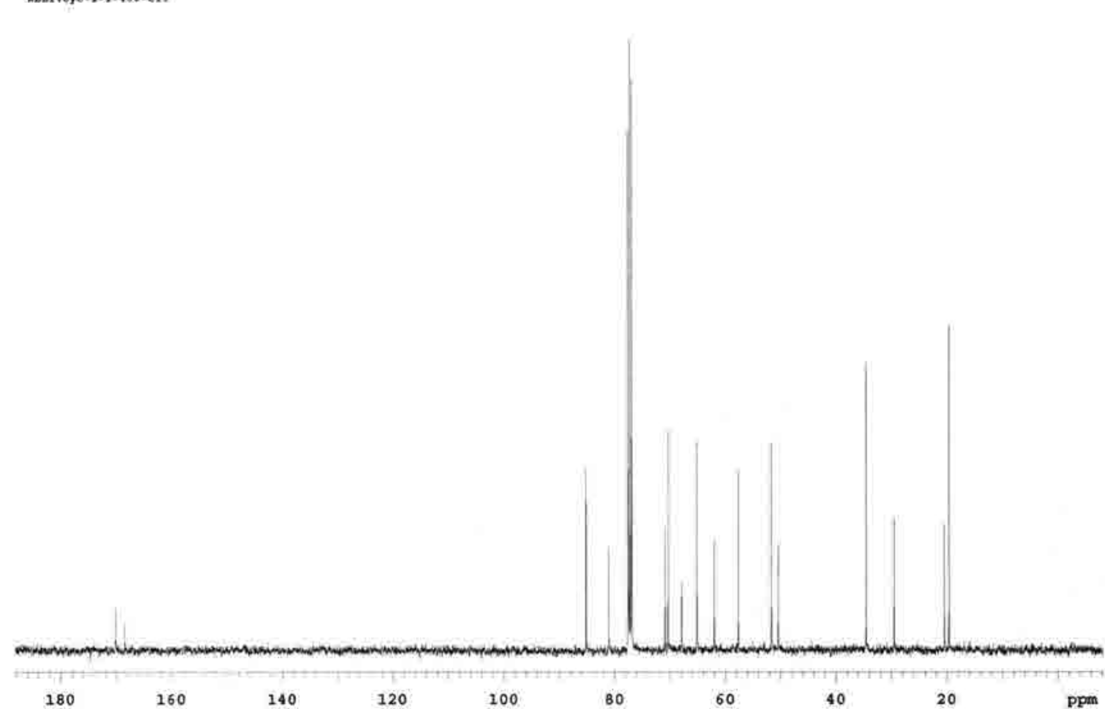


RELI118-COSY (400 MHz).

179: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.49 (buried dd, *J* = 10.8, 1.6 Hz, 1H), 4.00 (dd, *J* = 10.8, 2 Hz, 3.89 (br s, 1H), 3.80 (q, *J* = 6 Hz), 3.82-3.78 (buried m, 1H), 2.98 (d, *J* = 4.8 Hz), 1.87 (ddd, *J* = 12.4, 4.8, 3.2 Hz, 1H), 1.58 (dd, *J* = 12.4, 1.6 Hz, 1H), 1.15 (d, *J* = 6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz, 2:1 mixture): δ 168.44, 81.02, 70.86, 67.87, 61.95, 50.40, 29.48, 20.43.



^{1}HNMR, $CDCl_3$, 400 MHz, ~2:1 mixture **153**:**179**; filename: RELIVcyc-2-1-400

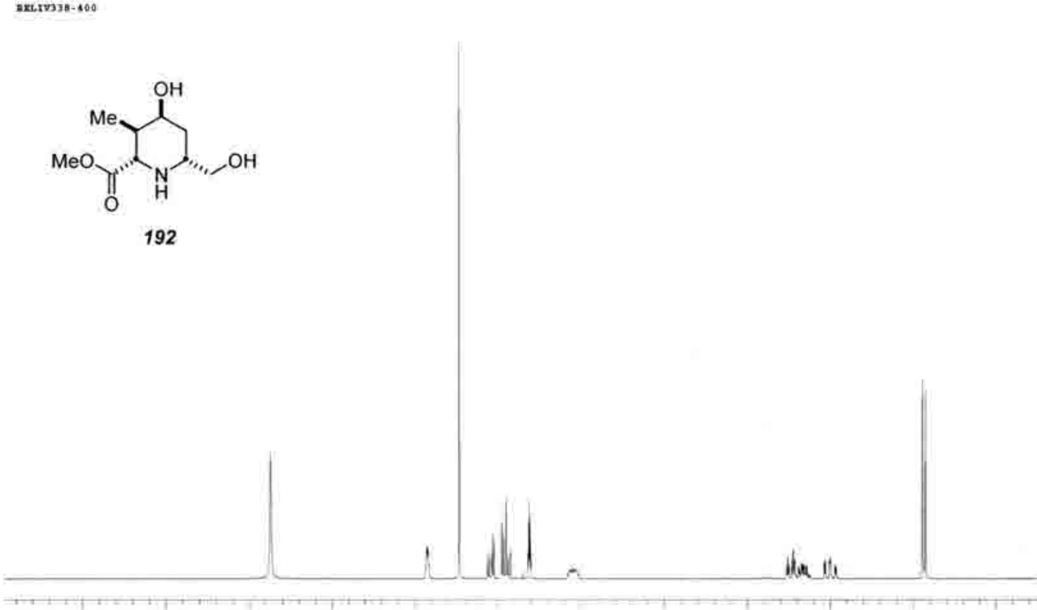


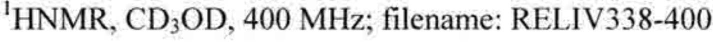
^{13}CNMR, $CDCl_3$, 100 MHz ~2:1 mixture **153**:**179**; filename: RELIVcyc-2-1-400-C13.

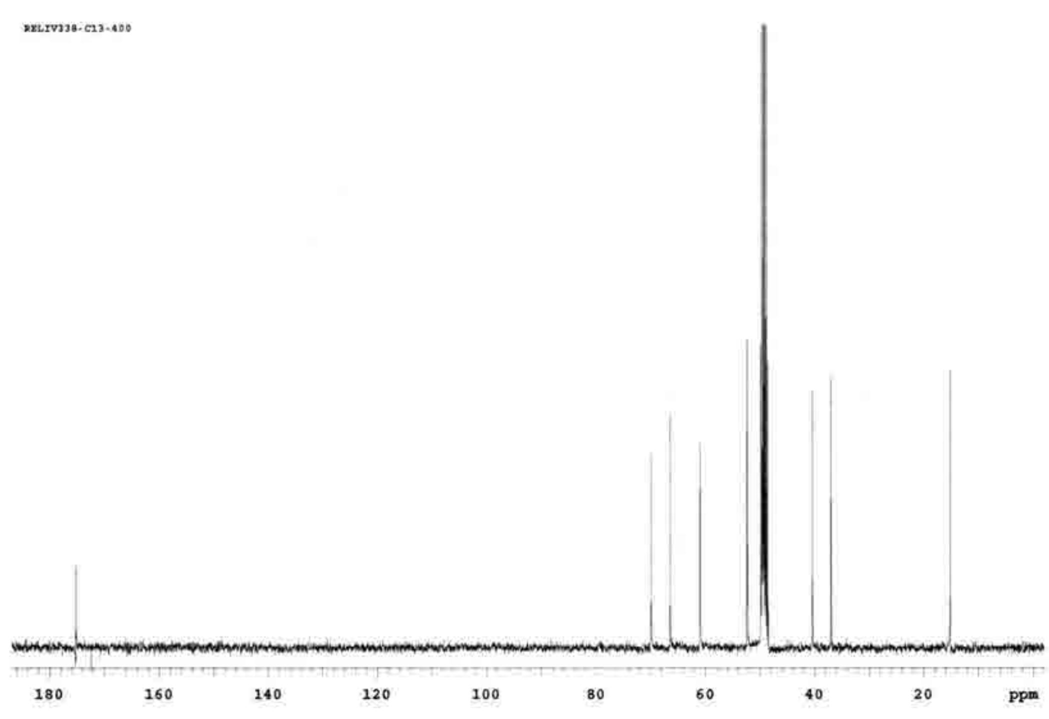
4-Hydroxy-6-hydroxymethyl-3-methyl-piperidine-2-carboxylic acid methyl ester (192).

153 → 192

To a solution of the isoxazolidine **153** (4.0 mg, 24 μmol) in MeOH (2 mL) was added 10% Pd/C (2 mg). The mixture was stirred under a hydrogen atmosphere for 12 h. The mixture was filtered through a short pad of celite with MeOH (5 mL). Concentration gave the hydroxy ester **192** (4.7 mg, 98%) as an analytically pure colourless oil. ^{1}HNMR (CD_3OD, 300 MHz): δ 3.92 (ddd, J = 3.0, 3.0, 2.4 Hz, 1H), 3.73 (s, 3H), 3.46 (d, J = 8.7 Hz, 1H), 3.45 (dd, J = 11, 5.7 Hz), 3.10-3.00 (m, 1H), 1.72 (ddd, J = 13, 2.6, 2.6, 1H), 1.66 (qd, J = 7.2, 2.4 Hz, 1H) 1.50 (ddd, J = 12.3, 12.3, 2.6 Hz, 1H), 0.93 (d, J = 7.2 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 175.2, 69.8, 66.4, 60.9, 52.3, 40.3, 36.9, 15.1. IR: 3390 (br, m), 1734 (s), 1436, 1051, 702 (all m). HRMS (FAB+): Calc. For $C_9H_{18}NO_4$ [M+H]: 204.1237; Found 204.1236.




^{1}HNMR, CD_3OD, 400 MHz; filename: RELIV338-400

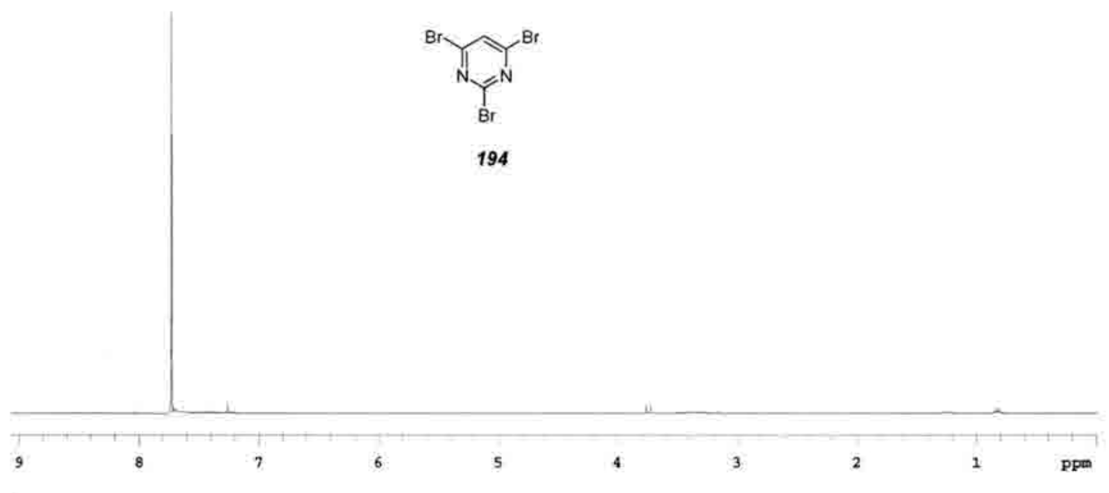


^{13}CNMR, CD_3OD, 100 MHz; filename: RELIV338-C13-400

2,4,6-Tribromo-pyrimidine (194).

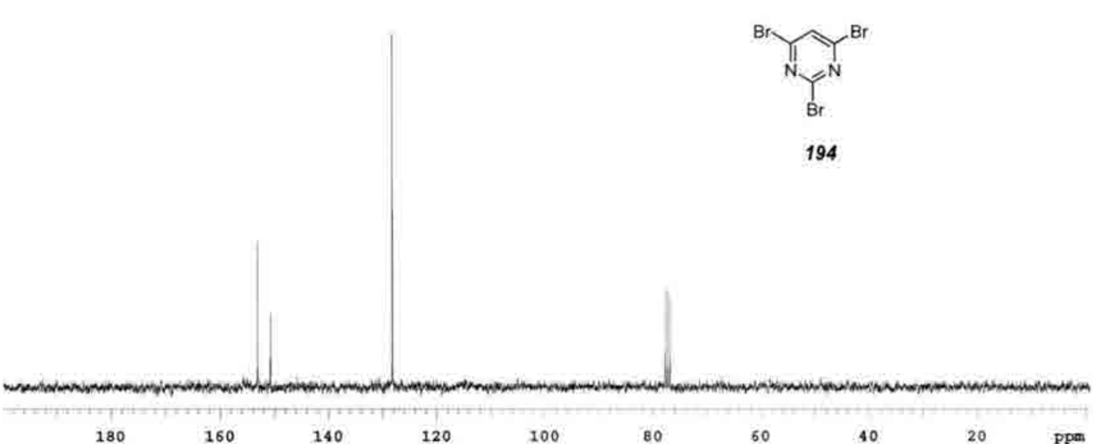
To a slurry of barbituric acid (2.79g, 21.8 mmol) and $POBr_3$ (25.0g, 87.2 mmol) in dry toluene (100 mL) was slowly added *N,N*-dimethylaniline (5.51 mL, 43.6 mmol). The mixture was refluxed for 2h and then allowed to cool to rt. The mixture was diluted with benzene (100 mL) and the organics washed with brine and dried (Na_2SO_4). After concentration the crude solid was recrystallized from pentane/$CHCl_3$ to give the tribromide as white needles (crop1 3.29g , 48%; crop2 0.83g, 12%; combined 60%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.73 (s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 148.46, 146.12, 123.61.

RELI196-2



^{1}HNMR, $CDCl_3$, 300 MHz; filename RELI196-2

RELI196-C13

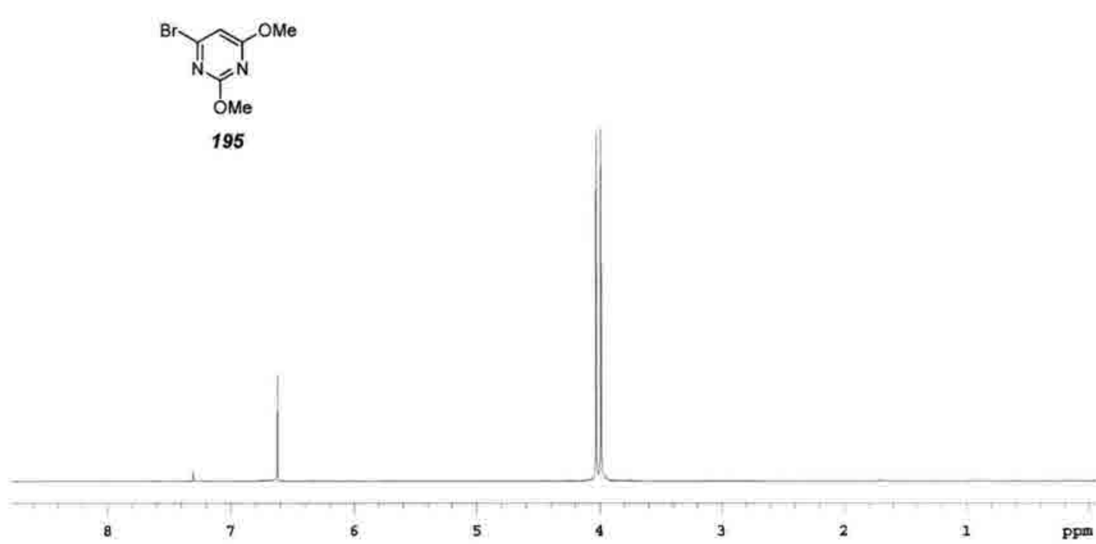


^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELI196-C13

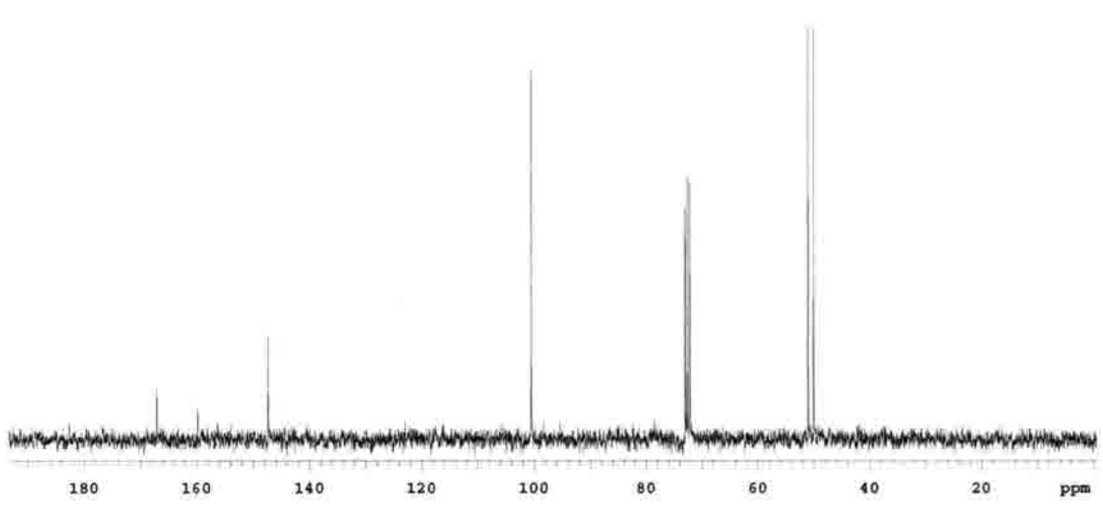
4-Bromo-2,6-dimethoxy-pyrimidine (195).

194 → 195

Sodium hydride (0.83g, 20.8 mmol) was added portion-wise to methanol at 0°C. After dissolution was complete the mixture was added dropwise to a solution of the bromopyrimidine (3.0g, 9.47 mmol) in 3:1 MeOH:THF (50 mL). After stirring for 10h the mixture was concentrated *in vaccuo* and taken up in Et_2O (100 mL). The organics were washed with H_2O, brine, and dried (Na_2SO_4). After concentration the crude solid was recrystallized from pentane to give the dimethoxy pyrimidine as long white needles m.p. 87-88 °C (lit. 90-91°C), (1.49 g, 72%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.62 (s, 1H), 4.03 (s, 3H), 3.99 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.72, 164.51, 152.02, 105.17, 55.63, 54.63.



^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELII330-2

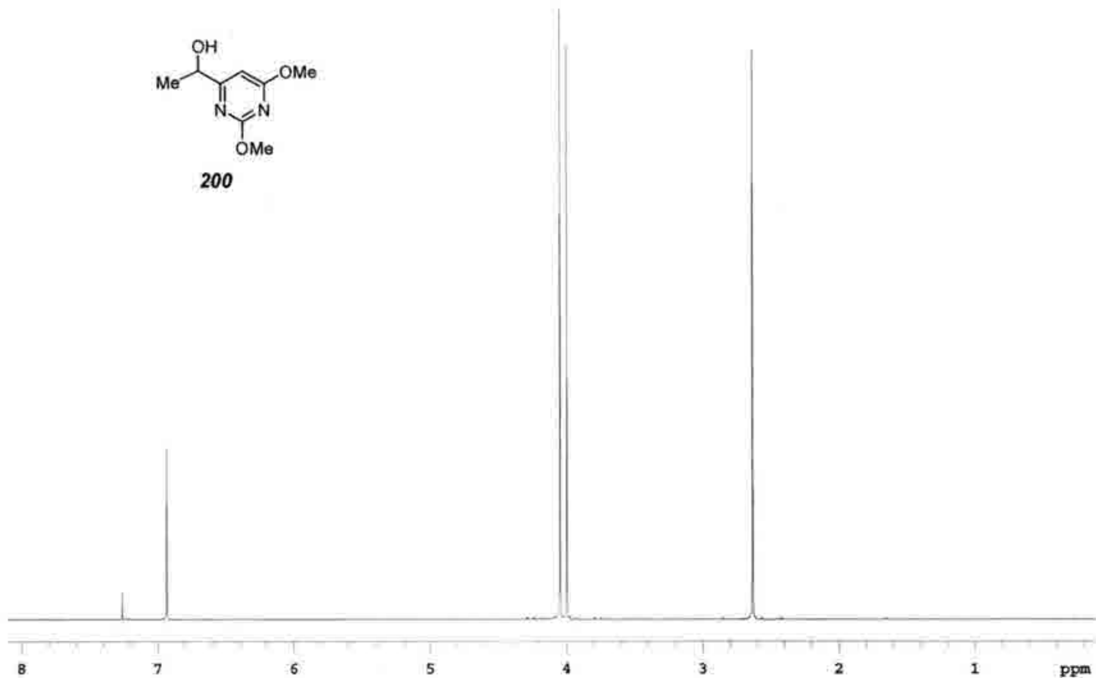


^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELI330-C13-2

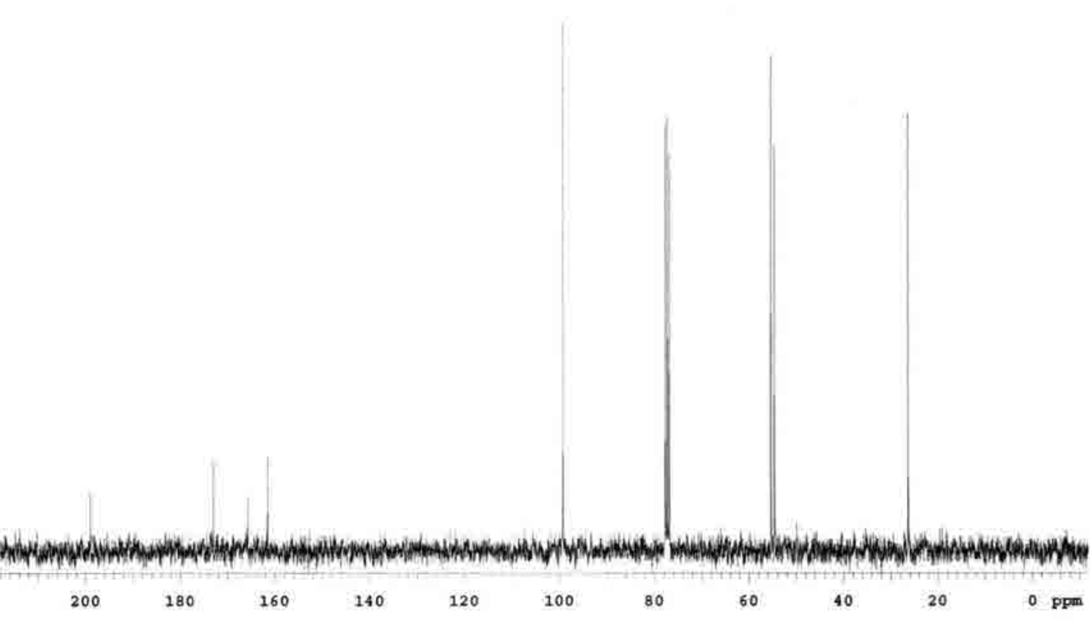
1-(2,6-Dimethoxy-pyrimidin-4-yl)-ethanol (200).

OH
Br OMe Me OMe
N N N N
OMe OMe
195 **200**

To a solution of the pyrimidine (250 mg, 1.14 mmol) in dry THF (10 mL) at -80°C under an argon atmosphere was added a 1.7M solution *t*-BuLi in pentane(1.48 mL, 2.51 mmol). After 5 min. acetaldehyde (70 μL, 1.25 mmol) was added rapidly. The mixture was allowed to stir at -80°C for 0.5h, quenched with sat. NH_4Cl (1 mL), and allowed to warm to rt. The mixture was diluted with Et_2O (20 mL) and sat. $NaHCO_3$ (10 mL). The organics were then washed with brine and dried (Na_2SO_4). The crude oil was purified on silica gel (2:1 hex:EtOAc) to give the alcohol as a colourless oil (197 mg, 94%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.33 (s, 1H), 4.66 (app quintet, J = 5.7 Hz, 1H), 3.96 (s, 3H), 3.93 (s, 3H), 3.68 (d, J = 5.1 Hz, 1H), 1.42 (d, J = 6.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.29, 172.19, 164.93, 96.78, 68.75, 54.97, 54.15, 23.53. IR (dep. $CDCl_3$) 3347 (s), 2933 (m), 1673, 1598, 1353, (all s).



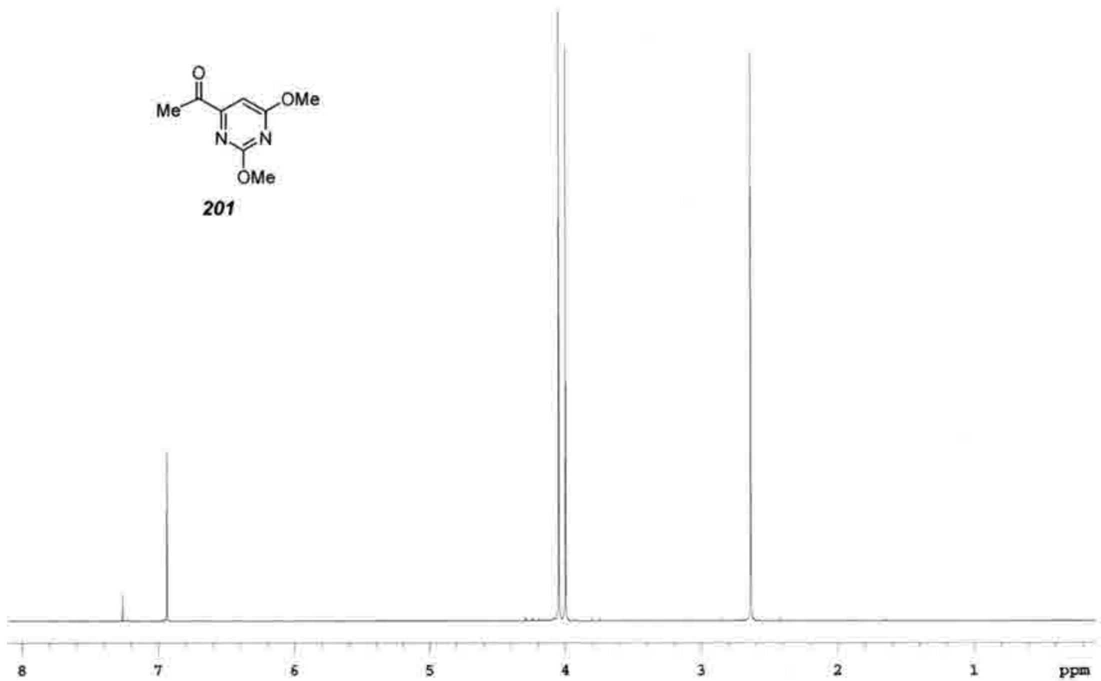
^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELI330-1



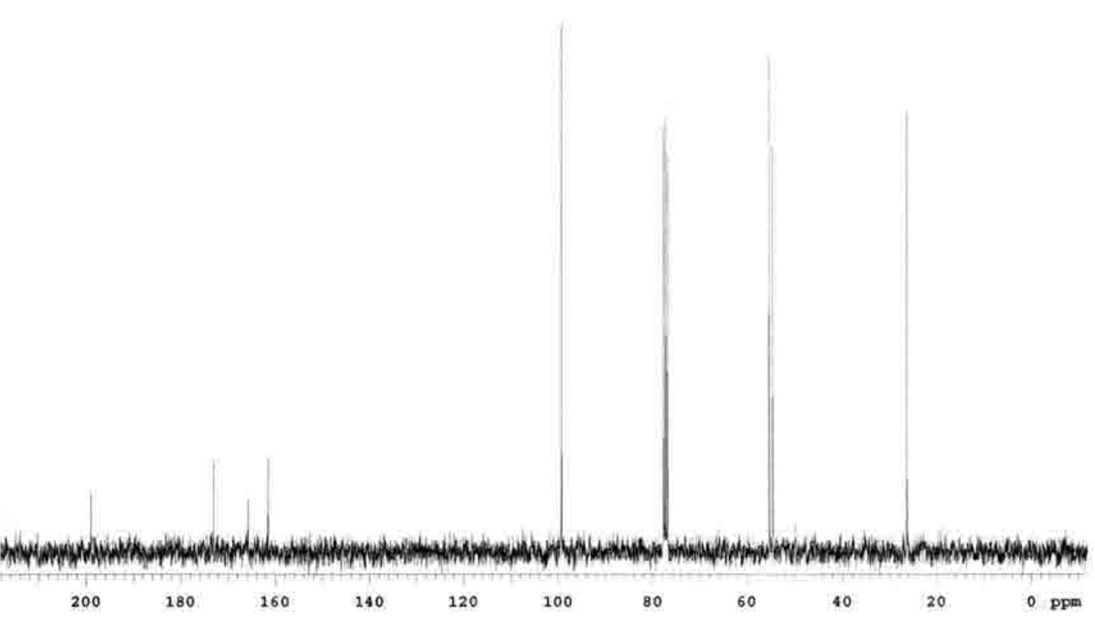
^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELI330-C13

1-(2,6-Dimethoxy-pyrimidin-4-yl)-ethanone.

To a solution of oxalyl chloride (70 μL, 0.77 mmol) in CH_2Cl_2 (5 mL) under argon at -78°C was added DMSO (0.10 mL, 1.40 mmol). The mixture was stirred at -78°C for 5 min. and then a solution of the alcohol (129 mg, 0.70 mmol) in CH_2Cl_2 (1 mL) was added. After 30 min. Et_3N (0.49 mL, 3.50 mmol) was added and the mixture allowed to warm to rt. The mixture was diluted with Et_2O (60 mL) and the organics washed with H_2O, brine, and dried (Na_2SO_4). The crude solid was recrystallized from pentane to give the ketone as a white powder (95 mg, 75%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.94 (s, 1H), 4.05 (s, 3H), 3.99 (s, 3H), 2.64 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 198.94, 173.04, 165.75, 161.55, 99.25, 55.30, 54.61, 26.32. IR (dep. $CDCl_3$) 1709, 1560 (both s), 1485, 1362, 1344 (all m).



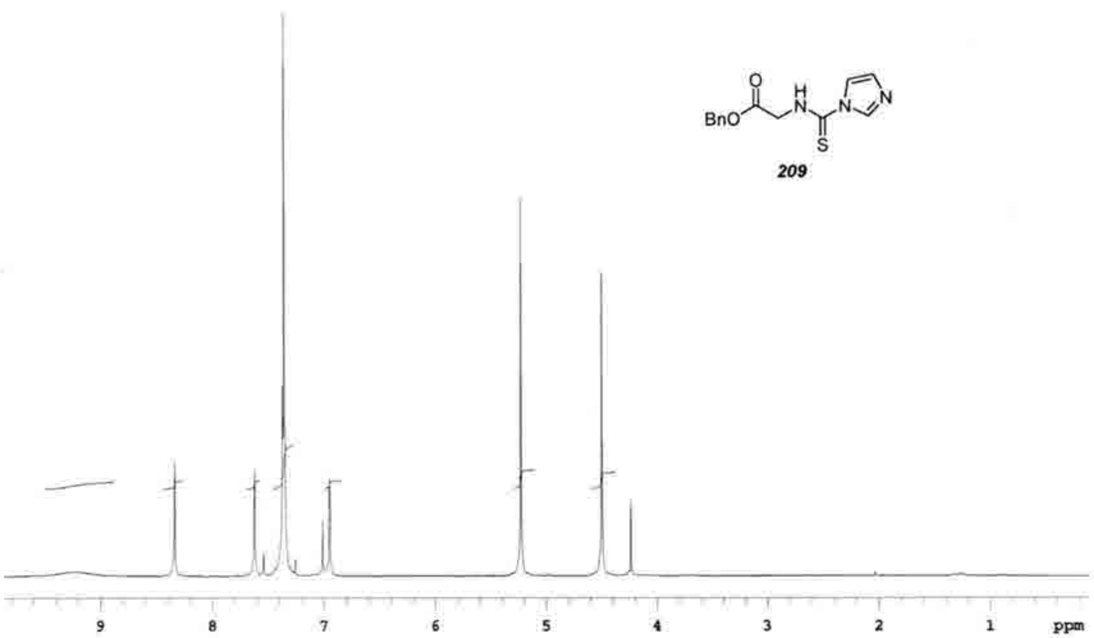
^{1}HNMR, $CDCl_3$, 300 MHz; filename RELI332-1



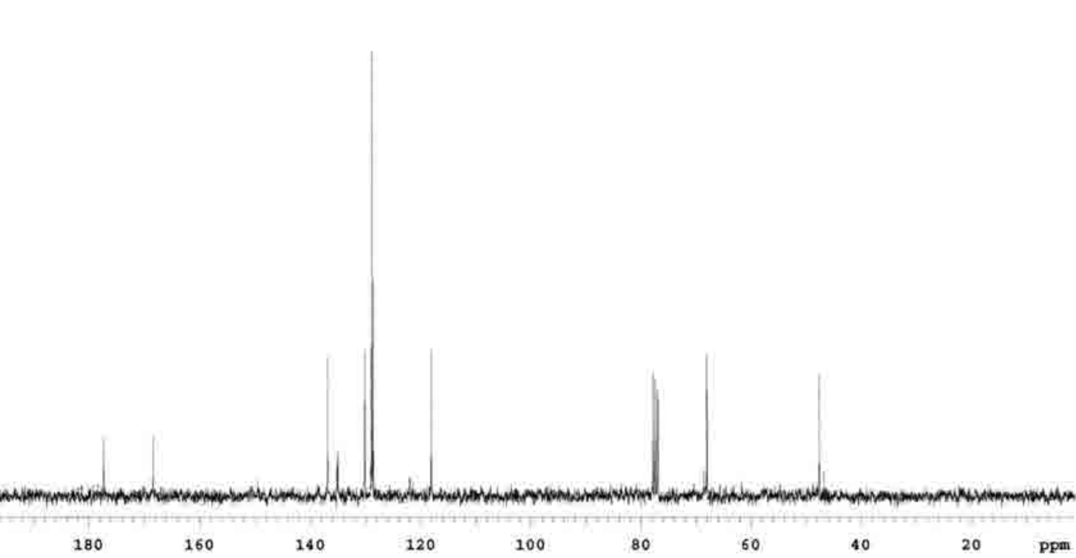
^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELI332-C13

[(Imidazole-1-carbothioyl)-amino]-acetic acid benzyl ester.

To a solution of Gly-*O*-Bn hydrochloride (**208**) (50 mg, 0.25 mmol) and triethylamine (0.05 mL) in MeCN (10 mL) at 0 ℃ was added dropwise a solution of 1,1′-thiocarbonyldiimidazole (46 mg, 0.26 mmol) in MeCN (1 mL). After 0.5h the mixture was concentrate *in vacuuo*and taken up in EtOAc (20 mL), washed with water, sat. $NaHCO_3$, brine, and dried (Na_2SO_4). The resulting oil was purified by flash chromatography to give **209** as a colourless oil (63 mg, 92%).^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.50 (s, 2H), 5.23 (s, 2H), 6.95 (s, 1H), 7.40-7.23 (m, 5H), 7.62 (s, 1H), 8.34 (s, 1H), 9.20 (br s, 1H). ^{13}CNMR ($CDCl_3$, 75 MHz): δ 177.21, 168.26, 136.77, 134.93, 130.06, 128.77, 128.51, 118.02, 67.87, 47.45. IR (Dep. $CDCl_3$): 2935, 2077 (both m), 1747 (s), 1550, 1474, 1291 (all m), 1192 (s).



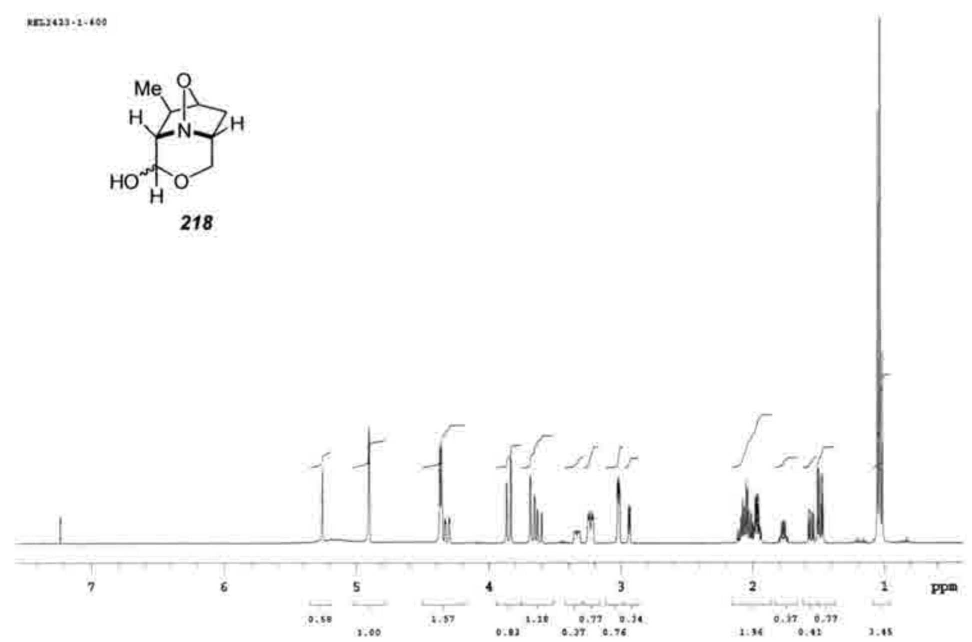
^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELII479-1



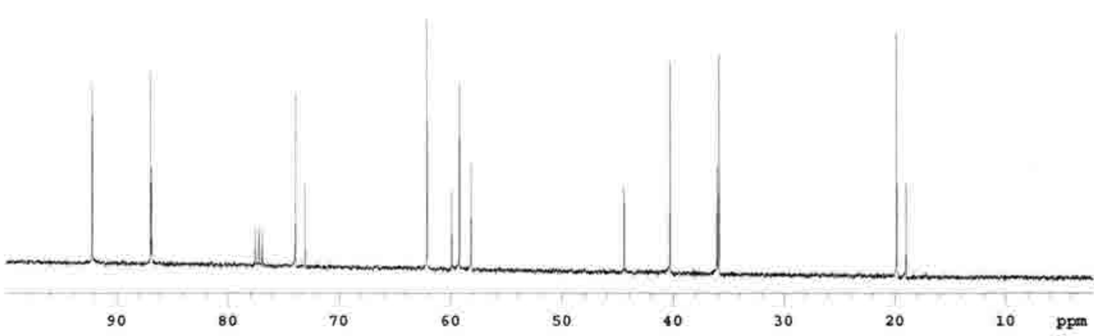
^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELI479-C13

2-Methyl-5,9-dioxa-8-aza-tricyclo[5.2.1.0^{3,8}]decan-4-ol (218).

To a solution of the isoxazolidine (167 mg, 0.99 mmol) in CH_2Cl_2 (20 mL) at –78 °C under argon was added DIBAL-H (1M / toluene, 0.99 mL, 0.99 mmol) over 0.5 h. The mixture was stirred for an additional 1 h, quenched with water (0.2 mL), allowed to warm to rt, and stirred for 2 h. The mixture was filtered through celite and concentrated. The resulting solid was recrystallized from $CHCl_3$ / pentane to give the lactol as white prisms (147 mg, 87 %). ^{1}HNMR ($CDCl_3$, 400MHz): [~2:1 mixture of anomers] δ 5.28 (s), 4.93 (d, J = 2.4 Hz), 4.39 (app d, J = 5.2 Hz), 4.34 (dd, J = 12.4, 2.0 Hz), 3.88 (dd, J = 12.8, 1.2 Hz), 3.69 (dd, J = 12.4, 1.2 Hz), 3.64 (dd, J = 12.4, 0.8 Hz), 3.35 (ddd, J = 10.8, 4.4, 2.0 Hz), 3.25 (ddd, J = 10.4, 4.4, 2.4 Hz), 3.04 (dd, J = 4.4, 2.4 Hz), 2.96 (d, J = 4.4 Hz), 2.14-2.01 (m), 1.99 (qd J = 6.8, 4.4 Hz), 1.79 (qd, J = 6.8, 4.4 Hz), 1.58 (dd, J = 11.2, 4.8 Hz), 1.51 9dd, J = 11.2, 4.8 Hz), 1.07 (d, J = 7.2 Hz), 1.05 (buried d, J = 7.2 Hz). ^{13}CNMR ($CDCl_3$ 100 MHz): [~2:1 mixture of anomers] δ 92.24, 86.99, 86.88, 73.98, 73.09, 62.12, 59.90, 59.19, 58.17, 44.47, 40.30, 36.06, 35.88, 19.87, 19.01. IR (Dep. $CDCl_3$): 3406, 3131 (br, s), 2965, 2930 (both s), 1452, 1124, 1092, 985, 710 (all m). HRMS (FAB+): Calc. For $C_8H_{14}NO_3$ [M+H]: 172.0974; Found 172.0976.



^{1}HNMR, $CDCl_3$, 400 MHz; filename: RELI423-400-1



^{13}CNMR, $CDCl_3$, 100 MHz; filename: RELI423-C13-400

7-Hydroxy-5-hydroxymethyl-8-methyl-hexahydro-imidazo[1,5-a]pyridine-3-thione (220).

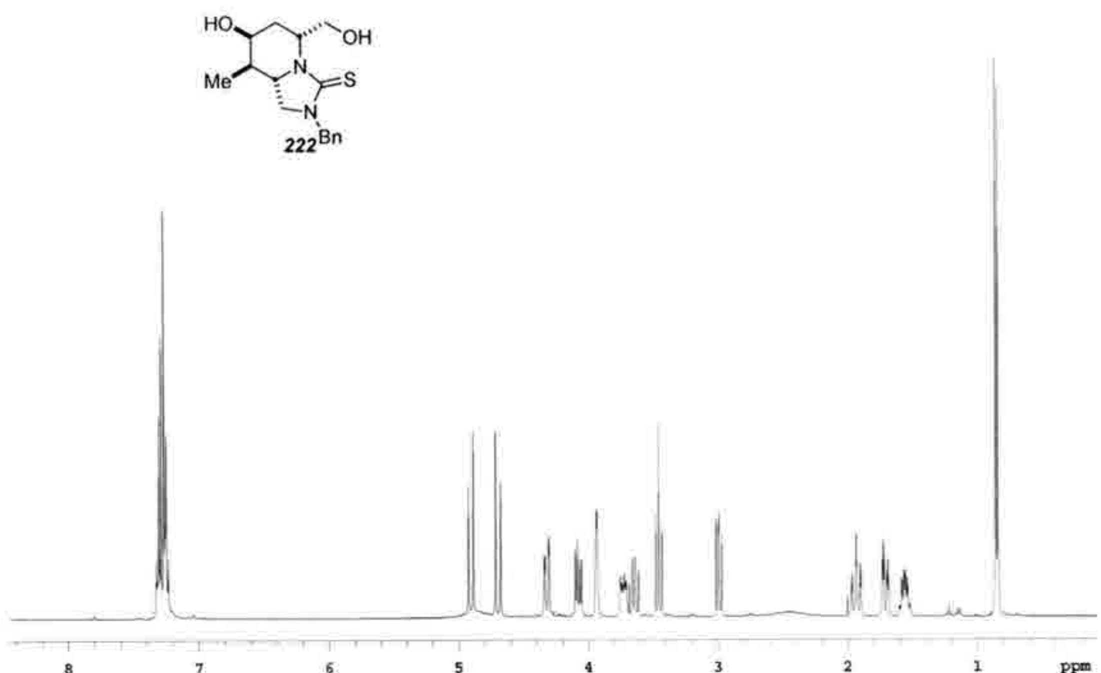
The lactol (32 mg, 0.19 mmol) was dissolved in 1:1 MeOH:NH_4OH (10 mL). 10% Pd/C (20mg) was added and the solution saturated with hydrogen. The mixture was allowed to stir under an argon atmosphere for 2d, then concentrated, taken up in MeOH (10 mL) and filtered through celite. Concentration gave 30 mg of an oily solid which was used without further purification. HRMS (FAB+): Calc. For $C_8H_{19}N_2O_2$ [M+H]: 175.1447; Found 175.1442. The residue was taken up in DMF (1 mL) and DMAP (2.5 mg, 0.02 mmol, 10mol %) was added. 1,1′ thiocarbonyldiimidazole in DMF (0.5 mL) was added over 2h. The DMF was removed under vacuum and the residue purified on silica gel (EtOAc, 5% iPrOH) to give the thiourea as a colourless oil (28 mg, 76 %). ^{1}HNMR (CD_3OD, 300MHz): δ 4.20 (dd, J = 12.9, 3.9 Hz, 1H), 4.06 (dd, J = 12.9, 5.7 Hz, 1H), 3.94 (dd, J = 3.3, 3.3 Hz, 1H), 3.82 (ddd, J = 19.2, 9.3, 0.6 Hz, 1H), 3.70 (ddd, J = 2.7, 2.7, 6.0 Hz, 1H), 3.61(dd, J = 9.6, 9.6 Hz, 1H), 3.20 (dd, J = 9.6, 8.1 Hz, 1H), 1.90 (ddd, J = 13.8, 11.4, 3.0 Hz, 1H), 1.80-1.65 (m, 2H), 0.95 (d, J = 6.6 Hz, 3H). HRMS (FAB+): Calc. For $C_9H_{17}N_2O_2$ [M+H]: 217.1011; Found 217.1015.

2-(Benzylamino-methyl)-6-hydroxymethyl-3-methyl-piperidin-4-ol (221).

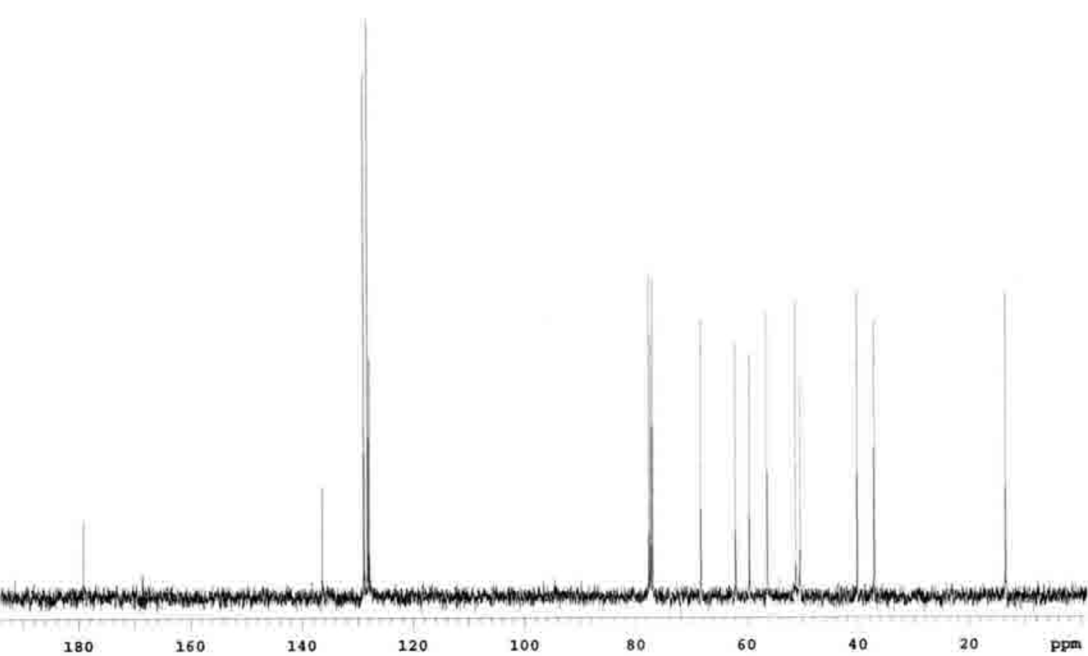
A solution of the tricyclic isoxazolidine **24** (114 mg, 0.67 mmol) and benzylamine (144 mg, 1.35 mmol. 2.0eq.) in PhMe (50 mL) was heated to 200 °C for 12h. The mixture was then concentrated and re-disolved in THF (25 mL). Lithium aluminumhydride (507 mg, 13.4 mmol, 20 eq.) was then added and the mixture refluxed for 16h. The reaction was cooled to 0 °C and quenched with 0.51 mL H_2O, 0.51 mL 10% NaOH, 1.53 mL H_2O succesively. The mixture was filtered though celite with THF and concentrated. The crude oil was purified by flash cromatography (20% MeOH/CH_2Cl_2) to give the diamine as a colourless oil (90 mg, 51%). ^{1}HNMR ($CDCl_3$, 400 MHz): 7.39-7.20 (m, 5H), 3.86 (dd, J=5.2, 2.4 Hz, 1H), 3.76 (1/2 ABq, J = 13.2 Hz, 1H), 3.69 (1/2 ABq, J = 14 Hz, 1H), 3.54 (dd, J = 11.2, 3.6 Hz, 1H), 3.37 (dd, J = 11.2, 7.2 Hz, 1H), 3.10-3.02 (m, 1H), 2.80 (dd, J = 11.6, 2.8 Hz, 1H), 2.73 (ddd, J = 8.0, 8.0, 2.8 Hz, 1H), 2.47 (dd, J = 11.6, 8.0 Hz, 1H), 1.65 (ddd, J = 14.0, 2.8, 2.8, 1H), 1.46-1.34 (m, 2H), 0.89 (d, J = 6.8 Hz, 3H).^{13}CNMR ($CDCl_3$, 100 MHz): 140.61, 128060, 128.39, 127.17, 70.51, 66.66, 54.99, 54.40, 52.293, 51.67, 38.98, 36.23, 14.47.

2-Benzyl-7-hydroxy-5-hydroxymethyl-8-methyl-hexahydro-imidazo[1,5-a]pyridine-3-thione

To a solution of the diamine 34 (13 mg, 49 μmol) in acetonitrile (1.5 mL) at 0 °C was added a solution of TCDI (9mg, 52 μmol) in acetonitrile (1 mL). After 0.5h the mixture was concentrated, taken up in EtOAc (25 mL) and washed with sat. $NaHCO_3$ and brine. The crude residue was purified by flash chromatography (1:1 hex : EtOAc) to give the thiourea 35 as a colourless oil (12 mg, 80%). ^{1}HNMR ($CDCl_3$, 300 MHz): 7.39-7.20 (m, 5H), 4.96 (1/2 ABq, J = 15.0 Hz, 1H), 4.74 (1/2 ABq, J = 15.0 Hz, 1H), 4.50-4.40 (m, 1H), 4.15-4.05 (m, 1H), 4.00 (apparent d, J = 2.7 Hz, 1H), 3.81-3.74 (m, 1H), 3.67 (dd, J = 10, 8.1 Hz, 1H), 3.50 (dd, J = 9.6, 9.6 Hz, 1H), 3.03 (dd, J = 9.6, 8.1 Hz, 1H), 2.03 (ddd, J = 14.4, 2.7, 2.4 Hz, 1H), 1.75 (ddd, J = 14.4, 2.4, 2.4 Hz, 1H), 1.66-1.58 (m, 1H), 0.90 (d, J = 6.9 Hz). HRMS (FAB+): Calc. For $C_{16}H_{23}N_2O_2S_1$[M+H]: 307.1480; Found 307.1486.



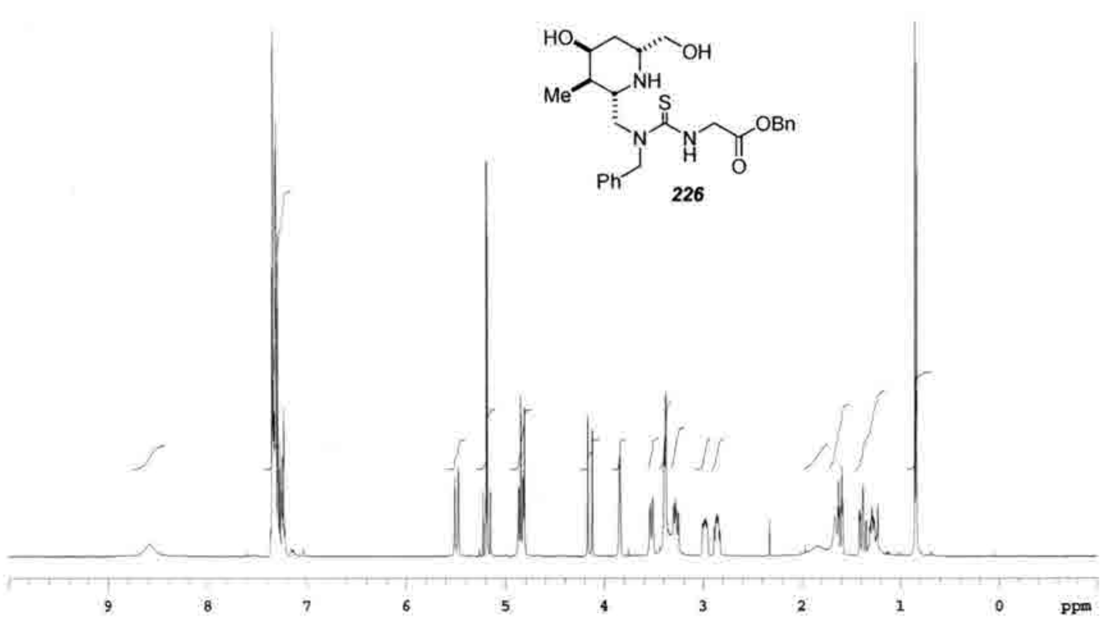
^{1}HNMR, $CDCl_3$, 400 MHz; filename: RELII397-400



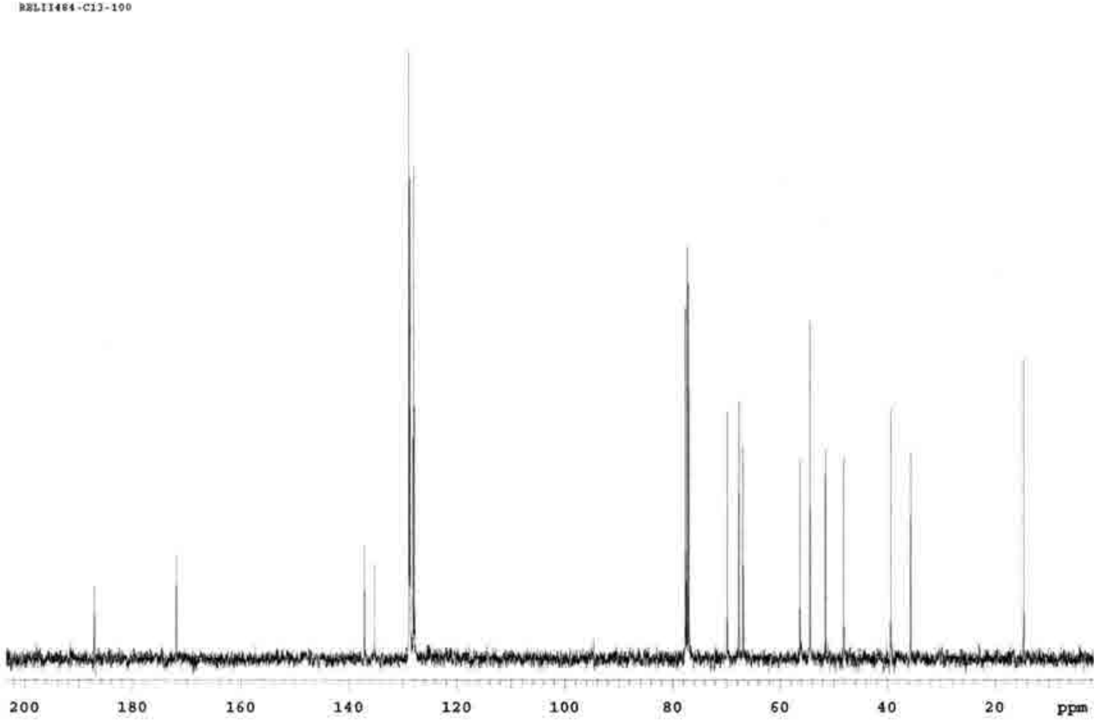
^{13}CNMR, $CDCl_3$, 100 MHz; filename: RELII397-C13-400

[3-Benzyl-3-(4-hydroxy-6-hydroxymethyl-3-methyl-piperidin-2-ylmethyl) thioureido]-acetic acid benzyl ester (226).

To a solution of the diamine (16 mg, 0.061 mmol) in MeCN (2 mL)was added the imidaloium xanthate (17 mg, 0.061 mmol). The mixture was concentrated and purified by flash chromatography (20% MeOH/CH_2Cl_2) to give the thiourea as a colourless oil (22 mg, 77%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.84 (d, J = 6.8 Hz, 3H), 1.28 (m, 1H), 1.38 (app. dd, J = 13.6, 2.0 Hz, 1H), 1.60 (ddd, 13.6, 3.2,3.2 Hz, 1H), 2.90-2.82 (m, 1H), 3.02-2.94 (m, 1H) , 3.28 (dd, J = 6.8, 11 Hz, 1H, 3.52 (dd, J = 2.4, 10 Hz, 1H), 3.82 (br d, J = *2.0 Hz, 1H),* 4.14 *(½ ABq,* J = 18 Hz, 1H), 4.81 (½ ABq, J = 15 Hz, 1H), 4.84 (½ ABq, J = 18 Hz, 1H), 5.16 (½ ABq, J = 12.4 Hz, 1H), 5.20 (½ ABq, J = 12.4 Hz, 1H), 5.50 (½ ABq, J = 15 Hz, 1H), 7.4-7.2 (m, 10H), 8.6 (br s, 1H). ^{13}CNMR ($CDCl_3$ 100 MHz): δ 187.03, 171.88, 137.11, 135.29, 128.85, 128.81, 128.68, 128.59, 127.99, 127.77, 69.76, 67.59, 66.78, 56.19, 54.32, 51.42, 48.04, 39.29, 35.61, 14.62. HRMS (FAB+): Calc. For $C_{25}H_{34}N_3O_4S_1$ [M+H]: 472.2270; Found: 472.2255.



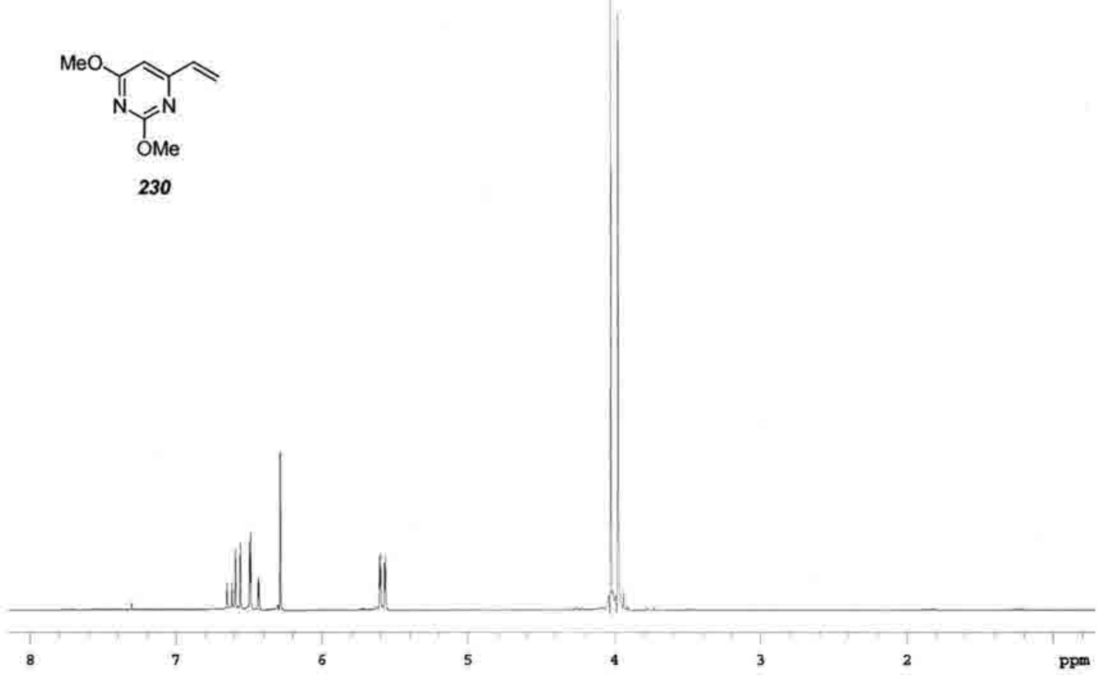
^{1}HNMR; $CDCl_3$, 400 MHz; filename: RELII484-400



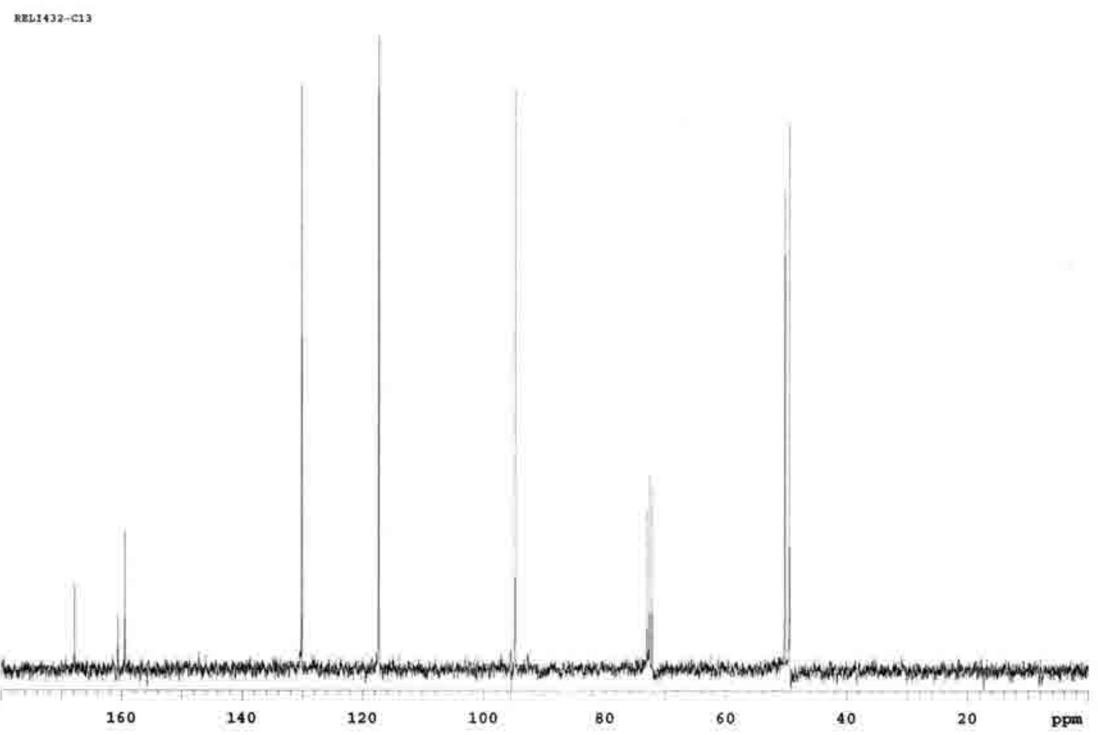
^{13}CNMR, $CDCl_3$, 100 MHz; filename: RELII484-C13-100

2,4-Dimethoxy-6-vinyl-pyrimidine (230).

To a solution of vinyl magnesium bromide (1M/THF, 9.48 mL, 9.48 mmol) in THF (20 mL) under argon at –78 °C was added zinc chloride (1M/Et_2O, 11.37 mL, 11.37 mmol). The mixture was stirred for 0.5h and then cannulated into a solution of the arylbromide (830 mg, 3.79 mmol) and $Pd(PPh_3)_4$ (44 mg, 38μmol, 1 mol%) in THF (50 mL). The mixture was stirred for 12h., diluted with ether (50 mL), washed with water, bicarb, brine, and dried (Na_2SO_4). The concentrated organics were purified by chromatography (6:1 hexanes : ethyl acetate) to give the vinyl pyrimidine as a colourless oil (572 mg, 91%). ^{1}HNMR ($CDCl_3$, 300MHz): δ 6.56 (dd, J = 17.1, 10.2 Hz, 1H), 6.42 (dd, J = 17.1, 2.1 Hz, 1H), 6.24 (s, 1H), 5.54 (dd, J = 10.4, 2.1 Hz, 1H), 3.98 (s, 3H), 3.94 (s, 3H). ^{13}CNMR ($CDCl_3$ 75 MHz): δ 172.43, 165.25, 164.08, 134.78, 121.95, 99.40, 54.78, 54.02. IR (Dep. $CDCl_3$): 2989, 2954 (both m), 1585, 1560, 1372, 1339, 1205 (all s). HRMS (FAB+): Calc. For $C_8H_{11}N_2O_2$ [M+H]: 167.0821; Found 167.0817.



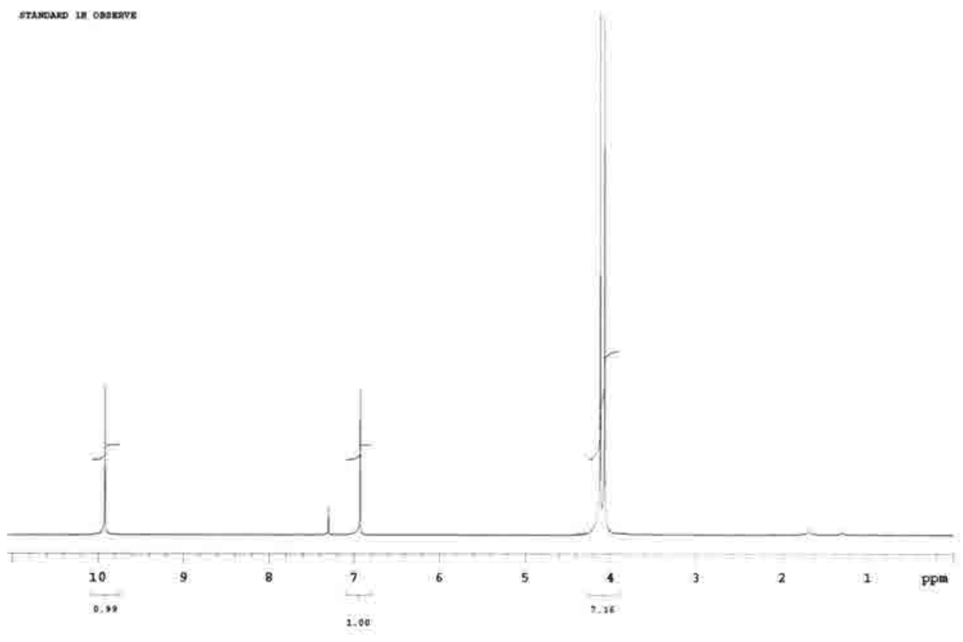
^{1}HNMR, $CDCl_3$, 300 MHz; filename RELI432-5



^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELI432-C13

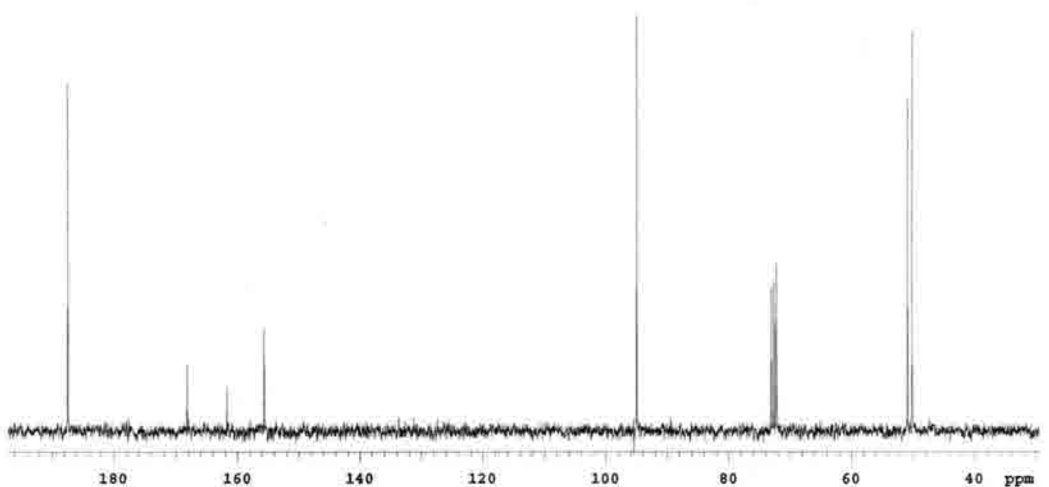
2,6-Dimethoxy-pyrimidine-4-carbaldehyde (59).

A solution of the vinyl pyrimidine (1.02 g, 6.14 mmol) in 2:1 MeOH:CH_2Cl_2 (20 mL) was cooled to –78 °C. Ozone was bubbled through the mixture until a blue colour was established. Argon was then bubbled through and Me_2S (1 mL) was added. The mixture was allowed to stir overnight and then concentrated. The residue was taken up in ether (100 mL) and washed with brine (2x50 mL). The organics were then dried (Na_2SO_4) and concentrated. The resulting solid was recrystallized from pet. ether/$CHCl_3$ to give the aldehyde as a white solid (865 mg, 84 %). ^{1}HNMR ($CDCl_3$, 300MHz): δ 9.88 (s, 1H), 6.89 (s, 1H), 4.07 (s, 3H), 4.02 (s, 3H). ^{13}CNMR ($CDCl_3$ 75 MHz): δ 192.11, 172.82, 166.33, 160.27, 99.61, 55.43, 54.72.



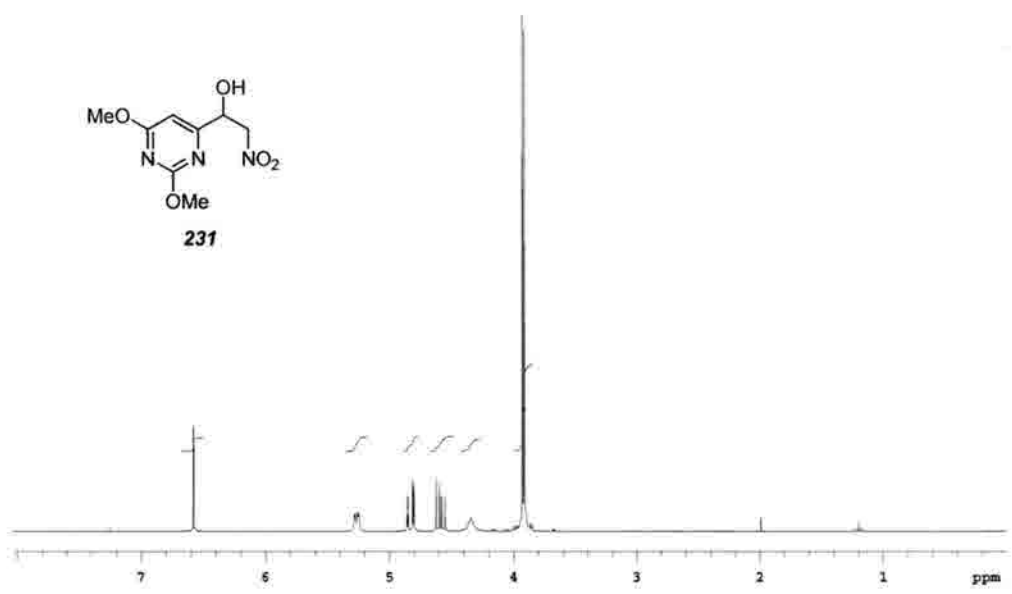
^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELI432-pyrald

REL2446-C13

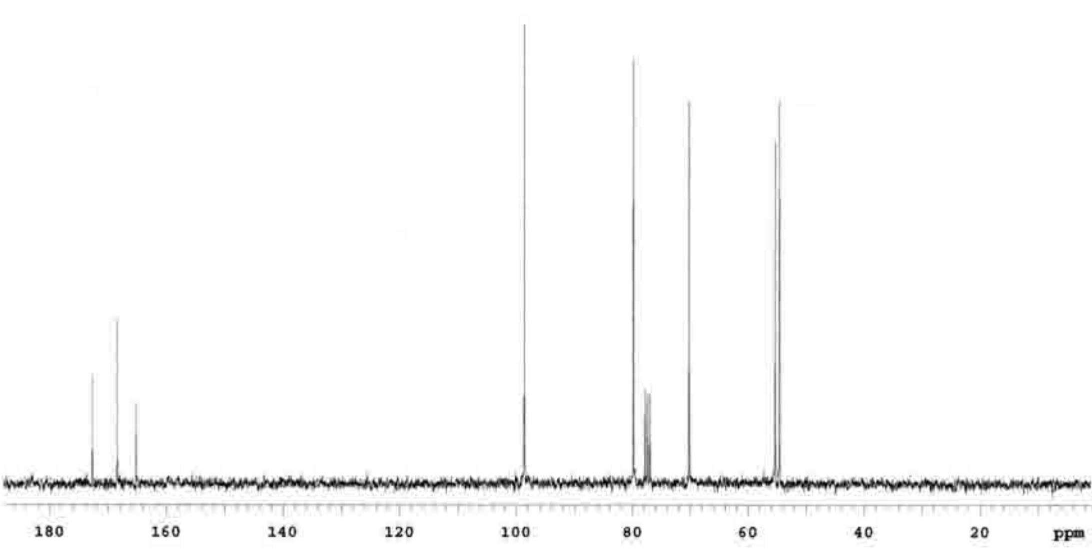


1-(2,6-Dimethoxy-pyrimidin-4-yl)-2-nitro-ethanol (231)

To a solution of triehtylamine (50 μL, 36 μmol, 10 mol%) in 5 : 1 CH_2Cl_2 : $MeNO_2$ (6 mL) at 0°C was added a solution of the aldehyde in CH_2Cl_2 (5 mL) over 2.5h. The mixture was stirred for a additional 1h, concentrated, and purified on silica gel (2 : 1 hex : EtOAc) to give the nitronol as a colourless oil (71 mg, 87 %).^{1}HNMR ($CDCl_3$, 300MHz): δ 6.58 (s, 1H), 5.29, (dd, J = 6.9, 2.4 Hz, 1H), 4.84 (dd, J = 13.5, 3.0 Hz, 1H), 4.63 (dd, J = 13.5, 8.1 Hz, 1H), 3.98 (s, 3H), 3.98 (s, 3H). ^{13}CNMR ($CDCl_3$ 75 MHz): δ 172.65, 167.81, 167.24, 98.67, 79.75, 70.10, 55.27, 54.49. IR (Dep. $CDCl_3$): 3367 (br, w), 2954 (w), 1598 (m), 1556 (s), 1376 (m), 1357 (s), 1204, 1092 (both w). HRMS (FAB+): Calc. For $C_8H_{12}N_3O_5$ [M+H]: 230.0777; Found 230.0777.



^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELI413-1



^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELI413-1-C13

2-Amino-1-(2,6-dimethoxy-pyrimidin-4-yl)-ethanol (235)

A solution of the nitronol (61 mg, 0.28 mmol) and 10% Pd/C (20 mg) in MeOH (5 mL) was placed under an atmosphere of hydrogen and stirred for 24h. The mixture was filtered through celite and concentrated. The resulting solid was triturated with diethyl ether (2 x 2 mL) to give the amino alcohol as a white solid (50 mg, 95 %). ^{1}HNMR (CD_3OD, 300MHz): δ 6.63 (s, 1H), 4.69 (dd, J = 7.8, 4.2 Hz, 1H), 3.97 (s, 6H), 3.26 (dd, J = 12.9, 3.6 Hz, 1H), 3.00 (dd, J = 12.9, 7.5 Hz, 1H). ^{13}CNMR (CD_3OD, 75 MHz): δ 173.98, 173.48, 166.52, 98.87, 72.25, 55.47, 54.75, 46.49. HRMS (FAB+): Calc. For $C_8H_{14}N_3O_3$ [M+H]: 200.1035; Found 200.1030.

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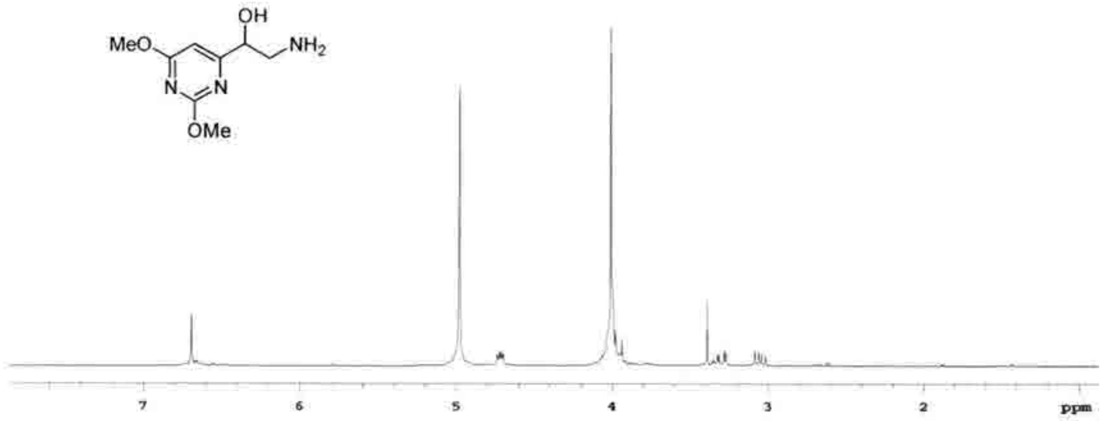
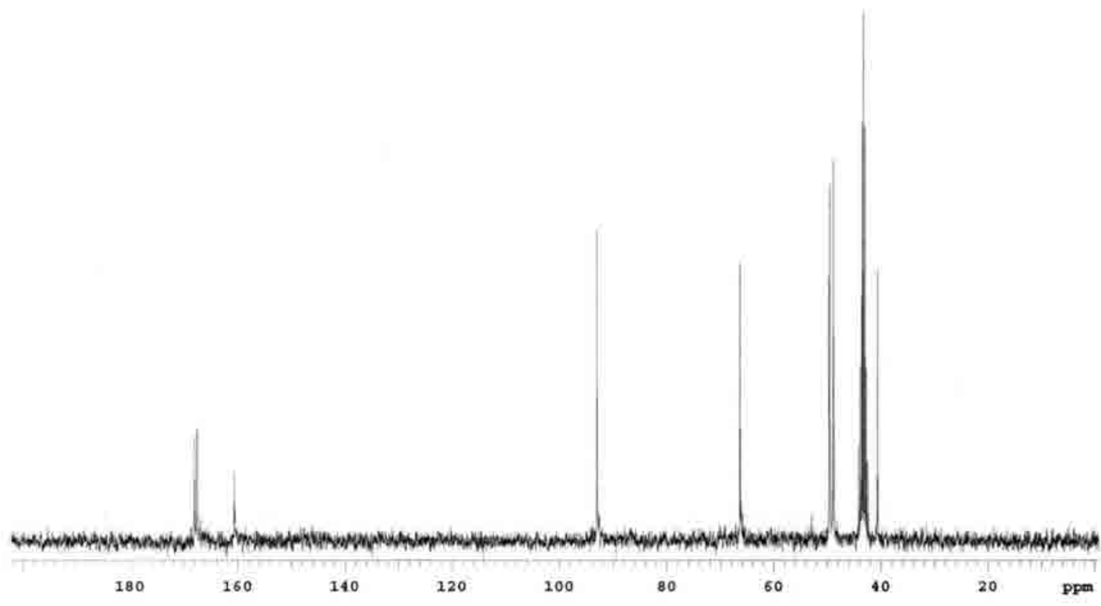


^{1}HNMR, CD_3OD, 300 MHz; filename RELII021-2

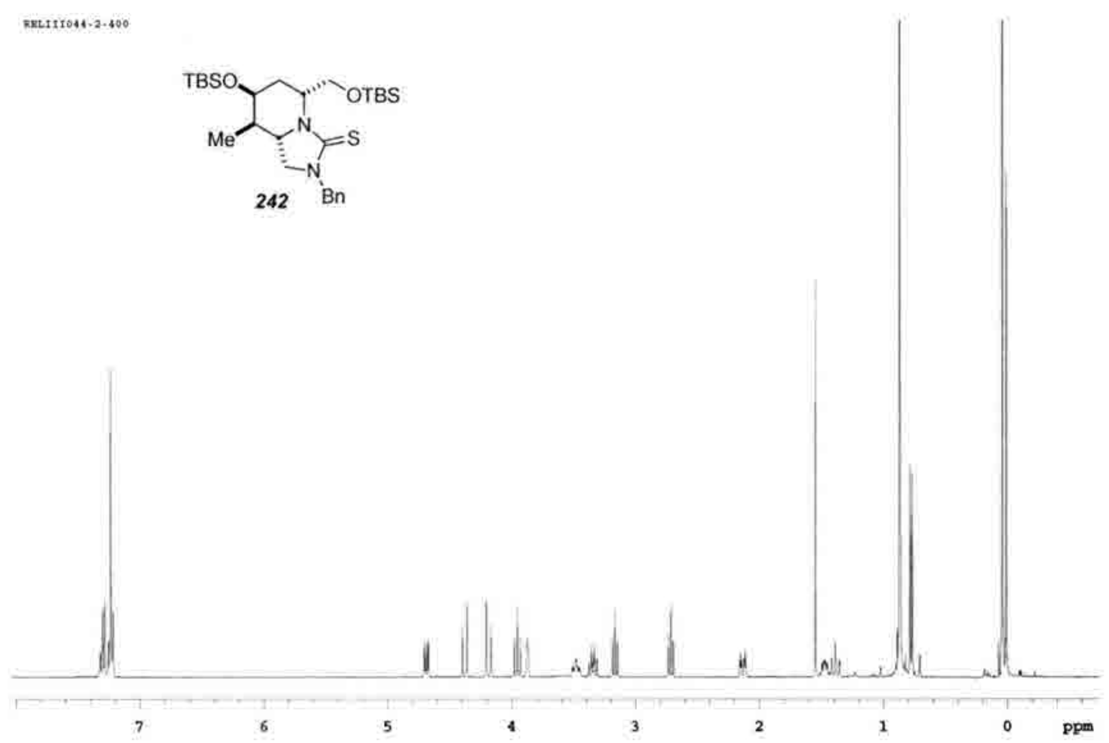


^{13}CNMR, CD_3OD, 75 MHz; filename: RELII021-C13

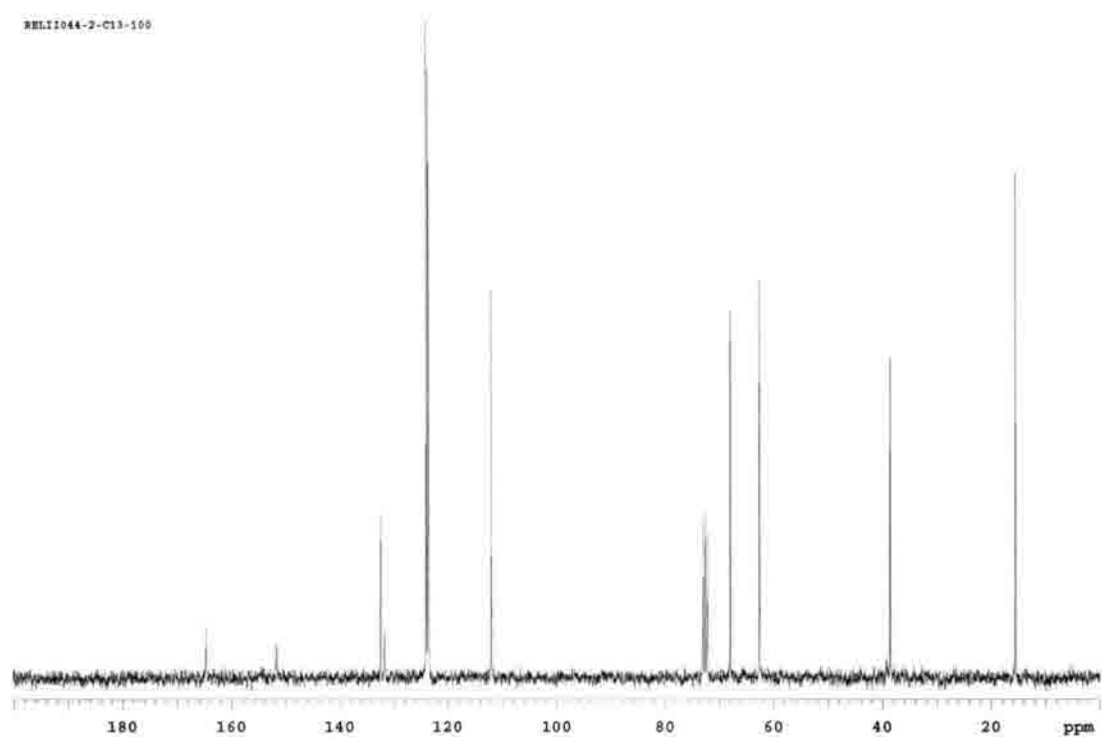
2-Benzyl-7-(tert-butyl-dimethyl-silanyloxy)-5-hydroxymethyl-8-methyl-hexahydro-imidazo[1,5-a]pyr idin-3-one (242).

To a solution of the diol in THF (3 mL) under argon at 0 °C was added NaHMDS (0.11 mL, 0.11 mmol). After 0.5h. TBSOTf (0.2 mL, 0.11 mmol) was added and the mixture stirred for an additional 2h. The reaction was quenched with sat. NH_4Cl and diluted with Et_2O (20 mL) and washed with sat. $NaHCO_3$, brine and dried (Na_2SO_4). The resultant oil was purified by flash chromatography (9:1 hexanes:EtOAc) to give XX as a film (20 mg, 77%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.01 (s, 3H), 0.04 (s, 9H), 0.78 (d, J = 6.8 Hz, 3H), 0.89 (s, 18H), 1.38 (dd, J = 2.0,12.0 Hz, 1H), 1.47 (dqd, J = 2.0, 6.8, 15 Hz, 1H), 2.13 (ddd, J = 3.6, 3.6, 14 Hz, 1H), 2.71 (dd, J = 8,8 Hz, 1H), 3.17 (dd, J = 8.0, 8.0 Hz, 1H),

3.34 (ddd, J = 8.0,8.0,10 Hz, 1H), 3.48 (m, 1H), 3.87 br s, 1H), 3.96 (dd, J = 9.6, 9.6 Hz, 1H), 4.18 (½ ABq, J = 15Hz, 1H), 4.38 ((½ ABq, J = 15Hz, 1H), 4.69 (dd, J = 3.6, 10 Ha, 1H), 7.32 (m, 5H). ^{13}CNMR ($CDCl_3$ 100 MHz): δ 160.48, 137.46, 128.44, 127.99, 127.87, 69.51, 63.24, 54.50, 51.89, 47.96, 47.38, 39.77, 37.27, 25.85, 25.76, 18.13, 18.04, 13.70, -4.51, -5.07, -5.40, -5.49. IR (Dep. $CDCl_3$): 1701 (s), 1425 (w), 1251, 1084, 837 (all m). HRMS (FAB+): Calc. For $C_{28}H_{51}N_2O_3Si_2$ [M+H]: 519.3438; Found: 519.3421.



^{1}HNMR, $CDCl_3$, 400 MHz; filename: RELII044-2-400



^{13}CNMR; $CDCl_3$, 100 MHz; filename: RELII044-2-C13-100

2-Benzyl-7-hydroxy-5-hydroxymethyl-8-methyl-hexahydro-imidazo[1,5-a]pyridin-3-one (239).

To a solution of the diamine (53 mg, 0.20 mmol) in MeCN (5 mL) at 0°C was added dropwise a solution bis-*p*-nitrophenylcarbonate (61 mg, 0.20 mmol) in MeCN (2 mL). After the addition was complete (~0.5 h) the mixture was stirred for an additional 0.5 h and concentrated. The residue was taken up in EtOAc and washed 5x 9%$NaHCO_3$, brine, and dried (Na_2SO_4). The crude residue was purified on silica gel with 10% $MeOH/CH_2Cl_2$ to give the urea as a clear oil (41 mg, 75%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.90 (d, J = 6.9 Hz, 3H), 1.57-1.44 (m, 1H), 1.63 (app d, J = 14 Hz, 1H), 1.72 (br d, J =

14 Hz, 1H), 1.97 (br s, 1H), 2.80 (dd, J = 8.4, 8.4 Hz, 1H), 3.31 (dd J = 8.4, 8.4 Hz, 1H), 3.59-3.43 (m, 2H), 3.91-3.72 (m, 3H), 3.94 (br s, 1H), 4.22 (½ABq, J = 15.9 Hz, 1H), 4.49 (½ABq, J = 15.9 Hz, 1H), 5.79 (dd, J = 9.3, 5.4 Hz, 1H), 7.4-7.2 (m, 5H). ^{13}CNMR ($CDCl_3$ 75 MHz): δ 160.78, 137.00, 128.79, 128.79, 128.17, 127.63, 68.73, 64.81, 54.43, 53.40, 48.33, 48.18, 40.11, 36.55, 13.30.

2-Benzyl-7-hydroxy-8-methyl-3-oxo-octahydro-imidazo[1,5-a]pyridine-5- carbaldehyde (255).

HO, Me, OH, N, O, N, Bn, **239** → HO, Me, O, H, N, O, N, Bn, **255**

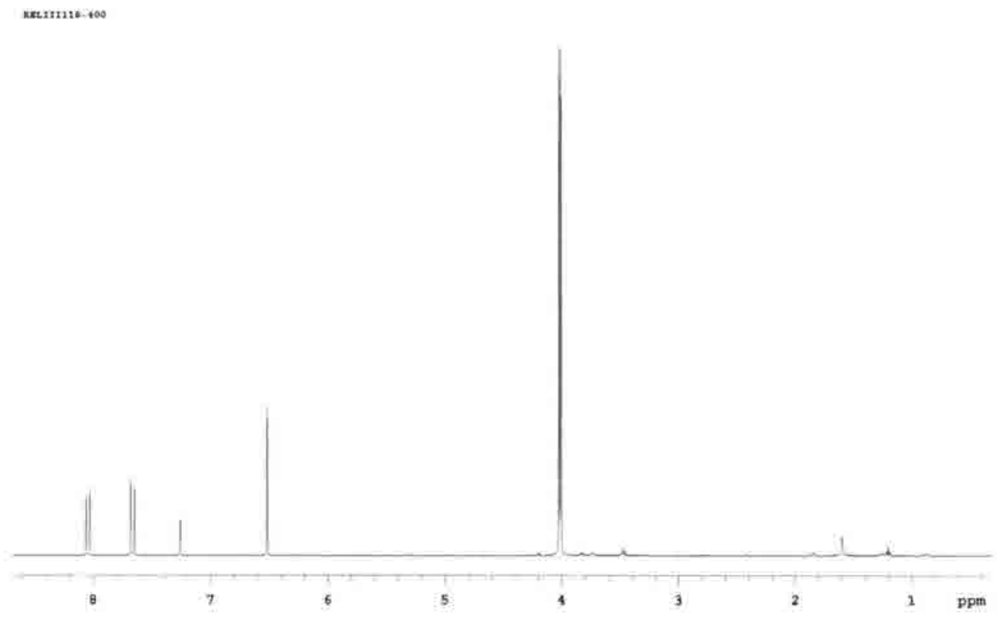
To a solution of the diol (70 mg, 0.24 mmol) in $CHCl_3$ (2 mL, freshly distilled from $CaSO_4$) was added TEMPO (15 mg, 0.096 mmol), $PhI(OAc)_2$ (116 mg, 0.36 mmol), and MsOH (0.23 mg, 2.4 μmol). The mixture was stirred for 12h then diluted with EtOAc and the organics washed with sat. $Na_2S_2O_3$, sat. $NaHCO_3$, brine, and dried (Na_2SO_4). The crude mixture was purified on silica gel eluting with EtOAc / 5% iPrOH to give the aldehyde (51 mg, 73%) as a colourless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (d, J = 6.8 Hz, 3H), 1.55 (m, 1H), 1.65 (dd, J = 12.4, 12.4 Hz, 1H), 1.89 (br d, J = 12.4 Hz, 1H),

2.87 (dd, $J = 8.8, 8.8$ Hz, 1H), 3.29 (dd, $J = 8.8, 8.8$ Hz, 1H), 3.42 (ddd, $J = 10.4, 8.8, 8.8$ Hz, 1H), 3.85 (dd, $J = 12.2, 2.5$ Hz, 1H), 4.01 (br s, 1H), 4.24 (½ABq, $J = 15.6$ Hz, 1H), 4.42 (½ABq, $J = 15.6$ Hz, 1H), 7.4-7.2 (m, 5H), 9.82 (d, $J = 2.5$ Hz, 1H). ^{13}CNMR ($CDCl_3$ 100 MHz): δ 198.42, 160.66, 136.82, 128.81, 128.21, 127.72, 68.16, 57.38, 53.35, 48.05, 47.94, 38.36, 32.90, 13.32.

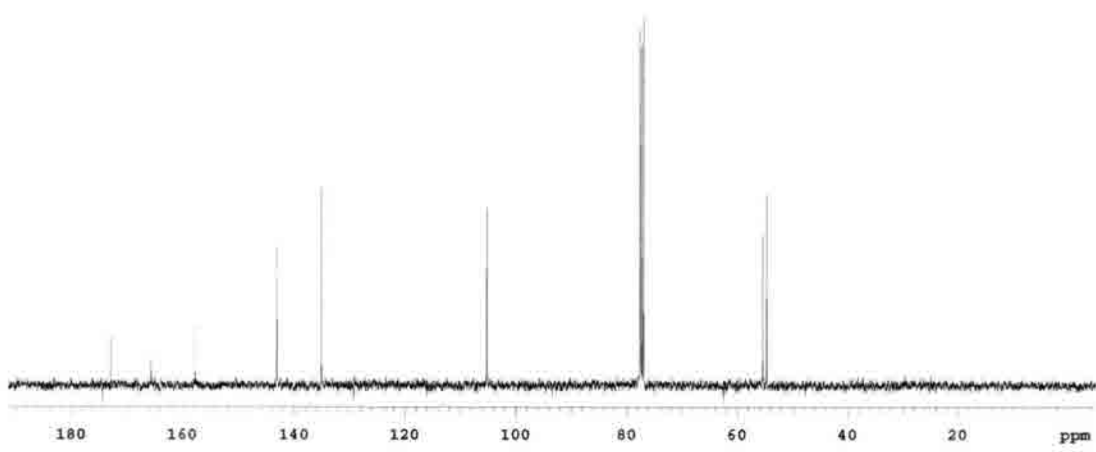
(E)-2,4-dimethoxy-6-(2-nitrovinyl)pyrimidine (271).

A solution of oxallyl chloride (120 μL, 1.43 mmol) in CH_2Cl_2 (5 mL) under argon was cooled to -78 °C. DMSO (150 μL, 1.43 mmol) was added, and the mixture stirred for 15 min. A solution of the nitroalcohol **231** (164 mg, 0.716 mmol) in CH_2Cl_2 (1 mL) was added dropwise over 5 min. After 30 min. Et_3N (0.50 mL, 3.6 mmol) was added and the reaction warmed to rt. After 30 min sat. NH_4Cl (3 mL) was added. The mixture was diluted with Et_2O (20 mL) and the organics washed 1 x 10% HCl, 1 x sat. $NaHCO_3$, brine, and dried (Na_2SO_4). The crude mixture was purified on silica eluting with 3:1

hexanes:EtOAc to give the nitroalkene **271** as a white solid (139 mg, 92%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.03 (d, J = 13.2 Hz, 1H), 7.65 (d, J = 13.2 Hz, 1H), 6.52 (s, 1H), 4.03 (s, 3H), 4.01 (s, 3H). ^{13}CNMR ($CDCl_3$ 100 MHz): δ 172.88, 165.67, 157.78, 143.14, 135.11, 105.21, 55.34, 54.61.



^{1}HNMR, $CDCl_3$, 400 MHz; filename: RELIII118-400



^{13}CNMR, $CDCl_3$, 100 MHz, filename: RELIII118-C13-400

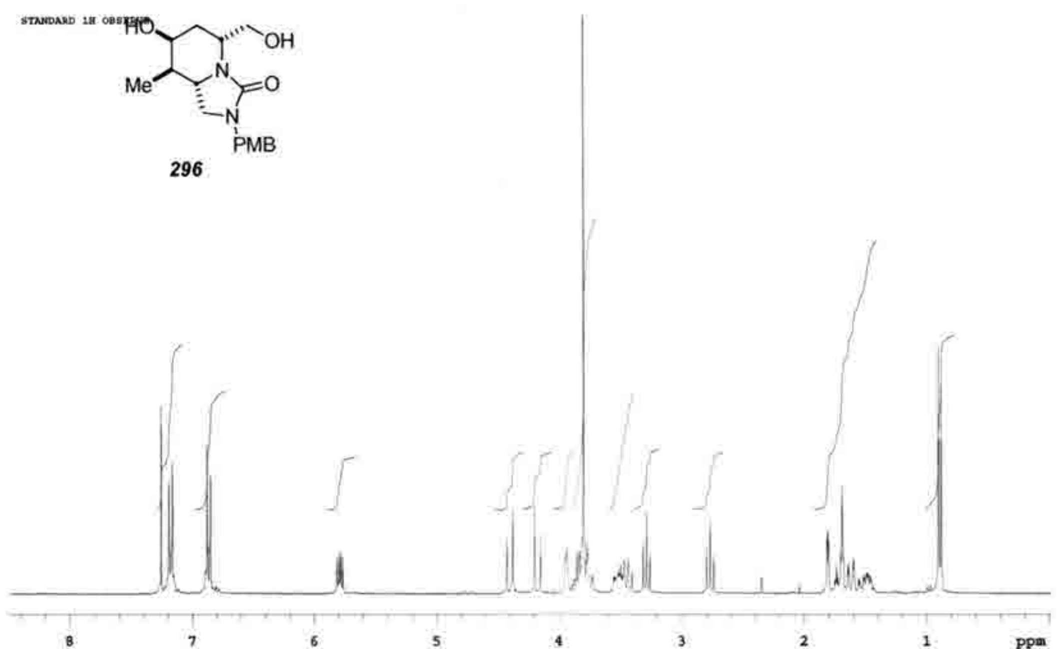
2,4-Dimethoxy-6-(2-nitro-ethyl)-pyrimidine (257).

MeO NO2 N N OMe 271 → MeO N N NO2 OMe 257

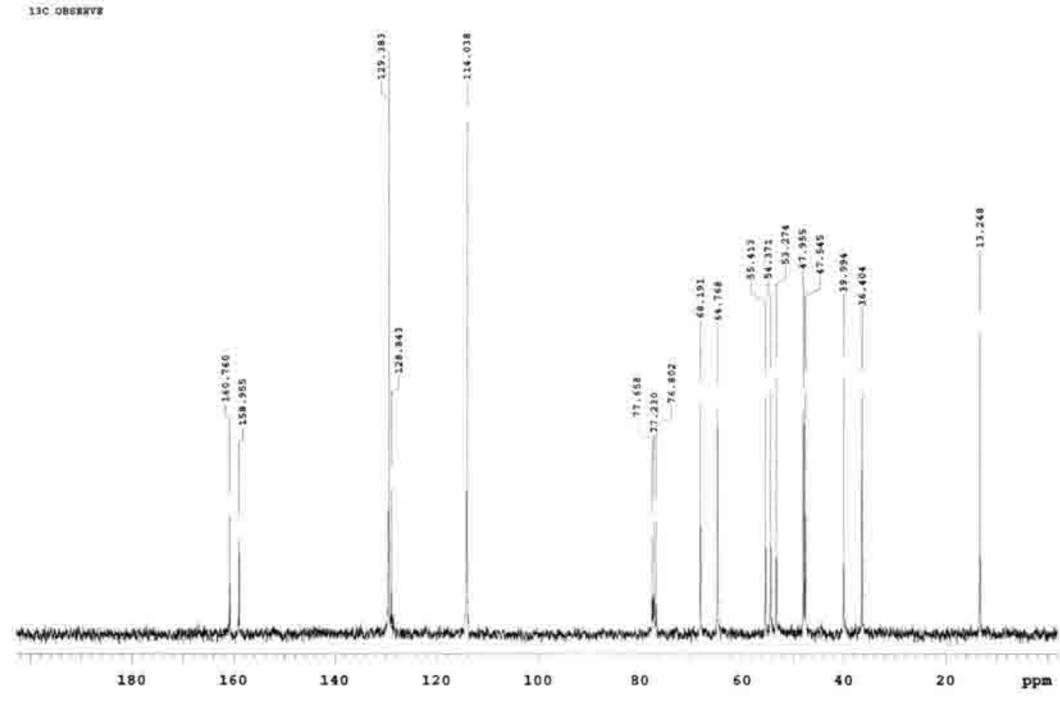
To a suspension of $NaBH_4$ (5 mg, 0.073 mmol) in dioxane (1 mL) and EtOH (0.3 mL) was added dropwise a solution of the nitroalkene (7 mg, .033mmol) in dioxane (1 mL). After stirring for 1 h the excess borohydride was quenched by the adition of AcOH. The mixture was concentrated, taken up in Et_2O and washed with sat. $NaHCO_3$, brine, and dried Na_2SO_4. The mixture was purified on silica gel with 6:1 hexanes : EtOAc to give the nitroalkane (5 mg, 71%) as a clear oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.25 (t, J = 6.4 Hz, 2H); 3.92 (br s, 6H); 4.80 (t, J = 6.4 Hz, 2H); 6.25 (s, 1H). ^{13}CNMR ($CDCl_3$ 100 MHz): δ 172.26; 166.27; 165.58; 100.87; 72.45; 55.00; 54.11; 33.63. IR (dep. $CDCl_3$) 1597, 1569, 1555 (all s); 1462 (m); 1363 (s). HRMS (FAB+): Calc. For $C_8H_{12}N_3O_4$ [M+H]: 214.0827; Found: 217.0824.

7(S)-Hydroxy-5(R)-hydroxymethyl-2(S)-(4-methoxy-benzyl)-8(S)-methyl-hexahydro-imidazo[1,5-a]pyridin-3-one (296).

To a solution of the lactol (15 mg, 0.88 mmol) in EtOAc (3 mL) was added *p*-methoxybenzyl amine (17 mg, 0.12 mmol). The solution was degassed with argon and then 10% Pd/C (15 mg) was added. The solution was then purged with H_2 and stirred under a hydrogen atmosphere for 12 h. The mixture was filtered and concentrated. The crude oil was dissolved in MeCN (5 mL) and cooled to 0 °C. A solution of *bis-p*-nitrophenyl carbonate (32 mg, 0.11 mmol) in MeCN (5 mL) was added dropwise over 15 min. After stirring an additional 0.5 h the mixture was concentrated, taken up in EtOAc (20 mL) and the organics washed 3 x 9% Na_2CO_3, 1 x brine and dried (Na_2SO_4). The crude material was purified on silica gel eluting with EtOAc / 5% *i*PrOH to give the urea as a clear oil (19 mg, 67 %). $[\alpha]^{25}_{D} = +37.7°$ (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.18 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 5.80 (dd, J = 9, 5 Hz, 1H), 4.4 (1/2 ABq, J = 15 Hz, 1H), 4.19 (1/2 ABq, J = 15 Hz, 1H), 3.94 (br dd, J = 2.4, 2.4 Hz, 1H), 3.90-3.72 (buried m, 3H), 3.80 (s, 3H), 3.51 (dddd, J = 9, 5, 3,3 Hz, 1H), 3.45 (ddd, J = 10, 9, 9 Hz, 1H), 3.28 (dd, J = 9, 9 Hz, 1H), 2.76 (dd, J = 9, 9, Hz, 1H), 1.82 (d, J = 3 Hz, 1H), 1.72 (ddd, J = 14, 3, 3 Hz, 1H), 1.62 (ddd, J = 12, 12, 2 Hz, 1H), 1.48 (ddd, J = 14, 6, 3 Hz, 1H), 0.89 (d, J = 6 Hz, 3H). ^{13}CNMR ($CDCl_3$ 75 MHz): δ 160.76, 158.96, 129.38, 114.04, 68.19, 64.77, 55.41, 54.37, 53.27, 47.96, 47.55, 39.99, 36.40. IR (Dep. $CDCl_3$): 3385, 2933 (both m), 1664, 1513, 1246 (all s). HRMS (FAB+): Calc. for $C_{17}H_{24}N_2O_4$ [M+H]: 322.1814; Found: 321.1811.



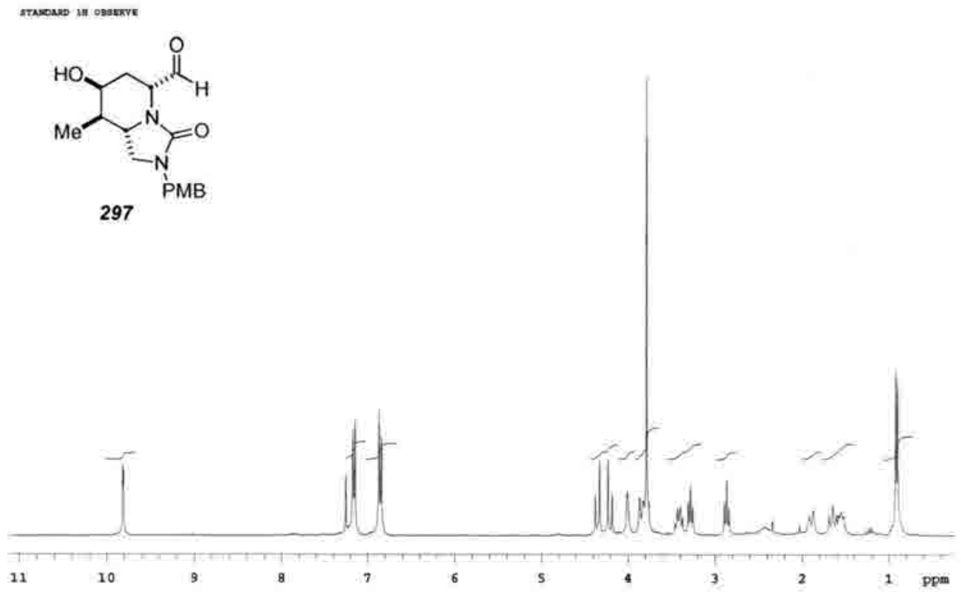
^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELIII474-1



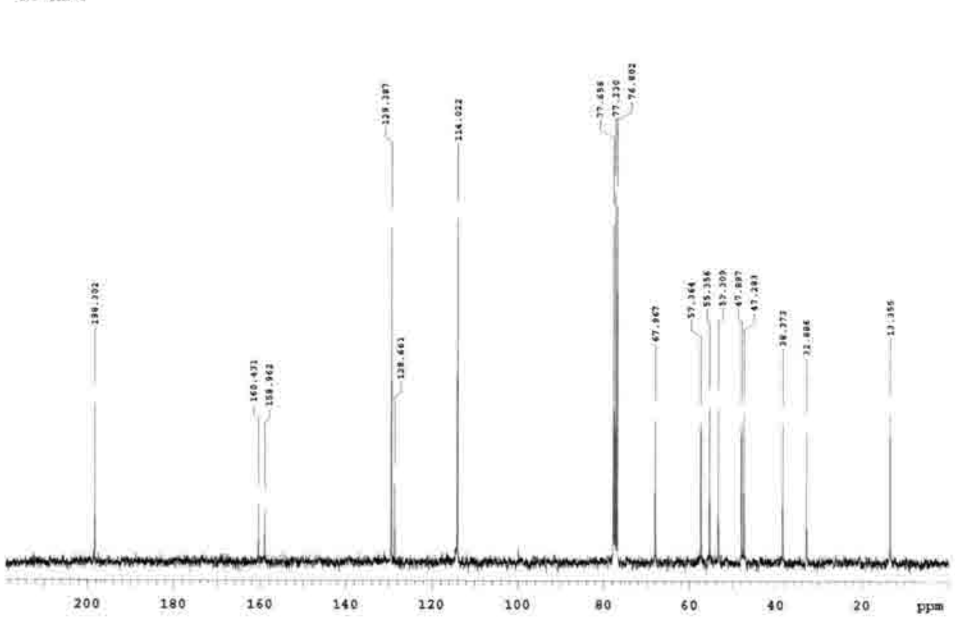
^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELIII471-C13

7(S)-Hydroxy-2(S)-(4-methoxy-benzyl)-8(S)-methyl-3-oxo-octahydro-imidazo[1,5-a]pyridine-5(R)-carbaldehyde

To a solution of the diol (211 mg, 0.66 mmol) in $CDCl_3$ (3 mL) was added $PhI(OAc)_2$ (318 mg, 0.99 mmol) and TEMPO (41 mg, 0.26 mmol). Methane sulfonic acid (0.63 mg, 7 μmol, 1 mol%) was then added as a solution in $CDCl_3$. The mixture was stirred for 3h, diluted with EtOAc (30 mL) and the organics washed with sat. $Na_2S_2O_3$, sat. $NaHCO_3$, brine, and dried (Na_2SO_4). The resulting oil was purified on silica gel eluting with EtOAc / 5% *i*PrOH) to give the aldehyde as a white foam (156 mg, 75%). $[\alpha]^{25}_{D} = + 84.8°$ (*c* 1.13, $CHCl_3$) ^1H NMR ($CDCl_3$, 300 MHz): δ 9.81 (d, *J* = 2.1 Hz, 1H), 7.16 (d, *J* = 8.1 Hz, 2H), 6.86 (d, *J* = 8.1, 2H), 4.36 (1/2 ABq, *J* = 15 Hz, 1H), 4.20 (1/2 ABq, *J* = 15 Hz, 1H), 4.00 (br s, 1H), 3.82 (buried m, 1H), 3.79 (s, 3H), 3.40 (ddd, *J* = 10.5, 9, 9 Hz, 1H), 3.28 (dd, *J* = 9, 9 Hz, 1H), 2.86 (dd, *J* = 9, 9 Hz, 1H), 1.90 (br d, *J* = 13.5 Hz, 1H), 1.64 (dd, *J* = 12, 12 Hz, 1H), 1.54 (br dd, *J* = 9, 9 Hz, 1H), 0.90 (d, *J* = 6.6 Hz). ^{13}CNMR ($CDCl_3$ 75 MHz): δ 198.30, 160.43, 158.96, 129.39, 128.66, 114.02, 67.97, 57.36, 55.36, 53.31, 47.89, 47.28, 38.37, 32.89, 13.36. IR (Dep. $CDCl_3$): 3431, 2878 (both m), 1727, 1682, 1513, 1448, 1246 (all s). HRMS (FAB+): Calc. for $C_{17}H_{22}N_2O_4$ [M+H]: 319.1657; Found: 319.1664.



^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELIII475-1

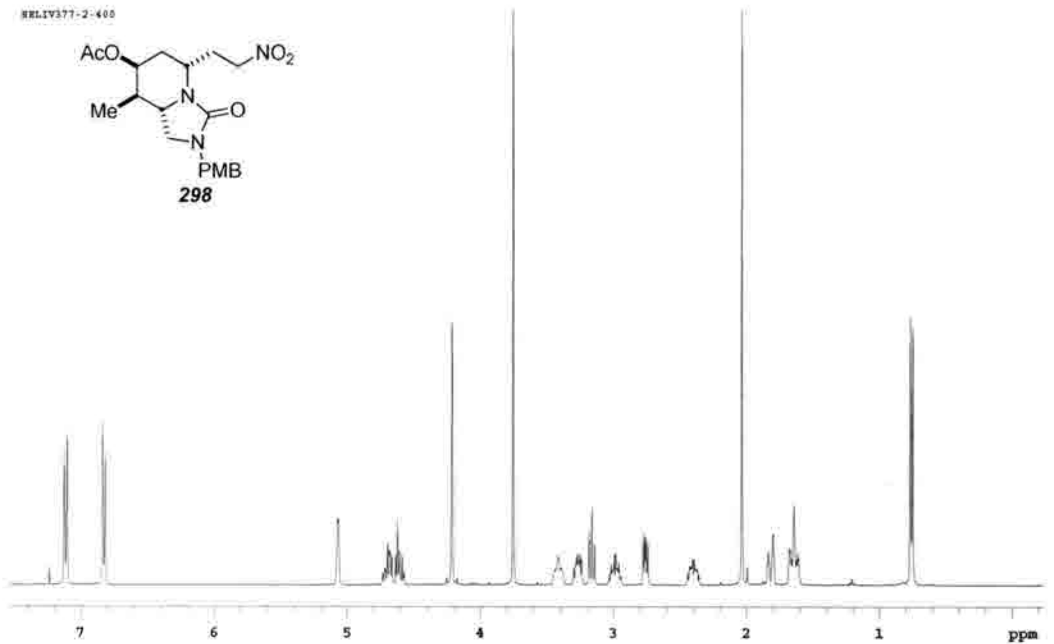


^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELIII475-C13

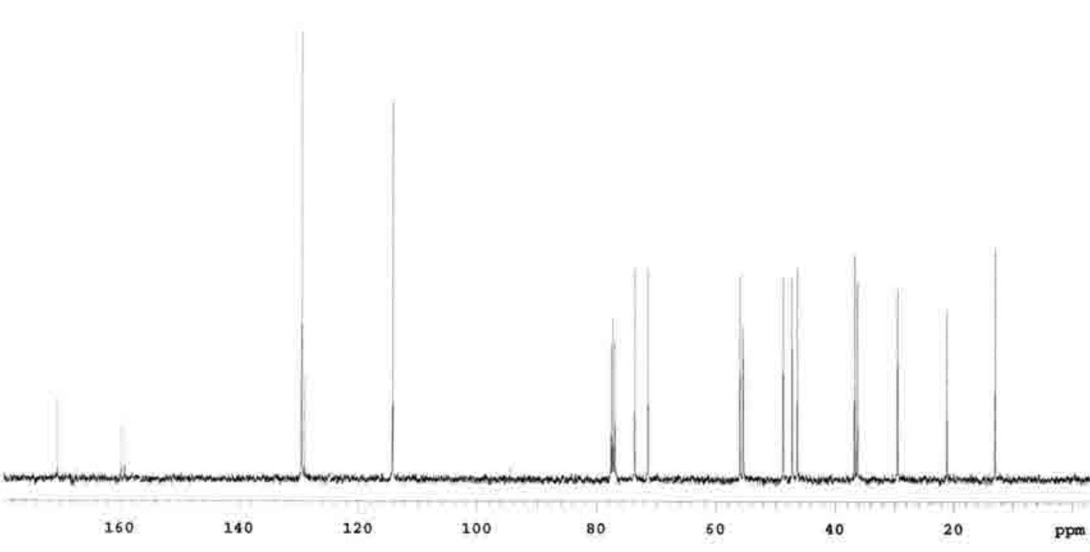
Acetic acid 2-(4-methoxy-benzyl)-8-methyl-5-(2-nitro-ethyl)-3-oxo-octahydro-imidazo[1,5-a]pyridin-7-yl ester (298).

A solution of nitromethane in THF (10 : 1, 20 mL) under argon was cooled to 0 °C. A 1.6 M solution of *n*BuLi (3.5 mL, 5.66 mmol) was added slowly (*caution!* highly exothermic) over 20 min. The mixture was stirred an additional 15 min and a solution of the aldehyde (180 mg, 0.57 mmol) in THF added. The reaction was allowed to proceed for 12 h, quenched with sat. NH_4Cl and extracted 3 x 10 mL EtOAc. The combined organics were washed brine and dried (Na_2SO_4). The crude oil was purified on silica eluting with 1 : 1 hexanes : EtOAc then EtOAc / 5% *i*PrOH to give the diastereomeric nitroalcohols (183 mg, 84%). To a solution of these nitroalcohols (41 mg, 0.11 mmol) and *N,N*-dimethylaminopyridine (3 mg, 0.025 mmol, 20 mol%) in CH_2Cl_2 under an argon atmosphere was added acetic anhydride (0.10 mL, 1.1 mmol). After stirring for 12 h the mixture was concentrated, taken up in EtOH (3 mL) and added dropwise to a slurry of $NaBH_4$ (101 mg, 2.67 mmol) in EtOH (5 mL). The mixture was stirred for 2 h and quenched by the addition of 50% AcOH / H_2O (0.4 mL). The mixture was concentrated under reduced pressure and partitioned between H_2O and EtOAc. The aqueous phase was extracted again with EtOAc and the combined organics washed with sat. $NaHCO_3$, brine, and dried (Na_2SO_4). The crude oil was purified on silical gel eluting with 1:1 hexanes : EtOAc to give the nitroalkane as a colourless oil (40 mg, 87%). $[\alpha]^{25}_{D} = +15.2°$ (*c* 1.00, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.12 (d, *J* = 8 Hz, 2H), 6.87 (d, *J* = 8 Hz, 2H), 5.12 (br d, *J* = 6.8, 3 Hz, 1H), 4.72 (ddd, *J* = 13.6, 8.4, 5.6 1H), 4.61 (ddd, *J* = 13.6, 5.6,

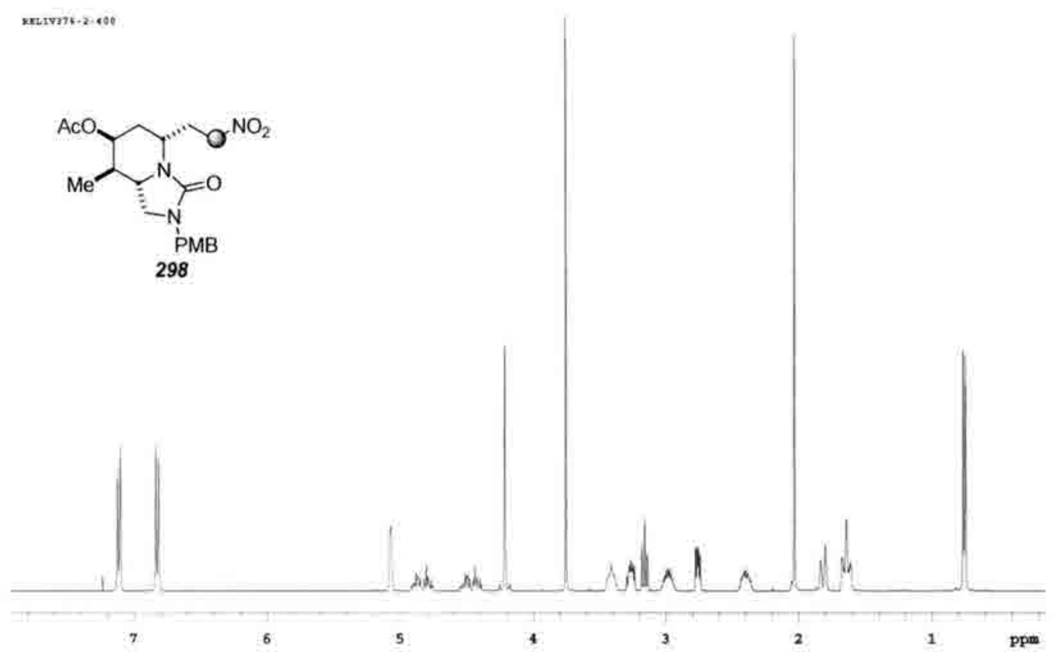
5.6 Hz), 4.23 (s, 2H), 3.78 (s, 3H), 3.43 (dddd, *J* = 10.8, 10.8, 3, 3 Hz, 1H), 3.28 (ddd, *J* = 9, 8, 5.6 Hz, 1H), 3.18 (dd, *J* = 8, 8 Hz, 1H), 2.78 (dd, *J* = 8, 5 Hz, 1H), 2.41 (dd, *J* = 13.6, 8, 5, 5 Hz, 1H), 2.05 (s, 3H), 1.83 (ddd, *J* = 12, 3, 3 Hz, 1H), 1.70-1.60 (m, 2H), 0.78 (d, *J* =6.8 Hz, 3H). ^{13}CNMR ($CDCl_3$, 100 MHz) δ 170.36, 159.72, 159.07, 129.46, 128.99, 114.17, 73.68, 71.46, 56.06, 55.50, 48.81, 47.34, 46.45, 36.83, 36.40, 29.63, 21.34, 13.28. IR (Dep. $CDCl_3$): 2937 (m), 1737, 1693, 1550, 1513 (all s), 1442, 1374, 1351 (all m), 1242 (s). HRMS (FAB+): Calc. for $C_{20}H_{28}N_3O_6$ [M+H]: 406.1978; Found: 406.1969.



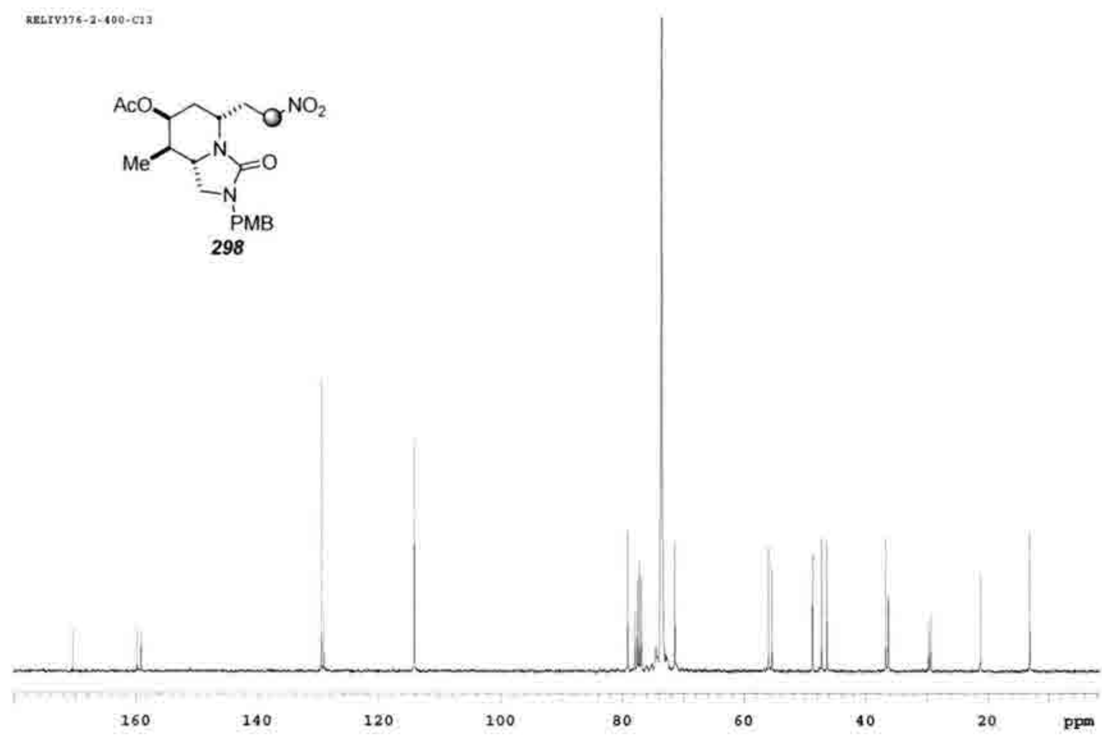
^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELIV377-2-400



^{13}CNMR, $CDCl_3$, 100 MHz; filename: RELIV377-2-400-C13



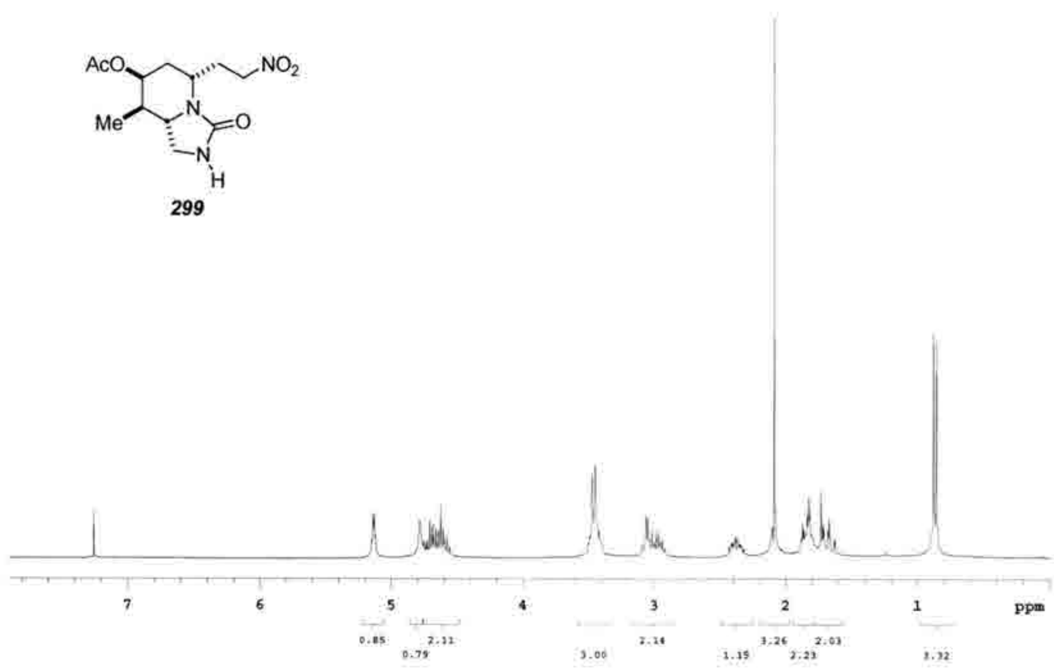
^{1}HNMR, $CDCl_3$, 400 MHz; filename: RELIV376-2-400



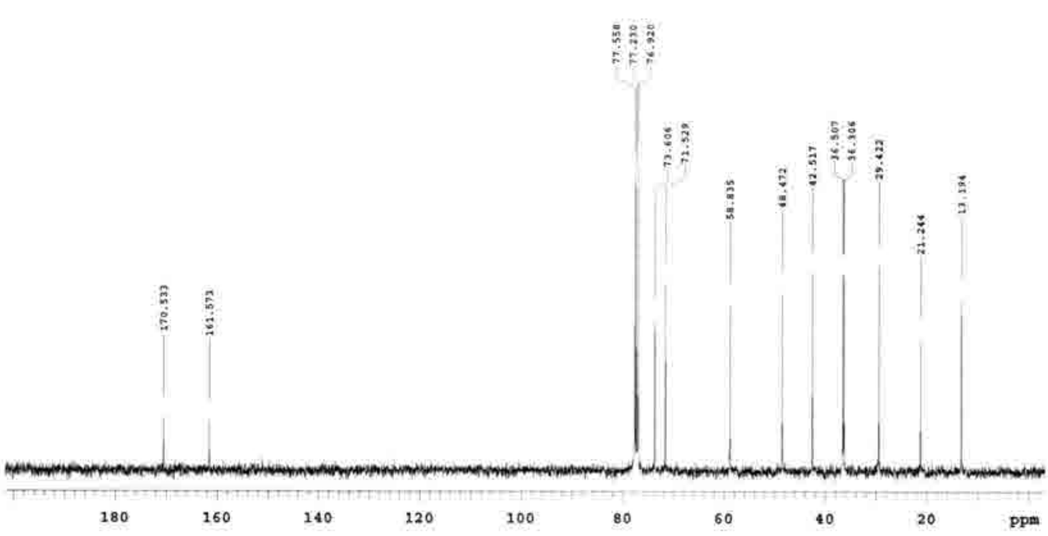
^{13}CNMR, $CDCl_3$, 100 MHz, filename: RELIV376-2-400-C13

Acetic acid 8-methyl-5-(2-nitro-ethyl)-3-oxo-octahydro-imidazo[1,5-a]pyridin-7-yl ester (299).

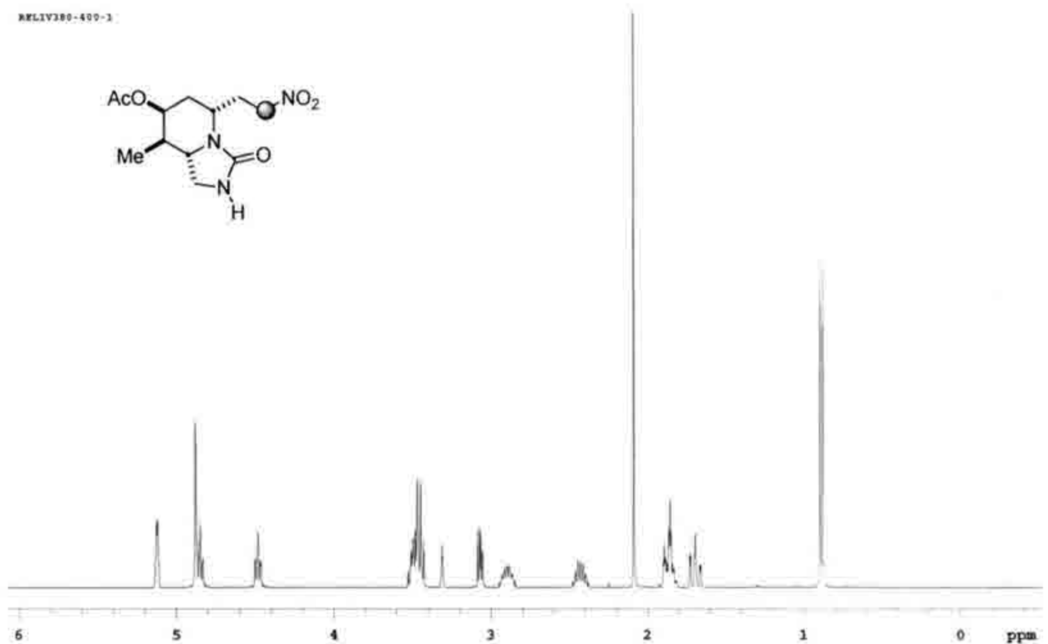
The protected urea (25 mg, 0.062 mmol) was dissolved in trifluoroacetic acid (1.5 mL). The mixture was refluxed for 1 h and concentrated under reduced pressure. The purple residue was taken up in EtOAc (10 mL) and washed 1 x H_2O, 1 x sat. $NaHCO_3$, 1 x brine, and dried (Na_2SO_4). The crude residue was purified on silica gel eluting with EtOAc / 5% *i*PrOH to give the urea (14 mg, 80%) as a white solid. $[\alpha]^{25}_{D} = +17.3°$ (*c* 1.00, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz): δ 5.14 (dd, *J* = 6, 3 Hz, 1H), 4.80 (br s, 1H), 4.76-4.54 (m, 2H), 3.52-3.38 (m, 3H), 3.1-2.9 (m, 2H), 2.37 (dddd, *J* = 15, 6, 6, 3 Hz, 1H), 2.09 (s, 3H), 1.86 (ddd *J* = 12, 3, 3 Hz, 1H), 1.84-1.78 (buried m, 1H), 1.66 (ddd, *J* = 12, 3, 3 Hz, 1H), 0.87 (d, *J* = 6 Hz, 3H). ^{13}CNMR ($CDCl_3$ 100 MHz): δ 170.53, 161.57, 73.61, 71.53, 58.84, 48.47, 42.52, 36.51, 36.61, 29.42, 21.24, 13.19. IR (Dep. $CDCl_3$): 3269, 2939 (both w), 1736, 1698, 1550 (all s), 1436, 1374 (both m), 1242 (s). HRMS (FAB+): Calc. for $C_{12}H_{20}N_3O_5$ [M+H]: 286.1403; Found: 286.1409.



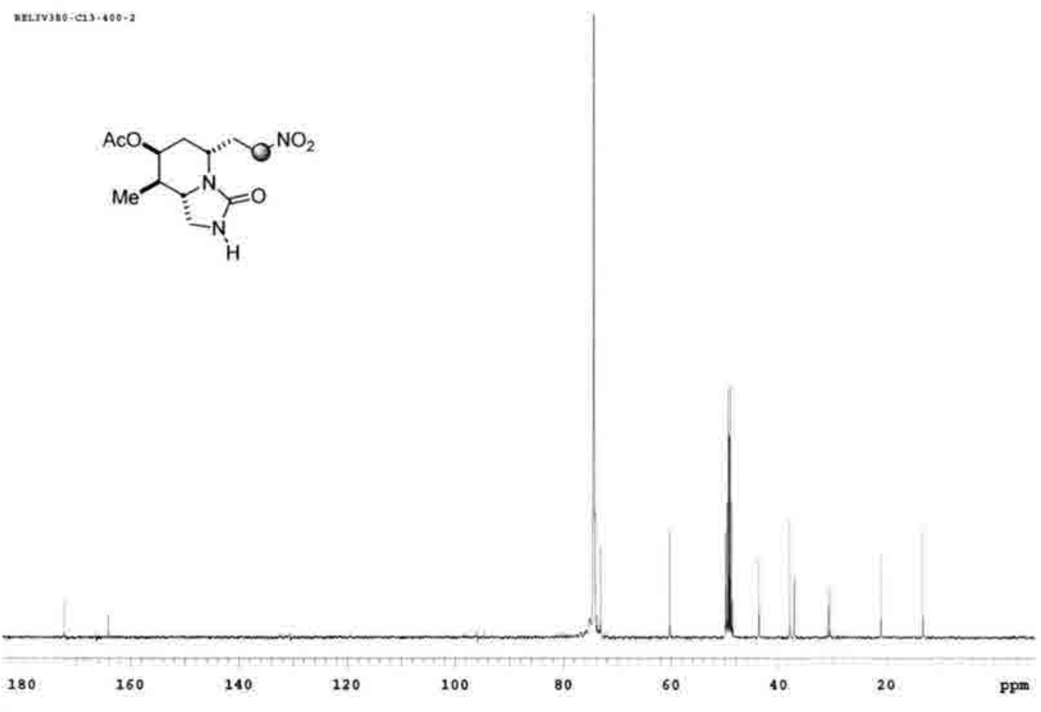
^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELIII476-3



^{13}CNMR, $CDCl_3$, 100 MHz; filename: RELIII476-400-C13



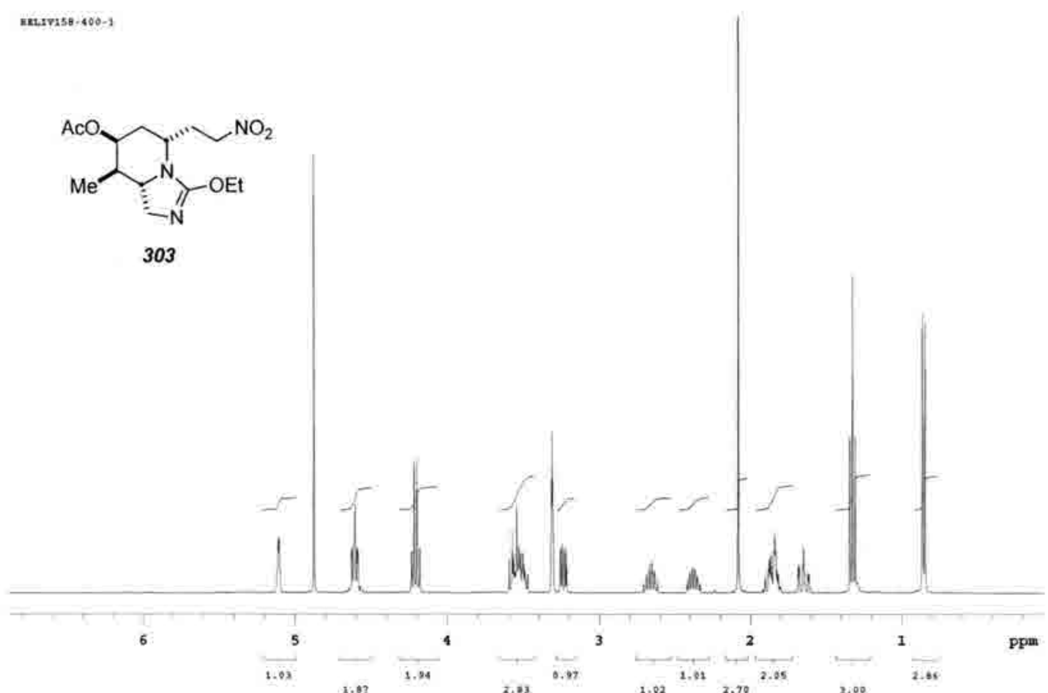
^{1}HNMR, CD_3OD, 400 MHz; filename: RELIV380-400-1



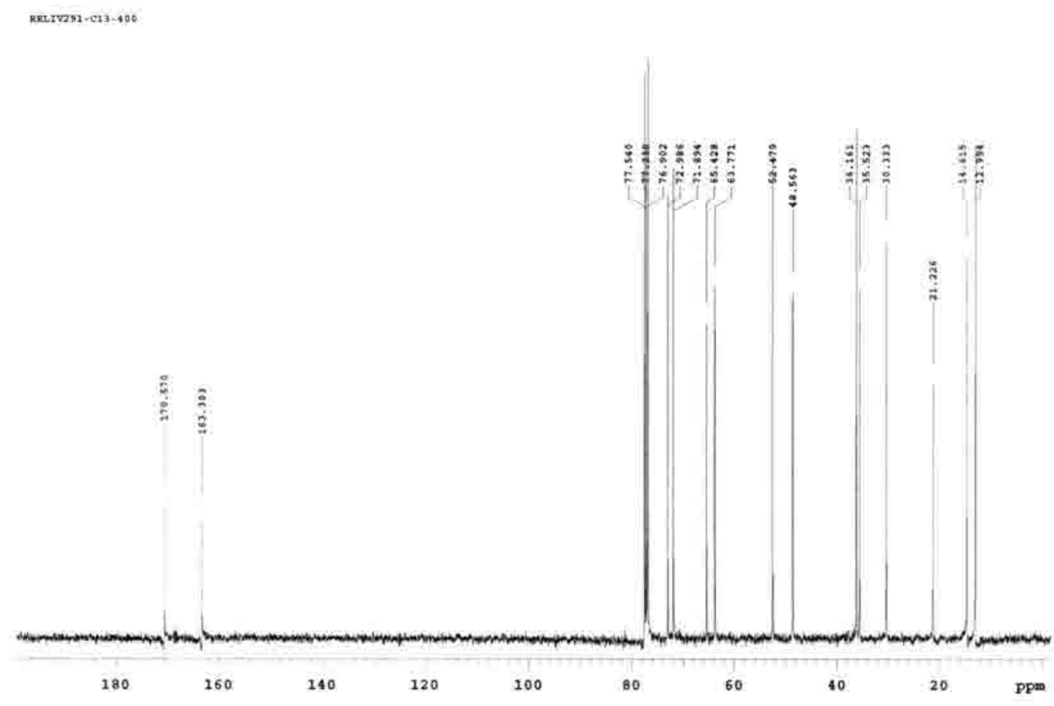
^{13}CNMR, CD_3OD, 100 MHz; filename: RELIV380-C13-400-2

(5S,7S,8R,8aS)-3-ethoxy-8-methyl-5-(2-nitroethyl)-1,5,6,7,8,8a-hexahydroimidazo[1,5-a]pyridin-7-yl acetate (303).

To a solution of the urea **299** (58 mg, 0.20 mmol) under argon in CH_2Cl_2 (10 mL) was added Cs_2CO_3 (650 mg, 2.0 mmol) and triethyloxonium tetrafluoroborate (386 mg, 2.0 mmol). The reaction was stirred at rt for 15 h and quenched by the addition of aq. 9% Na_2CO_3 (5 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organics were washed with brine and dried (Na_2SO_4). After concentration the crude mixture was purified on silica gel with 10% MeOH / CH_2Cl_2 to give the isourea as a clear oil (49 mg, 78 %). $[\alpha]^{25}_D = +6.2°$ (*c* 1.00, $CHCl_3$). ^{1}H NMR (CD_3OD, 400 MHz): δ 5.22 (app br dd, J = 8.0, 2.8 Hz, 1H), 4.61 (ddd, J = 7.6, 7.6, 2.4 Hz, 1H), 4.21 (q, J = 7.2 Hz, 2H), 3.59-3.46 (m, 2H), 3.55 (buried dd, J = 11.6, 4.0 Hz, 1H), 3.25 (dd, J = 11.6, 4.8 Hz, 1H), 2.66 (dddd, J = 18, 8, 8, 8 Hz, 1H), 2.37 (dddd, J = 18, 8, 8, 6 Hz, 1H), 2.08 (s, 3H), 1.94-1.79 (m, 2H), 1.65 (ddd, J = 14, 12, 2 Hz, 1H), 1.32 (q, J = 7.2 Hz, 3H), 0.85 (d, J = 6.8 Hz, 3H). ^{13}CNMR ($CDCl_3$,100 MHz): δ 170.6, 163.4, 73.0, 71.9, 65.4, 63.8, 52.5, 48.6, 36.2, 35.5, 30.3, 21.2, 14.6, 12.9. IR (Dep. $CDCl_3$): 2963 (m), 1735 (s), 1622, 1550, 1436, 1372, 1334 (all m), 1228 (s). HRMS (FAB+): Calc. for $C_{14}H_{24}N_3O_5$ [M+H]: 314.1715; Found: 314.1710.



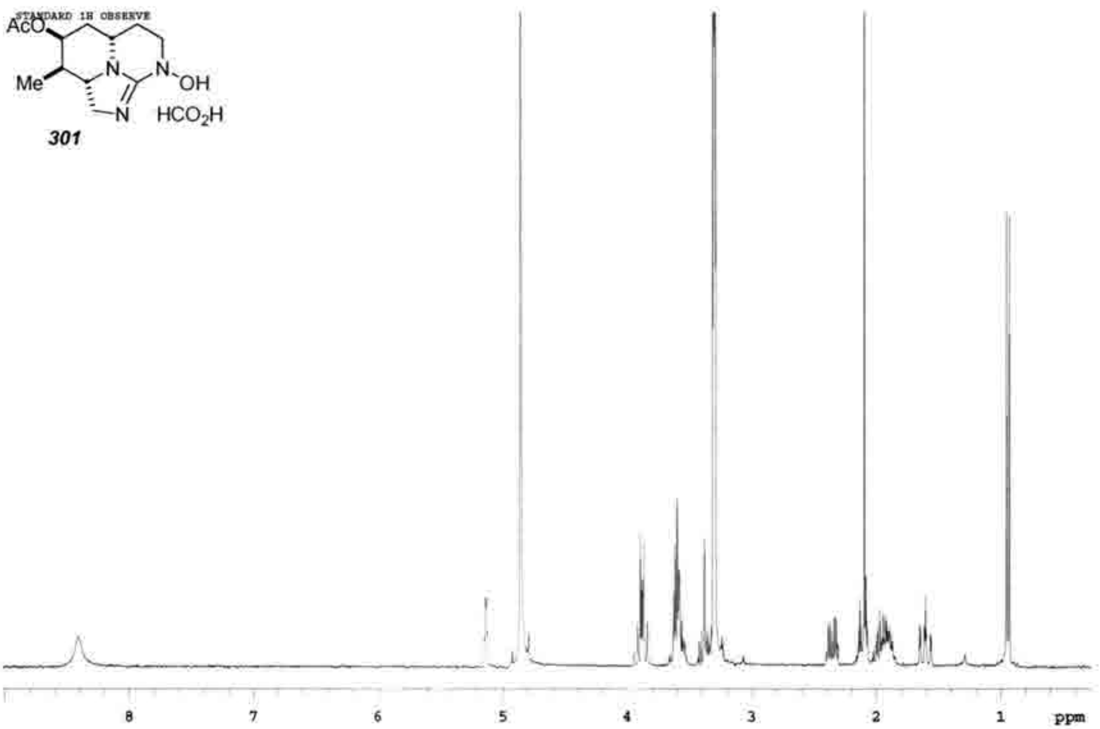
^{1}HNMR, CD_3OD, 400 MHz; filename: RELIV-291-400



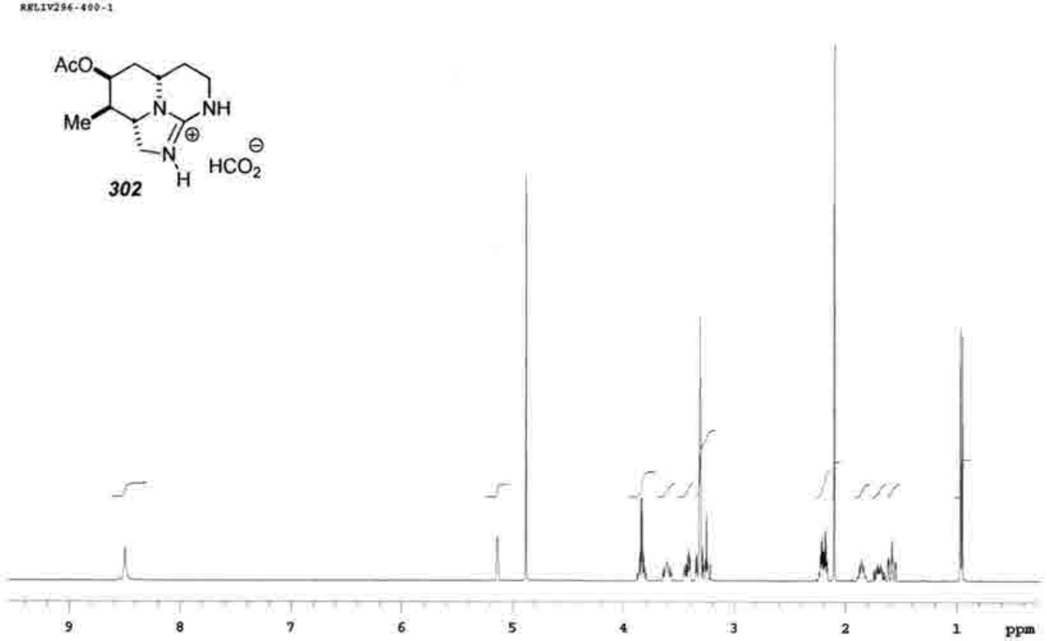
^{13}CNMR, CD_3OD, 100 MHz; filename: RELIV291-C13-400

Tricyclic guanidine (302).

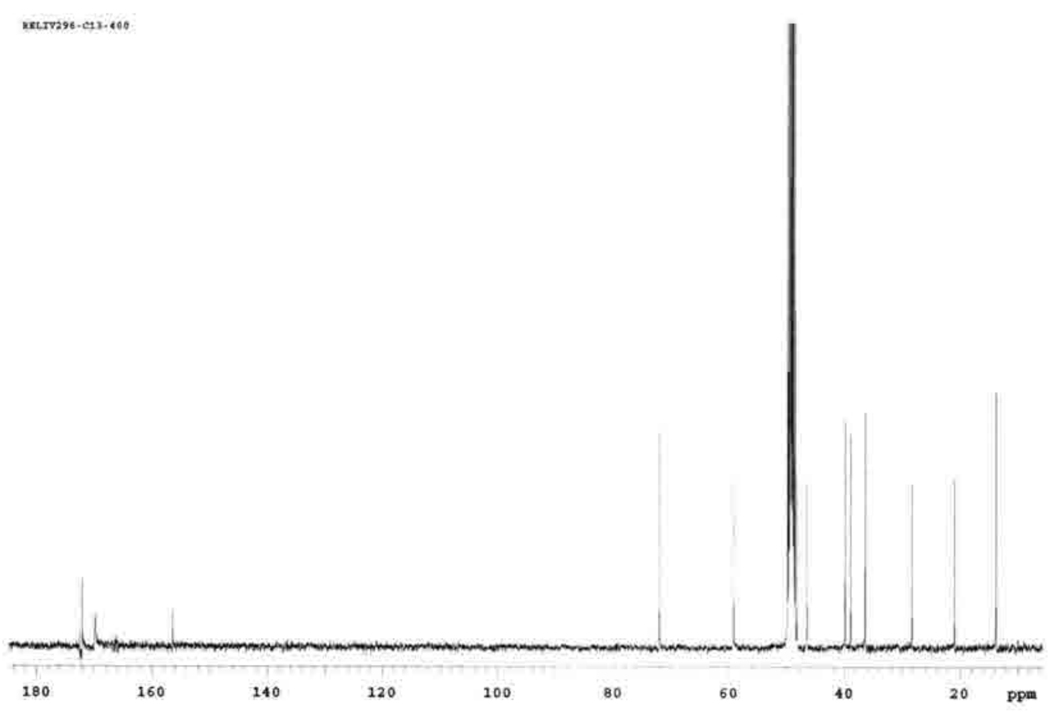
To a solution of the *O*-Et-isourea **303** (8.0 mg, 26 μmol) in 5% MeOH/HOAc (5 mL, to be 5 mM) was added $Pd(OH)_2$ (24 mg). The mixture was purged with hydrogen and the reaction stirred under an H_2 atmosphere for 12 h. The mixture was taken up in MeOH and filtered through a 0.45μm Acrodisc®. Concentration gave a clear oil that was purified on silica gel, eluting with 80 : 20 : 1 CH_2Cl_2 : MeOH : HCO_2H to give the tricyclic guanidine **302** as its formate salt (7 mg, 96%).



^{1}HNMR, CD3OD, 300 MHz; filename: RELIVNOH



^{1}HNMR, CD_3OD, 400 MHz; filename: RELIV296-400-1

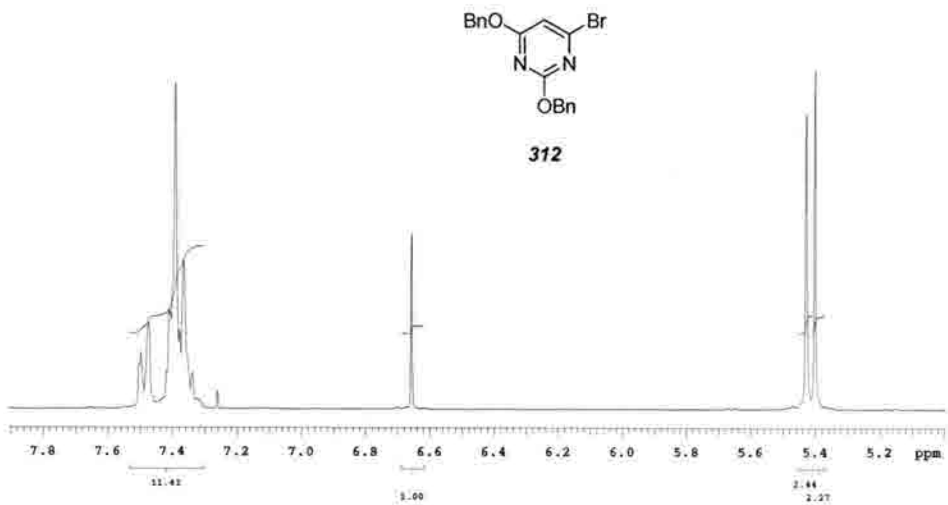


^{13}CNMR, CD_3OD, 100 MHz; filename: RELIV296-C13-400

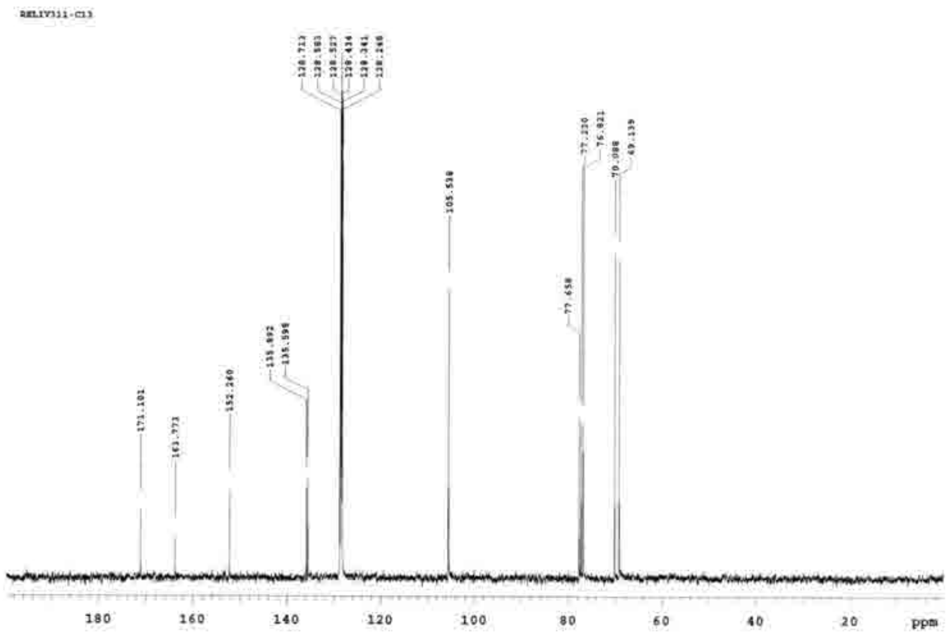
2,4-bis(benzyloxy)-6-bromopyrimidine (312).

Br Br N N Br **194** → BnO Br N N OBn **312**

To a solution of benzyl alcohol (0.11 mL, 1.03 mmol) in THF (0.5 mL) under an argon atmosphere at 0 °C was added a 1.6 M solution of *n*BuLi in hexanes (0.62 mL, 0.99 mmol). The mixture was stirred 10 min and DMF (5 mL) added. A solution of the tribromopyrimidine in DMF (1 mL) was added and the mixture stirred at 0 °C for 3h. The reaction was quenched with sat. NH_4Cl and diluted with H_2O (10 mL). The aqueous phase was extracted with Et_2O (3 x 10 mL) and the combined organics washed with brine and dried (Na_2SO_4). The crude oil was purified on silica gel eluting with 15 : 1 hexanes : EtOAc to give the dibenzyloxypyrimidine as a clear oil (137 mg, 80%). . ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.47-7.32 (m, 10H), 6.66 (s, 1H), 5.43 (s, 2H), 5.40 (s, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.10, 163.77, 152.26, 135.89, 135.60, 128.71, 128.58, 128.53, 128.43, 128.34, 128.25, 105.54, 70.09, 69.14. IR (Dep. $CDCl_3$): 2952 (w), 1549, 1404, 1323 (all s), 1130, 1003 (both m). HRMS (FAB+): Calc. for $C_{18}H_{16}N_2O_2{}^{81}Br_1$ [M+H]: 373.0375; Found 373.0363. Calc. for $C_{18}H_{16}N_2O_2Br_1$ [M+H]: 371.0395; Found 371.0383.



^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELIV311-final-1

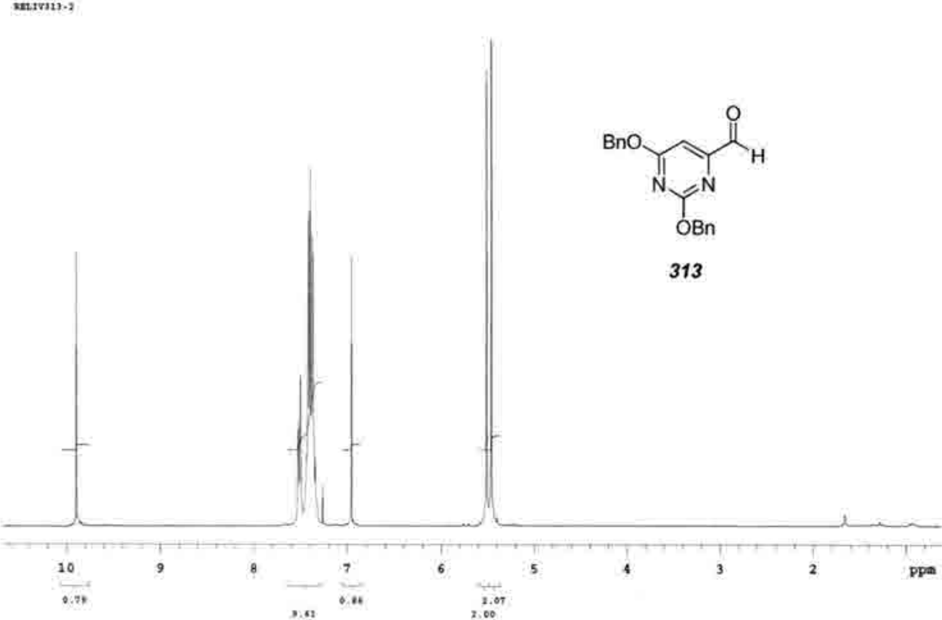


^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELIV311-C13-test1

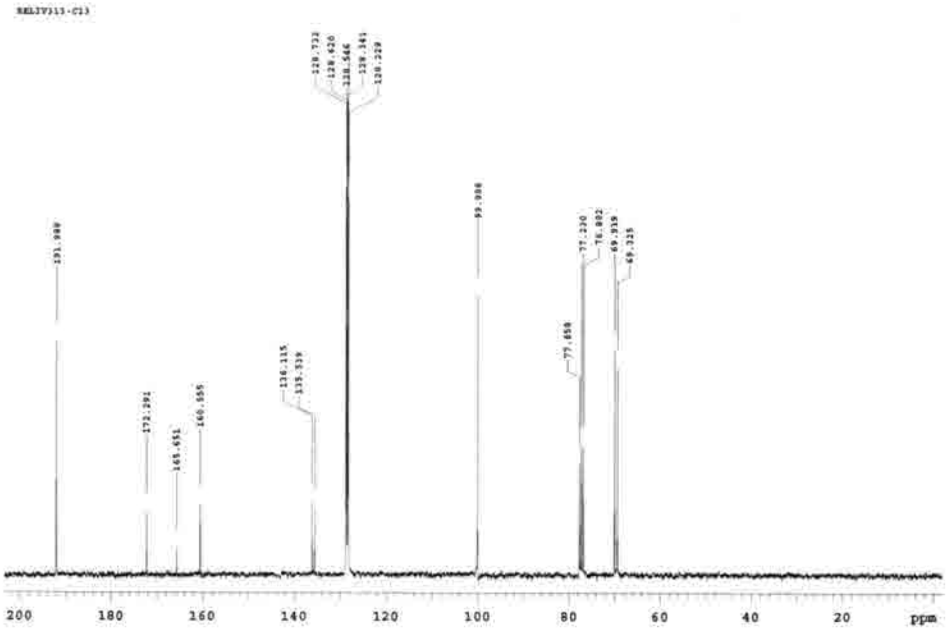
2,6-bis(benzyloxy)pyrimidine-4-carbaldehyde (313).

BnO Br N N OBn 312 → BnO O H N N OBn 313

To a solution of the pyrimidine (491 mg, 1.32 mmol) in Et_2O (30 mL) under argon at −100°C was added a 1.6 M solution of *n*BuLi in hexanes (1.07 mL, 1.71 mmol). The mixture was stirred for 20 min. and then DMF (0.51 mL, 6.60 mmol) was added. The mixture was allowed to warm to rt over 0.5 h and then refluxed briefly with a heat gun. After cooling to rt 10% HCl (5 mL) was added and the mixture stirred vigorously for 10 min. The mixture was partitioned between 10% HCl and Et_2O and the aqueous phase extracted again with Et_2O. The combined organics were washed with brine and dried (Na_2SO_4). The crude oil was purified on silica gel eluting with 9 : 1 hexanes : EtOAc to give the aldehyde as a clear oil (308 mg, 73%) which solidified upon standing. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.90 (s, 1H), 7.53-7.35 (m, 10H), 6.96 (s, 1H), 5.51 (s, 2H), 5.46 (s, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.99, 172.29, 165.65, 160.56, 136.12, 135.54, 128.73, 128.62, 128.55, 128.34, 128.23, 99.99, 69.94, 69.33. IR (Dep. $CDCl_3$): 2953, 2836 (both w), 1721, 1590, 1566, 1401, 1337 (all s), 1248, 1097 (both m). HRMS (FAB+): Calc. for $C_{19}H_{17}N_2O_3$ [M+H]: 321.1239; Found 321.1238.



^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELIV312-1



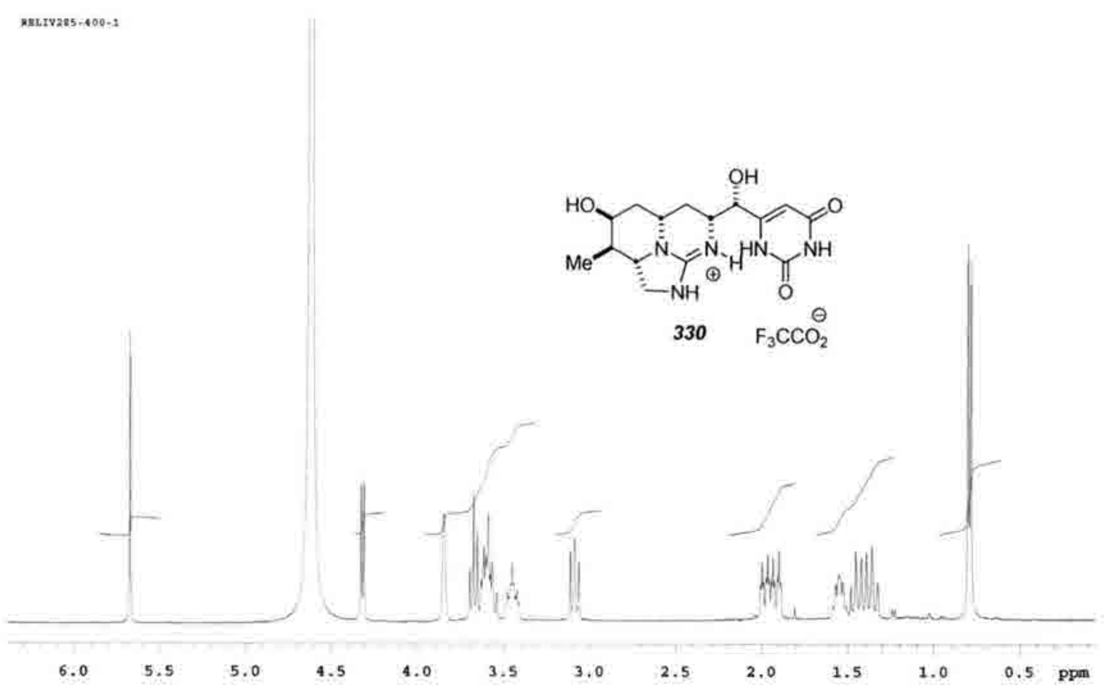
^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELIV312-C13

7-epi-cylindrospermopsin diol (330).

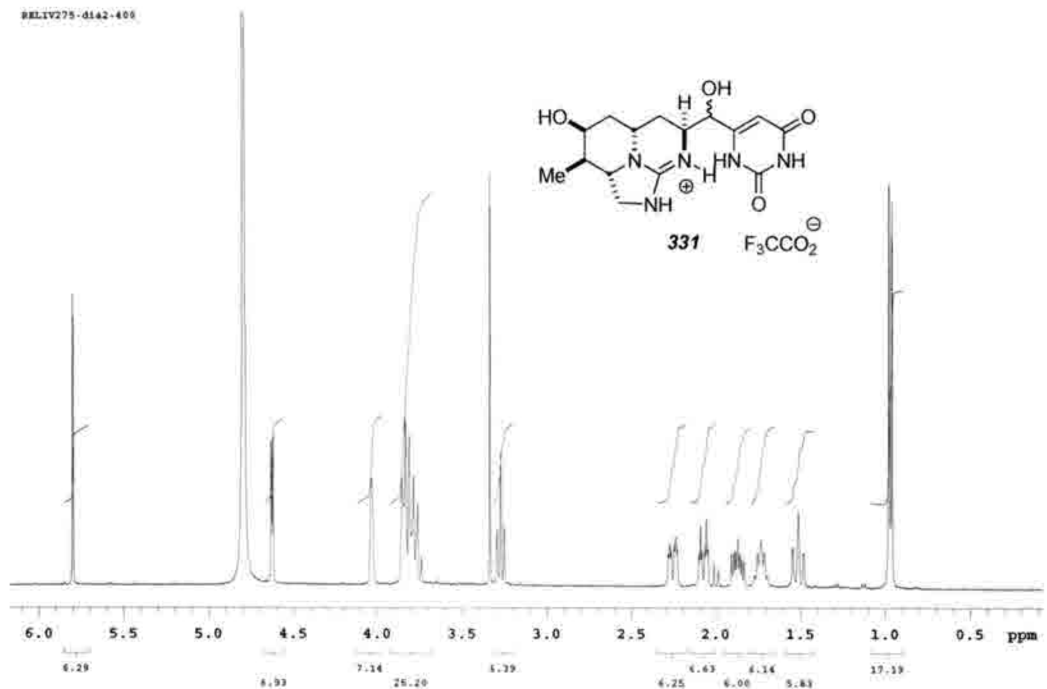
To a solution of the *O*-ethyl isourea (8.0 mg, 26 μmol) and pyrimidine aldehyde (5.2 mg, 31 μmol) in THF at -15 °C was added a 1 M solution of tetra-*n*-butylammonium fluoride (51 μL, 51 μmol). The reaction was allowed to proceed for 0.5 h and quenched with 20% AcOH / THF (0.5 mL). The mixture was concentrated and the crude oil dissolved in 5% AcOH / MeOH (5.1 mL, to be 5 mM) and the solution purged with argon. 20% $Pd(OH)_2$ on carbon (32 mg) was added and the solution purged with hydrogen. After stirring for 12 h under an H_2 atmosphere the mixture was filtered through a 0.45μm Acrodisc® and concentrated. Purification (to remove 6-hydroxymethyl pyrimidine and TBAF) by PTLC eluting with 20% MeOH / CH_2Cl_2 with 1% HCO_2H afforded an inseparable mixture (1 : 0.8) of the two C-7 diastereomers after stripping the silica with 20% abs. EtOH / CH_2Cl_2. This mixture was then refluxed in conc. HCl for 8 h and concentrated. Purification of the uracils was achieved by HPLC using a Waters Symmetry® C-18 colum (4.6 x 250 mm) eluting with 4% MeOH / H_2O with 1% TFA at 1.5 mL/min, monitoring at 263 nm to give 7-epi-cylindrospermopsin diol as a white solid (3.0 mg, 32%, t_R = 19.05 min) and the other C8 diastereomer also as a white crystalline solid (2.7 mg, 29 %, t_R = 23.53 min).

7-epi-cylindrospermopsin diol: $[\alpha]^{25}{}_{D} = -11.7°$ (*c* 0.06, H_2O); (lit $[\alpha]_D{}^{24} = -8.3°$ (*c* 0.06, H_2O)) see ref 67.

8-epi-cylindrospermopsin diol: : $[\alpha]^{25}{}_{D} = +70.0°$ (*c* 0.20, H_2O) ^{1}H NMR (D_2O, 400 MHz): δ 5.80 (s, 1H), 4.62 (d, *J* = 4.4 Hz, 1H), 4.04 (br s, 1H), 3.88-3.74 (m, 3H), 3.28 (app t, *J* = 8.4 Hz, 1H), 2.26 (ddd, *J* = 14, 4, 3 Hz, 1H), 2.07 (ddd, *J* = 14, 4, 4 Hz, 1H), 1.87 (ddd, *J* = 15, 10, 6 Hz, 1H), 1.78-1.68 (m, 1H), 1.52 (app t, *J* = 13 Hz, 1H), 0.97 (d, *J* = 7 Hz, 3H). HRMS (FAB+): Calc. for $C_{15}H_{22}N_5O_4$ [M+H]: 336.1672; Found: 336.1672.



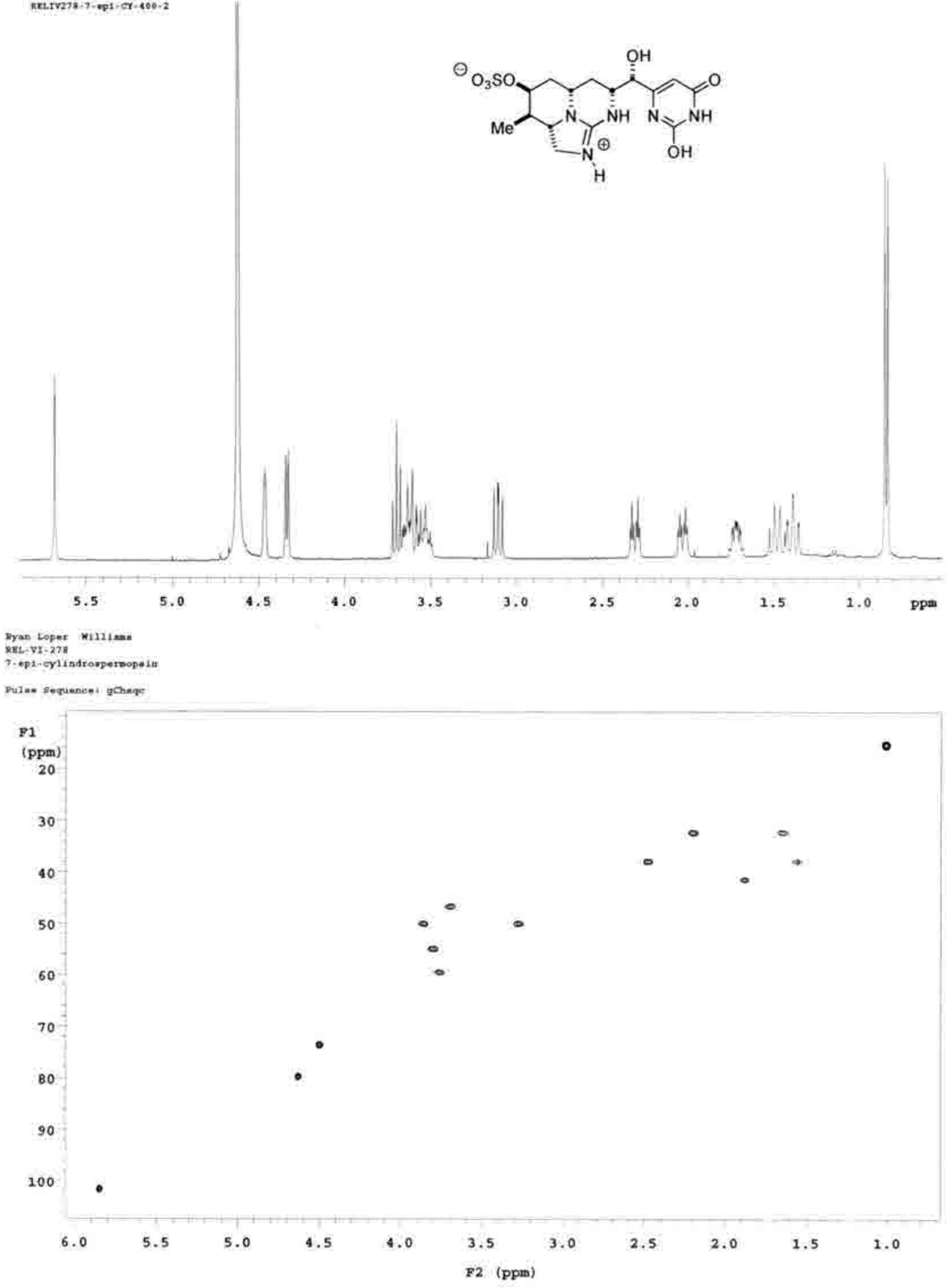
^{1}HNMR, D_2O, 400 MHz; filename: RELIV285-400-1.



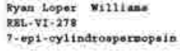
^{1}HNMR, D_2O, 400 MHz; filename: RELIV275-d2-400

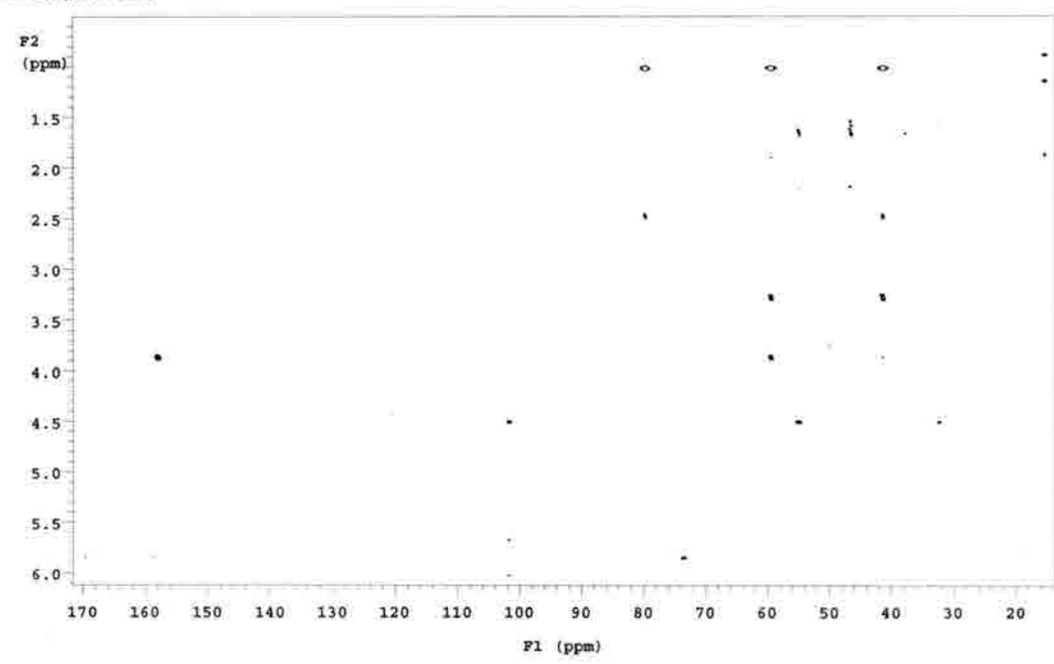
7-epi-cylindrospermopsin (23):

7-epi-cyclindrospermopsin diol (2.6 mg, 7.0 μmol) was co-concentrated with MeCN (2 x 5 mL) and PhMe (2 x 5 mL). The resulting solid was dried under vacuum for 0.5 h and placed under argon. DMF (0.4 mL) and activated, powdered 3Å molecular sieves (6 mg) were added and the mixture stirred for 15 min. To this solution was added solid SO_3·pyr (11 mg, 70 μmol) and the mixture stirred for 1 h. MeOH (0.1 mL) was added and the solvents removed *in vacuuo*. The mixture was taken up in MeOH and filtered through a 0.45μm Acrodisc®. Purificataion by HPLC on a Waters Symmetry® C-18 colum (4.6 x 250 mm) eluting with 2% MeOH / H_2O with 1% TFA at 1.5 mL/min, monitoring at 263 nm gave 7-epi-cylindrospermopsin (t_R = 9.22 min) as a white solid after lyophilization (1.7 mg, 59 %). This was preceded by its bis sulfate (t_R = 6.54 min) as a ~2:1 mixture.
$[\alpha]^{25}_D = -12.5°$ (*c* 0.04, H_2O); (lit $[\alpha]_D^{24} = -20.5°$ (*c* 0.04, H_2O), ^1H and ^{13}C NMR spectra agree with those reported. HRMS (FAB+): Calc. for $C_{15}H_{22}N_5O_7S$ [M+H]: 416.1240; Found: 416.1247.



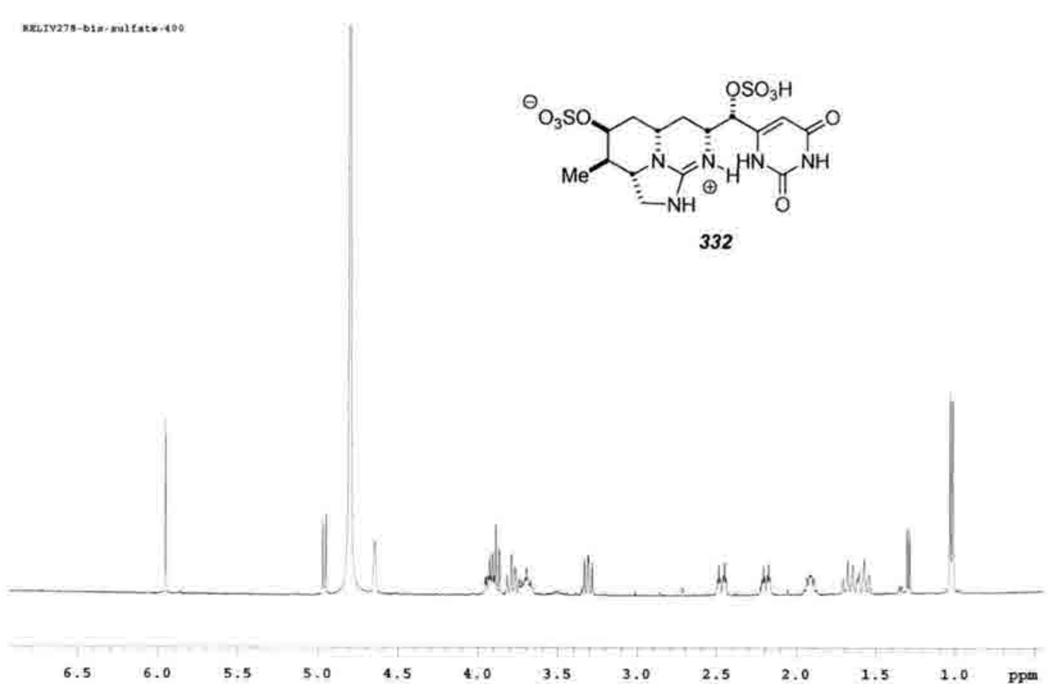
gHSQC, D2O, 500 MHz; filename: RELIV278-gCHSQC





gHMBC, D_2O, 500 MHz; filename: RELIV278-gHBMC

332:



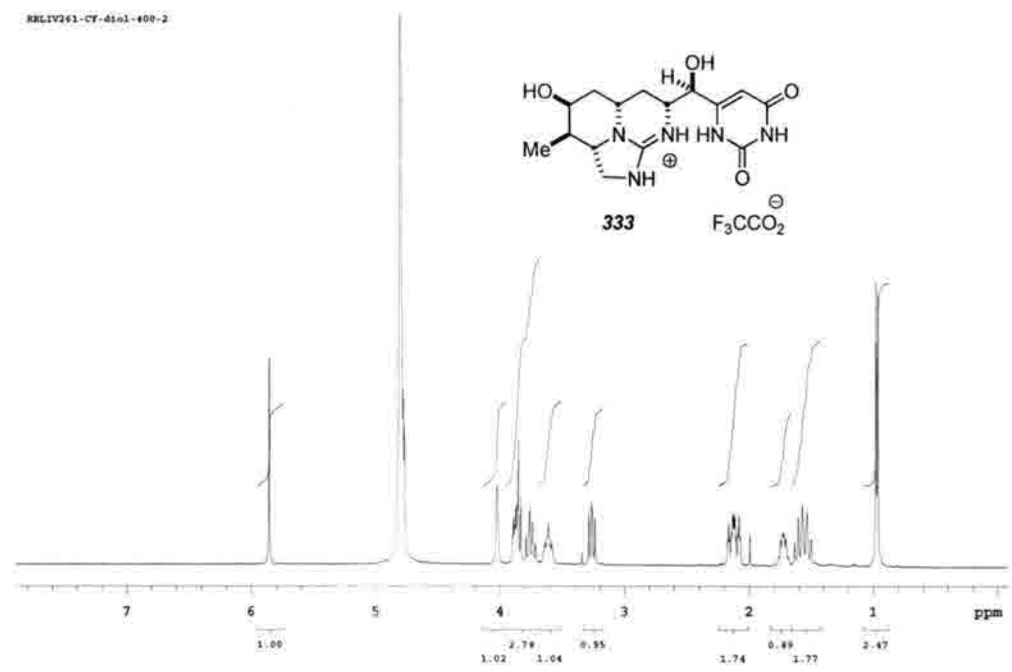
^{1}HNMR, D_2O, 400 MHz; filename: RELIV278-bis-sulfate-400

Cyilindrospermopsin **.**

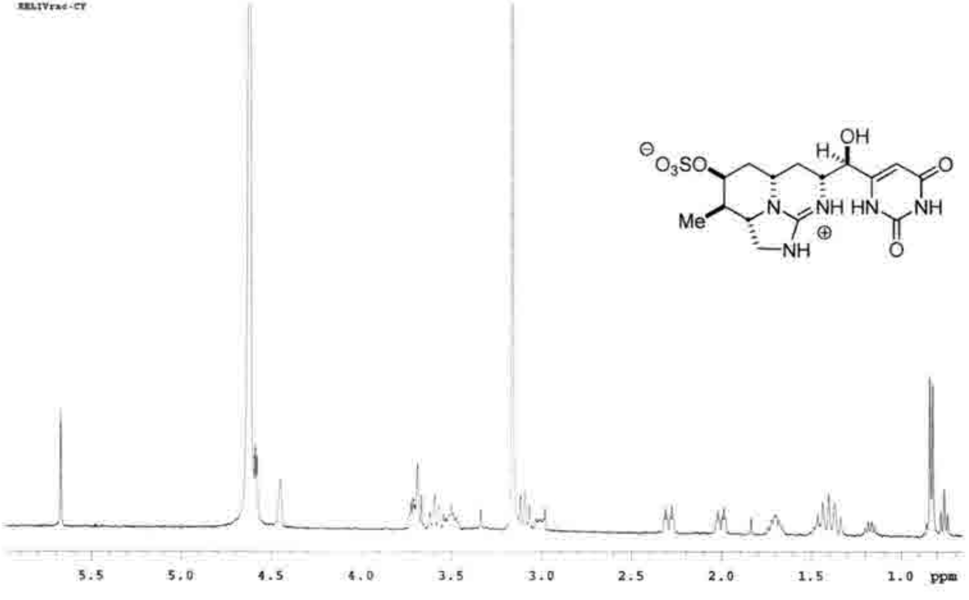
302 → 333 → 19 cylindrospermopsin

To a solution of **302** (4.5 mg, 14 μmol) and **313** (5.5 mg, 17 μmol) in THF (120 μL) at −15°C was added a 1 M solution of TBAF (14 μL, 14 μmol). The solution was stirred for 0.5 h and quenched with 20% AcOH/THF (0.2 mL). The mixture was concentrated and taken up in 5% AcOH/THF (3 mL) and $Pd(OH)_2$ (20%/C, 5 mg) added. The solution was purged with H_2 and stirred under an H_2 atmosphere for 12h. The mixture was taken up in MeOH and filtered through a 0.45μm Acrodisc®. Purificataion by HPLC on a Waters Symmetry® C-18 colum (4.6 x 250 mm) eluting with 8% MeOH / H_2O with 1% TFA at 1.5 mL/min, monitoring at 263 nm gave cylindrospermopsin diol (**333**) (t_R = 9.47 min) as a white solid after lyophilization (1.3 mg, 20 %). $[\alpha]^{25}_D = +7.7°$ (*c* 0.13, H_2O). **333** (1.3 mg, 2.89 μmol) was co-concentrated with MeCN (2 x 5 mL) and PhMe (2 x 5 mL). The resulting solid was dried under vacuum for 0.5 h and placed under argon. DMF (0.3 mL) and activated, powdered 3Å molecular sieves (6 mg) were added and the mixture stirred for 15 min. To this solution was added solid SO_3·pyr (4.6 mg, 29 μmol) and the mixture stirred for 1 h. MeOH (0.1 mL) was added and the solvents removed *in vacuuo*. The mixture was taken up in MeOH and filtered through a 0.45μm Acrodisc®. Purificataion by HPLC on a Waters Symmetry® C-18 colum (4.6 x 250 mm) eluting with 4% MeOH / H_2O with 1% TFA at 1.5 mL/min, monitoring at 263 nm gave cylindrospermopsin (t_R = 8.14 min) as a white solid after lyophilization (0.7 mg, 60 %).

This was preceded by its bis sulfate (t_R = 5.32 min) as a ~6:1 mixture. $[\alpha]^{25}_D$ = +8.0° (*c* 0.05, H_2O).



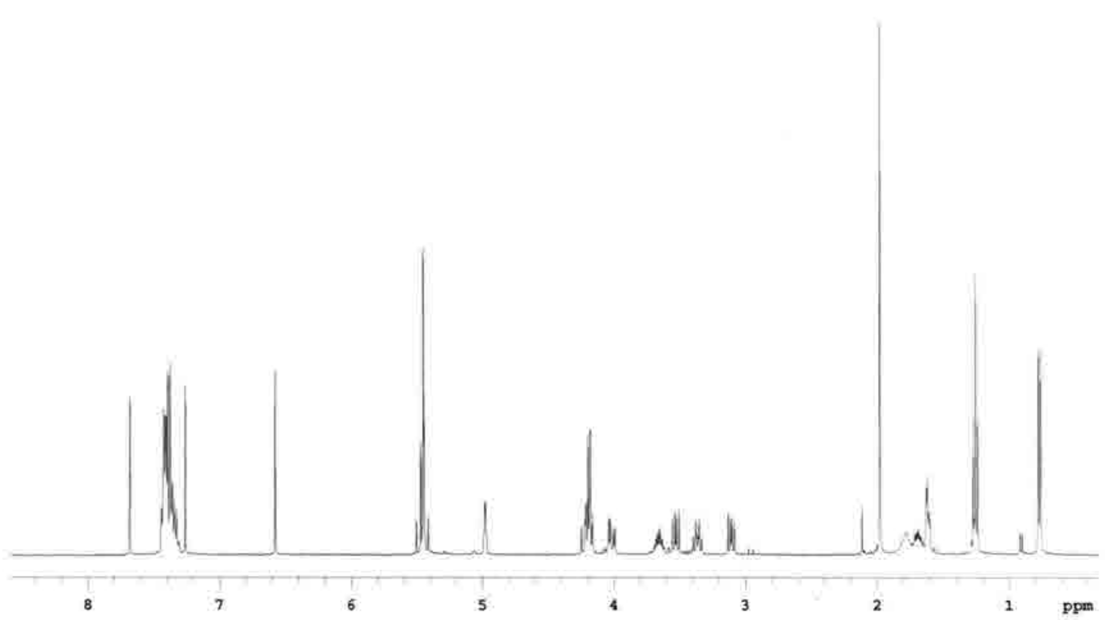
^{1}HNMR, D_2O, 400 MHz; filename: RELIV261-CY-diol-400-2



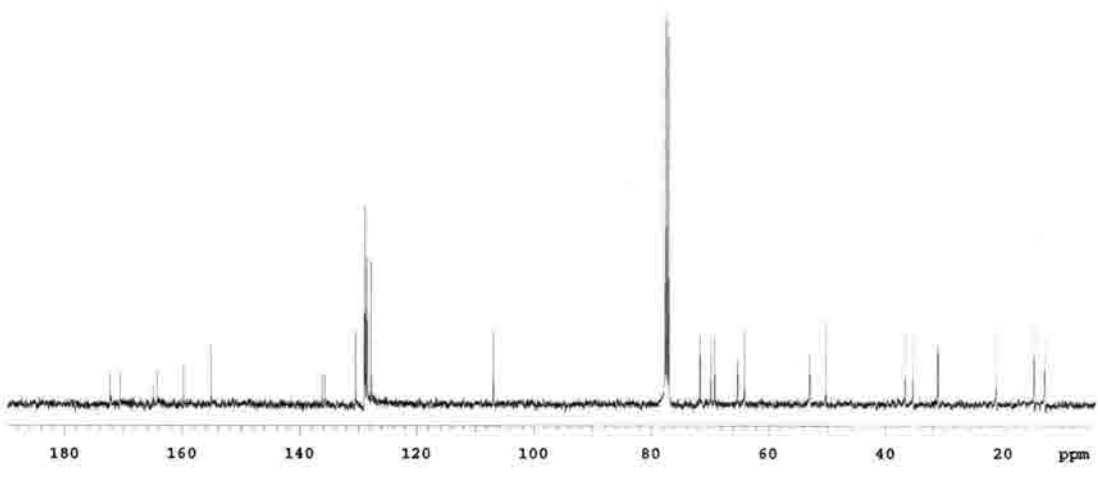
^{1}HNMR, D_2O, 400 MHz; filename: RELIV384-400-1

(5S,7S,8R,8aS)-5-((E)-3-(2,6-bis(benzyloxy)pyrimidin-4-yl)-2-nitroallyl)-3-ethoxy-8-methyl-1,5,6,7,8,8a-hexahydroimidazo[1,5-a]pyridin-7-yl acetate

To a solution of the isourea **rac-301** (23 mg, 73 μmol) and the pyrimidine aldehyde **313** (26 mg, 81 μmol, 1.1 eq.) in CH_2Cl_2 (1 mL) under argon was added Ac_2O (34 μL, 0.35 mmol, 5 eq.). CsF (110 mg, 0.73 mmol) was then added as a solid in one portion. The reaction was diluted with MeCN (3 mL) and the mixture stirred for 4h. The reaction was concentratede under reduced pressure, taken up in CH_2Cl_2 and filtered to remove the cesium salts. This mixture was again concentrated and purified on silica gel eluting with 10% MeOH / CH_2Cl_2 to give the nitroalkene **334** as a yellow oil (30 mg, 67%) as a single geometric isomer. *This compound is unstable, decomposing overnight at rt.* ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.68 (s, 1H), 7.48-7.30 (m, 10H), 6.58 (s, 1H), 5.52-5.40 (m, 4H), 4.98 (br 2, *J* = 3.2 Hz), 4.28-4.18 (m, 3H), 4.00 (dd, *J* = 14, 5 Hz, 1H), 3.66 (ddd, *J* = 15, 10, 5 Hz, 1H), 3.55 (dd, *J* = 10, 8 Hz, 1H), 3.40-3.30 (m, 1H), 3.12 (dd, *J* = 10, 8 Hz, 1H), 1.98 (s, 3H)1.78-1.64 (m, 1H), 1.62-1.60 (m, 2H), 1.25 (t, *J* = 7.2 Hz, 3H), 0.76 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.3, 170.6, 165.0, 164.3, 159.8, 155.2, 136.2, 135.7, 130.4, 128.9, 128.7, 128.5, 128.4, 127.8, 106.9, 71.6, 69.8, 69.1, 65.3, 64.1, 52.9, 50.2, 36.7, 35.4, 31.1, 21.2, 14.7, 13.0. HRMS (FAB+): Calc. for $C_{33}H_{38}N_5O_7$ [M+H]: (*m/z*) 616.2771; Found: (*m/z*) 616.2795.



^{1}HNMR, $CDCl_3$, 400 MHz; filename: RELIV385-400-1.



^{13}CNMR, $CDCl_3$, 100 MHz; filename: RELIV385-C13-400-1.

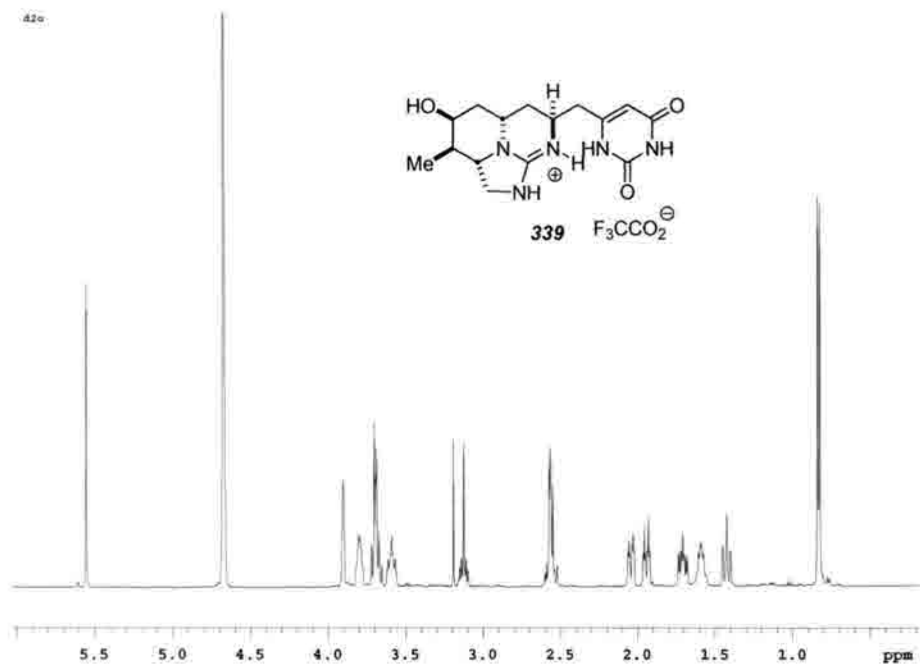
7-Deoxycylindrospermopsin diol

334 337 338 336 339

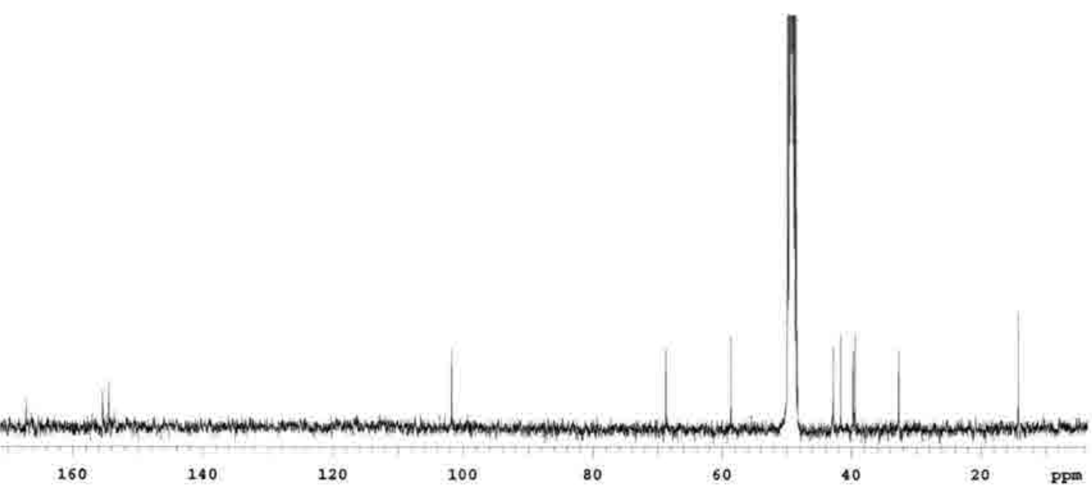
A solution of the nitroalkene **334** (18 mg, 29.2 μmol) in EtOH (0.5 mL) was added dropwise to a slurry of $NaBH_4$ (5 mg, 146 μmol) in EtOH (0.5 mL) over 20 min. After stirring for 1.5 h the reaction was quenched by the addition of 1:1 H_2O:AcOH (0.1 mL) and concentrated. The concentrate was diluted with 5% AcOH:MeOH (5.8 mL, to be 5 mM) and purged with argon. $Pd(OH)_2$ (20% / C, 6 mg) was added and the mixture stirred under a hydrogen atmosphere for 12 h, filtered through a 0.45μm Acrodisc® and concentrated. The residue was dissolved in conc. HCl and refluxed for 1h and concentrated. Purification of the uracils was achieved by HPLC using a Waters Symmetry® C-18 colum (4.6 x 250 mm) eluting with 8% MeOH / H_2O with 1% TFA at 1.5 mL/min, monitoring at 263 nm to give 7-deoxy-cylindrospermopsin diol as a white solid (3.7 mg, 38%, t_R = 22.1 min) preceded by the C8 diastereomer also obtained as a white crystalline solid (4 mg, 38 %, t_R = 12.6 min). A small sample of **339** (~1 mg) was recrystallized from methanol (layered with pentane) to give *X*-ray quality crystals.

339 (8S^*): ^{1}H NMR (D_2O, 500MHz): δ 5.68 (s, 1H), 4.03 (br s, 1H), 3.92 (m, 1H), 3.82 (dd, J = 9, 9 Hz, 1H), 3.78 (dd, J = 9,9 Hz, 1H), 3.72 (dddd, J = 11, 11, 4, 4 Hz, 1H), 3.25 (m, 1H), 2.71 (dd, J = 14, 5.5 Hz, 1H), 2.67 (dd, J = 14, 9 Hz, 1H), 2.16 (dt, J = 14, 4, 4 Hz, 1H), 2.06 (dt, J = 15, 3 Hz, 1H), 1.83 (ddd, J = 15, 11, 5 Hz, 1H), 1.72 (ddq, J = 14,

7, 3 Hz, 1H), 1.55 (ddd, J = 14, 14, 1.5 Hz, 1H), 0.95 (d, J = 7 Hz, 3H). HRMS (FAB+): Calc. for $C_{15}H_{22}N_5O_3$ [M+H]: (m/z) 320.1723; Found: (m/z) 320.1723.

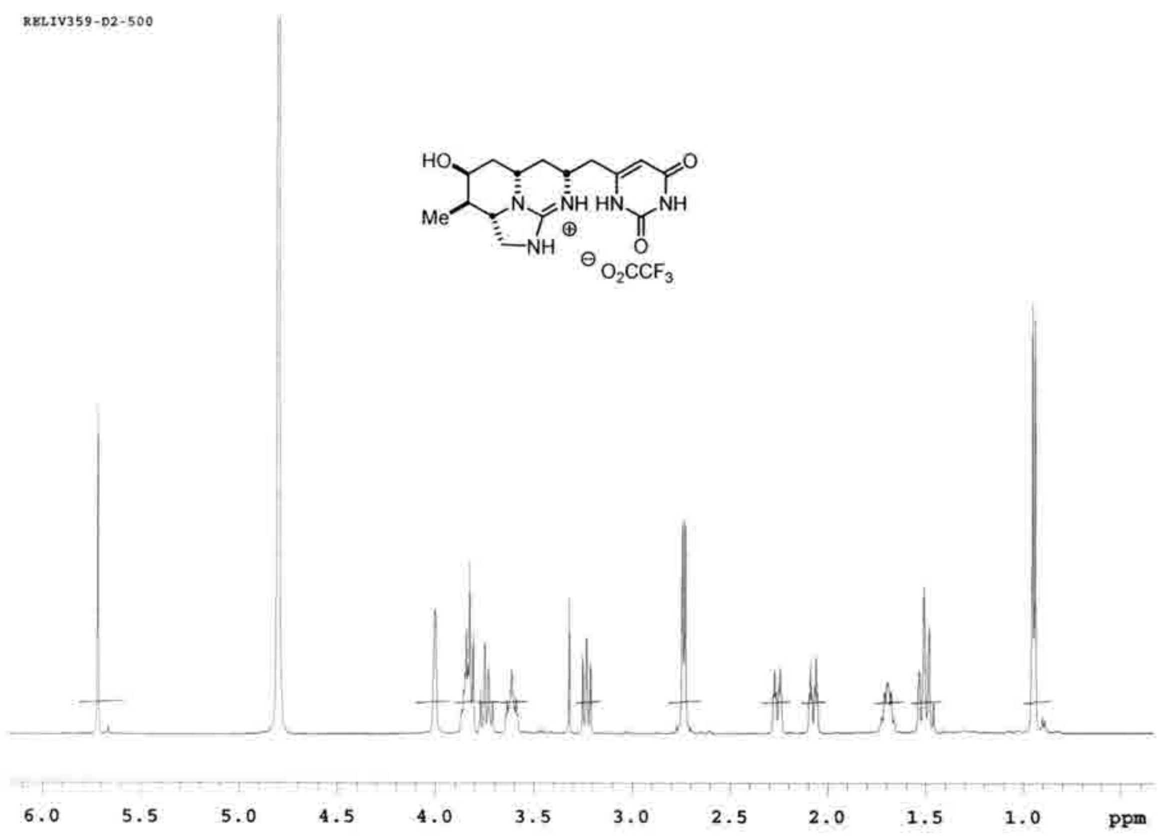


^{1}HNMR, D_2O, 500 MHz; filename: rmw_RELIV559-D1_2004-0501-01_h1



^{13}CNMR, 100 MHz, CD_3OD; filename: RELIV359-d1-HPLC-C13

336 ($8R^*$): ^{1}H NMR (D_2O, 500 MHz): δ 5.72 (s, 1H), 4.00 (br s, 1H), 3.86 (buried m, 1H), 3.82 (dd, *J* = 9.0, 9.0 Hz, 1H), 3.74 (dd, *J* =10,10 Hz, 1H), 3.61 (ddt, *J* = 11, 11, 3.5 Hz, 1H), 3.23 (dd, *J* = 10, 10 Hz, 1H), 2.73 (app d, *J* = 5 Hz, 1H), 2.26 (dt, *J* = 15, 5, 5 Hz, 1H), 2.07 (dt, *J* = 15, 3, 3, Hz, 1H), 1.70 (ddq, *J* = 9, 6.5, 2.5, 1H), 1.50 (app q, *J* = 11 Hz, 2H), 0.95 (d, *J* = 6.5 Hz, 3H). HRMS (FAB+): Calc. for $C_{15}H_{22}N_5O_3$ [M+H]: *(m/z)* 320.1723; Found: 320.1712.

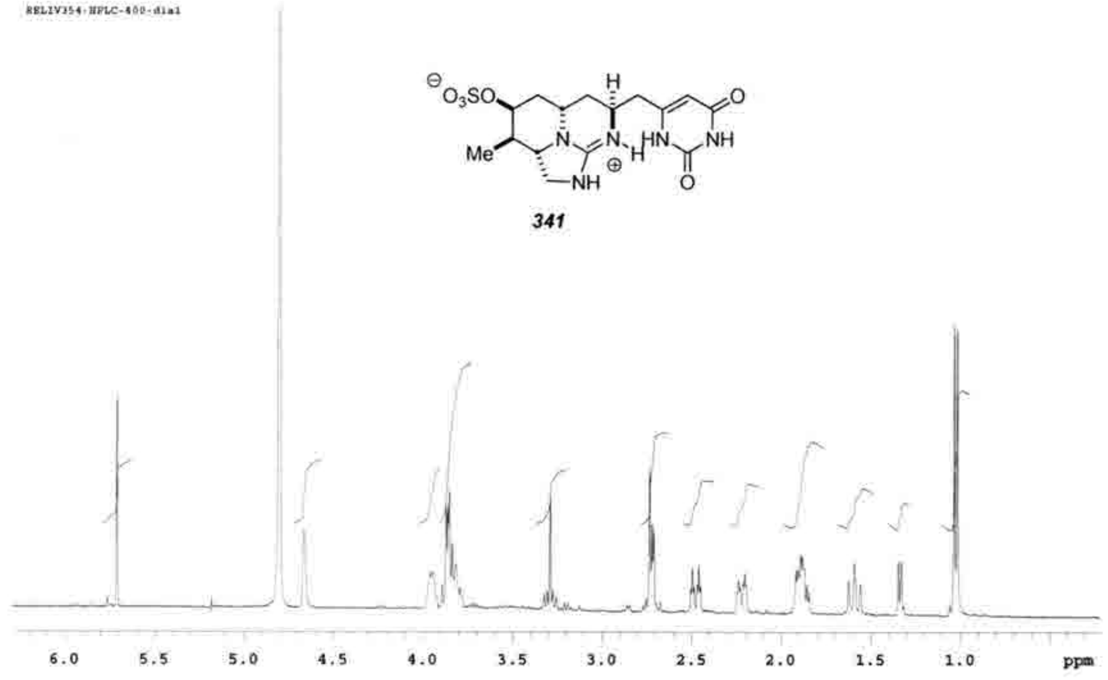


^{1}HNMR, D2O, 500 MHz; filename: rmw_RELIV559-D2_2004-0501-02_h1

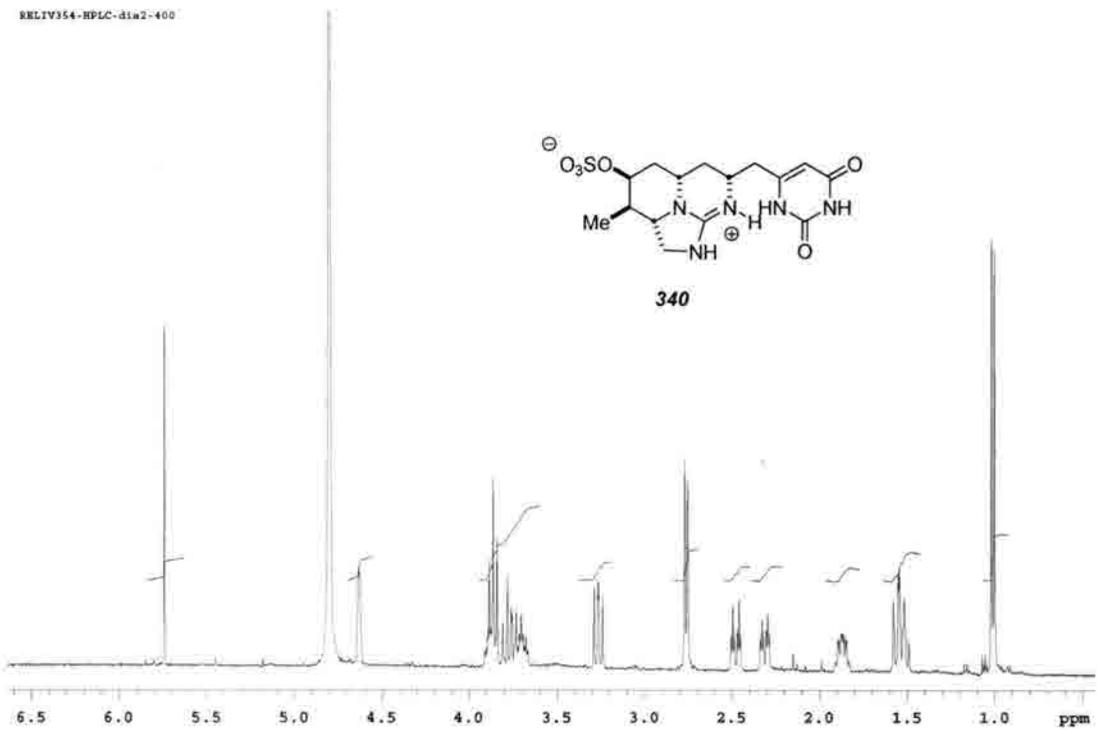
7-deoxycylindrospermopsin.

340 341

Alternatively a mixture of the C12-hydroxy uracils (3.2 mg, 7.9 μmol) can be directly sulfonated by treatment with SO_3·pyr (19 mg, 120 μmol) in DMF (300 μL). Purification of the uracils after concentration was achieved by HPLC using a Waters Symmetry® C-18 colum (4.6 x 250 mm) eluting with 8% MeOH / H_2O with 1% TFA at 1.5 mL/min, monitoring at 263 nm to give 7-deoxy-cylindrospermopsin as a white solid (1 mg, 33%, t_R = 8.25 min) preceded by the C8 diastereomer also obtained as a white crystalline solid (1 mg, 33 %, t_R = 4.91 min).



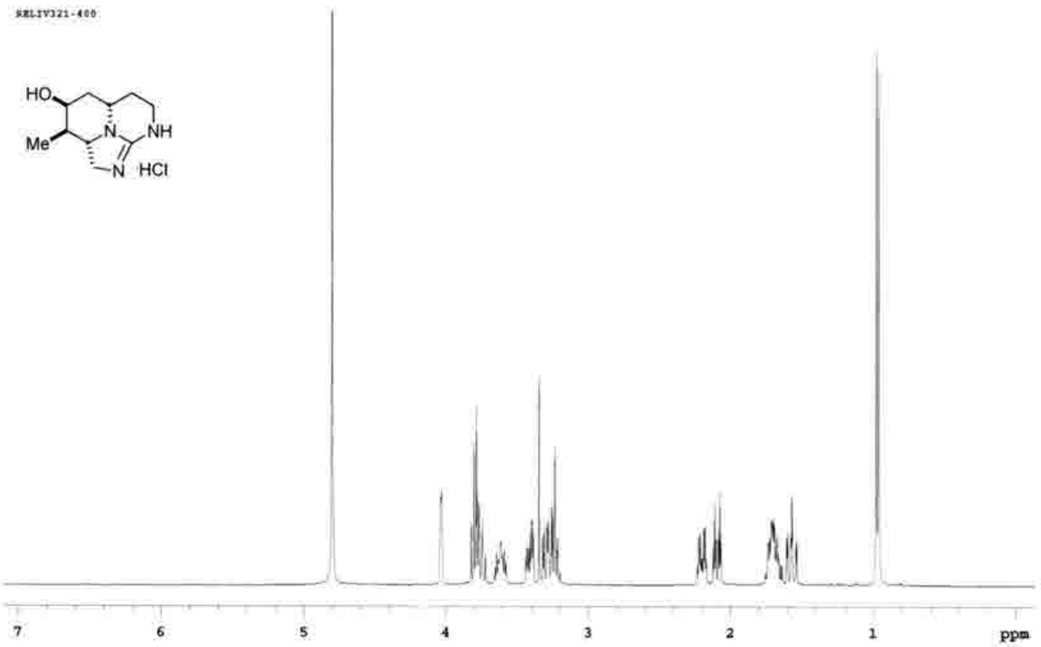
^{1}HNMR, D_2O, 400 MHz; filename: RELIV354-HPLC-dia1-400



^{1}HNMR, D_2O, 400 MHz; filename: RELIV354-HPLC-dia2-400

Tricyclic-guanidine-ol (342)

A solution of the acetylated guanidine (15 mg, 52.9 μmol) in conc. HCl was refluxed for 3h. The mixture was cooled and lyophilized to give the deacetylated guanidine hydrochloride as an amorphous solid (12 mg, ~*quant*).

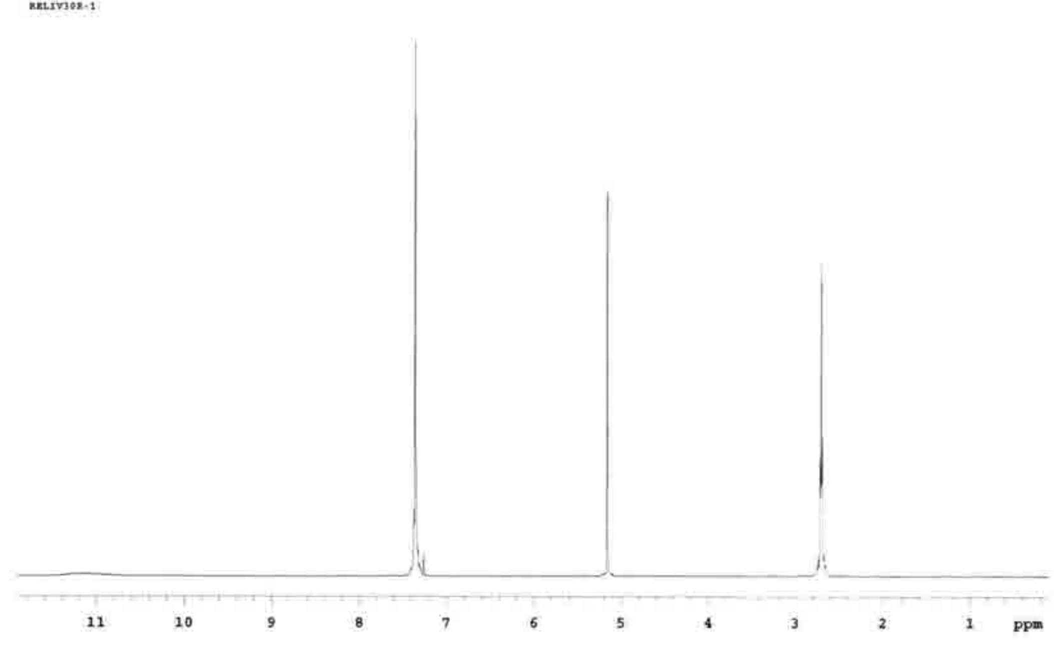


^{1}HNMR, D2O, 400 MHz; filename: RELIV321-400

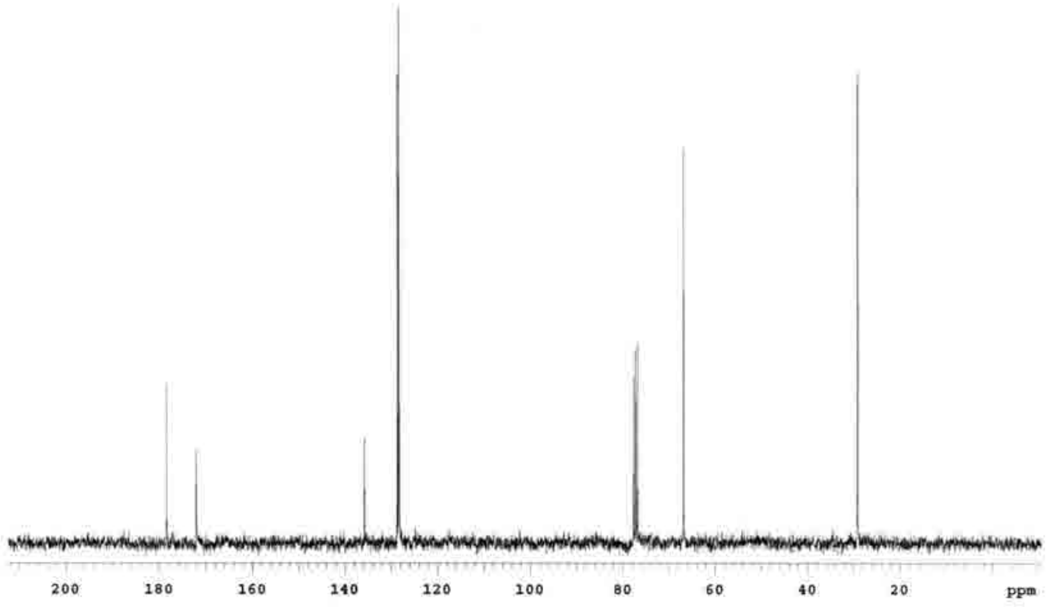
4-(benzyloxy)-4-oxobutanoic acid (344).

343 → 344

A slurry of succinic anhydride (1.50 g, 14.9 mmol) and benzyl alcohol (1.85 mL, 17.9 mmol) in PhMe (50 mL) was heated in a sealed tube at 200 °C for 12h. The mixture was cooled and partitioned between EtOAc and $NaHCO_3$. The organics were extracted further with 9% Na_2CO_3 (2 x 15 mL). The aqueous phase was acidified with $NaHSO_4$ to pH ~2 and extracted with CH_2Cl_2 (3 x 20 mL). The combined organics were washed with brine and dried (Na_2SO_4). Concentration gave an analytically pure oil which solidified upon standing (m.p. = 56-57 °C, 2.77 g, 89%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.10 (br s, 1H), 7.38-7.32 (m, 5H), 5.16 (s, 2H), 2.71 (app t, J = 2.4 Hz, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 178.39, 171.99, 135. 76, 128.68, 128.39, 128.30, 66.87, 29.21, 29.15. IR (Dep. $CDCl_3$): 3034 (br, m), 1713 (s), 1214, 1165(both s).



^{1}HNMR, $CDCl_3$, 300 MHz; filename: RELIV308-1



^{13}CNMR, $CDCl_3$, 75 MHz; filename: RELIV308-C13

5.3 Biological Protocols

Effects on Protein Synthesis

* All determinations of protein synthesis inhibition were conducted at the University of Southern California by Dr. Maria T.C. Runnegar and John Khulenkamp.

Materials

The culture medium for isolated rat hepatocytes: DME/F12 and sulfur amino acid-free DME were purchased from Gibco BRL Life Technologies. L-[^{35}S]Methionine or [35S]Protein labeling mix were purchased from Amersham Pharmacia Biotech and NEN Life Sciences respectively. LDH was measured using a kit from Sigma Chemicals (Lit DG1340-K). All other reagents were from Sigma Chemicals or from other routine commercial sources.

Synthetic Analogues

Synthetic materials were supplied from the above experimental protocols. Concentrations of **rac-301**, **rac-302**, and **rac-342** were determined by mass. Due to the limited quantities of **330-333**, **107**, **340**, and **341** concentrations were estimated using ε = 4600 $cm^{-1}M^{-1}$ at λ = 263 nm. Due to the extrapolation of CY's molar absorptivity, these concentrations are approximate.

In vitro protein synthesis

This was measured using the Rabbit Reticulocyte Lysate System of Promega (catalog number L4960). The incorporation of [^{35}S]-methionine into luciferase protein was used to measure protein synthesis. The effect of CY and related compounds was determined by comparing the incorporation of label with that of control incubations.

Hepatocyte cell culture

Isolation of rat hepatocytes was done aseptically according to the method of Moldeus et al.[5] Initial cell viability was 90% as determined by 0.2% Trypan blue exclusion. The plating medium was DME/F12 containing high glucose, 10% fetal bovine serum, insulin 1μg/mL, hydrocortisone (50nM) supplemented with 1 mM methionine. Cells (2 mL suspension of 0.8-1.0 x 106) were plated in 6-well cluster plates. (35 mm) pre-coated with rat tail collagen and incubated at 37 °C in 5% CO_2, 95% air. Cells were allowed to attach for two to three hours, the medium was changed to remove the fetal bovine serum and any unattached cells. Natural CY, synthetic EPI-CY and intermediates were added at the concentrations stated in the Results section. Cells were incubated for 20 hours followed by a two hour incubation in sulfur amino acid-free medium containing about 2 μCi of [^{35}S] methionine/mL to determine the effect of CY on protein synthesis. Aliquots of medium at the end of the 20 hour and the two hour incubations were taken for analysis and for counting of radioactivity.

Hepatocyte extraction

At the end of the incubation hepatocytes were washed in phosphate-buffered saline (PBS) followed by further washing in PBS containing 1 mM methionine. The cells were then scraped in 0.5 mL of PBS. Lactate dehydrogenase activity and protein levels were measured. 10% trichloroacetic acid (TCA) was added to an equivalent volume of hepatocyte extract to precipitate protein.

Measurement of the toxicity to hepatocytes of CY

Toxicity (cell lysis) due to CY was measured by the release of lactate dehydrogenase (LDH) from the cytosol into the medium.[6] LDH was measured in the medium and in the

cell extract. Percentage LDH release (cell death) was the LDH activity in the medium as a percentage of total LDH (cellular + medium).

Effect of CY on protein synthesis in hepatocytes

The protein precipitate obtained by centrifugation following addition of 10% TCA to the hepatocyte extract was resuspended in 5% TCA and centrifuged again. The cell pellet was then dissolved in 0.2 N NaOH and an aliquot used to determine the incorporation of [35S] methionine into protein.

Measurement of reduced glutathione (GSH)

Cellular GSH was measured in the 10% TCA supernatant of the cell extract by the method of Tietze.

5.4 References

[1] *A convenient method for estimation of alkyllithium concentrations.* Kofron, W. G.; Baclawski, L. M. *J. Org. Chem.* **1976**, 41, 1879-1880.

[2] *NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities.* Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**; *62*(21); 7512-7515.

[3] *A Practical Guide to First-Order Multiplet Analysis in ^{1}H NMR Spectroscopy.* Hoye, T.R.; Hansen, P.R.; Vyvyan, J.R. *J. Org. Chem.* **1994**; *59*(15); 4096-4103.

[4] for examples of tertiary amide rotamer separation on chiral stationary phases see: Clayden, J.; Pink, J.H, *Angew. Chem. Int. Ed. Eng.* **1998**, 1937-1939.

[5] *Isolation and use of liver cells.* Moldeus, P.; Hogberg, J.; Orrenius, S. *Methods Enzymol.* **1978**, *51*, 60-70.

[6] *The Role of Glutathione in the Toxicity of a Novel Cyanobacterial Alkaloid Cylindrospermopsin in Cultured Rat Hepatocytes.* Runnegar, M. T.; Kong, S. M.; Zhong, Y. Z.; Ge, J. L.; Lu, S. C. *Biochem. Biophys. Res. Comm.* **1994**, *201*, 235-241.

[7] *Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues.* Tietze, F. *Anal. Biochem.* **1969**, *27*, 502-522.

Appendix 3: X-ray crystallographic data:

Compound 153

Table 7. Crystal data and structure refinement for smmrw19.

Identification code	rw19 (smmrw19)	
Empirical formula	C8 H10 N O3	
Formula weight	168.17	
Temperature	298(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)/n	
Unit cell dimensions	a = 7.6507(2) Å	α= 90°.
	b = 10.5383(4) Å	β= 96.443(2)°.
	c = 9.8111(4) Å	γ = 90°.
Volume	786.03(5) Å^3	
Z	4	
Density (calculated)	1.421 Mg/m^3	
Absorption coefficient	0.110 mm^{-1}	
F(000)	356	
Crystal size	0.100 x 0.350 x 0.450 mm^3	
Theta range for data collection	2.85 to 23.30°.	
Index ranges	-8<=h<=7, -8<=k<=11, -10<=l<=10	
Reflections collected	3533	
Independent reflections	1137 [R(int) = 0.0267]	
Completeness to theta = 23.30°	99.8 %	
Absorption correction	SADABS	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	1137 / 0 / 110	
Goodness-of-fit on F^2	1.009	
Final R indices [I>2sigma(I)]	R1 = 0.0345, wR2 = 0.0972	
R indices (all data)	R1 = 0.0445, wR2 = 0.1048	
Extinction coefficient	0.040(7)	
Largest diff. peak and hole	0.127 and -0.134 e.Å^{-3}	

Table 8. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for smmrw19. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
N(1)	9304(2)	4399(1)	6852(1)	43(1)
O(1)	10506(2)	5285(1)	7625(1)	50(1)
O(2)	8925(2)	1702(1)	6556(1)	54(1)
O(3)	10639(2)	1714(1)	4919(1)	62(1)
C(1)	13738(3)	4424(2)	6422(2)	67(1)
C(2)	12351(2)	3688(2)	7079(2)	45(1)
C(3)	11710(2)	4354(2)	8300(2)	47(1)
C(4)	10480(2)	3485(2)	8981(2)	50(1)
C(5)	8787(2)	3618(2)	7989(2)	46(1)
C(6)	7897(3)	2430(2)	7429(2)	56(1)
C(7)	10024(2)	2297(2)	5801(2)	43(1)
C(8)	10563(2)	3638(2)	6131(2)	38(1)

Table 9. Bond lengths [Å] and angles [°] for smmrw19.

N(1)-O(1)	1.4613(18)
N(1)-C(5)	1.476(2)
N(1)-C(8)	1.4916(19)
O(1)-C(3)	1.454(2)
O(2)-C(7)	1.338(2)
O(2)-C(6)	1.446(2)
O(3)-C(7)	1.200(2)
C(1)-C(2)	1.516(2)
C(2)-C(3)	1.516(2)
C(2)-C(8)	1.566(2)
C(3)-C(4)	1.520(2)
C(4)-C(5)	1.536(2)
C(5)-C(6)	1.500(2)
C(7)-C(8)	1.497(2)
O(1)-N(1)-C(5)	99.84(12)
O(1)-N(1)-C(8)	100.82(11)
C(5)-N(1)-C(8)	107.47(13)
C(3)-O(1)-N(1)	97.73(12)
C(7)-O(2)-C(6)	119.80(14)
C(1)-C(2)-C(3)	113.79(15)
C(1)-C(2)-C(8)	111.79(15)
C(3)-C(2)-C(8)	98.45(13)
O(1)-C(3)-C(2)	101.34(13)
O(1)-C(3)-C(4)	102.42(13)
C(2)-C(3)-C(4)	109.84(15)
C(3)-C(4)-C(5)	100.73(13)
N(1)-C(5)-C(6)	109.75(14)
N(1)-C(5)-C(4)	104.55(13)
C(6)-C(5)-C(4)	118.16(15)
O(2)-C(6)-C(5)	113.89(15)
O(3)-C(7)-O(2)	118.79(16)
O(3)-C(7)-C(8)	121.22(16)
O(2)-C(7)-C(8)	119.84(14)
N(1)-C(8)-C(7)	115.67(13)
N(1)-C(8)-C(2)	105.51(12)
C(7)-C(8)-C(2)	111.20(13)

Symmetry transformations used to generate equivalent atoms:

Table 10. Anisotropic displacement parameters ($Å^2 x 10^3$) for smmrw19. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2 a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
N(1)	48(1)	41(1)	40(1)	0(1)	4(1)	7(1)
O(1)	62(1)	36(1)	51(1)	-2(1)	4(1)	5(1)
O(2)	63(1)	47(1)	53(1)	-5(1)	11(1)	-9(1)
O(3)	74(1)	58(1)	57(1)	-18(1)	19(1)	0(1)
C(1)	53(1)	70(1)	79(2)	-7(1)	14(1)	-9(1)
C(2)	43(1)	39(1)	50(1)	2(1)	0(1)	2(1)
C(3)	52(1)	43(1)	42(1)	-2(1)	-6(1)	1(1)
C(4)	68(1)	47(1)	33(1)	0(1)	3(1)	2(1)
C(5)	52(1)	50(1)	38(1)	-2(1)	12(1)	2(1)
C(6)	57(1)	65(1)	50(1)	-4(1)	16(1)	-10(1)
C(7)	47(1)	46(1)	35(1)	-1(1)	1(1)	3(1)
C(8)	45(1)	39(1)	32(1)	4(1)	6(1)	5(1)

Table 11. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for smmrw19.

	x	y	z	U(eq)
H(1A)	14801	4457	7042	100
H(1B)	13325	5270	6218	100
H(1C)	13968	4009	5589	100
H(2A)	12767	2830	7328	53
H(3A)	12654	4722	8936	56
H(4A)	10314	3775	9896	59
H(4B)	10903	2616	9027	59
H(5A)	7937	4103	8455	55
H(6A)	7638	1900	8190	68
H(6B)	6787	2656	6910	68
H(8A)	10725	4070	5269	46

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Table 1. Crystal data and structure refinement for rwccd22.

Identification code	rwccd22	
Empirical formula	C7 H9 N O3	
Formula weight	155.15	
Temperature	177(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)/c	
Unit cell dimensions	a = 7.6847(12) Å	α= 90°.
	b = 5.7091(9) Å	β= 94.016(3)°.
	c = 15.670(3) Å	γ = 90°.
Volume	685.80(19) $Å^3$	
Z	4	
Density (calculated)	1.503 Mg/m^3	
Absorption coefficient	0.119 mm^{-1}	
F(000)	328	
Crystal size	0.20 x 0.30 x 0.40 mm^3	
Theta range for data collection	3.59 to 23.32°.	
Index ranges	-8<=h<=8, -6<=k<=6, -17<=l<=17	
Reflections collected	4104	
Independent reflections	993 [R(int) = 0.0347]	
Completeness to theta = 23.32°	99.7 %	
Absorption correction	SADABS	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	993 / 0 / 100	
Goodness-of-fit on F^2	1.044	
Final R indices [I>2sigma(I)]	R1 = 0.0408, wR2 = 0.1055	
R indices (all data)	R1 = 0.0429, wR2 = 0.1076	
Largest diff. peak and hole	0.209 and -0.307 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for rwccd22. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
N(1)	1956(2)	4137(2)	3298(1)	29(1)
O(1)	517(1)	4676(2)	3825(1)	30(1)
O(2)	4416(1)	363(2)	3182(1)	47(1)
O(3)	6211(2)	2084(2)	4131(1)	54(1)
C(1)	653(2)	2619(2)	4373(1)	31(1)
C(2)	553(2)	626(3)	3729(1)	33(1)
C(3)	1388(2)	1805(3)	2971(1)	30(1)
C(4)	2886(2)	597(3)	2592(1)	42(1)
C(5)	4795(2)	2060(3)	3752(1)	38(1)
C(6)	3398(2)	3768(3)	3971(1)	33(1)
C(7)	2483(2)	2870(3)	4767(1)	38(1)

Table 3. Bond lengths [Å] and angles [°] for rwccd22.

N(1)-O(1)	1.4595(15)
N(1)-C(3)	1.4811(19)
N(1)-C(6)	1.4901(19)
O(1)-C(1)	1.4544(17)
O(2)-C(5)	1.336(2)
O(2)-C(4)	1.449(2)
O(3)-C(5)	1.203(2)
C(1)-C(7)	1.503(2)
C(1)-C(2)	1.519(2)
C(2)-C(3)	1.544(2)
C(3)-C(4)	1.499(2)
C(5)-C(6)	1.508(2)
C(6)-C(7)	1.561(2)
O(1)-N(1)-C(3)	99.73(10)
O(1)-N(1)-C(6)	100.59(10)
C(3)-N(1)-C(6)	107.71(11)
C(1)-O(1)-N(1)	97.96(9)
C(5)-O(2)-C(4)	119.24(12)
O(1)-C(1)-C(7)	101.04(12)
O(1)-C(1)-C(2)	102.37(11)
C(7)-C(1)-C(2)	110.29(13)
C(1)-C(2)-C(3)	100.43(12)
N(1)-C(3)-C(4)	109.54(12)
N(1)-C(3)-C(2)	104.70(11)
C(4)-C(3)-C(2)	118.44(14)
O(2)-C(4)-C(3)	113.64(13)
O(3)-C(5)-O(2)	119.09(15)
O(3)-C(5)-C(6)	120.93(15)
O(2)-C(5)-C(6)	119.72(14)
N(1)-C(6)-C(5)	115.86(12)
N(1)-C(6)-C(7)	104.96(11)
C(5)-C(6)-C(7)	109.74(13)
C(1)-C(7)-C(6)	99.44(11)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x 10^3$) for rwccd22. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2 a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
N(1)	28(1)	32(1)	28(1)	1(1)	4(1)	1(1)
O(1)	31(1)	30(1)	31(1)	0(1)	4(1)	6(1)
O(2)	32(1)	47(1)	65(1)	-10(1)	10(1)	7(1)
O(3)	26(1)	57(1)	77(1)	10(1)	-5(1)	0(1)
C(1)	35(1)	32(1)	27(1)	3(1)	8(1)	3(1)
C(2)	31(1)	31(1)	38(1)	-1(1)	8(1)	-2(1)
C(3)	30(1)	35(1)	26(1)	-4(1)	1(1)	-1(1)
C(4)	39(1)	49(1)	39(1)	-11(1)	10(1)	-1(1)
C(5)	26(1)	38(1)	51(1)	7(1)	5(1)	-3(1)
C(6)	28(1)	32(1)	38(1)	-1(1)	-4(1)	-2(1)
C(7)	44(1)	41(1)	27(1)	0(1)	-3(1)	7(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for rwccd22.

	x	y	z	U(eq)
H(1A)	-255	2541	4800	37
H(2A)	-668	152	3575	40
H(2B)	1232	-753	3943	40
H(3A)	452	2049	2503	36
H(4A)	2505	-982	2396	51
H(4B)	3207	1488	2084	51
H(6A)	3956	5313	4114	39
H(7A)	2968	1353	4977	45
H(7B)	2555	4027	5239	45

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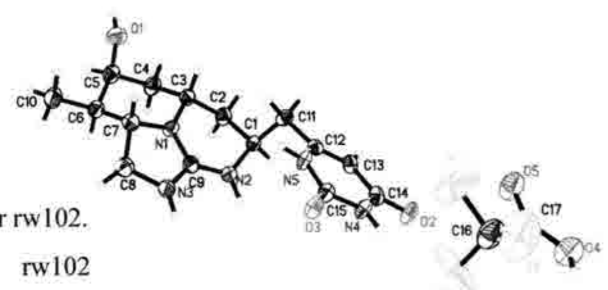

Table 1. Crystal data and structure refinement for rw102.

Identification code	rw102	
Empirical formula	C17 H23 F3 N5 O5	
Formula weight	434.40	
Temperature	168(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)/n	
Unit cell dimensions	a = 11.120(2) Å	α= 90°.
	b = 10.3102(19) Å	β= 108.003(3)°.
	c = 17.352(3) Å	γ = 90°.
Volume	1892.1(6) $Å^3$	
Z	4	
Density (calculated)	1.525 Mg/m^3	
Absorption coefficient	0.132 mm^{-1}	
F(000)	908	
Crystal size	0.20 x 0.08 x 0.02 mm^3	
Theta range for data collection	1.94 to 20.81°. Crystal did not diffract beyond this.	
Index ranges	-11<=h<=11, -10<=k<=10, -17<=l<=17	
Reflections collected	9169	
Independent reflections	1983 [R(int) = 0.0959]	
Completeness to theta = 20.81°	99.9 %	
Absorption correction	SADABS	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	1983 / 0 / 299	
Goodness-of-fit on F^2	0.910	
Final R indices [I>2sigma(I)]	R1 = 0.0603, wR2 = 0.1393	
R indices (all data)	R1 = 0.0842, wR2 = 0.1548	
Extinction coefficient	0.028(3)	
Largest diff. peak and hole	0.234 and -0.331 e.$Å^{-3}$	

Comment: F1, F2, F3, are disordered and are modeled as partial atoms.

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for rw102. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(1)	9048(3)	9716(3)	1787(2)	39(1)
O(2)	9170(3)	-25(3)	3956(2)	37(1)
O(3)	10320(3)	3515(3)	5567(2)	33(1)
O(4)	7942(3)	375(4)	10121(2)	46(1)
O(5)	7281(4)	1879(3)	9170(2)	45(1)
N(1)	10595(4)	7048(4)	2737(2)	25(1)
N(2)	10886(4)	4882(4)	3107(2)	30(1)
N(3)	12099(4)	6581(4)	3864(2)	33(1)
N(4)	9714(4)	1759(4)	4746(2)	28(1)
N(5)	9374(4)	3816(4)	4217(2)	27(1)
C(1)	9755(4)	4491(4)	2459(3)	26(1)
C(2)	9530(5)	5444(4)	1748(3)	31(1)
C(3)	9571(5)	6860(4)	1988(3)	28(1)
C(4)	9829(5)	7742(5)	1353(3)	35(1)
C(5)	10129(5)	9148(5)	1657(3)	33(1)
C(6)	11232(5)	9199(5)	2430(3)	29(1)
C(7)	10999(4)	8333(4)	3082(3)	28(1)
C(8)	12170(5)	7996(5)	3797(3)	39(2)
C(9)	11187(5)	6111(5)	3238(3)	28(1)
C(10)	11528(5)	10581(5)	2746(3)	40(1)
C(11)	8628(4)	4319(4)	2777(3)	27(1)
C(12)	8912(4)	3337(5)	3444(3)	24(1)
C(13)	8823(4)	2051(5)	3328(3)	28(1)
C(14)	9224(4)	1181(5)	3998(3)	27(1)
C(15)	9841(5)	3069(5)	4886(3)	28(1)
C(16)	8518(7)	2547(6)	10449(4)	47(2)
C(17)	7838(5)	1499(5)	9845(3)	34(1)
F(1)	8230(20)	2480(30)	11141(16)	94(8)
F(1A)	8070(20)	2700(30)	11033(19)	51(7)
F(2)	7974(16)	3767(18)	10171(12)	89(6)
F(2A)	8637(12)	3634(13)	10112(8)	56(3)
F(3)	9620(50)	2810(40)	10620(30)	100(20)
F(3A)	9769(19)	2237(11)	10739(12)	74(4)

Table 3. Bond lengths [Å] and angles [°] for rw102.

O(1)-C(5)	1.416(6)	C(4)-C(5)	1.543(7)
O(2)-C(14)	1.246(5)	C(5)-C(6)	1.513(7)
O(3)-C(15)	1.226(6)	C(6)-C(7)	1.525(7)
O(4)-C(17)	1.245(6)	C(6)-C(10)	1.525(7)
O(5)-C(17)	1.209(6)	C(7)-C(8)	1.534(7)
N(1)-C(9)	1.330(6)	C(11)-C(12)	1.497(6)
N(1)-C(3)	1.452(6)	C(12)-C(13)	1.341(6)
N(1)-C(7)	1.466(6)	C(13)-C(14)	1.426(7)
N(2)-C(9)	1.312(6)	C(16)-F(3)	1.20(5)
N(2)-C(1)	1.461(6)	C(16)-F(1A)	1.27(2)
N(3)-C(9)	1.327(6)	C(16)-F(1)	1.34(2)
N(3)-C(8)	1.468(6)	C(16)-F(2A)	1.290(15)
N(4)-C(15)	1.372(6)	C(16)-F(3A)	1.36(2)
N(4)-C(14)	1.378(6)	C(16)-F(2)	1.414(19)
N(5)-C(15)	1.355(6)	C(16)-C(17)	1.533(8)
N(5)-C(12)	1.371(6)	F(1)-F(1A)	0.31(7)
C(1)-C(11)	1.528(6)	F(1A)-F(2)	1.83(4)
C(1)-C(2)	1.537(6)	F(2)-F(2A)	0.787(16)
C(2)-C(3)	1.515(6)	F(2A)-F(3)	1.44(6)
C(3)-C(4)	1.523(7)	F(3)-F(3A)	0.64(5)
C(9)-N(1)-C(3)	125.5(4)	C(2)-C(3)-C(4)	111.9(4)
C(9)-N(1)-C(7)	111.2(4)	C(3)-C(4)-C(5)	112.5(4)
C(3)-N(1)-C(7)	122.9(4)	O(1)-C(5)-C(6)	109.9(4)
C(9)-N(2)-C(1)	120.8(4)	O(1)-C(5)-C(4)	109.8(4)
C(9)-N(3)-C(8)	110.1(4)	C(6)-C(5)-C(4)	111.5(4)
C(15)-N(4)-C(14)	125.5(4)	C(5)-C(6)-C(7)	111.1(4)
C(15)-N(5)-C(12)	124.1(4)	C(5)-C(6)-C(10)	112.1(4)
N(2)-C(1)-C(11)	111.2(4)	C(7)-C(6)-C(10)	110.4(4)
N(2)-C(1)-C(2)	109.2(4)	N(1)-C(7)-C(6)	109.1(4)
C(11)-C(1)-C(2)	114.6(4)	N(1)-C(7)-C(8)	101.6(4)
C(3)-C(2)-C(1)	114.3(4)	C(6)-C(7)-C(8)	115.9(4)
N(1)-C(3)-C(2)	108.9(4)	N(3)-C(8)-C(7)	103.7(4)
N(1)-C(3)-C(4)	107.3(4)	N(2)-C(9)-N(3)	125.7(5)

N(2)-C(9)-N(1)	122.7(5)
N(3)-C(9)-N(1)	111.6(4)
C(12)-C(11)-C(1)	110.9(4)
C(13)-C(12)-N(5)	119.4(4)
C(13)-C(12)-C(11)	124.3(4)
N(5)-C(12)-C(11)	116.1(4)
C(12)-C(13)-C(14)	120.6(5)
O(2)-C(14)-N(4)	118.9(4)
O(2)-C(14)-C(13)	125.6(4)
N(4)-C(14)-C(13)	115.4(4)
O(3)-C(15)-N(5)	123.3(4)
O(3)-C(15)-N(4)	122.0(4)
N(5)-C(15)-N(4)	114.6(5)
F(3)-C(16)-F(1A)	114(3)
F(3)-C(16)-F(1)	108(3)
F(1A)-C(16)-F(1)	13(3)
F(3)-C(16)-F(2A)	71(2)
F(1A)-C(16)-F(2A)	112.2(19)
F(1)-C(16)-F(2A)	122.6(14)
F(3)-C(16)-F(3A)	28(2)
F(1A)-C(16)-F(3A)	109.9(18)
F(1)-C(16)-F(3A)	99.1(14)
F(2A)-C(16)-F(3A)	97.8(11)
F(3)-C(16)-F(2)	100(2)
F(1A)-C(16)-F(2)	86(2)
F(1)-C(16)-F(2)	98.9(14)
F(2A)-C(16)-F(2)	33.4(8)
F(3A)-C(16)-F(2)	127.9(11)
F(3)-C(16)-C(17)	125(2)
F(1A)-C(16)-C(17)	113.6(13)
F(1)-C(16)-C(17)	111.9(13)
F(2A)-C(16)-C(17)	113.6(7)
F(3A)-C(16)-C(17)	108.6(8)
F(2)-C(16)-C(17)	109.0(9)
O(5)-C(17)-O(4)	129.3(5)
O(5)-C(17)-C(16)	115.5(5)
O(4)-C(17)-C(16)	115.2(5)
F(1A)-F(1)-C(16)	70(8)
F(1)-F(1A)-C(16)	97(8)
F(1)-F(1A)-F(2)	144(8)
C(16)-F(1A)-F(2)	50.3(14)
F(2A)-F(2)-C(16)	64.6(19)
F(2A)-F(2)-F(1A)	100(2)
C(16)-F(2)-F(1A)	43.5(11)
F(2)-F(2A)-C(16)	82(2)
F(2)-F(2A)-F(3)	126(4)
C(16)-F(2A)-F(3)	51.6(19)
F(3A)-F(3)-C(16)	91(7)
F(3A)-F(3)-F(2A)	145(8)
C(16)-F(3)-F(2A)	58(2)
F(3)-F(3A)-C(16)	61(6)

Table 4. Anisotropic displacement parameters ($Å^2$x 10^3) for rw102. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2 a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(1)	36(2)	42(2)	38(2)	7(2)	11(2)	6(2)
O(2)	59(3)	16(2)	34(2)	-2(2)	11(2)	-3(2)
O(3)	55(2)	22(2)	22(2)	-3(2)	11(2)	-1(2)
O(4)	58(3)	42(3)	28(2)	-9(2)	-2(2)	-5(2)
O(5)	58(3)	36(2)	33(2)	3(2)	0(2)	-8(2)
N(1)	32(2)	22(2)	18(2)	1(2)	2(2)	0(2)
N(2)	27(3)	26(3)	33(3)	5(2)	3(2)	0(2)
N(3)	31(3)	32(3)	31(3)	3(2)	0(2)	-5(2)
N(4)	44(3)	18(3)	26(3)	2(2)	16(2)	4(2)
N(5)	42(3)	15(2)	27(3)	2(2)	16(2)	0(2)
C(1)	26(3)	18(3)	32(3)	-3(2)	7(3)	0(2)
C(2)	34(3)	31(3)	27(3)	3(2)	9(2)	-3(2)
C(3)	29(3)	28(3)	26(3)	6(2)	7(2)	-1(2)
C(4)	43(3)	32(3)	28(3)	6(2)	9(3)	-1(3)
C(5)	39(3)	31(3)	32(3)	6(2)	16(3)	-1(3)
C(6)	32(3)	27(3)	34(3)	1(2)	16(3)	0(2)
C(7)	30(3)	28(3)	27(3)	-7(2)	10(2)	1(2)
C(8)	37(3)	34(4)	41(3)	-1(3)	4(3)	-1(3)
C(9)	26(3)	31(4)	31(3)	2(3)	14(3)	-2(3)
C(10)	45(3)	31(3)	49(4)	2(3)	21(3)	-2(3)
C(11)	31(3)	23(3)	25(3)	-2(2)	6(2)	3(2)
C(12)	23(3)	25(3)	28(3)	-4(2)	12(2)	0(2)
C(13)	29(3)	26(3)	28(3)	-3(3)	9(2)	-7(2)
C(14)	32(3)	28(4)	29(3)	-3(3)	20(3)	-4(3)
C(15)	34(3)	25(3)	31(4)	2(3)	19(3)	3(3)
C(16)	62(5)	40(5)	32(4)	5(3)	2(4)	-4(4)
C(17)	38(3)	29(4)	32(4)	4(3)	9(3)	0(3)
F(1)	152(15)	70(9)	49(8)	-16(6)	14(7)	-21(7)
F(1A)	51(9)	68(14)	50(15)	-47(12)	38(10)	-21(9)
F(2)	112(14)	32(6)	85(10)	-22(6)	-24(10)	23(10)
F(2A)	80(10)	26(6)	45(5)	1(4)	-8(7)	-2(7)
F(3)	50(20)	100(40)	160(50)	-110(40)	50(30)	-50(30)
F(3A)	47(5)	64(8)	95(6)	-24(7)	-2(4)	-19(6)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for rw102.

	x	y	z	U(eq)
H(1A)	8562	9992	1345	59
H(4D)	8296	397	10625	69
H(2A)	11372	4286	3414	36
H(3A)	12590	6112	4261	40
H(4A)	9966	1244	5170	34
H(5A)	9367	4662	4283	33
H(1B)	9936	3624	2261	31
H(2B)	10179	5292	1475	37
H(2C)	8694	5259	1351	37
H(3B)	8751	7109	2071	33
H(4B)	9080	7745	863	42
H(4C)	10552	7392	1199	42
H(5B)	10354	9658	1231	40
H(6A)	11993	8858	2305	35
H(7A)	10333	8724	3286	34
H(8A)	12956	8258	3684	47
H(8B)	12138	8427	4300	47
H(10A)	11696	11121	2326	60
H(10B)	12274	10575	3228	60
H(10C)	10804	10934	2886	60
H(11A)	8422	5160	2980	33
H(11B)	7882	4035	2329	33
H(13A)	8489	1715	2794	34

Appendix 2: Publications

Pergamon

Tetrahedron Letters 42 (2001) 769–771

TETRAHEDRON LETTERS

Construction of the A-ring of cylindrospermopsin via an intramolecular oxazinone-*N*-oxide dipolar cycloaddition

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Abstract—The efficient synthesis of an A-ring synthon for the marine hepatatoxin cylindrospermopsin has been achieved. The key step features an intramolecular oxazinone *N*-oxide/alkene dipolar cycloaddition resulting in the establishment of the three contiguous stereogenic centers in the A-ring from one pre-existing stereogenic center in a single step. © 2001 Elsevier Science Ltd. All rights reserved.

Cylindrospermopsin (**1**) was first isolated in 1992, from the marine cyanobacterium *Cylindrospermopsis raciborskii*.[1a] Following the initial discovery of **1** it has also been isolated from *Aphanizomenon* ovalisporum[1b] and *Umezikia natans*.[1c] The family has recently been expanded with the isolation of 7-*epi*-cylindrospermopsin (**2**)[2a] and deoxycylindrospermopsin (**3**).[2b] These compounds have exhibited hepatotoxic activity ($LD_{50}=0.5$–0.2 mg/kg in mice for **1**) and are partially responsible for the acute toxicity of algae blooms.[3] Much effort has been dedicated to the detection of these toxins in water supplies as outbreaks of both human and animal hepatoenteritis have become a serious problem.[4a] Cylindrospermopsin, along with mycrocrystin heptapeptides, have been implicated in the death of at least 50 people in Cararu, Brazil who consumed contaminated drinking water.[4b] Although the exact mode of action has not been elucidated for these compounds, it has been shown that cultured rat hepatocytes experience an inhibition of glutathione synthesis prior to cell death.[3,5]

1, cylindrospermopsin

2, R = OH
3, R = H

In addition to cylindrospermopsin's intriguing biological activity, the incorporation of six stereogenic centers, the zwitterionic guanidinium and sulfate units, and a densely functionalized tricyclic core have made these molecules attractive synthetic targets. A racemic total synthesis of **1** has recently been accomplished[6] and several synthetic strategies have appeared in the literature.[7] We envisioned that the three contiguous stereogenic centers of the 4-hydroxypiperidine moiety in the A-ring of **1** could be set in a single intramolecular cycloaddition reaction.

The intramolecular 1,3-dipolar cycloaddition (1,3-DC) of nitrones has become a powerful tool to access architecturally complex heterocycles.[8] Nonetheless, the use of functionalized nitrone precursors, in particular α-alkoxycarbonylnitrones, has found little application in the synthesis of natural products.[9] This is in part due to poor stereoselectivity arising from nitrone *E*/*Z* isomerization in acyclic systems. The ability of the 1,3-DC reaction to set three contiguous stereogenic centers, with the possibility of post cycloaddition manipulation of the carbonyl functionality, makes α-alkoxycarbonylnitrones functionally rich synthons for heterocyclic ring construction. The development of geometrically (*E*/*Z*) constrained nitrones, from chiral 1,4-oxazin-2-ones (oxazinone *N*-oxides), have shown excellent regio- and diastereoselectivity in intermolecular 1,3-DC reactions, but have remained unexplored in an intramolecular fashion.[10]

The synthesis of the requisite oxazinone *N*-oxide **10**, began with the esterification of (±)-3-buten-2-ol (**4**) with *N*-*t*-Boc-Gly to afford the allylic ester **5** (Scheme 1).[11]

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Scheme 1.

The dianion of **5**, generated by the addition of sodium bis(hexamethylsilyl)amide, smoothly underwent [3,3]-sigmatropic Claisen rearrangement to give the crotyl glycine derivative **6** in quantitative yield.[12]

The optically active *N*-*t*-Boc crotylglycine[13] derivative **6**, could alternatively be prepared by enolate alkylation of the commercially available oxazinone **11** affording **12** as a single stereoisomer in 64% yield.[14] Birch reduction of **12** afforded **6** in 57% yield and >99:1 er. Preliminary results have shown that rearrangement of the ester **5** derived from *R*-(−)-3-buten-2-ol proceeds in 91.5:8.5 er.

Reduction of **6** with lithium aluminum hydride generated the protected amino alcohol **7** in 62–83% yield.[15] Removal of the *t*-Boc group was effected by treatment with boron tifluoride etherate.[16] It should be noted that, the use of traditional Brønsted and Lewis acids led predominantly to urethane formation. Treatment of the free amino alcohol **8** with α-bromophenyl acetate in situ gave moderate yields of the oxazin-2-one **9**.[17]

Oxidation of the secondary amine (**9**) with Davis' oxaziridine led exclusively to the conjugated oxazinone-*N*-oxide **10** in 75% yield.[18] The dimerization of **9** to the corresponding diketopiperazine was rapid in polar solvents. Oxidation of the amine was therefore carried out immediately after isolation of **9**. The stability of nitrone **10** was surprising as no dimerization[9b] nor spontaneous cyclization was observed.

Exposure of the oxazinone-*N*-oxide **10** to elevated temperatures cleanly effected the 1,3-dipolar cycloaddition reaction to give the tricyclic isoxazolidine **13** in 78% isolated yield (Scheme 2). As expected from Oppolzer's work with intramolecular *N*-alkenylnitrone cycloadditions,[9b] the nitrone **10** added suprafacially to the alkene predominantly through the chair like *exo*-transition state $A^{\ddagger}$ to give **13**. Although the reaction produced **13** as a single diastereomer it was accompanied, as a 10:1 mixture, by the regioisomeric isoxazolidine **14** as evidenced by a quartet at 3.77 ppm (1H, $J=6.3$ Hz) in the

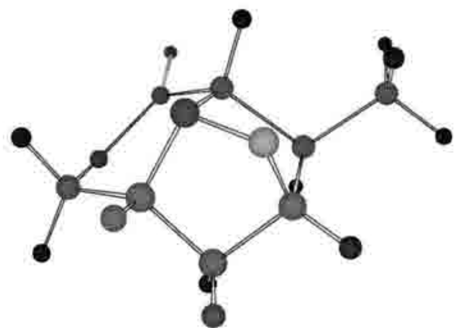

Figure 1. X-Ray structure for **13**.

Scheme 2.

^{1}H NMR spectrum for the alkoxy-methine proton. The stereochemistry of **13** was confirmed by single-crystal X-ray diffraction (Fig. 1). Presumably severe eclipsing interactions in the regioisomeric *exo*-transition state $B^{\ddagger}$ disfavor the formation of the tricyclic ring system in **14**.

Hydrogenolysis of **13** in the presence of methanol led directly to the ester **15**. NO bond cleavage in **13** leads to a highly strained bicyclic system. The resulting 1,3-diaxial disposition of the lactone renders the ensuing *trans*-esterification a facile process.

In summary, we have found that the use of α-alkoxycarbonylnitrones to be an efficient and highly stereoselective route to the 4-hydroxy piperidine moiety in the A-ring of cylindrospermopsin. Current work in our laboratory is directed toward the enantioselective total synthesis of cylindrospermopsin utilizing this general approach.

Acknowledgements

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Natural Product Synthesis

A Concise Asymmetric Synthesis of the Marine Hepatotoxin 7-Epicylindrospermopsin**

*Ryan E. Looper and Robert M. Williams**

The cyanobacterial toxin cylindrospermopsin (**1**) was isolated as the principal hepatotoxin from *Cylindrospermopsis raciborskii* in 1992 after suspicion of its involvement in an outbreak of hepatoenteritis that hospitalized 150 people on Palm Island, Australia.[1] It has since been isolated in Japan from *Umezakia natans* and in Israel from *Aphazinomenon ovalisporum*.[2] Following the discovery of the parent compound, 7-epicylindrospermopsin (**2**) was isolated from *A. ovalisporum* as a toxic minor metabolite.[3] The natural congener 7-deoxycylindrospermopsin (**3**) was initially isolated from *C. raciborskii* and has recently been co-isolated with **1** in China from *Raphidiopsis curvata*.[4] Cylindrospermopsin has been shown independently to be a potent hepatotoxin (LD_{50} – 0.2 mg kg^{-1} in mice), and 7-epicylindrospermopsin is equipotent with **1**, whereas **3** is nontoxic.[1,4a,5] It is thought that the toxicity of these substances results from a general inhibition of protein synthesis, but it is unclear whether this action occurs at the ribosomal or transcriptional level by the interaction of oxidized metabolites with DNA.[6,7] The threat posed to global public health by these molecules in drinking water and the isolation of *C. raciborskii* in several regions of the United States have prompted the National Toxicology Program (NTP) of the National Institutes of Health and the Unregulated Contaminant Monitoring Rule (UCMR) of the Environmental Protection Agency to elect **1** for toxicological and environmental evaluation.[8] The paucity of understanding of the biological functions of these agents has prompted us to develop an efficient and flexible synthetic approach that will be adapted to the production of uracil analogues to be deployed for biomechanistic evaluation.

1: R^1 = H, R^2 = OH
2: R^1 = OH, R^2 = H
3

These highly polar compounds pose a significant synthetic challenge as they contain almost as many heteroatoms as carbon atoms, and their structures include a zwitterionic guanidinium sulfate, a rare tetrasubstituted tricyclic guanidine, and a uracil moiety. Not surprisingly, these natural products have attracted the attention of synthetic chemists for over ten years.[9] Snider and co-workers completed the first racemic total synthesis of cylindrospermopsin in 20 steps eight years after its discovery.[10] However, their studies failed to illuminate the misassigned stereogenic center at C7. The configuration at that center was corrected by Weinreb and co-workers in an elegant 30-step racemic, stereoselective synthesis of **2**, thus validating the illustrated structures.[11] Shortly thereafter White and Hansen completed the first asymmetric total synthesis of **2** in 28 steps and confirmed the absolute stereochemistry as 7*S*, 8*R*, 10*S*, 12*S*, 13*R*, 14*S*.[12]

We envisaged that the three contiguous stereogenic centers of the A ring of cylindrospermopsin could be constructed by a single intramolecular 1,3-dipolar cycloaddition of the nitrone **4** to yield the tricyclic compound **5** (Scheme 1). At the onset of our work, the absolute stereochemistry of these natural products was not known. Retrosynthetic analysis suggested that the nitrone **4** could be derived from a simple amino acid, namely (*R*)- or (*S*)-crotylglycine, so that **1** could be constructed from a precursor with a single stereogenic center, which would provide the inherent flexibility to produce either enantiomer of the natural product. The two remaining, remote stereogenic centers at C7 and C8 and their respective oxidation states define this family in terms of both structure and function. Considering this, it was anticipated that a nitroaldol (Henry reaction) strategy would permit access to both C7 epimers (**1** and **2**), and through reductive nitromethylation provide access to **3**.

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Scheme 1. Retrosynthesis of the cylindrospermopsins. PMB = p-methoxybenzyl.

To this end, the protected crotylglycine derivative **11** was prepared from our oxazinone template **10** (Scheme 2).[13] Thus, alkylation of **10** with (*E*)-crotyl iodide gave the corresponding crotylated lactone in 92% yield with > 99% *ee*.[14] Reductive removal of the chiral auxiliary with lithium in ammonia gave **11** in moderate to good yield. Acidic removal of the Boc group with concomitant methyl ester formation followed by reduction with lithium aluminum hydride gave the optically pure alcohol **12**. This substance was then transformed into the free morpholinone in a one-pot procedure by treatment with bromophenyl acetate.[15] Oxidation of the secondary amine was most conveniently effected by treatment with purified MCPBA in dichloromethane to give the oxazinone *N*-oxide **4** in 84% yield.[16] Exposure of **4** to elevated temperatures gave the tricyclic isoxazolidine **5** in 78% yield as a 10:1 mixture with the minor product assumed to arise from an *endo* approach of the alkene to the nitrone.[9,17] We also observed that the treatment of **4** with scandium triflate at room temperature for up to three days generated **5** in an improved 12:1 mixture. The relative stereochemistry of **5** was confirmed by X-ray crystallography.[9]

Scheme 2. a) KHMDS, (*E*)-crotyl iodide, THF, −78°C, 92%, > 99% *ee*; b) Li, NH_3, THF, EtOH, 68–87%; c) AcCl, MeOH, 0°C→RT; d) $LiAlH_4$, THF, 65% (2 steps); e) $BrCH_2CO_2Ph$, iPr_2NEt, MeCN, 63–80%; f) MCPBA, Na_2HPO_4, CH_2Cl_2, −78°C, 84%; g) PhMe, 200°C (sealed tube), 78%. KHMDS = potassium hexamethyldisilazide, MCPBA = 3-chloroperoxybenzoic acid.

The installation of N16 proved difficult as it necessitated a simultaneous oxidation-state change at C15. It was eventually found that a two-step protocol in which the lactone carbonyl group was first reduced to the corresponding lactol with DIBAL-H fulfilled this requirement (Scheme 3). Tandem reductive amination/N–O bond cleavage gave a free diamine which was more readily handled as the urea **13** after in situ treatment with bis(*p*-nitrophenyl)carbonate.[18] Selective oxidation of the primary alcohol with TEMPO gave the sensitive aldehyde **6** in 75% yield.[19] Most common oxidation protocols showed selectivity for the secondary hydroxy group or led to epimerization of the sensitive α-ureidoaldehyde. Eventually it was found that the addition of methanesulfonic acid (1 mol%) accelerated the reaction to give **6** without attendant epimerization, as evidenced by ^{1}H NMR NOE experiments. The aldehyde **6** underwent nitromethylation upon treatment with the lithium salt of nitromethane in THF. The resulting nitroalcohol was peracetylated with acetic anhydride, and the nitroalkene was then reduced to afford the nitroalkane **14** in 67% yield. The PMB group was removed cleanly in TFA at reflux to give an 80% yield of the urea poised for alkylative activation.[20] Attempts to methylate the oxygen atom of the urea gave an unstable compound that decomposed. Fortunately, *O*-ethylation with triethyloxonium tetrafluoroborate gave the satisfactorily stable isourea **15** (78% yield), which could be subjected to subsequent reactions.[21]

Scheme 3. a) DIBAL-H, CH_2Cl_2, −78°C, 87%; b) $PMBNH_2$, H_2/Pd/C, EtOAc, then $(p\text{-}NO_2PhO)_2CO$, MeCN, 81%; c) TEMPO, $PhI(OAc)_2$, MsOH (1 mol%), $CDCl_3$, 75%; d) $MeNO_2$, *n*BuLi, THF, room temperature, 84%; e) Ac_2O, DMAP, CH_2Cl_2, then $NaBH_4$, EtOH, 67%; f) TFA (neat), reflux, 80%; g) Et_3OBF_4, Cs_2CO_3, CH_2Cl_2, 78%. DIBAL-H = diisobutylaluminum hydride, DMAP = 4-dimethylaminopyridine, MsOH = methanesulfonic acid, TEMPO = 2,2,6,6-tetramethylpiperidine *N*-oxide, TFA = trifluoroacetic acid.

Initial attempts to effect the nitroaldol reaction yielded essentially equimolar amounts of all four C7/C8 diastereomers.[22,23] The treatment of **15** and 2,6-dimethoxypyrimidine-4-carbaldehyde with 2 equivalents of tetra-*n*-butylammonium fluoride for short reaction times, followed by reductive guanidinylation of the nitro group, gave the best selectivities (Scheme 4).[24,25] These conditions afforded a kinetic 1:0.8 mixture in favor of the diastereomer required for the syn-

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Scheme 4. a) 2,6-Dimethoxypyrimidine-4-carbaldehyde, TBAF (2.0 equiv), −15°C, THF; b) $Pd(OH)_2$, H_2, 5% AcOH/MeOH; c) conc. HCl, reflux, 32% for **16**, 29% for **17** (3 steps); d) SO_3·pyridine, DMF, MS (3 Å), 59%. DMF = *N*,*N*-dimethylformamide, TBAF = tetra-*n*-butylammonium fluoride.

thesis of **2**. The other diastereomer in the mixture led to **17**, which is epimeric at C7 with a diastereomer described by Snider and co-workers (relative configuration not assigned).[10] It was found that the nitroaldol reaction must be quenched with acetic acid and the products immediately subjected to reduction conditions. Isolation of the products after treatment with TBAF provided ~1:1:1:1 mixtures of the nitroalcohols, thus indicating that the reaction is indeed highly reversible. At this stage the diastereomeric dimethoxypyrimidines were inseparable. Acidic hydrolysis of the pyrimidines gave a separable mixture of **16** (32% yield from **15**) and **17** (29%).[10-12] In our hands, the sulfonation of the C12 hydroxy group proved capricious under the conditions reported in the literature.[10-12] The use of sulfur trioxide/pyridine complex in DMF with 3-Å molecular sieves gave **2** reproducibly in 59% yield,[26] along with the corresponding bis(sulfate) (2:1 ratio by HPLC), as previously observed by others.[11,12,27]

The asymmetric synthesis of **2** detailed herein represents the shortest successful route toward this family of natural products. 7-Epicylindrospermopsin has thus been obtained in only eighteen steps with few protecting-group manipulations from commercially available **10**. Investigations directed at controlling the diastereoselectivity of the nitroaldol process to afford cylindrospermopsin (**1**) are ongoing and will be reported in due course. The uracil moiety in **1** and **2** has been deemed essential for their hepatotoxicity.[28] We believe that the incorporation of the uracil synthon at a late stage in the synthesis should render our strategy amenable to the production of uracil analogues for evaluation of their biological and hepatotoxic activity.

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1 cylindrospermopsin: R^1=H, R^2=OH, R^3=H
2 7-*epi*-cylindrospermopsin: R^1=OH, R^2=R^3=H
3 5-chlorocylindrospermopsin; R^1=H, R^2=OH, R^3=Cl
4 cylindrospermic acid
5 7-deoxycylindrospermopsin
(proposed tautomer)

Scheme 1. Structures of the cylindrospermopsins and chlorination products.

Natural Product Synthesis

Synthesis of the Putative Structure of 7-Deoxycylindrospermopsin: C7 Oxygenation Is Not Required for the Inhibition of Protein Synthesis**

*Ryan E. Looper, Maria T. C. Runnegar, and Robert M. Williams**

Cylindrospermopsin (**1**) and its naturally occurring epimer, 7-*epi*-cylindrospermopsin (**2**; Scheme 1), are attracting increasing attention as threats to public health.[1] These alkaloids are hepatotoxic metabolites of the cyanobacterium *Cylindrospermopsis raciborskii* and of three other types of cyanobacteria, and they pose a serious public-health problem when they occur in water supplies. Both **1** and **2** are potent inhibitors of protein synthesis both in vitro (IC_{50} = 200 and 480 nM, respectively) and in vivo (IC_{50} = 1.28 and 2.66 μM, respectively).[2] A number of researchers have shown that these compounds inhibit the translation of mRNA into protein.[2,3] Despite two decades of research, the exact mechanism of this inhibition remains unknown. Investigations into the removal of these toxins from water supplies have led to the isolation of both 5-chlorocylindrospermopsin (**3**) and cylindrospermic acid (**4**).[4] Both of these by-products were shown to be nontoxic by mouse bioassay. Furthermore, a related metabolite (**5**), described as 7-deoxycylindrospermopsin, has been isolated and shown to be nontoxic.[5] Interestingly, **5** is thought to exist as a mixture of tautomers (which are illustrated in Scheme 1), as its ^{1}H NMR spectrum lacked a signal for the vinylic proton on the uracil unit and the resonances were generally broad. These structure–activity relationships have led to conjecture about the steric and electronic requirements of the uracil unit and its role in the toxicity of these compounds.[1-4]

Our continuing interest in the synthesis of these intriguing natural products and elucidation of their mode of action led us to pursue the synthesis of 7-deoxycylindrospermopsin for several reasons:[6] First, the absorption maximum (λ_{max}) for **5** at 263 nm, which is consistent with the presence of a uracil unit and not the tautomers shown in Scheme 1.[7] Second, the configuration of C7 arises biosynthetically from the methyl group of an acetate unit;[8] thus, the synthesis of 7-deoxycylindrospermopsin would allow us to explore its intermediacy in the biogenesis of **1** and **2**. Last, the involvement of cytP450 oxidases in the toxicity of **1** suggests a possible oxidation event at C7,[9] thus potentially explaining the redundancy of the configuration of C7 and also implicating 7-deoxycylindrospermopsin as a potential toxin if it intercepted a common metabolic pathway.

Noting that the optical rotation of **5** has not been reported, we began by synthesizing it in a racemic fashion (Scheme 2). Diimide-mediated coupling of 3-buten-2-ol with *N*-Boc-glycine (**6**) afforded an allylic ester that underwent a smooth enolate Claisen rearrangement on treatment with 2.2 equivalents of NaHMDS.[10] Reduction of the acid and acidic removal of the Boc group gave crotyl glycinol salt **7** in good yield. Isourea **10** was obtained in nine further steps.[6b] A highlight of this sequence is the intramolecular dipolar cycloaddition to afford **8**, in which three contiguous stereogenic centers were constructed[6a] in ring A in a selectivity of approximately 10:1. An acid-mediated oxidation employing tetramethylpiperidine-1-oxyl allowed a selective oxidation of

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Scheme 2. a) 3-Buten-2-ol, DIC, DMAP, CH_2Cl_2 (96%); b) 2.2 equiv NaHMDS, THF, 0°C→RT (99%); c) EtOCOCl, Et_3N, THF, $NaBH_4$, H_2O; d) AcCl, MeOH (60%, 2 steps); e) $BrCH_2CO_2Ph$, iPr_2NEt, MeCN (63–80%); f) mCPBA, Na_2HPO_4, CH_2Cl_2, −78°C (84%); g) PhMe, 200°C, sealed tube, (78%); h) DIBAL-H, CH_2Cl_2, −78°C (87%); i) $pMBNH_2$, H_2/Pd/C, EtOAc then $(p\text{-}O_2NPhO)_2CO$, MeCN (81%); j) TEMPO, $PhI(OAc)_2$, 1 mol% MsOH, $CDCl_3$ (75%); k) $MeNO_2$, nBuLi, THF, RT (84%); l) Ac_2O, DMAP, CH_2Cl_2 then $NaBH_4$, EtOH (67%); m) TFA (neat), reflux (80%); n) Et_3OBF_4, Cs_2CO_3, CH_2Cl_2 (78%). DIC = diisopropylcarbodiimide, DMAP = 4-dimethylaminopyridine, HMDS = hexamethyldisilazide, Ac = acetyl, mCPBA = 3-chloroperoxybenzoic acid, DIBAL-H = diisobutylaluminum hydride, pMB = *para*-methoxybenzyl, TEMPO = tetramethylpiperidine-1-oxyl, MsOH = methanesulfonic acid, TFA = trifluoroacetic acid, Boc = *tert*-butoxycarbonyl.

the hydroxymethyl group, thus affording good yields of the sensitive ureido aldehyde **9**.[11]

Intrigued by the possibility of conducting a reductive guanidinylation sequence and simultaneously unmasking the uracil unit, we first synthesized the dibenzyloxypyrimidine aldehyde **12** (Scheme 3): A slightly modified procedure permitted the substitution of 2,4,6-tribromopyrimidine (**11**) with benzyl ether groups,[12] and formylation of the lithiated pyrimidine with dimethylformamide (DMF) afforded **12** in good yield. Treatment of **10** with **12** in the presence of acetic anhydride and excess cesium fluoride couples these two units directly and allows dehydration to occur in a single operation, thus affording **13** in 67% yield. This nitroalkene was produced as a single geometric isomer, which is presumed to be the *E* isomer from studies of the NOE interactions of the pyrimidine proton. Attempts to reduce the nitroalkene directly to the corresponding saturated amine led predominantly to hydrolysis of the presumed enamine intermediate.

To obviate this reactivity, sodium borohydride was employed to reduce **13** to the nitroalkane. Hydrogenolysis of this mixture reduced the nitroalkene, thus effecting reductive guanidinylation, and cleanly cleaved the benzyl ether groups to afford the uracil/guanidine compounds in an approximate 1:1 mixture of isomers. Brief exposure of this mixture to HCl facilitated the removal of the acetate group at C12 to give **14** and **15**. The configuration of **15** was ultimately determined by X-ray crystallographic analysis.[13] It should be noted that the guanidine moieties were obtained as trifluoroacetate salts after purification by HPLC. This method of purification has been used in all of the previous syntheses of **2**.[6b,14] The small amount of synthetic material produced did not allow this counterion to be detected by ^{13}C NMR spectroscopy, but it is presumed to accompany the non-zwitterionic guanidines purified by HPLC with trifluoroacetic acid (TFA) in the eluent.

The reductive guanidinylation sequence is sufficiently clean to effect the direct sulfonation of **14** and **15** at O12 to give sulfates **16** and **17** in a combined yield of 66% from **13** after a single purification by HPLC. In the ^1H NMR spectrum of **16**, the uracil proton at C5 is clearly evident at δ = 5.72 ppm, and the resonances are well defined and closely match those of **1**, except for the methylene protons at C7. In an attempt to reconcile these differences, this spectrum was compared with the spectrum that had led to the elucidation of the structure of **5**. However, it is clear that the natural material is a mixture of compounds, and it was not possible to conclude whether **16** was a minor component of that mixture.

Most significantly, our synthetic 7-deoxycylindrospermopsin (**16**) proved to be a potent inhibitor of protein synthesis in vitro, as measured in the rabbit reticulocyte lysate system.[9b] Protein synthesis was completely inhibited at 12 μM and partially inhibited at 500 nM. This effect on protein synthesis was also evaluated in whole cells (Table 1).[2] As seen, **16** completely inhibits protein synthesis at 10 μM, thus displaying a potency that is within an order of magnitude of natural **1**. Synthetic **16** also inhibits the synthesis of glutathione (GSH), in a similar fashion to **1**.[3,9a]

Scheme 3. a) BnOH, nBuLi, THF/DMF (80%); b) nBuLi, Et_2O, DMF, then 10% HCl (73%); c) **12**, CsF, Ac_2O, MeCN (67%); d) $NaBH_4$, EtOH then $Pd(OH)_2$, H_2, 5% AcOH/MeOH; e) HCl (conc.), reflux for 0.5 h (**14** = 33%, **15** = 30%; 2 steps); f) SO_3·py, DMF, 3-Å MS (**16** = 33% from **13**, **17** = 33% from **13**). Bn = benzyl, py = pyridine, MS = molecular sieves.

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Table 1: Inhibition of protein synthesis in rat hepatocytes.

Compound	Conc. [μM][a]	Protein [%][b]	GSH [%][c]
1	0.20	58	100
	1.03	15	80
	1.55	7	60
16	2	64	100
	5	28	95
	10	8	45
17	40	73	100
	80	56	100

[a] Calculated from $\varepsilon = 4600\,\text{mol}^{-1}\,\text{dm}^3\,\text{cm}^{-1}$ at 263 nm. [b] Measured by the incorporation of [^{35}S]methionine into protein (given as % of control values). [c] Measured as nmol of GSH per mg of protein (given as % of control values).

Contrary to previously reported findings,[5] we found that a sample of natural 7-deoxycylindrospermopsin also inhibits protein synthesis with potency similar to the synthetic 7-deoxycylindrospermopsin (**16**). These two materials were shown by HPLC analysis to be identical, thus corroborating the natural occurrence of **16**.[15,16]

The synthesis detailed herein should cast doubt on the notion of uracil tautomers in the purported structure of 7-deoxycylindrospermopsin. It appears unlikely that an oxygenation event at C7 or C8 occurs in the metabolism of **1**, **2**, or **16** (to generate an enol ketone), as the C8 diastereomer **17** is two orders of magnitude less toxic. Compounds **14** and **16** have been labeled with $^{13}CH_3NO_2$ to investigate their intermediacy in the biosynthesis of **1** and **2**. These studies will be reported in due course.

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[15] Co-HPLC-injection of **16** and natural 7-deoxycylindrospermopsin (Agilent zorbax C18 column (4.6 × 150 mm) with MeOH/TFA/H_2O (0.04:0.001:1) as the elutant at 2.0 mL min^{-1} with monitoring at 262 nm) gave a single peak at 5.48 min.
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Syntheses of the cylindrospermopsin alkaloids

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Abstract—An intramolecular 1,3-dipolar cycloaddition has efficiently constructed the A-ring portions of the cylindrospermopsin alkaloids. A nitro-aldol addition of an elaborated nitroalkane to a pyrimidine aldehyde followed by an intramolecular reductive guanidinylation has enabled the syntheses of all three alkaloids in this family in 18–19 steps. We report the first asymmetric synthesis of cylindrospermopsin, unambiguously assigning its absolute configuration.
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1. Introduction

Among the many toxic metabolites produced by cyanobacteria, the hepatotoxins pose the greatest threat to human health.[1] The peptidal toxins, microcystin-LR (**1**, $LD_{50}=$ 50 μg/kg) is an example of the cyclic hepta-peptides first isolated from *Microcytis aeruginosa* (Fig. 1).[2] This family of toxins has been implicated in the elevated occurrence of liver cancer in China, where surface water is relied upon.[3] They are also the only toxins implicated in human fatalities, tragically in the death of 60 people who received microcystin contaminated water at a hemodialysis center in Carauru, Brazil.[4] These peptides have been shown to be highly liver specific due to their active uptake into

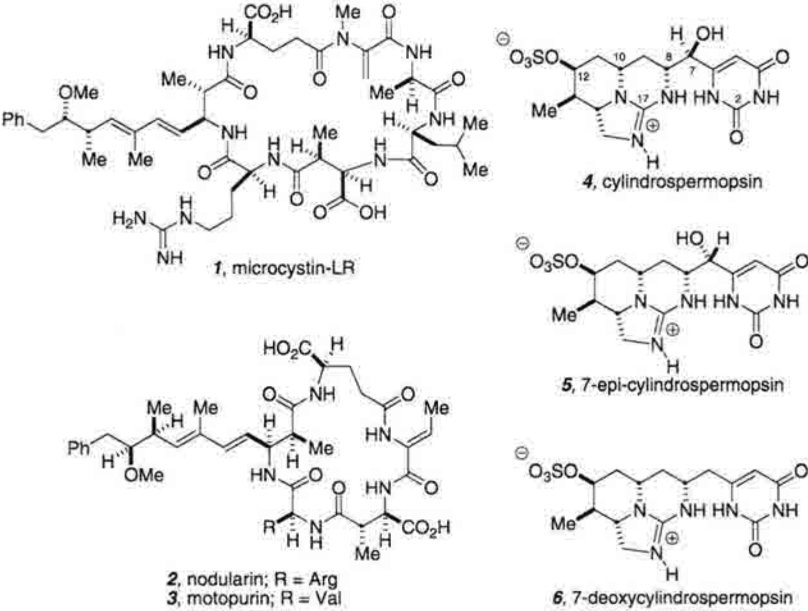

Figure 1. Hepatotoxic cyanobacterial metabolites.

Keywords: Cycloaddition; Guanidine; Alkaloid; Cyanobacteria; Hepatotoxin.
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hepatocytes via members of the organic anion transporting polypeptide family.[5] More importantly they have been shown to be potent inhibitors of the protein phosphatases PP1 and PP2A.[6] Nodularin (**2**) and motopurin (**3**) are related cyclic pentapeptides, with **3** remaining one of the most potent inhibitors of these phosphatases ($IC_{50} < 1.0$ nM).[7] Inhibition of these enzymes is thought to cause hyperphosphorylation of cytoskeletal proteins leading to the disruption of the hepatic architecture resulting in cell death of hepatocytes and liver hemorrhage.

Cylindrospermopsin (**4**) was isolated as the principal hepatotoxin from *Cylindrospermopsis raciborskii* in 1992 after suspicion of its involvement in an outbreak of hepatoenteritis that hospitalized 150 people on Palm Island, Australia.[8,9] It has since been isolated in Japan from *Umezakia natans*[10] and Israel from *Aphanizomenon ovalisporum*.[11] Following the discovery of the parent compound, 7-*epi*-cylindrospermopsin (**5**) was isolated from *A. ovalisporum* as a toxic min or metabolite.[12] 7-Deoxy-cylindrospermopsin (**6**) was initially isolated from *C. raciborskii* and has recently been co-isolated with **4** in China from *Raphidiopsis curvata*.[13] Cylindrospermopsin has been shown to be a potent hepatotoxin (LD_{50} = 0.2 mg/kg in mice), **4** is equipotent with **5** while **6** was thought to be non-toxic.[9,13a,14] Unlike **1–3**, the cylindrospermopsins do not inhibit PP1 or PP2A. Their toxicity appears to result at least in part from the inhibition of protein synthesis. The translation step of protein synthesis is inhibited by the cylindrospermopsins, but the mechanism of this inhibition is not yet known.[15] Cylindrospermopsin has also been shown, in vitro, to be a non-competitive inhibitor ($K_I = 10\ \mu M$) of the uridine monophosphate (UMP) synthase complex, although in vivo assays do not support a general inhibition of UMP synthesis.[16]

The threat posed to global public health by these molecules in drinking water and the isolation of *C. raciborskii* in several regions of the United States has prompted the NIH's national toxicology program (NTP) and the EPA's unregulated contaminant monitoring rule (UCMR) to elect **4** for toxicological and environmental evaluation.[17]

The intriguing biogenesis[18] and challenging structural features of the cylindrospermopsin alkaloids have garnered intense synthetic investigation.[19] Snider and co-workers completed the first racemic total synthesis of cylindrospermopsin 8 years after its discovery.[19h] Their accomplishments however, failed to illuminate the missasigned stereocenter at C7, elegantly corrected by Weinreb in a racemic but highly stereocontrolled synthesis of **5** validating the illustrated structures.[19j,k] Shortly thereafter Hansen and White were able to complete an asymmetric total synthesis of **5**, confirming the absolute stereochemistry as 7*S*, 8*R*, 10*S*, 12*S*, 13*R*, 14*S*.[19l,n]

2. Synthetic considerations

At the onset of this project little was known about the mechanism of action of this family of hepatotoxins. This encouraged us to develop an efficient and flexible synthesis of **4**. We were intrigued by the observations that while **4** and **5** are toxic and **6** is not. Cytochrome P450 oxidation had been purported to mediate their toxicity.[14c] We thought that an oxidation event at C7 or C8 may produce the enol-guanidine **7** (Fig. 2), alternatively C15 oxidation may generate the guanidinimine **8**. Both of these intermediates are potentially redundant through extensive tautomerization, and both are electrophilic intermediates, perhaps responsible for the observation that oxidized metabolites of **4** may alkylate DNA.[14d] These considerations helped guide our synthetic investigations. We envisaged a late stage guanidine installation via a reductive guanidinylation of the nitronol **9**. This nitro-aldol disconnection might lead to both the *anti* and *syn* C7 diastereomers required for the synthesis of **4** and **5**, respectively.[20] Further, diastereomers at C8 would allow us to test the possibility that a C7/C8 oxidation event generates an identical metabolite (i.e., **7**).

The three contiguous stereocenters in the A-ring were to be created through an intramolecular nitrone dipolar cycloaddition producing **10**.[21] The ultimate starting point of the synthesis would then be either antipode of the simple crotyl glycine derivative **11**. This was desirable as the absolute configurations of **4–6** were unknown and could not be discerned from their biogenesis. Although the absolute configuration of **4** has been inferred, it was confounding that **4** isolated from *C. raciborskii* and that isolated from

Figure 2. Synthetic strategy.

A. ovalisporum were characterized with opposite signs of optical rotation.

3. Results and discussion

3.1. Synthesis of a common precursor

We investigated two strategies to obtain **11** (Scheme 1). The first began with *rac*- or (*R*)-3-buten-2-ol (**12**), which was coupled to *N*-Boc-Gly to give the ester **13**. Enolate-Claisen rearrangement of **13** gave good yields of **11** and was used to generate large quantities of racemic material for initial synthetic explorations.[22] Rearrangement of the optically pure ester through the chelated *Z*-enolate gave (*R*)-**11** in 92:8 er, with sodium being the most effective counterion. Unfortunately attempts to generate a non-chelated *E*-enolate and thus (*S*)-**11**, were ineffective merely eroding the selectivity for the *R*-enantiomer. Alternatively, the oxazinone **14** could be alkylated with crotyl iodide to give **15** as a single diastereomer.[23] Removal of the auxiliary with lithium in ammonia gave (*R*)-**11** in >99:1 er.[24] Similar results were obtained for the synthesis of (*S*)-**11**. Able to deliver both antipodes with higher optical purity, the oxazinone became the preferred method for the preparation of **11**.

Removal of the *t*-Boc group in **11** with concomitant methyl ester formation followed by reduction with lithium aluminumhydride gave the optically pure crotylglycinol (Scheme 2). This was then transformed into the free morpholinone **16** in a one-pot procedure by treatment with α-bromophenyl acetate.[25] It was found imperative that the aminoalcohol be distilled prior to use, trace amounts of water effect the annulation dramatically, and the use of the hygroscopic hydrochloride salt results in considerably lower yields. By introducing the lactone, we were confident that we could obviate dipole isomerization, which leads to diminished selectivity.[26] Oxidation of the secondary amine was most conveniently effected by treatment with purified *m*CPBA in dichloromethane to give an 84% yield of the oxazinone-*N*-oxide **17**.[27] Pleasingly, exposure of **17** to elevated temperatures gave the tricyclic isoxazolidine **18** in 78% isolated yield as a 10:1 mixture contaminated with **19**, arising from *endo*-approach of the alkene to the dipole. While treatment of **17** with scandium triflate can produce the tricycle as an improved 12:1 mixture, the reaction takes up to 3 days to reach completion at ambient temperatures. The relative stereochemistry of **18** was secured by X-ray crystallography.[19i]

Having established the stereochemistry in the A-ring, we needed to install N16 (Scheme 3). The lactone in **18** could be opened with benzylamine to give **20**, however, purification on silica gel returned **18**. To obviate this reactivity the intermediate amide and *N,O*-bond could be reduced with lithium aluminum hydride to afford the diaminodiol **21**. While benzylamine proved a convenient way to introduce a protected nitrogen, we were concerned about its orthogonality with the nitro group. *para*-Methoxybenzylamine cleanly underwent the addition to **18**, but to our surprise we were unable to effect the reduction of this more electron rich amide. We then examined a preemptive oxidation state change for C15. Thus **18** was reduced with diisobutylaluminum hydride to give the lactol

12 → 13: *N-t*-Boc-Gly, DIC, DMAP, CH_2Cl_2, 0 °C, 96%
13 → (*R*)-11: NaHMDS, THF, 0 °C-rt, ~quant.
14 → 15: Me–CH=CH–CH$_2$X, KHMDS, THF, -78 °C - rt; X = Cl (64%), X = I (92%)
15 → (*R*)-11: Li, NH_3, THF, EtOH, 68-87%

Scheme 1. Preparation of crotyl glycine.

11 → 16: 1. AcCl, MeOH; 2. $LiAlH_4$, THF; 3. PhOC(O)CH$_2$Br, *i*Pr2NEt, MeCN; 41-52% *3 steps*
16 → 17: *m*CPBA, Na_2HPO_4, CH_2Cl_2, -78°C, 84%
17 → 18 + 19: PhMe, 200°C, sealed tube, 78%; **18** *X-ray* 10 : 1 **19** *NOE's*

Scheme 2. Construction of the A-ring.

Scheme 3. N16 introduction.

22 in 87% yield. Reductive amination of 22 with either $BnNH_2$ or $PMBNH_2$ proceeded smoothly to afford 21 or 23, respectively. To aid purification, these diaminodiols were immediately converted to the thiourea using 1,1′-thiocarbonyldiimidazole or the urea using bis-*p*-nitrophenylcarbonate giving 24–26 in good yields.

Attempts to selectively oxidize the primary alcohol in the thiourea were unsuccessful. Interestingly, this system suffers from similar reactivity, used productively, in both Weinreb's and White's syntheses.[19j,l] Treatment of 24 with oxalyl chloride and DMSO surprisingly returned the chloromethyl urea 27, presumably from preferential activation of the thiourea (Scheme 4, Eq. 1). This was also observed when treating 27 with mercuric chloride and the correspoding acetoxymethyl urea was observed when treating the thiourea with Dess–Martin periodinane or $PhI(OAc)_2$/TEMPO. Efforts to introduce productive nucleophiles (i.e., a C1–N synthon) such as cyanide or nitromethane enolates in the presence or mercuric salts failed, prompting us to rely on the ureas. Attempts to oxidize the hydroxymethyl group in 25 utilizing Swern, Dess–Martin, or Ley oxidations actually showed selectivity for the secondary alcohol. Initial experiments utilizing the hindered nitroxyl oxidant, TEMPO, were promising but many of the reported conditions resulted in epimerization of the sensitive ureidoaldehyde.[28] Using $PhI(OAc)_2$ as the reoxidant proved promising as it produced no epimerization, however, the reaction failed to surpass ~30% conversion by ^1H NMR.[29] We were able to show that the rate of oxidation or conversion was independent of the concentration of TEMPO, $PhI(OAc)_2$, or substrate. This suggested that disproportionation of the nitroxyl radical to the active oxoammonium salt may be the problematic step. This equilibrium should be affected by the addition of acid,[30] and indeed the addition of 1 mol% methanesulfonic acid resulted in complete conversion of the primary alcohol by ^1H NMR and ~75% isolated yields.[31] Our concerns that the aldehyde would epimerize to the more stable axial configuration, avoiding pseudo $A^{1,3}$ strain with the urea, were negated by NOE correlations in 28.

Homologation of the aldehydes 28 or 29 by the addition of lithiated nitromethane provided an inseparable (~1.7:1) diastereomeric mixture of nitroalcohols (Scheme 5). Treatment of this mixture with acetic anhydride served both to protect the secondary alcohol and dehydrate the nitroalcohol. Fortunately this provided a single diastereomer of the nitroalkene, assuring us that epimerization of the aldehyde had not occurred. This nitroalkene was reduced

Scheme 4. Oxidation of the ureas.

Scheme 5. Successful reductive guanidinylation.

in situ with sodium borohydride to give the homologated nitroalkanes **30** and **31** in reasonable yield for the two steps. Reduction of the nitro group in **30** provided an amine that failed to cyclize to the guanidine **32**. Attempts to force this guanidinylation with heat, Lewis acids or protic acids were unsuccessful. This forced us to pursue the deprotection of the *p*-methoxybenzyl group in **31**, anticipating the activation of the urea as an *O*-alkylisourea. Refluxing **31** in neat trifluoroacetic acid[32] cleanly provided the free urea that could be *O*-alkylated with methyl or ethyl Meerwein's salts in the presence of an inorganic base. The *O*-Me isourea could be synthesized, however, this proved to be unstable, returning the urea after nucleophilic displacement of the methyl group.[33] A slightly more sterically hindered *O*-ethyl isourea was superior and stable to subsequent reaction conditions. Hydrogenolysis of **33** cleanly gave the tricyclic guanidine **35** in 96% isolated yield. Interestingly, the intermediate *N*-hydroxy guanidine **34** could be isolated if the reduction was interrupted after 0.5 h and conducted without the addition of protic acid. Conducting the reduction in the presence of acetic acid accelerated the reduction of **34**, rendering it virtually undetectable.

3.2. Synthesis of 7-*epi*-cylindrospermopsin

Having a substrate that successfully participated in the reductive guandinylation we were poised to construct the C7–C8 bond. Initial attempts to effect the nitro-aldol led to disappointing selectivities, yielding equimolar amounts of all four C7–C8 diastereomers. It was found imperative that the nitro-aldol reaction be quenched with AcOH and reduced. Treatment of **33** and 2,6-dimethoxypyrimidine-4-carbaldehyde (**36**)[34] with 2 equiv of tetra-*n*-butylammonium fluoride for short reaction times gave the best selectivities after reductive guanidinylation giving a 1:0.8 (**37**:**38**) mixture favoring the diastereomer required for the synthesis of 7-*epi*-cylindrospermopsin (Scheme 6). If the nitro-aldol products are purified without an acid quench, a ~1:1:1:1 mixture of diastereomers is formed, indicating that the reaction is indeed highly reversible. Thus, all reactions were quenched with 20% AcOH in THF and immediately subjected to reductive guanidinylation. At this stage the diastereomeric dimethoxypyrimidines were inseparable. Acidic hydrolysis of the pyrimidines gave a separable mixture of **39** (32% yield from **33**) and **40** (29%), isolated as their trifluoroacetate slats after purification.[19m] The use of sulfultrioxide–pyridine complex in DMF with 3 Å molecular sieves reproducibly gave **5** in 59% yield also as previously obtained as a ~2:1 mixture with its bis-sulfate.[19j–l] Synthetic **5** had spectroscopic properties identical to those reported. The optical rotation also agreed well: $[\alpha]_D^{25}$ −12.5 (*c* 0.04, H_2O); lit. $[\alpha]_D^{24}$ −20.5 (*c* 0.04, H_2O).[12]

Attempts to control this nitro-aldol process through the use of chiral Lewis acids that have been employed in the asymmetric additions of nitromethane or silylnitronates to aldehydes proved futile.[35] This is in part due to the extreme electrophilicity of the pyrimidine aldehyde **36**, which commonly underwent rapid disproportionation, returning the corresponding pyrimidinemethanol.[36] Cinchonidinium fluoride catalysts also provided equimolar mixtures and typically <10% conversion.[37]

Scheme 6. Synthesis of 7-*epi*-cylindrospermopsin.

33 → 41 CHO; 1. 1.0 eq.TBAF THF, -15 °C, 0.5h. 2. H_2 / $Pd(OH)_2$ MeOH, 5% HOAc 3. conc. HCl reflux (0.5h) (0.9 : 0.9 : 1 : 0.5 dr) → 42, 20% $F_3CCO_2^{\ominus}$ + 43 $F_3CCO_2^{\ominus}$ + 39 + 40; SO_3·pyr DMF, 3Å MS 60% (6 : 1 bis-sulfate) → 4, cylindrospermopsin

Scheme 7. Synthesis of cylindrospermopsin.

3.3. Synthesis of cylindrospermopsin

At this juncture we were intrigued by the possibility of conducting our reductive guanidinylation sequence while simultaneous unmasking the uracil. The di-benzyloxypyrimidine aldehyde **41** was synthesized (Scheme 7).[38] Treatment of **33** with **41** and 1.0 equiv TBAF for 0.5 h followed by reductive guanidinylation gave an extremely clean mixture of diastereomers by ^{1}H NMR, indicating that the benzyl groups are efficiently cleaved under the reducing conditions. Although we had experienced partial cleavage of the acetate group under hydrogenolysis conditions at higher hydrogen pressures, we were unable to drive this cleavage to completion. Thus it remained necessary to expose the mixture to concd HCl briefly (0.5 h). At this stage we could correlate all the diastereomers, with **42** and **43** being identical to the racemic diastereomers synthesized by Snider and Xie. Although this 3-step reaction sequence produces a ~1:1:1:0.5 mixture of **42**:**43**:**39**:**40** the overall chemical yield is excellent with **42** isolated in 20% yield after HPLC purification. Sulfonation, again with sulfur trioxide–pyridine complex, gives cylindrospermopsin in 60% yield, representing the first asymmetric synthesis of **4**.

Interestingly, synthetic cylindrospermopsin carrying the 7*R*, 8*R*, 10*S*, 12*S*, 13*R*, 14*S* configuration exhibits an $[\alpha]_D^{25}$ +7.7 (*c* 0.05, H_2O). The natural material first isolated from *C. raciborskii* displays an opposite rotation; $[\alpha]_D^{25}$ −30.1 (*c* 0.1, H_2O).[9] From *A. ovalisporum*, however, the optical rotation is consistent with synthetic **4**: $[\alpha]_D^{25}$ +12.5 (*c* 0.6, H_2O).[12] It would seem unlikely that the two metabolites would carry opposite absolute configurations as the polyketide synthetases involved in their biogenesis are highly conserved.[39] To reconcile these differences in optical rotation, Circular dichroism (CD) spectra were obtained in water of natural **4** obtained from *C. raciborskii* and compared to that of synthetic **4** at ~44 μg/mL (Fig. 3). Natural cylindrospermopsin displayed a Cotton effect at 264 nm ($\Delta\varepsilon = -6.949$) and 228 nm ($\Delta\varepsilon = -4.243$). Synthetic **4** showed identical Cotton effects at 264 nm ($\Delta\varepsilon = -8.797$) and 229 nm ($\Delta\varepsilon = -4.432$). Although it is unclear what caused the erroneous optical rotation for **4** isolated from *C. raciborskii* it is now clear that cylindrospermopsin does indeed carry the 7*R*, 8*R*, 10*S*, 12*S*, 13*R*, 14*S* configuration from both organisms (*C. raciborskii* and *A. ovalisporum*).

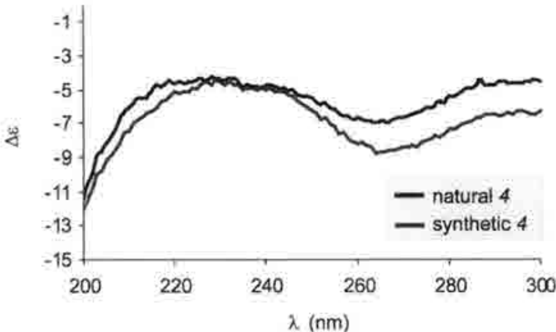

Figure 3. CD spectra of natural and synthetic **4**.

3.4. Synthesis of 7-deoxycylindrospermopsin

Having completed the syntheses of the two oxygenated cylindrospermopsin alkaloids we next focused on the synthesis of **6** (Scheme 8).[19c] We were intrigued that **6** was thought to exist as a mixture of unconjugated uracil tautomers, as the ^{1}H NMR spectrum lacked the vinyllic uracil proton, yet it displayed a λ_{max} = 263 nm, consistent with the presence of a fully conjugated uracil.[13]

Treatment of the racemic isourea (*rac*-**33**) with the aldehyde **41**, acetic anhydride, and cesium fluoride affords the nitroalkene **44** in 67% yield. Although fluoride promoted coupling and subsequent acetic anhydride mediated eliminations of nitroalcohols are known, they generally require two distinct steps and require a molar excess of the nitroalkane partner.[40] This sequence generates **44** in a single operation with only 1 equiv of both the aldehyde and the nitroalkane, making this protocol amenable to complex molecule synthesis. The nitoalkene is thought to carry the *E* geometry around the tri-substituted double bond. It was

Scheme 8. Synthesis of 7-deoxycylindrospermopin.

hoped that **44** could be directly reduced to **45** [via the intermediate ene-guanidine]. However, subjection of **44** to the reductive guanidinylation conditions returns a complex mixture, containing products arising from hydrolysis of the intermediate enamine prior to ring closure. To circumvent this hydrolysis, **44** was subjected to a one-pot conjugate reduction/reductive guanidinylation sequence giving a 1:1 mixture of diastereomers. Again the acetates could be cleaved by brief heating in HCl to give **45** and **46**. The relative stereochemistry of these uracils was secured by X-ray analysis of **46**.[41] Again, the reductive guanidinylation sequence was clean enough that sulfonation could be executed immediately, also uncomplicated by the need to selectively sulfonate the C12 hydroxyl group. Thus racemic **6** and **47** were obtained in 66% combined yield over the three steps. Co-HPLC-injection of synthetic **6** and natural 7-deoxycylindrospermopsin produced a single peak, corroborating both the structure of **6** and its natural occurrence. Further the ^{1}H NMR spectrum of **6** (Fig. 4) clearly shows the vinyllic uracil proton at 5.72 ppm. To reconcile these differences, we compared the spectrum that led to the elucidation of 5′s structure.[13a] However, it is clear that the natural material is a mixture of compounds, and we could not conclude whether **16** was a minor component of that mixture.

3.5. Inhibition of protein synthesis

Having completed the total syntheses of all the cylidrospermosin alkaloids, we were able to examine the feasibility of our biomechanistic hypothesis for the intermediacy of **7** or **8**. While synthetic **4** was a potent inhibitor of protein synthesis in hepatocytes (4% of control at 3.3 μM), the C8 diastereomer (**38**) required a concentration of 320 μM to achieve the same level of inhibition.[14] Two orders of magnitude less toxic, this suggests that they are not processed through a common metabolic intermediate. Most significantly, our synthetic **6** also proved to be a potent inhibitor of protein synthesis, contrary to previous results.[19b] Protein synthesis was completely inhibited at 12 μM, in vitro, and at 10 μM in whole cells; displaying potency within an order of magnitude of **4**. Resembling intoxication by **4**, synthetic **6** also inhibits the synthesis of glutathione (GSH).[15c] The deoxygenated C8 diastereomer **47**, also required a 100-fold increase in concentration to elicit these effects. These results suggest that substitution at C7 is not requisite for the toxicity of these alkaloids, and that a common oxidized metabolite at C8 is not involved. In agreement with previous studies, intermediates lacking the uracil (i.e., **35**) showed greatly diminished toxicity.[14] However, the *N*-hydroxyguanidine **34** was shown to inhibit protein synthesis on a dose dependent manner at millimolar concentrations, whereas, **35** did not.

4. Conclusion

The synthetic approach detailed herein has provided an efficient and flexible route to these natural products. This strategy has enabled the first enantioselective synthesis of cylindrospermopsin and corroborated the absolute configuration of this natural product. It also permitted the first synthesis of 7-deoxycylindrospermopsin and corrected both structural and toxicological misconceptions. We are further exploiting this synthetic strategy, guided by the preliminary toxicological data, to investigate alternative N18 or C15 oxidation events and their manifestation in the toxicity of the cylindrospermopsin alkaloids.

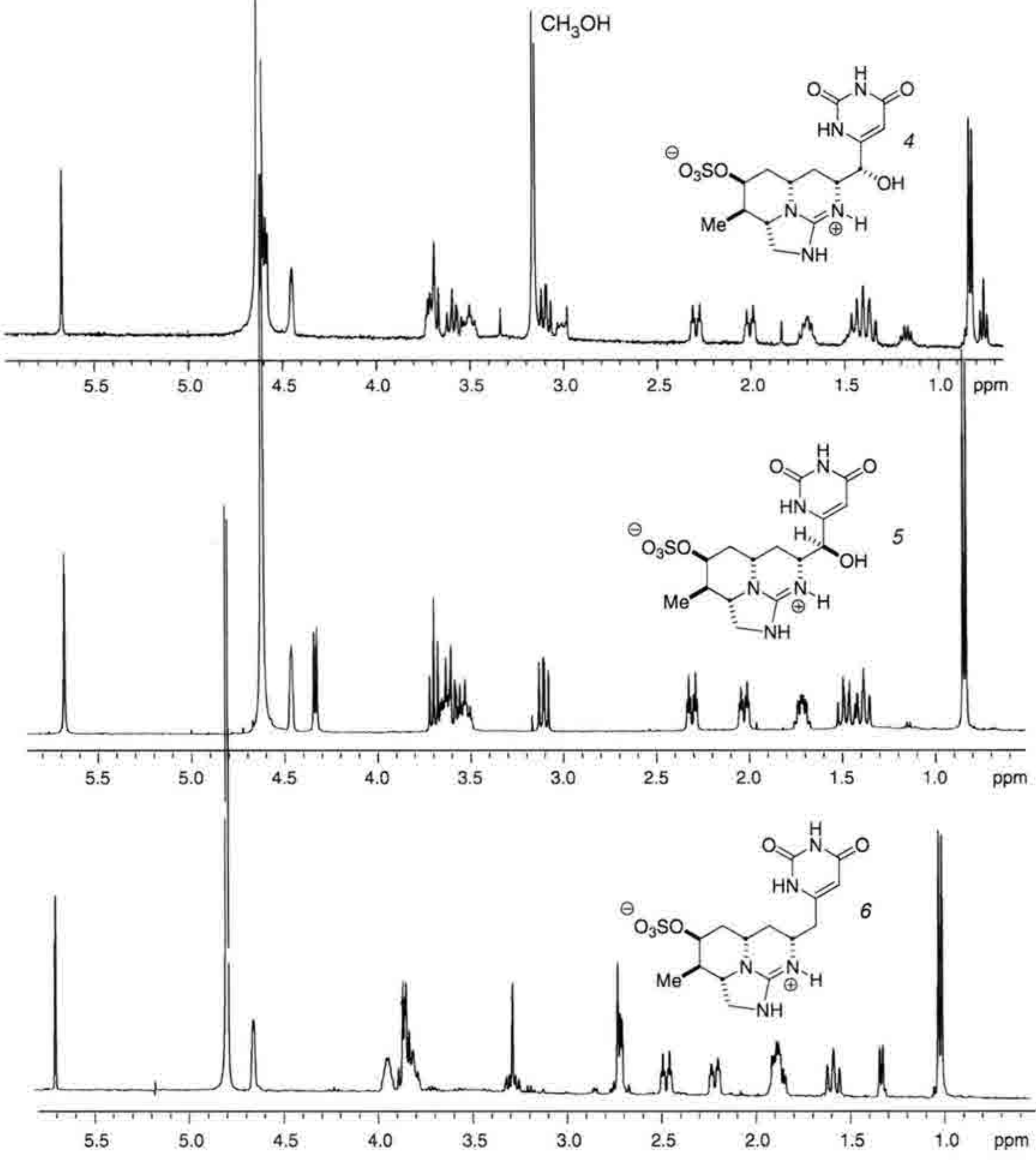

Figure 4. ^{1}H NMR of the synthetic cylindrospermopsins in D_2O (CH_3OH used as internal reference); (1) **4**, (2) **5**, (3) **6**.

5. Experimental

5.1. General

Dichloromethane, diisopropylamine, triethylamine, and *N,N*-diisopropylethylamine were distilled from CaH_2 immediately prior to use. Tetrahydrofuran, diethylether, toluene, and dimethylformamide were degassed with argon and passed through a solvent purification system (Meyer of Glass Contour) containing either alumina or molecular sieves. Flash chromatography was performed on Merk silica gel Kieselgel 60 (230–400 mesh) from EM science with the indicated solvent. ^{1}H NMR spectra were recorded on Varian 300, 400, or 500 MHz spectrometers. The chemical shifts (δ) of proton resonances are reported relative to $CHCl_3$, DMSO-d_5, HOD, or HD_2COD, and *J*-values reported in Hertz.[42] ^{13}C NMR spectra were recorded at 75, 100, or 125 MHz. The chemical shifts of carbon resonances are reported relative to the deuterated solvent peak, except those in D_2O, which are referenced to methanol. IR spectra were recorded on a Nicolet Avatar 320-FT IR spectrometer (Dep = deposited). Mass spectra were obtained on a Fisons VG Autospec. Optical rotations were obtained with a 2 mL, 1 dm cell on a Rudolf Research Autopol III polarimeter operating at 589 nm. $CHCl_3$ was distilled from $CaCl_2$ for optical rotations where indicated. HPLC data was obtained on a Waters 600 HPLC system Interfaced with Varian Dynamax Integration software using the indicated column and eluent conditions. Melting points are uncorrected.

5.1.1. 3-(*R*)-But-2-enyl-2-oxo-5-(*R*),6-(*S*)-diphenyl-morpholine-4-carboxylic acid *tert*-butyl ester (15). To a solution of NaI (6.00 g, 40.0 mmol) in MeCN (30 mL) under an argon atmosphere was added TMSCl (5.08 mL, 40.0 mmol) dropwise over 10 min. H_2O (0.36 mL, 20.0 mmol) was then added followed by crotyl alcohol (3.40 mL, 40.0 mmol). After 30 min the reaction was diluted with H_2O (100 mL) and extracted 3×50 mL hexanes. The combined organics were washed with satd $Na_2S_2O_3$, brine, and dried ($MgSO_4$). The organics were then concentrated under aspirator pressure to ~1/4 volume. To this solution, under an argon atmosphere, was added the oxazinone **14** (5.66 g, 16.0 mmol) and THF (100 mL). The mixture was cooled to −78 °C and a 0.5 M solution of KHMDS in PhMe (32.0 mL, 16.0 mmol) was added dropwise over 10 min. After 0.5 h the reaction was quenched with satd NH_4Cl and diluted with Et_2O. The organics were washed with satd $Na_2S_2O_3$, brine, and dried (Na_2SO_4). Concentration of the organics afforded a white solid, which was recrystallized from EtOH/H_2O. The white solid was dried at 60 °C to constant mass giving the crotyloxazinone (5.97 g, 92%, mp 138–141 °C). $[\alpha]_D^{25}$ +13.2 (*c* 1.00, $CHCl_3$). Optical purity was determined by HPLC, Chiracel OD-H column eluting with 97:3 hexanes/*i*PrOH at 1 mL/min: (* indicates minor rotamer): 3(*S*), 5(*S*), 6(*R*) t_R=5.78*, 6.26 min; 3(*R*), 5(*R*), 6(*S*) t_R=7.66*, 9.35 min.[43] ^1H NMR ($CDCl_3$, 400 MHz, 273 K): (mixture of rotamers, * indicates minor rotamer where discernable) δ 7.28–7.10 (m, 6H), 7.05 (t, *J*=7 Hz, 2H), 6.94 (d, *J*=7 Hz, 2H), 6.55 (t, *J*=8 Hz, 2H), 6.00* (br d, *J*=2 Hz, 1H), 5.92 (br d, *J*=3 Hz, 1H), 5.7–5.5 (m, 2H), 5.19* (d, *J*=2 Hz, 1H), 5.05 (app t, *J*=7 Hz, 1H), 4.96 (d, *J*=3 Hz, 1H), 4.88* (dd, *J*=6, 8 Hz, 1H), 2.80 (br t, *J*=6 Hz, 2H), 1.70 (overlapping d, *J*=5 Hz, 3H), 1.43* (s, 9H), 1.08 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz, 273 K): (major rotamer) δ 169.5, 153.9, 136.8, 134.7, 130.7, 128.7, 128.3, 127.9, 127.8, 127.7, 126.7, 125.2, 81.3, 79.1, 61.5, 57.2, 37.7, 28.0, 18.2. IR (Dep. $CDCl_3$): 2975 (w), 1752, 1700 (both s), 1388, 1166, 700 (all m). HRMS (FAB+): Calcd for $C_{25}H_{29}NO_4$ (*m*/*z*) 407.2097; Found (*m*/*z*) 407.2094.

5.1.2. 2-(*R*)-*tert*-Butoxycarbonylamino-hex-4-(*E*)-enoic acid ((*R*)-11). A flame dried flask fitted with a CO_2 condenser was charged with flattened lithium metal (660 mg, 95.7 mmol) under argon. Ammonia (50 mL) was condensed into the flask at −78 °C and the blue slurry stirred for 15 min. A solution of the oxazinone **15** (3.00 g, 7.36 mmol) in THF (10 mL) and EtOH (1.29 mL, 22.08 mmol) was added dropwise over 5 min. The cooling bath was removed and the mixture allowed to reflux at −33 °C for 0.5 h. The reaction was quenched by the careful addition of NH_4Cl and the ammonia allowed to evaporate. The resulting residue was taken up in satd $NaHCO_3$ (100 mL) and extracted Et_2O (2×50 mL). The aqueous layer was acidified to pH 2 with $NaHSO_4$ and extracted 3× CH_2Cl_2 (50 mL). The combined organics were washed with brine and dried (Na_2SO_4). Concentration gave the acid as a light yellow oil (1.12 g, 67%), which was used without further purification. Note: smaller reaction scale (~1 mmol) resulted in increased ~80% yields. $[\alpha]_D^{25}$ −4.30 (*c* 1.0, $CHCl_3$). Optical purity can be determined by HPLC on the free amino acid after hydrolysis with concd aqueous HCl, Crownpak CR column eluting with aqueous $HClO_4$ (pH 1) at 0.8 mL/min: 2(*R*) t_R=3.95 min.; 2(*S*) t_R=5.71 min. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.25 (br s, 1H), 5.60 (dq, *J*=15.0, 6.3 Hz, 1H), 5.40–5.24 (m, 1H), 5.00 (d, *J*=7.7 Hz, 1H), 4.34 (br m, 1H), 2.58–2.40 (m, 2H), 1.66 (dd, *J*=6.3, 0.9 Hz, 3H), 1.44 (s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.2, 155.7, 130.5, 124.5, 80.5, 52.2, 35.4, 28.6, 18.3. IR (Dep. $CDCl_3$): 3330 (m, br); 2978 (m); 1716 (s, br); 1508 (m); 1165 (s). HRMS (FAB+): Calcd for $C_{11}H_{20}NO_4$ [M+H]: (*m*/*z*) 230.1392; Found (*m*/*z*) 230.1393.

5.1.3. *tert*-Butoxycarbonylamino-acetic acid 1-methyl-allyl ester (13). To a solution of 3-buten-2-ol (**12**, 2.00 g, 27.7 mmol), 4-dimethylamino pyridine (10 mol%, 346 mg, 2.77 mmol), and *N*-*tert*-butoxycarbonyl glycine (5.35 g, 30.5 mmol) in CH_2Cl_2 (50 mL) was added diisopropylcarbodiimide (4.78 mL, 30.5 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The mixture was stirred for 2 h and filtered through Celite with CH_2Cl_2 (100 mL). The combined organics were washed with 10% HCl, satd $NaHCO_3$, brine, and dried (Na_2SO_4). The concentrated organics were purified by flash chromatography (6:1 hexanes/EtOAc) to give the ester as a colourless oil (6.12 g, 96%). If the ester was derived from (*R*)-(−)-3-buten-2-ol $[\alpha]_D^{25}$ +17.9 (*c* 1.50, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz): δ 5.83 (ddd, *J*=17.3, 10.5, 6.6 Hz, 1H), 5.40 (qd (app quintet), *J*=6.6, 6.6 Hz, 1H), 5.25 (dd, *J*=17.2, 1.2 Hz, 1H), 5.15 (dd, *J*=10.5, 1.2 Hz, 1H), 5.00 (br s, 1H), 3.90 (app d, *J*=3.9 Hz, 2H), 1.45 (s, 9H), 1.33 (d, *J*=6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.7, 155.8, 137.2, 116.2, 80.1, 72.4, 42.8, 28.5, 20.1. IR (Dep. $CDCl_3$): 3381 (m); 2980 (m); 1751 (s, sh); 1719 (s); 1520 (m); 1368 (m); 1168 (s). HRMS (FAB+): Calcd for $C_{11}H_{20}NO_4$ [M+H]: (*m*/*z*) 230.1393; Found (*m*/*z*) 230.1392.

5.1.4. *rac*-2-*tert*-Butoxycarbonylamino-hex-4-(*E*)-enoic acid (11). To a solution of ester **13** (2.72 g, 11.9 mmol) in THF (30 mL) under an Ar atmosphere was added a 1 M solution of sodium bis(trimethylsilyl)amide in THF (2.2 equiv, 26.1 mL, 26.1 mmol) at 0 °C. The mixture was allowed to warm to rt. After 2 h the reaction was quenched with satd NH_4Cl (5 mL) and brought to pH 2 by the addition of 10% HCl. The mixture was extracted with Et_2O (3× 50 mL), the combined organics were washed with brine and dried (Na_2SO_4). Concentration gave **11** as a light yellow oil (2.69 g, 99%). All spectral characteristics agreed with (*R*)-**11**.

5.1.5. 5-(*R*)-But-2-enyl-morpholin-2-one (16). Acetyl chloride (1.39 mL, 19.5 mmol) was added dropwise to MeOH (40 mL) at 0 °C and the solution stirred for 15 min. A solution of the acid **11** (1.49 g, 6.49 mmol) in MeOH (3 mL) was added and the mixture allowed to reach rt and stirred an additional 12 h. The mixture was concentrated in vacuo and further concentrated after the addition of Et_2O (2×20 mL) and PhMe (1×50 mL). The crude solid was slurried in THF (50 mL) and $LiAlH_4$ (500 mg, 13.2 mmol) added in portions over 0.5 h at 0 °C. After stirring at rt for an additional 3 h the reaction was quenched by the sequential addition of H_2O (0.5 mL), 15% NaOH (0.5 mL), and H_2O (1.5 mL). The mixture was filtered through Celite with THF and concentrated. The crude oil was purified by Kugelhror distillation, collecting material between 80 and 100 °C

(0.5 mmHg) to give the amino alcohol as a clear oil (487 mg, 65%). $[\alpha]_D^{22}$ −14.3 (c 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.45 (dq, J=15, 6 Hz, 1H), 5.31 (dddq, J=15, 6, 6, 1.5 Hz, 1H), 3.59 (dd, J=11, 4 Hz, 1H), 3.24 (dd, J=11, 8 Hz, 1H), 2.78 (dddd, J=8, 6, 6, 4 Hz, 1H), 2.60 (br s, 3H), 2.06 (ddd, J=13, 6, 6 Hz, 1H), 1.86 (ddd, J=13, 6, 6 Hz, 1H), 1.61 (dd, J=6, 1.5 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 128.3, 127.4, 66.2, 52.6, 37.5, 18.2. IR (Dep. $CDCl_3$): 3335 (s), 1573, 1435, 1051, 968 (all m). HRMS (FAB+): Calcd for $C_6H_{13}NO$ [M+H]: (m/z) 116.1075; Found (m/z) 116.1080.

A solution of the amino alcohol (395 mg, 3.43 mmol) and $i$$Pr_2NEt$ (745 mg, 3.46 mmol, 1.01 equiv) in MeCN (40 mL) was added dropwise over 1 h to a solution of bromophenyl acetate in MeCN (131 mL, final concd to be 0.02 M). The mixture was stirred for an additional 4 h and concentrated. Purification on silica with a Na_2CO_3 pre-pad eluting with 5% iPrOH/EtOAc gave the morpholinone **16** as a colourless oil (335 mg, 63%). $[\alpha]_D^{22}$ −49.6 (c 1.00, $CHCl_3$). ^{1}H NMR (CD_3OD, 300 MHz): δ 5.58 (dq, J=15.0, 6.3 Hz, 1H), 5.43 (ddd, J=15.0, 6.6, 1.5 Hz, 1H), 4.38 (dd, J=10.9, 3.7 Hz, 1H), 4.07 (dd, J=10.9, 10.9 Hz, 1H), 3.62 (ABq, dd, J=18.1, 18.1 Hz, 2H), 3.04 (m, 1H), 2.14 (dd, J=6.6, 6.6 Hz, 2H), 1.68 (dd, J=6.3, 1.2 Hz, 3H). ^{13}C NMR (CD_3OD, 75 MHz): δ 170.8, 130.1, 126.9, 75.0, 52.2, 48.2, 35.6, 18.3. IR (Dep. CD_3OD): 3400 (br s), 2964 (s), 1636, 1404 (both m), 1063 (vs). HRMS (FAB+): Calcd for $C_8H_{14}NO_2$ [M+H]: 156.1025; Found 156.1025.

5.1.6. 5-(*R*)-But-2-enyl-4-oxy-5,6-dihydro-[1,4]oxazin-2-one (17). A solution of the oxazinone **16** (260 mg, 1.67 mmol) in CH_2Cl_2 (1 mL) was added dropwise over 5 min to a solution of purified mCPBA (636 mg, 3.69 mmol) and Na_2HPO_4 (1.18 g) in CH_2Cl_2 at −78 °C. The reaction was allowed to proceed for 0.5 h and quenched with satd $Na_2S_2O_3$. The mixture was partitioned between H_2O and Et_2O and the organics further washed with 9% Na_2CO_3, brine, and dried (Na_2SO_4). The crude oil was purified on silica eluting with 1:1 hexanes/EtOAc to afford the nitrone as a colorless oil (236 mg, 84%). $[\alpha]_D^{25}$ +4.00 (c 4.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.14 (s, 1H), 5.66 (dq, J=15.0, 6.5 Hz, 1H), 5.46–5.30 (m, 1H), 4.58 (dd, J=12.3, 3.9 Hz, 1H), 4.43 (dd, J=12.3, 3.9 Hz, 1H), 3.92 (dddd, J=9.3, 3.9, 3.9, 3.9 Hz, 1H), 2.82–2.70 (m, 1H), 2.61–2.49 (m, 1H), 1.69 (d, J=6.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 158.2, 132.3, 124.7, 123.3, 68.1, 65.6, 32.8, 18.3. IR (Dep. $CDCl_3$): 1715, 1556 (both s), 1209 (m), 1061, 968 (both w). HRMS (FAB+): Calcd for $C_8H_{12}NO_3$ [M+H]: 170.0818; Found 170.0817.

5.1.7. 2-(*S*)-Methyl-5-(*S*),9-(*R*)-dioxa-8-(*S*)-aza-tricyclo[5.2.1.0.^{3,8}]decan-4-one (18) The nitrone **17** (60 mg, 0.35 mmol) was dissolved in dry toluene (7 mL) to be 0.05 M. This solution was heated in a sealed tube at 200 °C (sand bath temperature) for 2.5 h. The mixture was then cooled and the solvent removed in vacuo. The crude organics were purified on silica eluting with 1:1 hexanes/EtOAc to afford the tricyclic isoxazolidine **18** (47 mg, 78%) as a colourless oil, which solidified upon standing. An analytical sample was recrystallized from pet. ether/CH_2Cl_2 (mp 78–80 °C). $[\alpha]_D^{25}$ +3.6 (c 0.52, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.56 (dd, J=12.3, 2.7 Hz, 1H), 4.53 (d, J=6.9 Hz, 1H), 4.45 (dd, J=12.3, 1.2 Hz, 1H), 3.58 (burried m, 1H), 3.58 (d, J=3.6 Hz, 1H), 2.30 (ddd, J=11.7, 10.8, 5.4 Hz, 1H), 2.08 (qd, J=6.9, 3.7 Hz, 1H), 1.56 (dd, J=12.0, 6.0 Hz, 1H), 1.22 (d, J=7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.9, 85.1, 70.4, 65.1, 57.7, 51.7, 34.7, 19.7. IR (Dep. $CDCl_3$): 2966 (w), 1746 (vs), 14548, 1404 (both w), 1227 (m), 1117 (w), 988 (m). HRMS (FAB+): Calcd for $C_8H_{12}NO_3$ [M+H]: 170.0817; Found 170.0812.

Compound **19**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.49 (buried dd, J=10.8, 1.6 Hz, 1H), 4.00 (dd, J=10.8, 2 Hz, 3.89 (br s, 1H), 3.80 (q, J=6 Hz), 3.82–3.78 (buried m, 1H), 2.98 (d, J=4.8 Hz), 1.87 (ddd, J=12.4, 4.8, 3.2 Hz, 1H), 1.58 (dd, J=12.4, 1.6 Hz, 1H), 1.15 (d, J=6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz, 2:1 mixture): δ 168.4, 81.0, 70.9, 67.9, 61.9, 50.4, 29.5, 20.4.

5.1.8. 2-(*S*)-Methyl-5(*S*),9-(*R*)-dioxa-8-aza-tricyclo-[5.2.1.0.^{3,8}]decan-4-ol (22) To a solution of the isoxazolidine (167 mg, 0.99 mmol) in CH_2Cl_2 (20 mL) at −78 °C under argon was added DIBAL-H (1 M/toluene, 0.99 mL, 0.99 mmol) over 0.5 h. The mixture was stirred for an additional 1 h, quenched with water (0.2 mL), allowed to warm to rt, and stirred for 2 h. The mixture was filtered through Celite and concentrated. The resulting solid was recrystallized from $CHCl_3$/pentane to give the lactol as white prisms (147 mg, 87%). ^{1}H NMR ($CDCl_3$, 400 MHz): [∼2:1 mixture of anomers] δ 5.28 (s), 4.93 (d, J=2.4 Hz), 4.39 (app d, J=5.2 Hz), 4.34 (dd, J=12.4, 2.0 Hz), 3.88 (dd, J=12.8, 1.2 Hz), 3.69 (dd, J=12.4, 1.2 Hz), 3.64 (dd, J=12.4, 0.8 Hz), 3.35 (ddd, J=10.8, 4.4, 2.0 Hz), 3.25 (ddd, J=10.4, 4.4, 2.4 Hz), 3.04 (dd, J=4.4, 2.4 Hz), 2.96 (d, J=4.4 Hz), 2.14–2.01 (m), 1.99 (qd, J=6.8, 4.4 Hz), 1.79 (qd, J=6.8, 4.4 Hz), 1.58 (dd, J=11.2, 4.8 Hz), 1.51 (dd, J=11.2, 4.8 Hz), 1.07 (d, J=7.2 Hz), 1.05 (buried d, J=7.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): [∼2:1 mixture of anomers] δ 92.2, 86.9, 86.9, 73.9, 73.1, 62.1, 59.9, 59.2, 58.2, 44.5, 40.3, 36.1, 35.9, 19.9, 19.0. IR (Dep. $CDCl_3$): 3406, 3131 (br, s), 2965, 2930 (both s), 1452, 1124, 1092, 985, 710 (all m). HRMS (FAB+): Calcd for $C_8H_{14}NO_3$ [M+H]: 172.0974; Found 172.0976.

5.1.9. 7(*S*)-Hydroxy-5(*R*)-hydroxymethyl-2(*S*)-(4-methoxy-benzyl)-8(*S*)-methyl-hexahydro-imidazo[1,5-*a*]pyridin-3-one (26). To a solution of the lactol (15 mg, 0.88 mmol) in EtOAc (3 mL) was added p-methoxybenzyl amine (17 mg, 0.12 mmol). The solution was degassed with argon and then 10% Pd/C (15 mg) was added. The solution was then purged with H_2 and stirred under a hydrogen atmosphere for 12 h. The mixture was filtered and concentrated. The crude oil was dissolved in MeCN (5 mL) and cooled to 0 °C. A solution of bis-p-nitrophenyl carbonate (32 mg, 0.11 mmol) in MeCN (5 mL) was added dropwise over 15 min. After stirring an additional 0.5 h the mixture was concentrated, taken up in EtOAc (20 mL) and the organics washed 3×9% Na_2CO_3, 1×brine and dried (Na_2SO_4). The crude material was purified on silica gel eluting with EtOAc/5% iPrOH to give the urea **26** as a clear oil (19 mg, 67%). $[\alpha]_D^{25}$ +37.7 (c 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.18 (d, J=8.4 Hz, 2H), 6.86 (d, J=8.4 Hz, 2H), 5.80 (dd, J=9, 5 Hz, 1H), 4.4 (1/2ABq, J=15 Hz, 1H), 4.19 (1/2ABq, J=15 Hz, 1H), 3.94 (br dd,

$J=2.4$, 2.4 Hz, 1H), 3.90–3.72 (buried m, 3H), 3.80 (s, 3H), 3.51 (dddd, $J=9$, 5, 3, 3 Hz, 1H), 3.45 (ddd, $J=10$, 9, 9 Hz, 1H), 3.28 (dd, $J=9$, 9 Hz, 1H), 2.76 (dd, $J=9$, 9 Hz, 1H), 1.82 (d, $J=3$ Hz, 1H), 1.72 (ddd, $J=14$, 3, 3 Hz, 1H), 1.62 (ddd, $J=12$, 12, 2 Hz, 1H), 1.48 (ddd, $J=14$, 6, 3 Hz, 1H), 0.89 (d, $J=6$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.8, 159.0, 129.4, 114.0, 68.2, 64.8, 55.4, 54.4, 53.3, 47.9, 47.6, 40.0, 36.4. IR (Dep. $CDCl_3$): 3385, 2933 (both m), 1664, 1513, 1246 (all s). HRMS (FAB+): Calcd for $C_{17}H_{24}N_2O_4$ [M+H]: 322.1814; Found: 321.1811.

5.1.10. 7(*S*)-Hydroxy-2(*S*)-(4-methoxy-benzyl)-8(*S*)-methyl-3-oxo-octahydro-imidazo[1,5-*a*]pyridine-5(*R*)-carbaldehyde (29). To a solution of the diol **26** (211 mg, 0.66 mmol) in $CDCl_3$ (3 mL) was added $PhI(OAc)_2$ (318 mg, 0.99 mmol) and TEMPO (41 mg, 0.26 mmol). Methanesulfonic acid (0.63 mg, 7 μmol, 1 mol%) was then added as a solution in $CDCl_3$. The mixture was stirred for 3 h, diluted with EtOAc (30 mL) and the organics washed with satd $Na_2S_2O_3$, satd $NaHCO_3$, brine, and dried (Na_2SO_4). The resulting oil was purified on silica gel eluting with EtOAc/5% *i*PrOH) to give the aldehyde as a white foam (156 mg, 75%). $[\alpha]_D^{25}$ +84.8 (*c* 1.13, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.81 (d, $J=2.1$ Hz, 1H), 7.16 (d, $J=8.1$ Hz, 2H), 6.86 (d, $J=8.1$ Hz, 2H), 4.36 (1/2ABq, $J=15$ Hz, 1H), 4.20 (1/2ABq, $J=15$ Hz, 1H), 4.00 (br s, 1H), 3.82 (buried m, 1H), 3.79 (s, 3H), 3.40 (ddd, $J=10.5$, 9, 9 Hz, 1H), 3.28 (dd, $J=9$, 9 Hz, 1H), 2.86 (dd, $J=9$, 9 Hz, 1H), 1.90 (br d, $J=13.5$ Hz, 1H), 1.64 (dd, $J=$ 12, 12 Hz, 1H), 1.54 (br dd, $J=9$, 9 Hz, 1H), 0.90 (d, $J=$ 6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 198.3, 160.4, 158.9, 129.4, 128.7, 114.0, 67.9, 57.4, 55.4, 53.3, 47.9, 47.3, 38.4, 32.9, 13.4. IR (Dep. $CDCl_3$): 3431, 2878 (both m), 1727, 1682, 1513, 1448, 1246 (all s). HRMS (FAB+): Calcd for $C_{17}H_{22}N_2O_4$ [M+H]: 319.1657; Found: 319.1664.

5.1.11. (5*S*,7*S*,8*R*,8a*S*)-2-(4-Methoxybenzyl)-8-methyl-5-(2-nitroethyl)-3-oxo-octahydroimidazo[1,5-*a*]pyridin-7-yl acetate (31). A solution of nitromethane in THF (10:1, 20 mL) under argon was cooled to 0 °C. A 1.6 M solution of *n*BuLi (3.5 mL, 5.66 mmol) was added slowly (caution! highly exothermic) over 20 min. The mixture was stirred an additional 15 min and a solution of the aldehyde **29** (180 mg, 0.57 mmol) in THF added. The reaction was allowed to proceed for 12 h, quenched with satd NH_4Cl and extracted with EtOAc (3×10 mL). The combined organics were washed brine and dried (Na_2SO_4). The crude oil was purified on silica eluting with 1:1 hexanes/EtOAc then EtOAc/5% *i*PrOH to give the diastereomeric nitro alcohol (183 mg, 84%). To a solution of the nitroalcohol (41 mg, 0.11 mmol) and *N*,*N*-dimethylaminopyridine (3 mg, 0.025 mmol, 20 mol%) in CH_2Cl_2 under an argon atmosphere was added acetic anhydride (0.10 mL, 1.1 mmol). After stirring for 12 h the mixture was concentrated, taken up in EtOH (3 mL) and added dropwise to a slurry of $NaBH_4$ (101 mg, 2.67 mmol) in EtOH (5 mL). The mixture was stirred for 2 h and quenched by the addition of 50% $AcOH/H_2O$ (0.4 mL). The mixture was concentrated under reduced pressure and partitioned between H_2O and EtOAc. The aqueous phase was extracted again with EtOAc and the combined organics washed with satd $NaHCO_3$, brine, and dried (Na_2SO_4). The crude oil was purified on silical gel eluting with 1:1 hexanes/EtOAc to give the nitroalkane **31** as a colorless oil (40 mg, 87%). $[\alpha]_D^{25}$ +15.2 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.12 (d, $J=8$ Hz, 2H), 6.87 (d, $J=8$ Hz, 2H), 5.12 (br d, $J=6.8$, 3 Hz, 1H), 4.72 (ddd, $J=13.6$, 8.4, 5.6 Hz, 1H), 4.61 (ddd, $J=13.6$, 5.6, 5.6 Hz), 4.23 (s, 2H), 3.78 (s, 3H), 3.43 (dddd, $J=10.8$, 10.8, 3, 3 Hz, 1H), 3.28 (ddd, $J=9$, 8, 5.6 Hz, 1H), 3.18 (dd, $J=8$, 8 Hz, 1H), 2.78 (dd, $J=8$, 5 Hz, 1H), 2.41 (dd, $J=$ 13.6, 8, 5, 5 Hz, 1H), 2.05 (s, 3H), 1.83 (ddd, $J=12$, 3, 3 Hz, 1H), 1.70–1.60 (m, 2H), 0.78 (d, $J=6.8$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.4, 159.7, 159.1, 129.5, 129.0, 114.2, 73.7, 71.5, 56.1, 55.5, 48.8, 47.3, 46.6, 36.8, 36.4, 29.6, 21.3, 13.3. IR (Dep. $CDCl_3$): 2937 (m), 1737, 1693, 1550, 1513 (all s), 1442, 1374, 1351 (all m), 1242 (s). HRMS (FAB+): Calcd for $C_{20}H_{28}N_3O_6$ [M+H]: 406.1978; Found: 406.1969.

5.1.12. (5*S*,7*S*,8*R*,8a*S*)-3-Ethoxy-8-methyl-5-(2-nitroethyl)-1,5,6,7,8,8a-hexahydroimidazo[1,5-*a*]pyridin-7-yl acetate (33). The protected urea **31** (25 mg, 0.062 mmol) was dissolved in trifluoroacetic acid (1.5 mL). The mixture was refluxed for 1 h and concentrated under reduced pressure. The purple residue was taken up in EtOAc (10 mL) and washed H_2O, satd $NaHCO_3$, brine, and dried (Na_2SO_4). The crude residue was purified on silica gel eluting with EtOAc/5% *i*PrOH to give the urea (14 mg, 80%) as a white solid. $[\alpha]_D^{25}$ +17.3 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.14 (dd, $J=6$, 3 Hz, 1H), 4.80 (br s, 1H), 4.76–4.54 (m, 2H), 3.52–3.38 (m, 3H), 3.1–2.9 (m, 2H), 2.37 (dddd, $J=15$, 6, 6, 3 Hz, 1H), 2.09 (s, 3H), 1.86 (ddd, $J=12$, 3, 3 Hz, 1H), 1.84–1.78 (buried m, 1H), 1.66 (ddd, $J=12$, 3, 3 Hz, 1H), 0.87 (d, $J=6$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.5, 161.6, 73.6, 71.5, 58.8, 48.5, 42.5, 36.5, 36.6, 29.4, 21.2, 13.2. IR (Dep. $CDCl_3$): 3269, 2939 (both w), 1736, 1698, 1550 (all s), 1436, 1374 (both m), 1242 (s). HRMS (FAB+): Calcd for $C_{12}H_{20}N_3O_5$ [M+H]: 286.1403; Found: 286.1409.

To a solution of the urea (58 mg, 0.20 mmol) under argon in CH_2Cl_2 (10 mL) was added Cs_2CO_3 (650 mg, 2.0 mmol) and triethyloxonium tetrafluoroborate (386 mg, 2.0 mmol). The reaction was stirred at rt for 15 h and quenched by the addition of aqueous 9% Na_2CO_3 (5 mL). The aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organics were washed with brine and dried (Na_2SO_4). After concentration the crude mixture was purified on silica gel with 10% $MeOH/CH_2Cl_2$ to give the isourea **33** as a clear oil (49 mg, 78%). $[\alpha]_D^{25}$ +6.2 (*c* 1.00, $CHCl_3$). ^{1}H NMR (CD_3OD, 400 MHz): δ 5.22 (app br dd, $J=8.0$, 2.8 Hz, 1H), 4.61 (ddd, $J=7.6$, 7.6, 2.4 Hz, 1H), 4.21 (q, $J=7.2$ Hz, 2H), 3.59–3.46 (m, 2H), 3.55 (buried dd, $J=11.6$, 4.0 Hz, 1H), 3.25 (dd, $J=11.6$, 4.8 Hz, 1H), 2.66 (dddd, $J=18$, 8, 8, 8 Hz, 1H), 2.37 (dddd, $J=18$, 8, 8, 6 Hz, 1H), 2.08 (s, 3H), 1.94–1.79 (m, 2H), 1.65 (ddd, $J=14$, 12, 2 Hz, 1H), 1.32 (q, $J=7.2$ Hz, 3H), 0.85 (d, $J=6.8$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.6, 163.3, 73.0, 71.9, 65.4, 63.8, 52.5, 48.6, 36.2, 35.5, 30.3, 21.2, 14.6, 13.0. IR (Dep. $CDCl_3$): 2963 (m), 1735 (s), 1622, 1550, 1436, 1372, 1334 (all m), 1228 (s). HRMS (FAB+): Calcd for $C_{14}H_{24}N_3O_5$ [M+H]: 314.1715; Found: 314.1710.

5.1.13. 7-*epi*-Cylindrospermopsin diol (37). To a solution of **33** (8.0 mg, 26 μmol) and pyrimidine aldehyde **36** (5.2 mg, 31 μmol) in THF at −15 °C was added a 1 M

solution of tetra-*n*-butylammonium fluoride (51 μL, 51 μmol). The reaction was allowed to proceed for 0.5 h and quenched with Twenty percentage AcOH/THF (0.5 mL). The mixture was concentrated and the crude oil dissolved in 5% AcOH/MeOH (5.1 mL, to be 5 mM) and the solution purged with argon. 20% $Pd(OH)_2$ on carbon (32 mg) was added and the solution purged with hydrogen. After stirring for 12 h under an H_2 atmosphere the mixture was filtered through a 0.45 μm Acrodisc® and concentrated. Purification (to remove 6-hydroxymethyl pyrimidine and TBAF) by PTLC eluting with 20% MeOH/CH_2Cl_2 with 1% HCO_2H afforded an inseparable mixture (1:0.8) of the two C-7 diastereomers after stripping the silica with 20% abs EtOH/CH_2Cl_2. This mixture was then refluxed in concd HCl for 8 h and concentrated. Purification of the uracils was achieved by HPLC using a Waters Symmetry® C-18 colum (4.6×250 mm) eluting with 4% MeOH/H_2O with 1% TFA at 1.5 mL/min, monitoring at 263 nm to give 7-*epi*-cylindrospermopsin diol as a white solid (3.0 mg, 32%, t_R = 19.05 min) and the other C8 diastereomer also as a white crystalline solid (2.7 mg, 29%, t_R = 23.53 min).

Compound **37**. ^{1}H and ^{13}C NMR agreed with those previously reported.[19m] $[\alpha]_D^{25}$ −11.7 (*c* 0.06, H_2O): (lit. $[\alpha]_D^{24}$ −8.3 (*c* 0.06, H_2O)):[12] **38**: $[\alpha]_D^{25}$ +70.0 (*c* 0.20, H_2O). ^{1}H NMR (D_2O, 400 MHz): δ 5.80 (s, 1H), 4.62 (d, *J* = 4.4 Hz, 1H), 4.04 (br s, 1H), 3.88–3.74 (m, 3H), 3.28 (app t, *J* = 8.4 Hz, 1H), 2.26 (ddd, *J* = 14, 4, 3 Hz, 1H), 2.07 (ddd, *J* = 14, 4, 4 Hz, 1H), 1.87 (ddd, *J* = 15, 10, 6 Hz, 1H), 1.78–1.68 (m, 1H), 1.52 (app t, *J* = 13 Hz, 1H), 0.97 (d, *J* = 7 Hz, 3H). HRMS (FAB+): Calcd for $C_{15}H_{22}N_5O_4$ [M+H]: 336.1672; Found: 336.1672.

5.1.14. 7-*epi*-Cylindrospermopsin (5). 7-*epi*-Cyclindrospermopsin diol **37** (2.6 mg, 7.0 μmol) was co-concentrated with MeCN (2×5 mL) and PhMe (2×5 mL). The resulting solid was dried under vacuum for 0.5 h and placed under argon. DMF (0.4 mL) and activated, powdered 3 Å molecular sieves (6 mg) were added and the mixture stirred for 15 min. To this solution was added solid SO_3·pyr (11 mg, 70 μmol) and the mixture was stirred for 1 h. MeOH (0.1 mL) was added and the solvents removed in vacuo. The mixture was taken up in MeOH and filtered through a 0.45 μm Acrodisc®. Purificataion by HPLC on a Waters Symmetry® C-18 colum (4.6×250 mm) eluting with 2% MeOH/H_2O with 1% TFA at 1.5 mL/min, monitoring at 263 nm gave 7-*epi*-cylindrospermopsin **5** (t_R = 9.22 min) as a white solid after lyophilization (1.7 mg, 59%). This was preceded by its bis sulfate (t_R = 6.54 min) as a ∼2:1 mixture. $[\alpha]_D^{25}$ −12.5 (*c* 0.04, H_2O); (lit. $[\alpha]_D^{24}$ −20.5 (*c* 0.04, H_2O),[3] ^{1}H and ^{13}C NMR spectra agree with those reported.[12] HRMS (FAB+): Calcd for $C_{15}H_{22}N_5O_7S$ [M+H]: 416.1240; Found: 416.1247.

5.1.15. 2,4-Bis(benzyloxy)-6-bromopyrimidine (41). To a solution of benzyl alcohol (0.11 mL, 1.03 mmol) in THF (0.5 mL) under an argon atmosphere at 0 °C was added a 1.6 M solution of *n*BuLi in hexanes (0.62 mL, 0.99 mmol). The mixture was stirred 10 min and DMF (5 mL) added. A solution of the tribromopyrimidine in DMF (1 mL) was added and the mixture stirred at 0 °C for 3 h. The reaction was quenched with satd NH_4Cl and diluted with H_2O (10 mL). The aqueous phase was extracted with Et_2O (3×10 mL) and the combined organics washed with brine and dried (Na_2SO_4). The crude oil was purified on silica gel eluting with 15:1 hexanes/EtOAc to give the dibenzyloxypyrimidine as a clear oil (137 mg, 80%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.47–7.32 (m, 10H), 6.66 (s, 1H), 5.43 (s, 2H), 5.40 (s, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.1, 163.8, 152.3, 135.9, 135.6, 128.7, 128.6, 128.5, 128.4, 128.3, 128.3, 105.5, 70.1, 69.1. IR (Dep. $CDCl_3$): 2952 (w), 1549, 1404, 1323 (all s), 1130, 1003 (both m). HRMS (FAB+): Calcd for $C_{18}H_{16}N_2O_2^{81}Br_1$ [M+H]: 373.0375; Found 373.0363. Calcd for $C_{18}H_{16}N_2O_2Br_1$ [M+H]: 371.0395; Found 371.0383.

5.1.16. Cylindrospermopsin (4). To a solution of **33** (4.5 mg, 14 μmol) and **41** (5.5 mg, 17 μmol) in THF (120 μL) at −15 °C was added a 1 M solution of TBAF (14 μL, 14 μmol). The solution was stirred for 0.5 h and quenched with 20% AcOH/THF (0.2 mL). The mixture was concentrated and taken up in 5% AcOH/THF (3 mL) and $Pd(OH)_2$ (20%/C, 5 mg) added. The solution was purged with H_2 and stirred under an H_2 atmosphere for 12 h. The mixture was taken up in MeOH and filtered through a 0.45 μm Acrodisc®. Purificataion by HPLC on a Waters Symmetry® C-18 colum (4.6×250 mm) eluting with 8% MeOH/H_2O with 1% TFA at 1.5 mL/min, monitoring at 263 nm gave cylindrospermopsin diol (**42**) (t_R = 9.47 min) as a white solid after lyophilization (1.3 mg, 20%). $[\alpha]_D^{25}$ +7.7 (*c* 0.13, H_2O). Compound **42** (1.3 mg, 2.89 μmol) was co-concentrated with MeCN (2×5 mL) and PhMe (2×5 mL). The resulting solid was dried under vacuum for 0.5 h and placed under argon. DMF (0.3 mL) and activated, powdered 3 Å molecular sieves (6 mg) were added and the mixture stirred for 15 min. To this solution was added solid SO_3·pyr (4.6 mg, 29 μmol) and the mixture stirred for 1 h. MeOH (0.1 mL) was added and the solvents removed in vacuo. The mixture was taken up in MeOH and filtered through a 0.45 μm Acrodisc®. Purification by HPLC on a Waters Symmetry® C-18 colum (4.6×250 mm) eluting with 4% MeOH/H_2O with 1% TFA at 1.5 mL/min, monitoring at 263 nm gave cylindrospermopsin **4** (t_R = 8.14 min) as a white solid after lyophilization (0.7 mg, 60%). This was preceded by its bis sulfate (t_R = 5.32 min) as a ∼6:1 mixture. ^{1}H and ^{13}C NMR agreed with those previously reported.[9,12,19h] $[\alpha]_D^{25}$ +8.0 (*c* 0.05, H_2O).

5.1.17. 5-((*E*)-3-(2,6-Bis(benzyloxy)pyrimidin-4-yl)-2-nitroallyl)-3-ethoxy-8-methyl-1,5,6,7,8,8a-hexahydroimidazo[1,5-*a*]pyridin-7-yl acetate (44). To a solution of the isourea **33** (23 mg, 73 μmol) and the pyrimidine aldehyde (26 mg, 81 μmol, 1.1 equiv) in CH_2Cl_2 (1 mL) under argon was added Ac_2O (34 μL, 0.35 mmol, 5 equiv). CsF (110 mg, 0.73 mmol) was then added as a solid in one portion. The reaction was diluted with MeCN (3 mL) and the mixture stirred for 4 h. The reaction was concentrated under reduced pressure, taken up in CH_2Cl_2 and filtered to remove the cesium salts. This mixture was again concentrated and purified on silica gel eluting with 10% MeOH/CH_2Cl_2 to give the nitroalkene as a yellow oil (30 mg, 67%) as a single geometric isomer. This compound is unstable, decomposing overnight at rt. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.68 (s, 1H), 7.48–7.30 (m, 10H), 6.58 (s, 1H), 5.52–5.40 (m, 4H), 4.98 (br 2, *J* = 3.2 Hz), 4.28–4.18 (m, 3H), 4.00 (dd, *J* = 14, 5 Hz, 1H), 3.66 (ddd, *J* = 15, 10, 5 Hz, 1H), 3.55

(dd, J=10, 8 Hz, 1H), 3.40–3.30 (m, 1H), 3.12 (dd, J=10, 8 Hz, 1H), 1.98 (s, 3H), 1.78–1.64 (m, 1H), 1.62–1.60 (m, 2H), 1.25 (t, J=7.2 Hz, 3H), 0.76 (d, J=6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.3, 170.6, 165.0, 164.3, 159.8, 155.2, 136.2, 135.7, 130.4, 128.9, 128.7, 128.5, 128.4, 127.8, 106.9, 71.6, 69.8, 69.1, 65.3, 64.1, 52.9, 50.2, 36.7, 35.4, 31.1, 21.2, 14.7, 13.0. HRMS (FAB+): Calcd for $C_{33}H_{38}N_5O_7$ [M+H]: (*m/z*) 616.2771; Found: (*m/z*) 616.2795.

5.1.18. 7-Deoxycylindrospermopsin diol (45). A solution of the nitroalkene **44** (18 mg, 29.2 μmol) in EtOH (0.5 mL) was added dropwise to a slurry of $NaBH_4$ (5 mg, 146 μmol) in EtOH (0.5 mL) over 20 min. After stirring for 1.5 h the reaction was quenched by the addition of 1:1 H_2O/AcOH (0.1 mL) and concentrated. The concentrate was diluted with 5% AcOH:MeOH (5.8 mL, to be 5 mM) and purged with argon. $Pd(OH)_2$ (20%/C, 6 mg) was added and the mixture stirred under a hydrogen atmosphere for 12 h, filtered through a 0.45 μm Acrodisc® and concentrated. The residue was dissolved in concd HCl and refluxed for 1 h and concentrated. Purification of the uracils was achieved by HPLC using a Waters Symmetry® C-18 colum (4.6× 250 mm) eluting with 8% MeOH/H_2O with 1% TFA at 1.5 mL/min, monitoring at 263 nm to give 7-deoxycylindrospermopsin diol **45** as a white solid (3.7 mg, 38%, t_R=22.1 min) preceded by the C8 diastereomer **46** also obtained as a white crystalline solid (4 mg, 38%, t_R= 12.6 min). A small sample of **45** (~1 mg) was recrystallized from methanol (layered with pentane) to give X-ray quality crystals. Compound **45** (8*S**): ^{1}H NMR (D_2O, 500 MHz): δ 5.68 (s, 1H), 4.03 (br s, 1H), 3.92 (m, 1H), 3.82 (dd, J=9, 9 Hz, 1H), 3.78 (dd, J=9, 9 Hz, 1H), 3.72 (dddd, J=11, 11, 4, 4 Hz, 1H), 3.25 (m, 1H), 2.71 (dd, J=14, 5.5 Hz, 1H), 2.67 (dd, J=14, 9 Hz, 1H), 2.16 (dt, J=14, 4, 4 Hz, 1H), 2.06 (dt, J=15, 3 Hz, 1H), 1.83 (ddd, J=15, 11, 5 Hz, 1H), 1.72 (ddq, J=14, 7, 3 Hz, 1H), 1.55 (ddd, J=14, 14, 1.5 Hz, 1H), 0.95 (d, J=7 Hz, 3H). HRMS (FAB+): Calcd for $C_{15}H_{22}N_5O_3$ [M+H]: (*m/z*) 320.1723; Found: (*m/z*) 320.1723. Compound **46** (8*R**): ^{1}H NMR (D_2O, 500 MHz): δ 5.72 (s, 1H), 4.00 (br s, 1H), 3.86 (buried m, 1H), 3.82 (dd, J=9.0, 9.0 Hz, 1H), 3.74 (dd, J=10, 10 Hz, 1H), 3.61 (ddt, J=11, 11, 3.5 Hz, 1H), 3.23 (dd, J=10, 10 Hz, 1H), 2.73 (app d, J=5 Hz, 1H), 2.26 (dt, J=15, 5, 5 Hz, 1H), 2.07 (dt, J=15, 3, 3, Hz, 1H), 1.70 (ddq, J=9, 6.5, 2.5 Hz, 1H), 1.50 (app q, J=11 Hz, 2H), 0.95 (d, J= 6.5 Hz, 3H). HRMS (FAB+): Calcd for $C_{15}H_{22}N_5O_3$ [M+H]: (*m/z*) 320.1723; Found: 320.1712.

5.1.19. 7-Deoxycylindrospermopsin (6). Alternatively a mixture of the C12-hydroxy uracils (3.2 mg, 7.9 μmol) can be directly sulfonated by treatment with SO_3·pyr (19 mg, 120 μmol) in DMF (300 μL). Purification of the uracils after concentration was achieved by HPLC using a Waters Symmetry® C-18 colum (4.6×250 mm) eluting with 8% MeOH/H_2O with 1% TFA at 1.5 mL/min, monitoring at 263 nm to give 7-deoxy-cylindrospermopsin **6** as a white solid (1 mg, 33%, t_R=8.25 min) preceded by the C8 diastereomer **47** also obtained as a white crystalline solid (1 mg, 33%, t_R=4.91 min). Compound **6**: ^{1}H NMR (D_2O, 400 MHz): δ 5.74 (s, 1H), 4.63 (br s, 1H), 3.92–3.85 (burried m, 1H), 3.86 (dd, J=8.9, 8.9 Hz, 1H), 3.78 (dd, J=10.7, 10.7 Hz, 1H), 3.70 (dddd, J=11.3, 11.3, 3.8, 3.8 Hz, 1H), 3.26 (dd, J=10.8, 8.9 Hz, 1H), 2.76 (app d, J= 6.8 Hz, 2H), 2.48 (ddd, J=14.3, 3.8, 3.8 Hz, 1H), 2.32 (ddd, J=13.2, 3.6, 3.6 Hz, 1H), 1.87 (ddd, J=8.9, 6.8, 2 Hz, 1H), 1.55 (app dd, J=13.2, 11.3 Hz, 1H), 1.01 (d, J=6.8 Hz, 3H). Calcd for $C_{15}H_{22}N_5O_6S$ [M+H]: (*m/z*) 400.1296; Found: 400.1282.

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Appendix 3: Research Proposal

30b. 1. Specific Aims

This proposal aims to develop an efficient and mild alternative to traditionally metal initiated oxidative radical cyclizations. Specifically this research will target; 1) The application of iodine(V) reagents for the oxidative radical cyclization of β-dicarbonyl compounds; 2) The design and synthesis of chiral iodine(V) reagents or complexes for the development of an (catalytic) enantioselective manifold for this reaction; and 3) the application of said reagents to the solid phase synthesis of a set of skeletally diverse small molecules.

30b 2. Background and Significance

30b 2.1. Reaction Development: Oxidative radical cyclizations and additions have proven valuable tools for carbon-carbon bond formation in synthetic chemistry. The ability of these carbon centered radicals to undergo domino reaction with high substrate-directed stereocontrol is often remarkable (Scheme 1). For example **1** undergoes tetra-cyclization to afford **2** as a single stereoisomer (out of 64 possible).[1] It is this reactivity, and the generation of highly reactive intermediates (radicals or cations) that has led us to consider this reaction for deployment in the preparation of skeletally diverse small molecules (*vide infra)*.

Scheme 1. Zoretic's Mn^{III} tetracyclization.

Traditionally, these reactions have been initiated by strong 1 e^- oxidants, commonly Mn^{III}, Ce^{IV}, Ag^{I} and Fe^{III}.[2] Of particular interest is the cyclization of activated methylene compounds such as β-diketones and β-ketoesters.[3,4] As shown in Scheme 2 these compounds smoothly form carbon centered radicals that can react both inter- and intra-molecularly with π-electrophiles. Reaction of **3** (Eq. 1) with $Mn(OAc)_3$ proceeds through two distinct single electron transfer (SET) steps to afford **4**. It has been shown that the rate determining step in this reaction is enolization of the carbonyl to generate the Mn enolate **5**.[5] SET to the Mn center is then followed by rapid addition of the radical to the pendant alkene to afford **7**. In turn this radical undergoes another SET to generate the cationic intermediate **8** which provides **4** after loss of a proton. In the reaction of dimedone (**9**) with phenylcyclohexene, cerium(IV) reagents are thought to behave in a similar fashion.[6] In this case the intermediate radical **13** generated by the intra-molecular addition is oxidized and trapped to generate the dihydrofuran **10**. While these reactions are synthetically useful for C-C bond formation, they often afford poor yields, require long reaction times under harsh conditions, and in the case of metal nitrates; often yield mixtures of organic nitrates and nitronic acids. Further, these unforgiving reaction conditions and overoxidation have prohibited the use of this reaction class on polymer supported substrates.

Scheme 1. Metal initiated oxidative radical cyclization/addition examples.

Hypervalent iodine reagents have enjoyed a renaissance in the synthetic community, shedding their traditional role as alcohol oxidants.[7a,b] Nicolaou and co-workers have recently described the IBX mediated dehydrogenation of ketones to yield enones (**14** → **15**) (Eq. 3, Scheme 3).[8a,b] Through the cyclopropane aldehyde **16** (Eq. 4), they were able to show that the reaction proceeds *via* an SET mechanism.[9] Of interest is the resemblance of this reaction course to the transformations in Eq.'s 1 and 2. For example the generic ketone/aldehyde **14** enolizes, then undergoes addition to the highly electrophilic iodine(V)center generating **18**. The first of two SET reactions occurs to give the radical cation **19**, in resonance with the α-cation **20**. Elimination of a β-hydrogen generates the unsaturation which is followed by a 2nd SET reaction to form the enone **15**.

Scheme 3. Nicolaou's proposed mechanism for the IBX mediated dehydrogenation of ketones.

In 1994, Schreiber and Meyer published a seminal paper on the acceleration of Dess-Martin periodinane (D-M) oxidations by water. [10] In this paper they also describe the oxidation of β-hydroxyester **21** to the tricarbonyl compound **23** (Scheme 4). Treatment of **21** with only one equivalent of D-M in CH_2Cl_2 they received an 86% yield of the β-ketoester **22** (entry 1). Exposure of **21** to excess D-M in the presence of 3.1 eq. of water

gives **23** in 80% yield (entry 2). However in the absence of water it requires 41h for the reaction to generate a 30% yield of **23** and it is accompanied by 20% of the α-chloro compound **25**. They suggest that **22** enolizes and attacks an I^{V} intermediate giving the iodinane enol **26**. This intermediate is then subject to allylic rearrangement (path ***a***) or allylic substitution (path ***b***). Path ***a*** then generates the iodinane ether **27** that gets oxidized to **23**. Following path ***b*** it was thought that water or chloride *ion* from solvent decomposition attacked **26**, accounting for the formation of **24** and **25** respectively.

Entry	D-M	H_2O	Pyr.	t(h)	22	23	24	25
1	1.2	-	-	0.08	86%			
2	5.5	3.1	2.3	4		80%		
3	5.5	-	2.3	12		70%		
4	5.5	-	-	41		30%		20%
5	4.2	4.0	-	1.3		65%	7%	

Scheme 4. Schreiber's oxidation of β-hydroxyesters.

In light of the recent experiments, i.e. the conversion **16** $\rightarrow$ **17**, it seems more plausible that the mechanism of this reaction follows the SET pathway (Scheme 5). Thus SET from the coordinated enol **26** would reversibly generate the radical cation **28**, that in the absence of water would eventually abstract a chlorine *atom* from the solvent (accounting for the 20% yield). Alternatively **28**, in resonance with **29**, in the presence of water undergoes addition to the cation giving **30** that leads to **23** and **24**.

Scheme 5. Plausible SET mechanism for the D-M oxidation of dicarbonyl compounds.

Recognizing the similarity in reaction mechanism between Eq.'s 4 and 1,2 and the plausibility of a methylene centered radical in the oxidation of **22**, the hypothesis becomes: If β-dicarbonyl compounds (lacking β-hydrogens, in contrast to **20**) are reacted with IBX, will an SET process ensue to generate compounds similar to those of the Mn^{III}, Ce^{IV} manifold (Scheme 6)? Thus β-ketoesters like **31** might undergo oxidative radical cyclization to afford the cyclic dicarbonyl compounds like **36**.

Scheme 6. Proposed IBX oxidative radical cyclization of unsaturated β-ketoesters.

The entrance of IBX into the oxidative radical cyclization manifold is considered to be of significant benefit for several reasons (Scheme 7). First, pre-coordination of the enol to the iodine(V) center should proffer the opportunity to control the stereo-selectivity of this reaction by chiral modification of the oxidant. Secondly, IBX esters have been developed and transferred to polymer support (i.e **37**).[11,12] These oxidants are stable on cross-linked polystyrene and show similar reactivity to the parent oxidant. Thus IBX esters should be compatible with the development of solid-supported library synthesis. Finally, as shown in the transformation **38** → **39**, IBX ligand modification can generate oxidants that are extremely efficient, undergo reaction at room temperature, and are tolerant of a wide range of functional groups. Most noteable is the reaction's tolerance of silyl ethers, another critical need for the application of this chemistry to library synthesis.

Scheme 7. Benefits of iodine(V) mediated oxidative radical cyclization reactions.

30b 2.1. Application to Diversity Oriented Synthesis:

Combinatorial synthesis has played a crucial role in both chemical biology and drug discovery. However the lack of skeletal diversity generated in such libraries has limited its widespread application in pharmaceutical and academic arenas.[13] Traditionally a molecule is subjected to sequential functionalization with building block (BB) libraries (Eq. 5, Scheme 8). Incorporating multiple reactive sites allows the application of split-pool synthesis, to generate multiplicative increases in appendage diversity. Applying this "one-synthesis, one-skeleton" approach, it is possible to generate libraries of hundreds of thousands of small molecules.[14] The apparent limitation of such a strategy is that this combination of molecules likely displays a relatively similar three dimensional morphology. In contrast, diversity oriented synthesis (DOS) is developing strategies by which skeletally distinct small molecules can be generated in short (3-5 step) reaction sequences. A powerful strategy is currently evolving hinged on the incorporation of appendages that pre-encode skeletal diversity (σ-elements, Eq. 6).[15] In such a system a combination of molecules, bearing multiple σ-elements, may be treated with a single chemical reagent. Depending of the chemical reactivity of the σ-elements, the reactive intermediates will be directed into skeletal differentiating pathways, leading to a variety of skeletally distinct products.

Scheme 8. Schematic comparison of combinatorics and σ-elements.

As previously stated, oxidative radical cylizations posses reactive intermediates, specifically radicals or cations (generated after oxidation of the cyclized radical). To harness these intermediates one needs functionality that will capture either the radical or the cation. This then offers the basis for a set of σ-elements (Scheme 9). If we append these σ-elements to a β-dicarbonyl framework then a matrix (σ_1 x σ_2) can be envisioned. If we first consider radical capture, it is expected that the dicarbonyl radical could cyclize on a pendant nitrile, alkene, or enol ether (σ_1), all of which have precedence in oxidative radical cyclizations.[2] In the present case σ_2 is a radical capture element (alkene). Thus the intermediate radical after cyclization should add in a *n-exo-trig* sense which would be oxidized and eliminate, furnishing the bicyclic enones (***A*** – ***C***). If σ_2 does not bear functionality for subsequent reactivity, the intermediate radical should be oxidized to the cation. In the case of the resultant iminyl radical, it should hydrolyze to give the trione ***D***. The alkyl cation should eliminate to give ***E***. The stabilized oxocabenium ion from cyclization on the enol ether should be trapped by the carbonyl and eliminate to give the furan ***F***.[16] Considering the possibility of intermolecular cation capture, the last σ element considered is an aromatic moiety. The cations thus formed should undergo Friedel-Crafts cyclization to give the tricycles **G – I**.[3] It is unclear whether this would formally be a cationic cyclization or radical addition to the aromatic core, delineated in large by the substituents on the arene. This framework is further supported for DOS by the linkage of σ_2. Oxidative radical cyclizations have been shown to work on β-ketoetser (x = O), β-ketoamides (X = N-BB), and β-diketones (X = CH_2), thus immediately predicating a 3x3x3 matrix.

Scheme 9. σ-element diversity in the oxidative radical cyclization manifold.

In short, it is thought that the oxidative radical cyclization of β-dicarbonyl compounds, containing three diversity elements will prove efficient in generating skeletally diverse

small molecules. For example if each σ element in Scheme 9 consists of only four structural analogues (i.e. aromatic or alkene substitution), varied in X, then a collection of four hundred and thirty two small molecules, of nine distinct skeletal families, will be produced. Further, it is hoped that new reaction methodology may be developed by the implementation of a solid support friendly radical initiator, namely IBX or derivatives thereof.

30b 3. Research Methods and Design

30b 3.1. Reaction Development: The first task at hand is to identify a general set of reaction conditions that can effect this transformation. To identify these conditions a set of substrates that have proven efficacious in oxidative radical cyclizations will be examined. From Snider's work with Mn^{III}, the β-ketoester **40** has been shown to undergo cyclization to **41** in 71% yield and produced as the sole product, although existing as a mixture of tautomers (Scheme 10).[17] It is important to identify substrates that will not be subject to competitive *6-exo / 7-endo* cyclization, at this point both **40** and **42** satisfy this need.[4] The cyclic β-ketoester **42** will be examined in parallel as the resulting product does not posses an α-hydrogen and should not be subject to either tautomerization or further oxidation. Additionally, α -alkyl substituents have shown to reduce the oxidation potential of the enol and thus facilitate the SET.[18] The choice of solvent is expected to have a dramatic influence on the reaction course and will comprise the first investigation. THF-DMSO mixtures have proven to be preferred for most IBX applications due to the increased oxidant solubility and coordination of the solvent to iodine center. However, other solvents especially protic/bisphasic mixtures may influence the reaction, especially the second SET event. They may initiate reductive termination of the cyclized radical or solvent addition to the intermediate cation. Assuming the identification of solvent conditions that will effect the IBX mediated transformation of **40** → **41** and **42** → **43** it will be important to assess the influence of additives on the reaction in particular Cu^{II}. Kochi has shown that $Cu(OAc)_2$ reacts with radicals (~10^6 s^1M^{-1}) to initially generate an alkyl Cu^{III} complex that β–hydride eliminates to give predominantly the less substituted alkene as in **41**,**43**.[19] Alternatively other Cu^{II} salts can undergo ligand transfer potentially effecting the outcome of the reaction.

CO₂Me, Me, **41**, 71% ← Mn(OAc)₃ / Cu(OAc)₂ — **40**, CO₂Me, Et
Et, CO₂Et, **44**, 4% + Me, CO₂Et, **43**, 78% ← Mn(OAc)₃ / Cu(OAc)₂ — **42**, Et, H, EtO₂C
IBX → **Solvent Screen**: THF, MeCN, refluxing EtOAc; THF-DMSO mixtures; biphasic-THF/H₂O → **Additive Screen**: Cu^II

Scheme 10. Initial substrate and condition screen.

Secondly an examination of hypervalent iodine reagents will be undertaken. Nicolaou has shown that ligation of the iodine (V) center produces differences in reactivity. As seen in figure 1 the use of the IBX·NMO (**44**) and IBX·MPO (**45**) complexes dramatically increases the rate of dehydrogenation of cyclooctanone.[8b] The origin of this rate acceleration is currently unknown. If ligation increases the rate of SET to the enol, we should see a similar rate acceleration when applied to the oxidative radical cyclizations.

If on the other hand the *N*-oxides merely facilitate elimination from the cation (Scheme 3) we should not see any rate enhancement, providing further mechanistic insight into this phenomenon. Thus the complexes **44** and **45** will be tested on **40** and **42**. Dess-Martin periodinane (DMP, **46**) will also be evaluated along with iodic acid (**47**) and iodine pentoxide (**38**) as these reagent may provide alternative coordination spheres for chiral ligation.[20] Most important will be the performance of the IBX ester **49** and the IBX amide **50**.[21,22] This I^{V} coordination sphere will be critical for chiral modification and its application to solid phase snthesis as discussed above. Finally an I^{III} reagent, IBDA (**51**) will be tested.

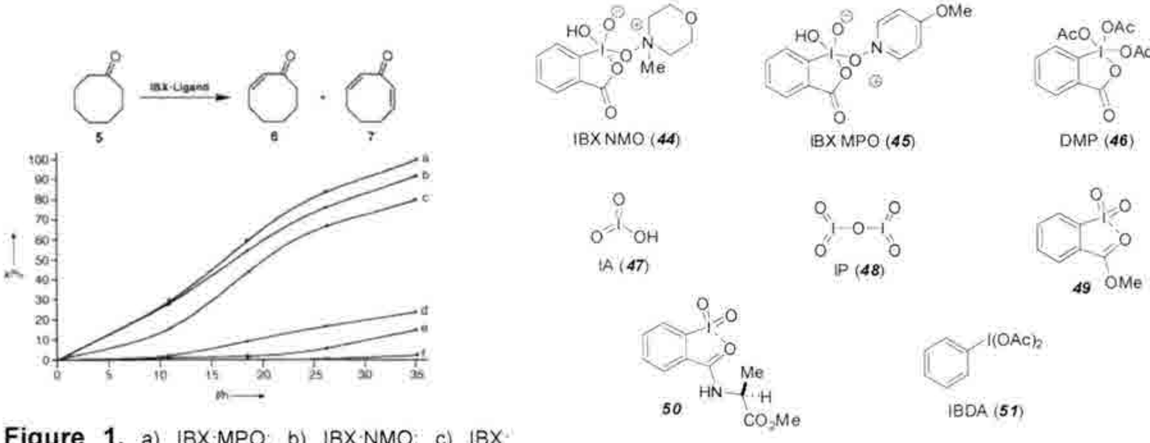

Figure 1. a) IBX·MPO; b) IBX·NMO; c) IBX·trimethylamine- *N*-oxide; d) IBX·DMSO formed by dissolution at 75 °C for 30 min; e) IBX·DMSO formed by dissolution at 25 °C; f) IBX·DMSO formed by dissolution at 90 °C for 20 min .

Figure 2. IBX derivates targeted for evaluation.

30b 3.2. Substrate scope: Having found optimal conditions for the IBX, or derivatives thereof, mediated oxidative radical cyclization of the malonate ester **40** and **42** the reaction will be examined for substrate scope, targeting the three functional elements outlined in Scheme 9. The first class of reaction to be studied will be that of sequential radical capture. The cinnamyl derived malonic acid **52** will serve as a starting point as it is readily available form the Claisen condensation of ethyl cinnamate and *t*BuOAc (Scheme 11).[23] This acid, **52**, is also targeted for its ability to stabilize the incipient radical after capture of the initial alkyl radical. Coupling of **52** with the known alcohols **53a-d** under the influence of *N,N*-diispropylcarbodiimide (DIC) and DMAP will give the esters **54a-d**.[24,25] These will then be evaluated for their ability to undergo the bis-cyclization reaction mediated by IBX, hopefully giving the expected products **55a-d**. It should also be noted that if it is found that α-substitution of the malonate derivatives is necessary, this may easily be accommodated by alkylation of **52**. The *N*-benzylamine series will be studied in parallel. It is expected that the amines **56a-d** will be available by activation and displacement of the alcohol function in **53a-d**. Again DIC mediated coupling to **52** should give the β-ketoamides **57a-d**. These will then be subjected to the IBX conditions to ensure the compatability of amides and particularly *N*-benzyl amides in the substrates.

Scheme 11. Radical capture substrates.

An easy adaptation of the above substrates should facilitate the examination of systems that have neither a cation or radical trap, thus promoting elimination or hydrolysis (i.e. intramolecular cation capture). Reduction of **62** will provide the saturated malonate **59** (Scheme 12).[26] Again, DIC mediated coupling of the alcohols **53a-d** should give the esters **60a-d** and the amines **56a-d** giving the amides **61a-d**. These will then be subjected to the cyclization conditions. The alkenes **60/61a** are expected to undergo oxidation/elimination to afford the exocyclic alkenes **62/63a**. The nitriles will be examined for their ability to hydrolyze, affording the triketones **62/63b**. It is expected that the enol ethers **60/51c** will undergo cyclization on the oxocarbenium ion by the carbonyl and suffer further elimination to generate the tri-substituted furans **62/63c**. The last substrates **60/61d** are expected to regenerate the cyclohexadienyl system (i.e. **62/63d**) after elimination from the allylic cation.

Scheme 12. Cation capture-elimination/hydrolysis substrates.

The last set of substrates to be examined are those containing an arylketone capable of trapping the cation in an intramolecular sense. The synthesis of these substrates will commence in two senses (Scheme 13). The first being the Aldol reaction of *t*BuOAc with the commercially available aldehydes **64/65**. Subsequent oxidation and deprotection will

furnish the acids **66/67**. The Aldol intermediates are of interest for the study of tandem reaction courses (see below). The acids may also be synthesized by Claisen condensation of the commercially available esters **68/69**. The electron donating/withdrawing nature of **66/57** will comprise an initial examination of the electronic requirements for this cyclization. It will also delineate the stereoselectivity of the cyclization, if there is difference in mechanism (i.e. radical addition to the arene vs. cation capture). Again coupling of the acids to the alcohols **43a-d** will furnish the esters **70a-d/71a-d**. When treated under the cyclization conditions it is hoped that the tricycles **72a-d/73a-d** will form.

Scheme 13. Arylketone cation capture substrates.

30b 3.3. Asymmetric variations: Upon definition of a class of substrates that undergo this transformation efficiently and stereoselectively, the development of chiral I^V reagents will be undertaken. Snider has shown that relatively distant chirality can control the enantioface from which the alkene approaches, as exemplified by the phenylmenthyl ester **74** (Scheme 14).[27] Firstly, the reagents developed by Zhdankin (**50**, **76**, **77**) will be evaluated for stereo-induction.[21] It is hoped that either distant chirality, or the potential hydrogen bonding of the amide to the second carbonyl may afford enantiofacilal discrimination in the cyclization event. This is an attractive approach as both enantiomers of the aminoacids are readily available from the chiral pool.

Scheme 14. Initial screening of Zhdankin's chiral I^V reagents.

The insolubility of periodinanes is attributed to the strong intramolecular I—O bonding that leads to polymeric structures. This can partially be broken up by ligation with DMSO, hence the preferred solvent for these reactions. Zhdankin has obtained an *X*-ray structure of the iodinane ester **49** complexed with DMSO (Figure 3).[22] As noted before *N*-oxides greatly enhance the reactivity of these complexes. The reason for this is unclear, and further it is unclear whether the *N*-oxide or DMSO is still coordinated to the iodine center upon substrate coordination. Thus several chiral additives will be tested to probe this coordination in the transition state. The enantiopure sulfonamide **78** is well known from Ellman's sulfinimine chemistry.[28] (-)-Menthyldiphenylphosphine oxide (**79**) and the *N*-oxide **80** derived from the proline amide are also known and will be examined.[29,30] If enantioinduction is achieved with these additives it would be of significant promise as a catalytic amount of "ligand" could be used if the rate enhancements are as great as shown in figure1. This application of ligand accelerated catalysis using the commercially available iodic acid (**47**) with **79-81** would also be studied.

49: I–O1 2.6979 Å ,I–O3 1.8061 Å, I–O3′ 2.7805Å, I–O4 1.7940 Å, I–O1S 2.7560 Å.

Figure 3. The use of chiral additives.

Thirdly a new generation of chiral iodinanes will be synthesized and their efficacy examined. Starting with either 2-iodobenzoic acid (**81**) or the bis-acid **84** coupling to (*S*)-phenylglycinol and dehydration should give the oxazoline **82** or the *C2*-symmetric bisoxazoline **85** (Scheme 15).[31] These are preferentially targeted as the phenyl oxazoline moiety is stable in asymmetric free-radical addition reactions, and should thus be competent in our system.[22] Oxidation of these iodoarenes may be effected under a variety of conditions as previously described: DMDO/acetone, NaOCl/AcOH, or $KBrO_3$ to give the I^V compounds **83/86**.[20,22] From the *X*-ray structure in figure 3, it is known that the sp^2-oxygen lone pair of the *ortho*-ester is effectively coordinated to the I^V center generating a pseudo benziodoxole ring (bond length = 2.6979 Å). It is then reasonably expected that the oxazoline should also coordinate to provide a rigid, chiral coordination sphere. Crude MM2 energy minimization calculations do show that the coordinated enol should enjoy facial discrimination, permitting alkene approach from the *si* face.

Scheme 15. Oxazoline iodinane complexes.

30b 3.4. Tandem reactions: A number of the products from the reactions outlined above become immediate substrates for subsequent well studied reactions. Thus the study of some tandem reactions will be undertaken, the first being the hetero-Diels Alder reaction. The oxidative radical cyclization/radical capture products, like that derived from **57a**, contain an exocyclic enone (i.e. **58a**, Scheme 16). This moiety is known to be a reactive diene, as it is forced to be in the *s*-cis conformation, and should undergo cycloaddition with electron rich dieneophiles like ethylvinyl ether to give the tricyclic acetal **87**.[32] The exocyclic alkene **63a** derived from **61a** might be forced to tautomerize to the ene-dione **88** and also react with ethvinyl ether to give **89**. Lastly the furan **63c** derived from the cyclization/elimination of **61c** should undergo normal Diels-Alder cycloaddition with the *N*-Et maleimide to give the tricyclic furan **90**.[33] The issue of tandem oxidation/cyclization will be addressed by the action of IBX on the aldol adduct **91**.

Scheme 16. Possible tandem reaction courses.

30b 3.4. Application to Diversity Oriented Synthesis: Lacking the knowledge of the particular building blocks, tandem reactions, or σ-elements that will be competent in this chemistry, it is not appealing to generate a specific DOS strategy that illustrates exact targets. It is however possible to create a general scheme for the transfer of this chemistry to solid phase (Scheme 17). Beginning with the macrobead (**93**) a library (BB_1) of alcohol containing an alkyl halide may be loaded to generate **94**.[34,35] Alkylation of **94**

with the amines bearing the σ-elements (σ_1). Coupling of the known thioester **95** or its acid chloride would introduce the malonate function, providing **95**.[36] Palladium mediated coupling of the thioester with aryl/vinyl tin reagents should introduce the second σ-element (σ_2).[37] Treatment of this library with the IBX conditions should afford the skeletally differentiated products, without harm to the silyl linkage or the macrobead itself. Further diversity may be easily be introduced to **97** by alkylation of the malonate with another (BB_2), or the same library (BB_1) o give the α-substituted collection **98**. This may then also be subjected to the IBX reaction.

Scheme 17. General outline for the application of this chemistry to DOS.

In summary, the application of the newly discovered reactivity of iodine(V) will be explored. Exploiting this chemistry in the oxidative radical cyclization of β-dicarbonyl compounds during which carbon-carbond bonds are formed, the possibility of asymmetric control arises. Further this chemistry will find application in the synthesis of a collection of skeletally diverse small molecules after reaction development is accomplished.

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