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Synthesis of Uracil Containing Precursors and Analogues Of Cylindrospermopsin

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Synthesis of Uracil Containing Precursors and Analogues Of Cylindrospermopsin

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the requirements for the degree of

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by

Hisham Saleh Fituri

Prifysgol Bangor • Bangor University

Contents

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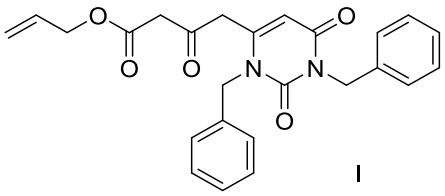
Abstract

The thesis covers three topics:

i) *Synthesis of the uracil D-ring precursor of the cylindrospermopsin alkaloids:* this study entailed the preparation of compounds **I** and **II** which were shown to be a RHS D-ring precursor in the synthesis of the cylindrospermopsin alkaloids. Compound **I** was prepared in 3 steps and in 24% overall yield from dibenzylurea whilst **II** was prepared from either diethyl 1,3-acetonedicarboxylate in 5 steps and 9% overall yield or barbituric acid in 5 steps and 16% overall yield.

ii) *Preparation of analogues of cylindrospermopsin:* the synthesis of the cylindrospermopsin analogue **III** was achieved in 6 steps and 12% overall yield from the literature compound 2,6-dimethoxypyrimidine-4-carboxaldehyde.

iii) *Preparation and enzymatic studies on 2.4-dinitrobenzamide pro-drugs:* the known pro-drugs **IV** (X = Cl, Br, I), were prepared and found to be good substrates for the enzyme NfsA NTR. These can thus be considered as alternative pro-drugs to the usually employed CB 1954 in combination with NfsA NTR for human chemotherapy.



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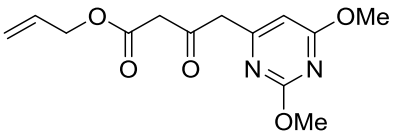

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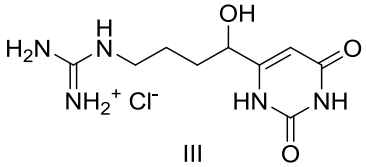

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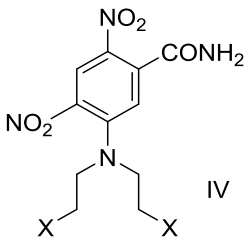


IV

Abbreviations

General

δ	chemical shift
Δ	heat, reflux
ACP	acyl carrier protein
ADEPT	antibody-directed enzyme prodrug therapy
CB1954	5-(1-aziridinyl)-2,4-dinitrobenzamide, 5-(Aziridin-1-yl)-2,4-dinitrobenzamide
CYN	cylindospermopsin
CI	chemical ionization
d	doublet
dd	doublet of doublets
DEPT	directed Enzyme Pro-drug Therapy
DNA	deoxyribonucleic acid
eqv.	molar equivalents
FMN	flavin mononucleotide
GDEPT	gene-directed enzyme prodrug therapy
GSH	glutathione
HRMS	high resolution mass spectrometry
h	hour(s)
HPLC	high performance liquid chromatography
Km	Michaelis constant (substrate concentration at half of V_{max})
LHS	left-hand side
MS	mass spectrometry
m	multiplet
NfsA	nodulation factor A
NfsB	nodulation factor B
NTR	nitroreductase

NMR	Nuclear Magnetic Resonance
ppm	parts per million
q	quartet
RBF	round bottomed flask
RHS	right-hand side
Rf	retention factor
rt	room temperature
s	singlet
SN 23862	5-(bis-(2-chloroethyl)amino)-2,4-dinitrobenzamide
t	triplet
TAP	tumour-activated pro-drugs
TLC	thin layer chromatography
V_{max}	maximum rate of reaction

Reagents

CDI	carbonyldiimidazole
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DCC	dicyclohexylmethanediimine
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
NADPH	Nicotinamide adenine dinucleotide phosphate
TBAF	tetra-*n*-butylammonium fluoride
TBSCl	*t*-Butyldimethylsilyl chloride
TFA	trifluoroacetic acid
TMSCl	trimethylsilyl chloride
p-TsOH	p-toluenesulfonic acid

Functional groups

Ac	Acetyl
Bn	Benzyl
Boc	*t*-Butoxycarbonyl
MOM	Methoxymethyl
TBS	*t*-Butyldimethylsilyl

Solvents

MeCN	acetonitrile
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EtOAc	ethyl acetate
THF	tetrahydrofuran

Section A**:**

Synthetic **A**nalogues of the Cylindrospermopsin **A**lkaloids

Introduction

The cylindrospermopsin alkaloids

Since 1979, when the alkaloid cylindospermopsin was associated with an outbreak of hepatoenteritis in Palm Island, Queensland, Australia, this family of compounds has been studied extensively because of their toxic effects.[1] The Palm Island hepatoenteritis outbreak affected 138 children, 10 adults and was caused by an algal bloom of the strain *Cylindrospermopsis raciborskii.*[1] Since then, other cyanobacteria species have also been shown to produce cylindospermopsin, most notably strains of *Umezakianatans* and *Aphanizomenon ovalisporum*[2] the production of CYN thus appears strain-specific, not species specific.

The guanidine functional group is prevalent throughout nature, being present in potent toxins produced by various species of the animal kingdom,[3] as well as the active sites of enzymes[4] and in the human body in the form of arginine and its metabolites. Several natural and synthetic guanidine derivatives have generated great interest, primarily because of their pronounced biological activities.[5] However, guanidine derivatives have also been shown to have applications in other fields such as catalysis,[6] peptidomimetics[7] and adhesives[8] as well as antifouling agents.[9]

The guanidine containing marine natural product cylindrospermopsin **1** was first isolated from the cyanobacterium *C.raciborskii* in 1992,[10] after suspicion of its involvement in an outbreak of hepatoenteritis on Palm Island, Australia.[11] Since then, it has also been isolated from several other sources[12] including fresh water lakes in various parts of Europe such as France, Germany, and the Czech Republic. Cylindrospermopsin and its diastereoisomer 7-*epi*-cylindrospermopsin **2** both display hepatotoxic,[13] cytotoxic,[14] neurotoxic,[15] and carcinogenic effects.[16] The toxicity of the deoxygenated metabolite 7-*deoxy*-cylindrospermopsin **3** remains controversial as it has been reported to be much less toxic than its counterparts[17] (Figure 1).

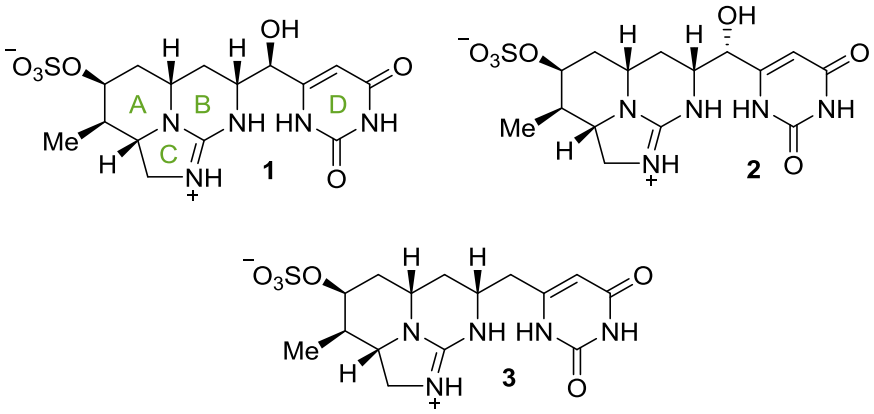


Figure 1. Main members of the cylindrospermopsin family.

Akinites (dormant cells that act as survival structures) of *C. raciborskii* can survive as spores for long periods in sediments. Akinites generally arise due to temperature fluctuation and rarely occur in tropical and subtropical zones; in such areas, *C. raciborskii* is perennial.[18] In more temperate zones akinites are necessary to provide new growth; germination occurs when temperatures rise to 22–24 ºC. The cells following germination eventually develop into trichomes[19] (Figure 2).

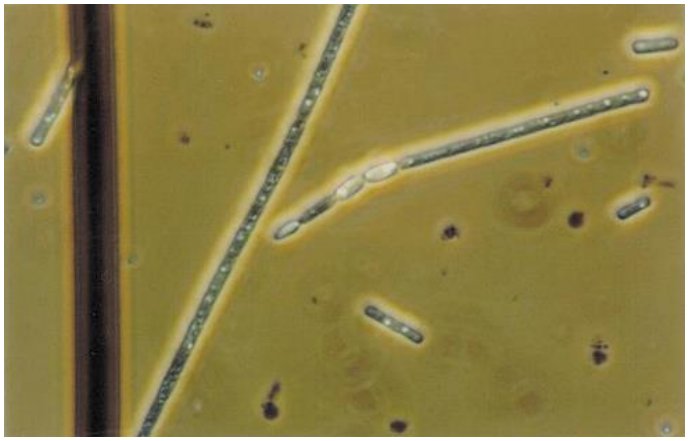


Figure 2. C. *raciborskiiakinites*[19] Source: Google images.

This work will focus on the synthesis of the uracil side-chain segment of the cylindrospermopsin alkaloids as well as developing a strategy for the synthesis of analogues of the natural compounds containing the uracil motif. As part of this work,

we will review guanidine containing natural products, alkaloids and synthetic methods to these metabolites.

Guanidine and natural occurring guanidine derivatives

Guanidine **4** is a crystalline, nitrogen containing, strongly basic compound that was first synthesised by the oxidation of guanine.[20] It is present in nature, for example, it is present in urine as a result of protein metabolism. Because of its basic nature, guanidine and many of its derivatives are generally found in nature in a protonated form. This is known as the guanidinium cation **5**, which has an acid dissociation (pK_a) value of 13.6.[20] The stability of the cation lies in the presence of three resonance forms, as shown in Figure 3,[21] which distribute the positive charge of the cation over the three nitrogen atoms.

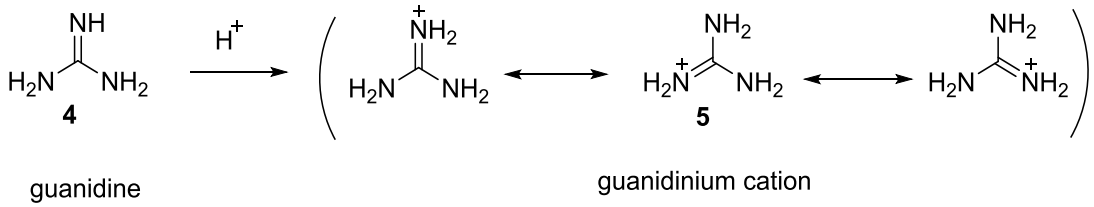


Figure 3. Guanidine and the guanidinium cation.

Substitution of guanidine is possible and obviously up to five substituients are possible to give a vast array of derivatives.[22] Guanidines have the general structure $(R^1R^2N)(R^3R^4N)C{=}N\text{-}R^5$ and as the central group has an imine, guanidines are related to amindines and to ureas (Figure 4).

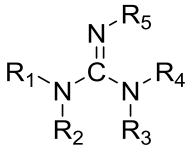


Figure 4. General structure of guanidines R = alkyl, aryl etc

Examples of guanidine containing natural products include arginine **6**,[23] one of the twenty common proteinogenic amino acids, saxitoxin **7**,[24] a shellfish toxin that induces paralysis, and triazabicyclodecene **8**,[25] an organic soluble base much used in chemistry to effect organic transformations e.g. aminolysis of esters (Figure 5).

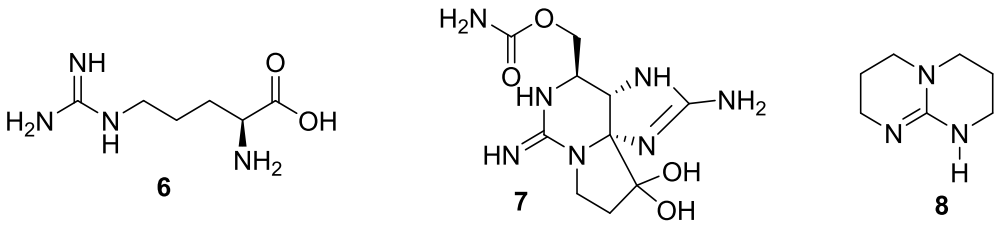


Figure 5. Structures of arginine **6**, and saxitoxin **7** and triazabicyclodecene **8.**

The guanidine functionality occurs in a wide variety of biologically active molecules.[3,4] The ubiquitous nature of arginine leads to it being a common structural feature in enzyme active sites.[4] Synthetic guanidine containing molecules also have wider importance as they have applications in catalysis,[6,24] adhesives,[8] and a range of pharmaceuticals.[24]

Cyanobacteria

Cyanobacteria, or blue-green algae, comprise a phylum of bacterial species, all of which are capable of photosynthesis[26] (Figure 6).

Figure 6. Cyanobacteria[26]

Today, cyanobacteria populate every area of the Earth. Some are extremophiles, surviving in extremes of heat or cold. Others prosper at moderate temperatures. Some species form colonies; others survive as single cells. Like other prokaryotes, all reproduce asexually, and all lack a cell nucleus. Cyanobacteria perform vital tasks for Earth's ecosystem. Aside from photosynthesis, many also fix

atmospheric nitrogen into ammonia, nitrates, or nitrites, thereby enabling the nitrogen to be available to plants.[27]

Nonetheless, today, cyanobacteria receive much negative publicity, largely because of their association with algal blooms, [28a] which represent the rapid reproduction of algal species within an area of water (freshwater or marine). Typically only one particular species is involved in any bloom and the rapid reproduction of this microscopic organism leads to visible discolouration of the water, which in the case of a cyanobacterium, turns the water a blue-green colour[28b] (Figure 7).

Figure 7. Algal bloom in a village river near Chengdu, Sichuan, China.[28c]

Paradoxically, the growth of algal blooms deprives the water of oxygen. This is because, although the algae reproduce rapidly, each organism is short-lived and the water in the bloom therefore becomes clogged with dead organic matter. As this dead matter decays it consumes free oxygen leading to *eutrophication*, which leads to the death of other life forms from hypoxia.[29]

Toxicity

Algal blooms have long been known to poison wildlife and such blooms are termed *harmful algal blooms* (HABs). This form of water poisoning was first noted in 1878, when George Francis reported in *Nature* a bloom in the Murray River estuary

on the border between New South Wales and Victoria, Australia.[30] This bloom had so poisoned the water that wildlife that drank the water subsequently died.[30] A more recent case in which algal blooms poisoned wildlife involved the death of 100 bottle nosed dolphins off the Florida coast in 2004.[31]

Algal blooms may affect any of the world's oceans. The Gulf of Mexico appears especially affected.[32] Algal blooms are also a problem in the Baltic Sea, where the resultant *eutrophication* is suspected to cause reproductive anomalies in fish.[33] Poisoning associated with cylindrospermopsin is also known to have affected aquatic environments as diverse as the Mediterranean and inland waters in Japan, Eastern Australia, Brazil, the USA, France and Central and Eastern Europe.[2] In addition, although wildlife poisoning is associated with HABs, poisoning may also occur in the absence of a bloom. This is because the toxins produced by the blooms may persist in the aquatic environment long after the bloom has disappeared;[1,2] moreover, some algal blooms are invisible from the surface.[2] About 2% of phytoplankton are known to produce toxins,[34] although not all involve cylindrospermopsin.

To an extent, algal blooms appear "natural", in that they appear to have occurred throughout history,[35] and in many cases their exact cause is unknown. Moreover, although their incidence appears to be becoming more frequent, this apparent rise may be a consequence of better environmental monitoring.[36,37] Nonetheless, anthropogenic causes are implicated, most notably leaching of fertilizers (especially phosphates) into waterways.[2,30,32] In addition, global warming is cited as a possible cause.[2] The effects of cylindrospermopsin producing bacteria is spreading. Initially, it appeared confined to tropical and sub-tropical regions, however it now appears to have spread to temperate regions.[2] Cylindrospermopsin **1** has been detected in countries as diverse as the U.S.A., Brazil, Thailand, Egypt and Japan, though the majority of detections have been in Australia.[38] On a country by country basis the most common source has been *C.raciborskii*, however several other bacterial species have been implicated and some of these seem restricted to only one country. For example, *Umezakianatans* (Japan), *Raphidiopsiscurvata* (China) and *Raphidiopsis mediterranea* (Australia).[38] Cylindrospermopsin has now been found in every continent except Antarctica and given the potential for cylindrospermopsin **1** pollution to cause harm, this is a matter for concern**.**

C. *raciborskii* blooms comprise subsurface trichomes in and below the euphotic zone (area of water that receives sufficient sunlight for photosynthesis to occur).[39] These blooms are associated with several environmental conditions; these include the water temperature, with the optimal range for growth being 25–30 °C (but growth is possible when the temperature range is 15–35 °C). Other factors include high pH, low water level, low water flow, high sulphate concentration, high incident sunlight, low levels of macrophytes (aquatic plants), and a low ratio of nitrogen to phosphorous.[40]

Cyanotoxins

There are three main classes of cyanotoxin associated with blue-green algae, cyclic peptides, alkaloids, and lipopolysaccharides. Table 1 lists the main examples, together with the mammalian organs mainly affected and the bacterial genera associated with them.[41]

Table 1. Classes of cyanotoxin

Class	Toxin	Organs mainly affected in mammals	Bacteria (genera)
Cyclic peptides	Microcystins	Liver	*Anabaena*, *Anabaenopsis*, *Microcystis*, *Nostoc*, *Planktothrix*, *Nostoc*, *Hapalosiphon*
	Nodularins	Liver	*Nodularia*
Alkaloids	Anatoxin-a	Nerve synapse	*Anabaena*, *Planktothrix*, *Aphanizomenon*
	Anatoxin-a(S)	Nerve synapse	*Anabaena*
	Aplysiatoxins	Skin	*Lyngbya*, *Schizothrix*, *Planktothrix*
	Cylindrospermopsins	Liver	*Cylindrospermopsis*, *Aphanizomenon*, *Umezakia*
	Lyngbyatoxin-a	Skin, gastro-intestinal tract	*Lyngbya*
	Saxitoxins	Nerve axons	*Anabaena*, *Aphanizomenon*, *Lyngbya*, *Cylindrospermopsis*
Lipopolysaccharides		Exposed tissue	All

Source: Adapted from Chorus and Bartram.[41]

It is apparent that the cyclindrospermopsins comprise only a sub-class of alkaloidal cyanotoxins and their main mode of action is upon the liver. However, as will be explained, they may be of special concern in that they may affect organs other than the liver.[41]

It is also clear from the table that cyanotoxins are varied in structure, and may induce a wide range of diseases. These include diverse cytotoxins, endotoxins,

hepatotoxis and neurotoxins and as such they comprise some of the most deadly naturally occurring toxins.[42] Aside from their importance to medical and environmental issues, cyanotoxins are so potent that they are of interest to the military, for purposes of chemical or biological warfare.[43] The toxins may affect humans or animals by ingestion of contaminated water, injection of contaminated animal protein, or simply by skin contact. They may also affect plants.[2]

However, as regards the cylindrospermopsin alkaloids, they appear to most severely affect organisms with complex metabolic systems, suggesting that the metabolites of the alkaloids may be responsible for their toxicity.[2]

Alkaloids

Alkaloids are a diverse group of naturally occurring products and all alkaloids contain nitrogen and most taste bitter when ingested by humans. They are synthesized by animals, bacteria, fungi and plants but are especially common in plants, although their concentration varies according to location within the plant.[44] Plants produce alkaloids for a variety of reasons but one main reason is for protection, as alkaloids are toxic and can protect plants from being eaten or from being attacked by parasitic organisms.[44] However alkaloids have many other uses, for example, they are also used by animals as neurotransmitters and hormones (see below).[44] The general classification of alkaloids is give in Table 2.[44]

Table 2. Alkaloid classification.

Class	Chemistry	Examples
True alkaloids	Most derive from amino acids. All possess nitrogen in the heterocycle	Atropine, nicotine, morphine (originate from amino acids), coneiceine (does not originate from amino acids)
Protoalkaloids	Contain nitrogen in a side chain, and derive from amino acids	Adrenaline, ephedrine, mescaline, dopamine
Polyamines	Derive from putrescine, spermidine, and spermine	Agrobactine
Peptide and cyclopeptides	Derived from amino acids,	Cyiclosporin
Pseudoalkaloids	Not derived from amino acids	Caffeine

Source: Adapted from Hesse.[44]

The table demonstrates that alkaloids are strongly implicated in narcotics (e.g., morphine) and other drugs (e.g. nicotine), hormones (e.g. adrenaline), and neurotransmitters (e.g., dopamine). They are also strongly implicated in disease. Ergoline, for example, is implicated in *ergotism*, a condition brought on by ingestion of fungal infected cereals and associated with delirium, pain, and in extreme form, gangrene of the extremities and (later) death. Ergotism also weakens the immune system[45] so much so that the spread of the Black Death in medieval Europe appears to have been facilitated by fungus on grain that weakened the immune systems of those who ingested it.[46]

This last point, as regards ergoline weakening the immune system, illustrates another problem with alkaloids. Although, as discussed above, alkaloid poisoning may precipitate specific organ failure (in the case of cylindrospermopsins, often liver failure), it may also have general debilitating effects. Some alkaloids are known teratogens (cause malformation of the foetus); for example, the alkaloid cyclopamine which is present in the leaves of corn lily, causes facial deformities in lambs born of

the ewes that chewed the leaves.[47] Alkaloids may also precipitate cancer.[44] Alkaloid poisoning may thus not only kill or debilitate directly, it may also have long-term insidious effects.

Despite, or perhaps because of, their complexity and their potentially toxic effects, alkaloids are of use to medicine and their application is diverse Table 3 gives some examples.[44]

Table 3. Alkaloids and medicine.

Alkaloid	**Condition treated**
Ajmaline	Arrhythmia
Vinblastine	Tumour
Vincamine	Hypertension
Codeine	Cough
Cocaine	Pain
Colchicine	Gout
Morphine	Pain
Reserpine	Hypertension
Quinidine	Arrhythmia
Quinine	Malaria
Emetine	Protozoan infection
Ergot alkaloids	Hypertension

Source: Adapted from Hesse.[44]

Not all alkaloids have psychoactive effects, however some non-psychoactive alkaloids may be used to synthesize psychoactive drugs, for example,

methamphetamine can be produced from ephedrine or pseudoephedrine. From this one may conclude, along with Hesse,[44] that alkaloids are both a blessing and a curse. They are a blessing in that some organisms need a specific alkaloid to survive and in that they have great potential use in medicine; they are a curse in that an alkaloid in an inappropriate environment may cause great damage, both to individual organisms and to ecosystems.

The cylindrospermopsin family of alkaloids

There are three members of the cylindrospermopsin family, cylindrospermopsin **1**,[10] 7-*epi*-cylindrospermopsin **2**,[48] and 7-*deoxy*-cylindrospermopsin **3**.[17,48] Common features in these metabolites are the presence of a sulfonated alcohol, a tricyclic guanidine core (the A, B, and C rings), and a uracil containing side chain (ring D). The metabolites differ only at the side chain with respect to the alcohol functions stereochemistry or absence. The metabolites are zwitterions, with a negatively charged sulfate group and a positively charged guanidinium ion. This zwitterionic nature leads to high water solubility. Cylindrospermopsin **1** is known to be very stable to a wide range of heat, light and pH conditions [49] (Figure 8).

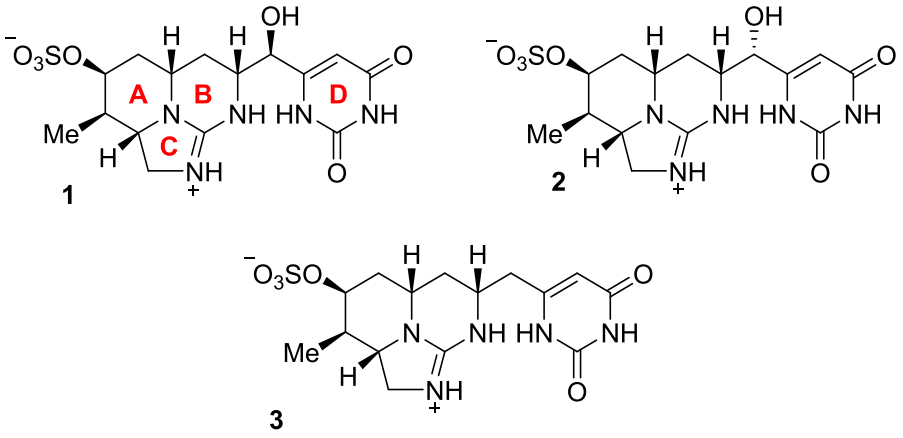


Figure 8. The cylindrospermopsin alkaloids.

Both cylindrospermopsin and 7-*epi*-cylindrospermopsin are cytotoxic,[14] hepatotoxic,[13] and neurotoxic.[15] Cylindrospermopsin has also been shown to be a

teratogen, at least to mice[50] and to be genotoxic.[51] There are also strong suggestions that cylindrospermopsin and 7-*epi*-cylindrospermopsin are carcinogens. The toxic effects of 7-*deoxy*-cylindrospermopsin are, as yet, uncertain.[2,16]

There is much uncertainty concerning such toxic effects, for example, Falconer and Humpage[16] used laboratory mice as test subjects to determine whether cylindrospermopsin (fed orally) induces cancer. The mice so fed did develop more tumours than did control mice; however, the difference was not statistically significant. There are also the problems of whether one can generalise from mice to other species, including humans, and of whether one can generalise from mice force-fed suspected carcinogens to mice living in the wild. It has long been known that numerous seemingly innocuous substances, including lettuce, orange juice, apple, and cinnamon,[32,52] produce cancer when they are force fed to mice, which suggests it is the force feeding that causes the cancer, not the substance.[53] Research also suggests considerable variation of cylindrospermopsin toxicity according to the animal model employed,[54] and indeed variations according to different members of the same species.[55] The choice of mice as a research animal may also be unfortunate in that it might be easier to induce cancer in them than it is in other species. In the case of DDT, for example, much research suggested the pesticide induced cancer in mice, especially when force fed (or injected) in large quantities, but the DDT seemed to have little to no deleterious effects on rats and golden hamsters.[52,56,57] Finally in this regard, to date, research has mostly concentrated on mammalian species, with a view to determining potential threats to humans, while very little has been done as regards the potential effects of cylindrospermopsin poisoning in plants or on ecosystems.[2]

Nonetheless, although the species-specificity of cylindrospermopsin toxins remains uncertain, as is the scale to which it poses a threat to human beings and wildlife, there is little doubt that it does constitute a threat, and plausibly a severe one. One reason for believing this is the mechanism of cylindrospermopsin action. It tends to inhibit protein synthesis,[58] thereby potentially damaging any body tissue and, in contrast to other cyanotoxins (e.g., microcystins), to affect a wide range of animal and plant species.[2] It also disrupts DNA synthesis, thereby enhancing its potential carcinogenic and teratogenic effects.[2]

A further reason for believing cylindrospermopsin constitutes an especially severe threat is that it has been shown to bio-accumulate within ecosystems.[40] For example it has been shown to bio-accumulate in crayfish,[59] mice,[60] eel-tailed catfish[61] and snails.[62] This raises the possibility, as Kinnear[2] suggests, of dangerous levels of cylindrospermopsin accumulating within terrestrial animals and plants, with species higher up the food chain being most severely affected.

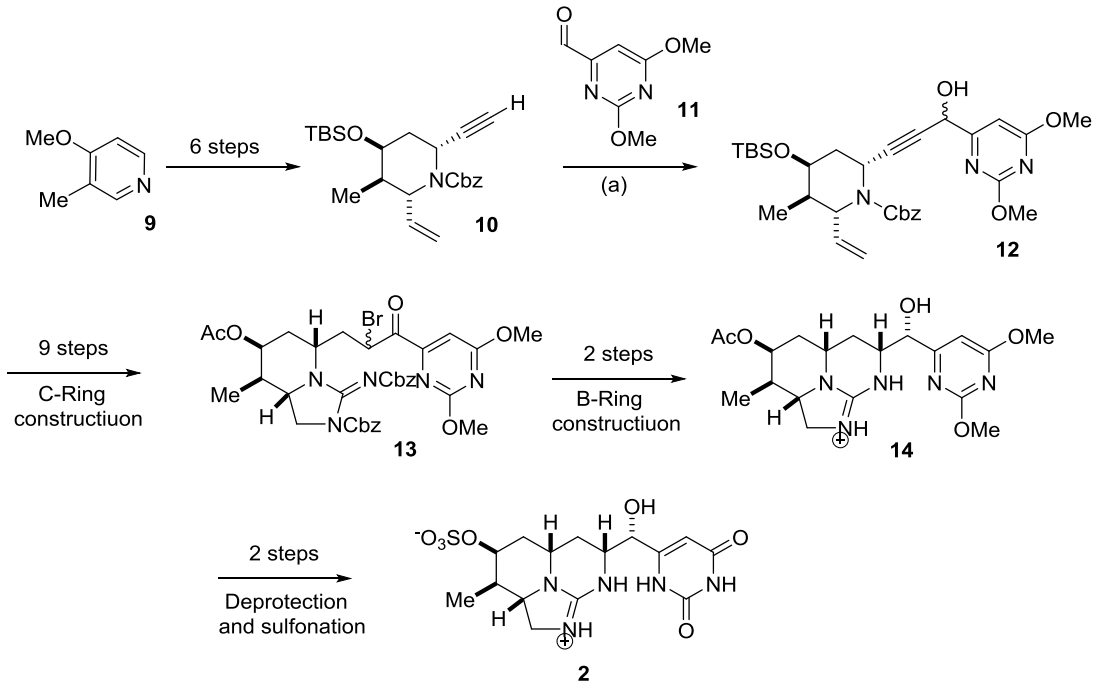
A third reason to believe cylindrospermopsin is potentially hazardous is simply its range. It is found across the globe and this global spread may have been very recent.[2] Also in this regard, it has now been found within several strains of bacterial species within several genera including *Raphidiopsis*, *Aphanizomenon, Anabaena*, *Umezakia* and *Lyngbya*[2] in addition to the original *Cylindrospermopsis.* The only good news is that it appears, thus far, that cylindrospermopsin is largely absent from the oceans and appears to be mainly a freshwater pollutant.[63]

Synthetic approaches to the cylindrospermopsin alkaloids

Four research groups have attempted the total synthesis of members of the cylindrospermopsin alkaloids, namely the Snider group, the Weinreb group, the White group and the Williams group. As this current work focuses on the synthesis of the uracil D-ring found in the Eastern half of the alkaloids and analogues of this portion of the molecule, this section will look at these syntheses and detail the approach to this segment of molecules. Two basic strategies can be visualised in order to incorporate the uracil ring system into the synthetic scheme, either at an early stage or at a later stage, with both these strategies having been adopted with success. We will consider the four major approaches in turn from this viewpoint.

The Snider synthesis

Snider and co-workers were the first group to report[64] the total synthesis of cylindrospermopsin **1** albeit in racemic form. Their approach was to prepare an A-ring precursor **10** from the pyridine **9** *via* conjugate addition chemistry then the magnesium acetylide of **10** was reacted with the aldehyde **11** to give the key intermediate **12** in which the D-ring has been introduced (Scheme 1)[65].

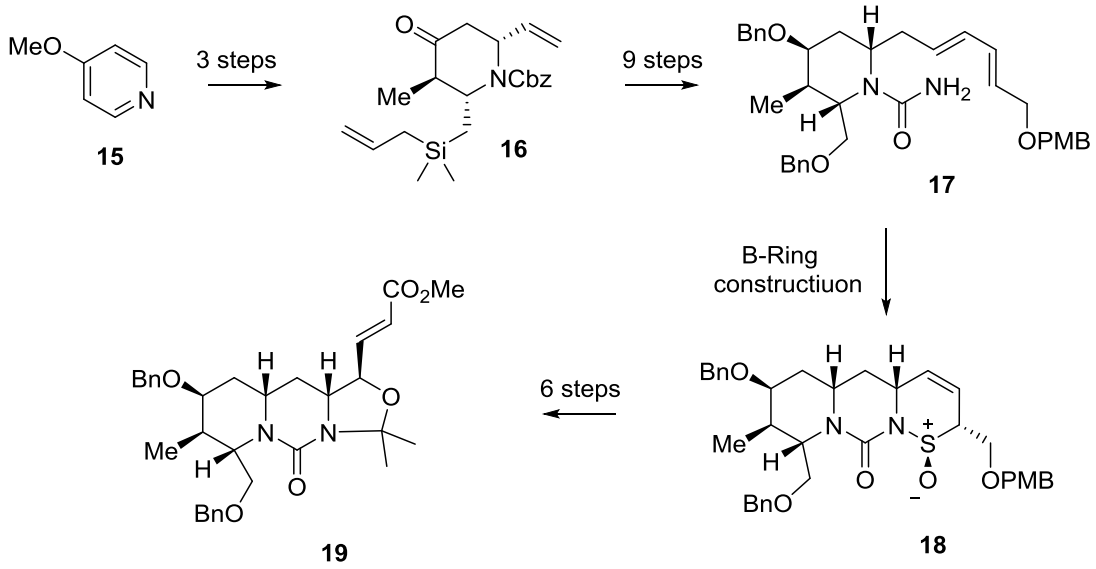


Scheme1. Snider's synthesis of (±) cylindrospermopsin **2**.[65] **(a)** EtMgBr, THF, 0 °C, then **11**, 83%

This methodology represents an early stage introduction of this the D-ring which remains protected throughout the synthesis in which the C- and B-rings are constructed. It is converted into the required uracil side chain only in the final deprotection and sulfonation steps. This sequence of reactions gave racemic cylindrospermopsin in 3.5% yield over 20 steps.

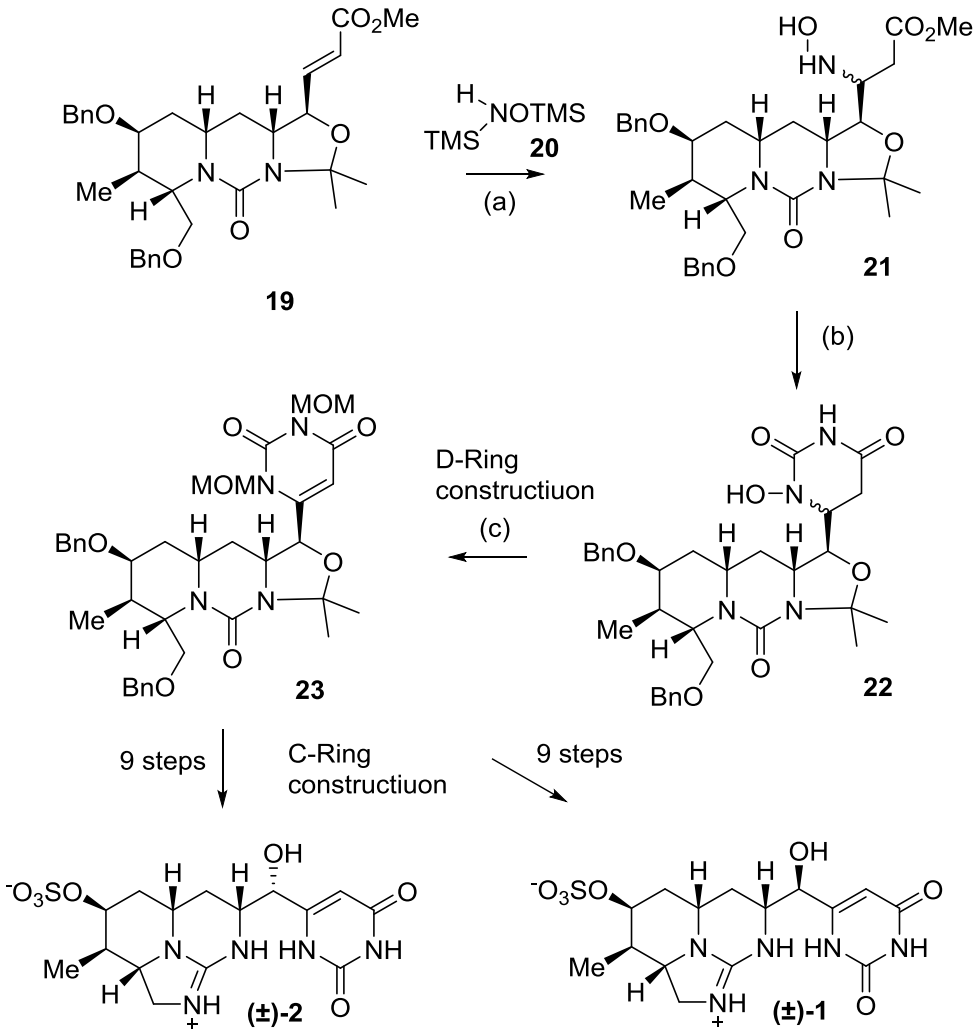
The Weinreb Approach

Weinreb and co-workers were next to report major progress on the synthesis of both cylindrospermopsin (1) and 7-*epi*-cylindrospermopsin (2)[66,67] and in doing so were able to assign the relative stereochemistry of the 2 metabolites. Their approach was similar to Snider's and involved the preparation of the A-ring precursor **16** from the pyridine **15** *via* conjugate addition chemistry, which was modified to the diene **17** over 8 steps. This was the precursor to the AB-ring intermediate **18** which was formed by a *N*-sulfinyl-hetero-Diels-Alder reaction.[68] This intermediate **18** was converted in turn to the conjugate ester **19** which was the key precursor to the synthesis of the uracil D-ring (Scheme 2).



Scheme2. Weinreb's synthesis of intermediate 19.[68]

The construction of the D-ring was achieved by reaction of ester **19** with *N*,*O*-bis-(trimethylsilyl) hydroxylamine[68] to give the conjugate addition product **21**. Treatment of **20** with phenyl chloroformate and ammonium hydroxide give **22**, which could be dehydrated on exposure to triflic anhydride in pyridine to give the uracil **23** (Scheme 3). Modification of **23** over 9 steps gave *epi*-cylindrospermopsin **2**, whilst cylindrospermopsin **1** could also be accessed over 9 steps via an epimerisation of the alcohol function.[67]

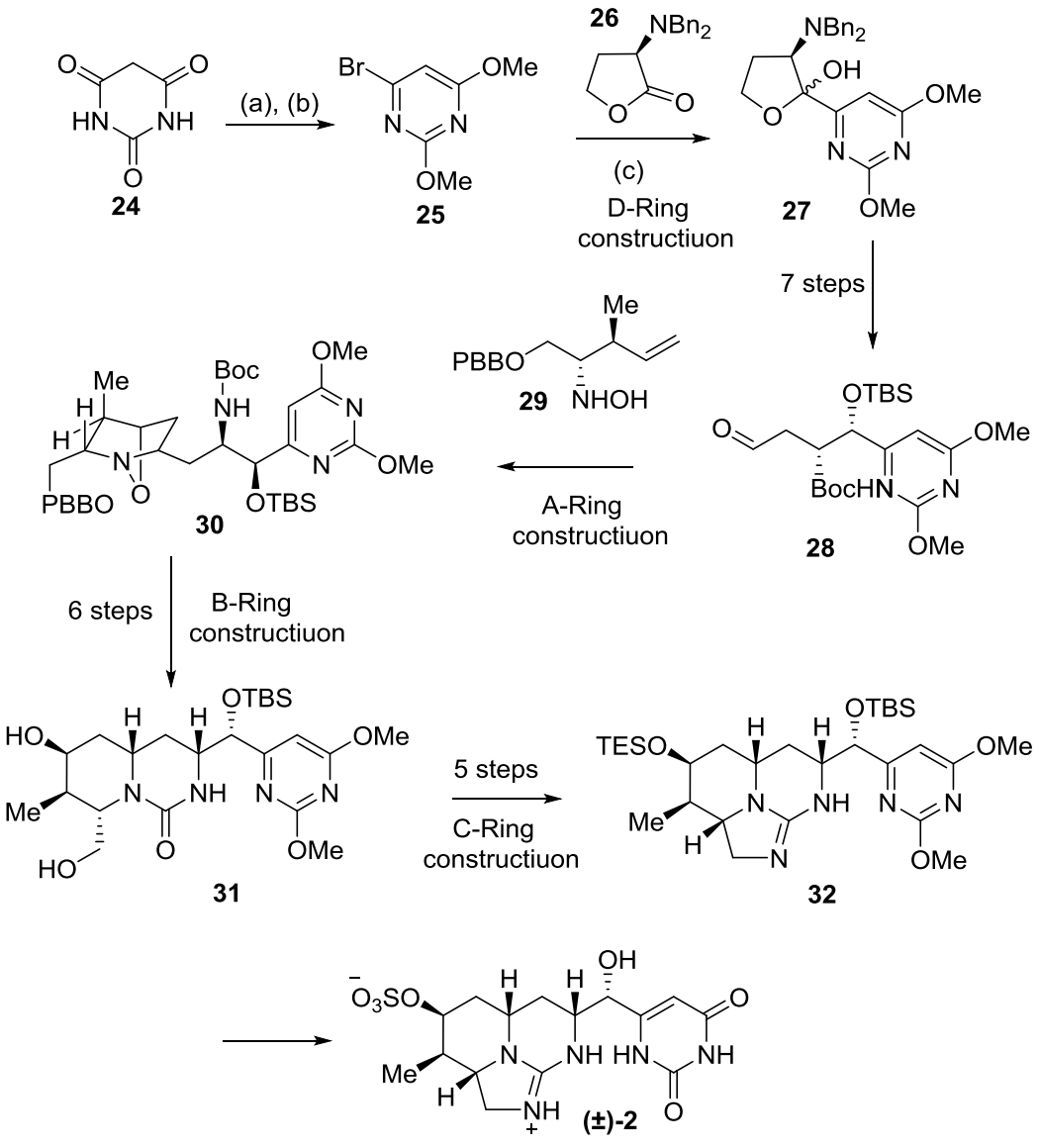


Scheme3. Weinreb's synthesis of *epi*-cylindrospermopsin **2** and cylindrospermopsin **1**.[67] (a) THF, EtOH, 82%; (b) (i) PhOCOCl, NEt_3, THF, (ii) NH_4OH, i-PrOH, 65%; pyridine, CH_2Cl_2, 73%; (c) (i) Tf_2O, pyridine, CH_2Cl_2, (ii) TMSCl, MOMCl, DIPEA, CH_2Cl_2, 80%.

The White synthesis

The next group to report work in this area were White and Hansen,[69,70] who detailed their synthesis of 7-*epi*-cylindrospermopsin **2**.[66] Their synthesis introduced the uracil D-ring in the initial steps beginning with barbituric acid **24**, which was converted into the bromide **25** then metallated and treated with lactone **26** to give the hemiketal **27**. This was then converted into aldehyde **28** over 7 steps, which on reaction with the hydroxylamine **29** give an intermediate nitrone which underwent 1,3-

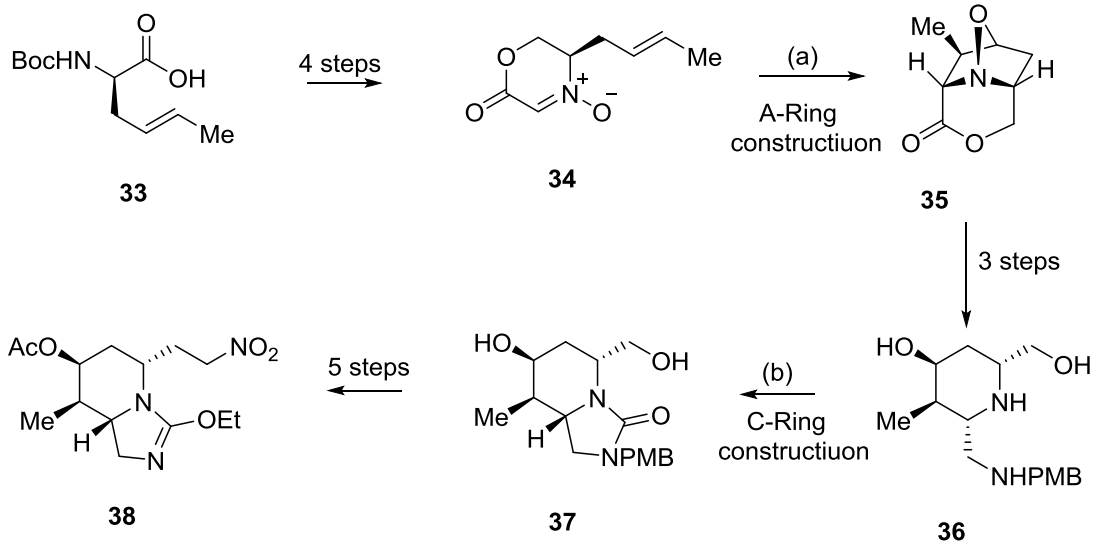
dipolar cycloaddition to give **30** as a 2:1 mixture of isomers. Modification of **30** and cyclisation gave the cyclic urea **31,** which represented an ABD model of the natural products. The final C-ring was introduced over 5 steps to give **32**, which was treated with acid to deprotect the silyl protecting groups and the pyrimidine to give the final product **2** after sulfonation. This overall process proceeded in 0.39% overall yield over 24 steps (Scheme 4).



Scheme4. White's synthesis of *epi*-cylindrospermopsin **2**.[69,70] (a) $POBr_3$, NEt_3, toluene, Δ, 99%; (b) NaOMe, MeOH, 84%; (c) n-BuLi, **26**, $CeCl_3$, Et_2O/THF, -78 ˚C to rt, 97%; MeOH, 84%.

The Williams synthesis

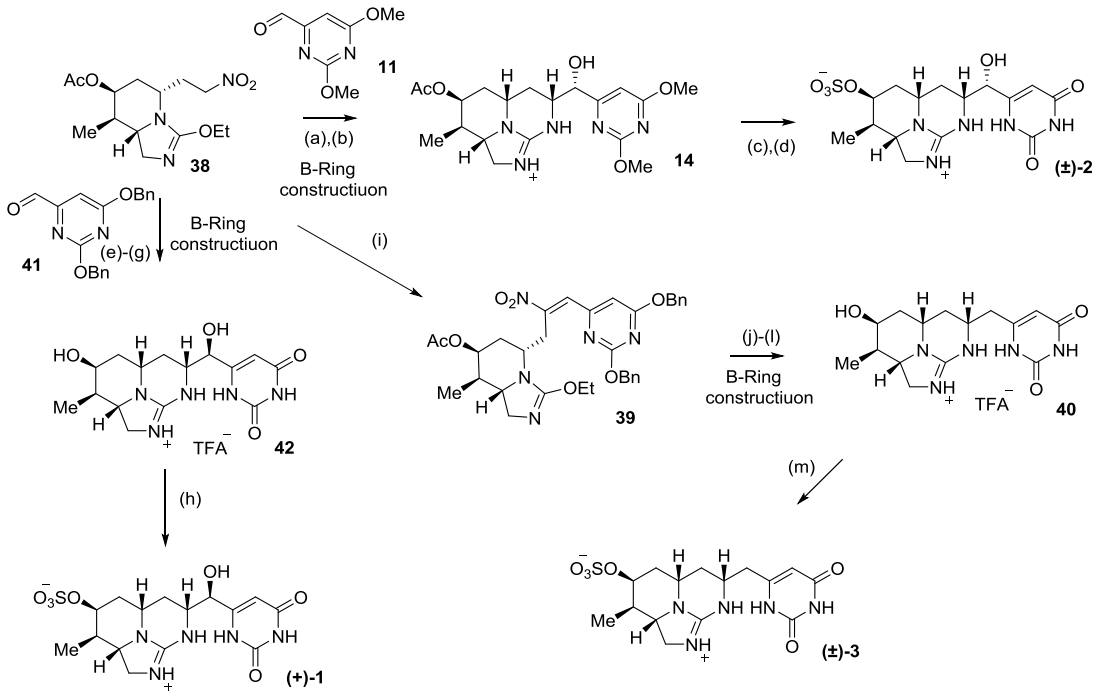
Perhaps the most synthetically elegant approach to the cylindrospermopsin alkaloids is that of Williams and co-workers, who reported[71,72,73] the total synthesis of all three of the metabolites. Their strategy is the most adaptable of those considered to date as it relies upon a central[71,72,73,74] synthetic precursor **38**, which is an AC-ring system model. Their synthesis begins with the protected amino acid **33**, prepared from glycine, which is converted over 4 steps to the oxazinone-*N*-oxide **34** which undergoes a 1,3-dipolar cycloaddition to give the tricyclic isoxazolidine **35** in 78% yield. Reduction of **35** with DIBAL followed by reductive amination with *p*-methoxybenzylamine gave the diol **36**. This diol was converted to bicyclic urea **37** in 67% yield, which was in turn converted to the key intermediate **38** over 5 steps[73,74] (Scheme 5).



Scheme 5. Williams synthesis of intermediate **38**.[73,74] (a) Toluene, 200 ˚C, sealed tube, 78%; (b) (p-$NO_2C_6H_4O)_2CO$, MeCN, 67%.

Williams reported the asymmetric synthesis of 7-*epi*-cylindrospermopsin **2** initially by reacting *O*-ethylisourea **38** under nitro-aldol conditions with aldehyde **11** and quenched this reaction with acetic acid followed by a reductive cyclisation step to give the tetracyclic guanidine **38** (plus isomers). Acidic deprotection and sulfonation of this intermediate gave 7-*epi*-cylindrospermopsin **2** in 59% yield over 2 steps.

Following this, Williams adapted the methodology to a synthesis of cylindrospermopsin **1**. Thus treatment of *O*-ethylisourea **38** with the dibenzylated pyrimidine aldehyde **41** in the presence of TBAF for 30 min, followed by reductive guanylation and then acidic deprotection led to the guanidine **42** (plus isomers) in 20% yield. Finally, sulfonation under previously reported conditions afforded cylindrospermopsin (**1**) in a 60% yield, representing the first asymmetric synthesis of this metabolite. Having completed the synthesis of the two oxygenated cylindrospermopsin alkaloids, work was directed toward the previously unprepared metabolite 7-*deoxy*-cylindrospermopsin **3**.[71,72,73] Thus treatment of racemic **38** with the aldehyde **41** in the presence of acetic anhydride and excess cesium fluoride coupled the two units together under aldol condensation conditions to give the nitroalkene **39** in 67% yield. Reduction of **39** with sodium borohydride followed by hydrogenation over Pearlman's catalyst and deprotection, gave **40** which was sulfonated to give racemic 7-*deoxy*-cylindrospermopsin **3** (Scheme 6).



Scheme 6. Willams synthesis of all three cylindrospermopsin alkaloids **1**-**3**.[71,72,73] (a) 2.0 eq. TBAF, THF, -15 ˚C, 15 min; (b) $Pd(OH)_2$, H_2, MeOH, 5% HOAc; (c) conc. HCl, Δ, 12 h, 32%; (d) SO_3.Py, 3Å, molecular sieves, DMF, 59%; (e) 1.0 eq. TBAF, -15 ˚C, 0.5 h; (f) $Pd(OH)_2$, H_2, MeOH, 5%

AcOH; (g) conc.HCl, Δ, 0.5 h, 20%; (h) SO_3.py, 3Å molecular sieves, DMF, 60%; (i) CsF, Ac_2O, MeCN, 67%; (j) $NaBH_4$, EtOH; (k) $Pd(OH)_2$, H_2, MeOH, 5% AcOH; (l) conc. HCl, Δ, 0.5 h; (m) SO_3.Py, 3Å molecular sieves, DMF, 33%.

Within this work Williams and co-workers reported the first 19-step enantioselective synthesis of cylindrospermopsin **1** in 0.34–0.57% overall yield, a 19-step synthesis of 7-*epi*-cylindrospermopsin 2 in 0.47–0.82% overall yield, and the first, 20-step, racemic synthesis of 7-deoxy-cylindrospermopsin **3** in 0.62–1.05% overall yield.

Conclusions on the reported total syntheses of the cylindrospermopsin alkaloids

From the synthetic work to date it is interesting to note that White's approach to the alkaloids introduces the D-ring into the synthesis at a very early stage and constructs the metabolites in the sequence D→AD→ABD→ABCD. Snider similarly incorporates the D-ring in a fairly early part of the synthesis and constructs the target in the sequence A→AD→ACD→ABCD. In contrast, Weinreb introduces the D-ring in a stepwise manner and constructs the metabolite in the sequence A→AB→ABD→ABCD and Williams incorporates the uracil D-ring in the very late stages of his synthesis, which takes the order A→AC→ACD→ABCD. In all the methodologies reported to date, the uracil D-ring is protected throughout the synthesis and is deprotected in the final steps. The use of an O-alkylated protecting group has been predominant with both -OMe and OBn being used by three groups whilst Weinreb utilized the *N*-MOM protecting group in his synthesis (Figure 9).

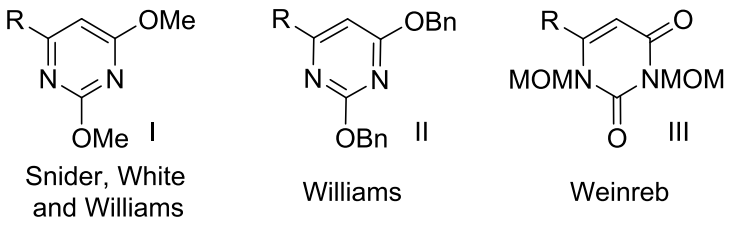
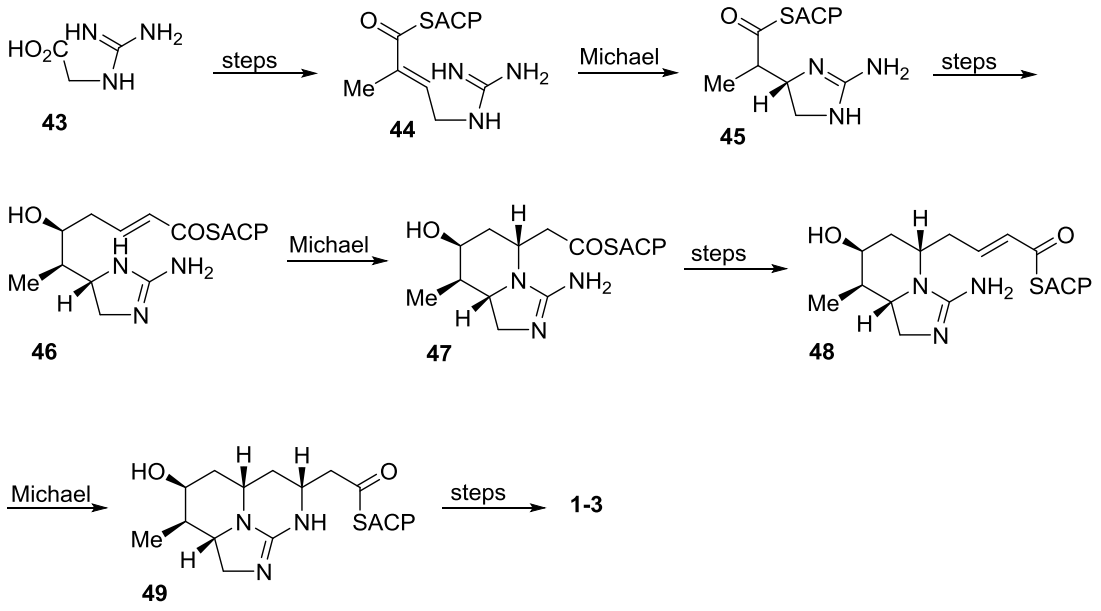


Figure 9. D-Ring protecting groups employed in the synthesis of the cylindrospermopsin alkaloids.

The synthetic approach adopted by the Murphy group

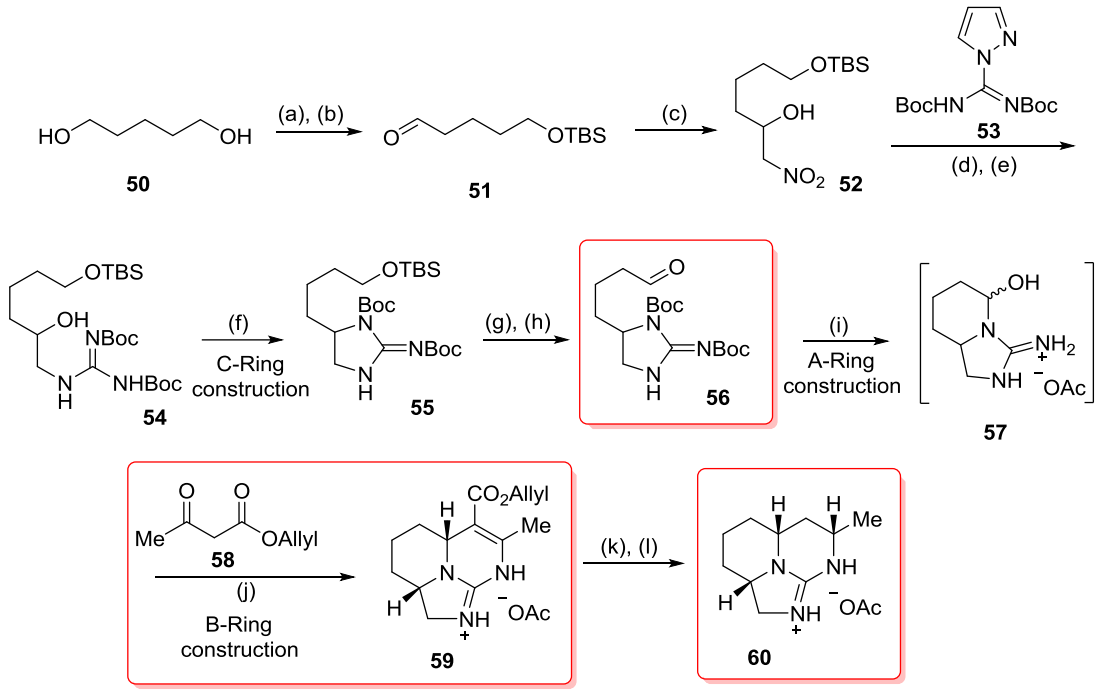
A recent report[75] on the biosynthesis of cylindrospermopsin suggests that it is produced in a step-wise manner from guanidino acetate **43**, with sequential polyketide extensions and Michael type ring closures to generate, in turn, the C ring (**44**→**45**), the A ring (**46**→**47**), and the B ring (**48** →**49**). A final series of polyketide extensions and an oxidation then provides the three main members of the cylindrospermopsin family **1**-**3** (Scheme 7).



Scheme 7. Biosynthesis of the cylindrospermopsin alkaloids[75]

This biosynthesis led Evans and Murphy[38] to suggest a potential biomimetic synthesis of the three alkaloids which might be achieved by using the chirality of the initial C ring **45** to generate the stereochemistry of the later A **47** and B **49** rings. A model study was performed by Evans and Murphy,[38] who prepared the aldehyde **58** from 1,5-pentanediol **50** over 9 steps and coupled this with allyl acetoacetate **58** under Biginelli conditions to generate the tricyclic guanidine **59** in 43% yield from **57**. The relative stereochemistry across the piperidine ring was found to be identical to that found in natural cylindrospermopsin **1**. Decarboxylation and reduction of the

intermediate enamine led to the saturated tricyclic model **60**, which possesses the correct stereochemistry across all three rings and represented a rapid method for the construction of this moiety (Scheme 8)[38]. It is interesting to note that this follows the biosynthetic hypotheses in that the sequence of the construction of the rings is C→AC→ACB→ABCD.



Scheme 8. Murphy's approach to the cylindrospermopsin alkaloids.[38]

(a)TBSCl, NaH, THF, 2h, 61%; (b) oxalyl chloride, DMSO, NEt_3, CH_2Cl_2, -78 °C, 3h, 89%; (c) $MeNO_2$, DIPEA, CH_2Cl_2, 36h, 81%; (d) $NiCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH, NEt_3, 0 °C, 3h: (e) **53**, 48h, 78% (2 steps from 52); (f) PPh_3, I_2, imidazole, CH_2Cl_2, -20 °C, 1h, 96%: (g) TBAF, THF, 0 °C–rt, 24h, 99%; (h) Dess-Martin periodinane, pyridine, CH_2Cl_2, 24h, 96%; (i) acetic acid, 24h; (j) morpholine acetate, **58**, Na_2SO_4, CF_3CH_2OH, 70 °C, 12d, 43%; (k) $Pd(PPh_3)_4$, pyrrolidine, THF/MeOH, 1h 30 min; (l) $NaBH_3CN$, AcOH/MeOH, 16h, 57% (2 steps from **60**).

Analogues of the cylindrospermopsin alkaloids

There have been attempts to link the toxicity of cylindrospermopsin **1** to specific features of its molecular structure. An initial study by Sukenik[48,77] suggested that the uracil moiety of **1** is at least partly responsible for its potency. He reasoned

that either competitive or inhibitory binding to a catalytic site is responsible[73] for the activity. Very little has been reported on the preparation of synthetic analogues of the natural products possibly because of their structural complexity; however, one report[17] on the synthesis of simple analogues sought to investigate the structural requirements for the activity. Within this paper[17] the unsulfonated analogues **42** and **61** were studied as was the tricyclic analogue **62**. In addition, the very simplified uracil analogue **63** was also studied as was the bicyclic sulfonate **64**, which lacks the uracil side chain. These compounds were prepared in the synthetic work of Snider (Figure 10).[65]

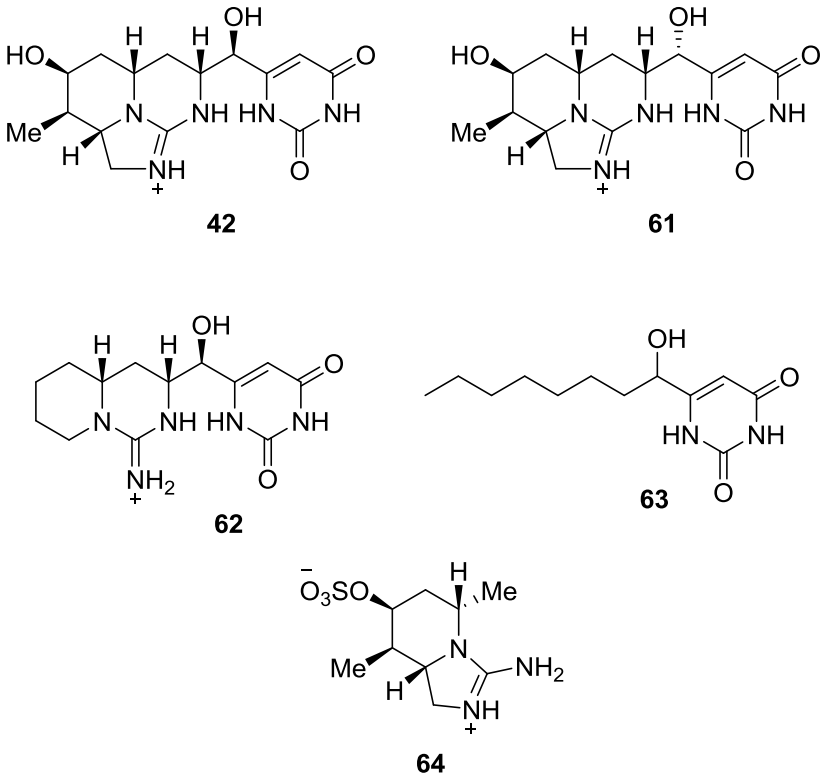


Figure 10. Cylindrospermopsin analogues.

Within this paper, the toxicity of racemic synthetic cylindrospermopsin **±1** was compared with that of native 7-*epi*-cylindrospermopsin **2**. Results suggested that the orientation of the hydroxyl group at C-7 was irrelevant as regards the biological activity or transport of toxin. In addition, comparison of the activity of **±1** and **2** with the corresponding unsulfonated diols ±**42** and **61** indicated that **±1** and **±42** both

inhibited protein synthesis with near identical IC_{50} values of 0.20 and 0.21μM. Furthermore, 7-*epi*-cylindrospermopsin **2** and the corresponding diol ±**42** each depleted cell GSH by similar amounts. This seems to suggest that the sulfate group plays no important role in the biological activity or uptake of the toxin. It was also reported that the AB model compound **62** had an inhibitory effect on protein synthesis, but only at concentrations 500-1000 fold higher than cylindrospermopsin **1**. In contrast, compounds **±63** and **±64** lacked any significant biological activity, at least in concentrations of up to 800 and 2000 μM. These results suggest that the presence of both the guanidine and the uracil moieties is essential for the biological activity observed in these compounds.[78] It was concluded that removal of either of the C-ring or the A-ring functionality reduces the toxicity of cylindrospermopsin more than 100-fold, and that the uracil ring is implicated in the biological activity and may be involved in transporting the metabolites into the cell.

The role of the hydroxy-group adjacent to the uracil was investigated by Shaw,[78] who studied the effects of 7-*deoxy*-cylindrospermopsin **3** in four different mammalian cell lines. They observed clear dose response curves for all cell lines, suggesting that **3** is at least as toxic as cylindrospermopsin **1**. Results also suggested that **1** and **3** inhibit protein synthesis in a similar manner having IC_{50} values of 340 and 220 nM respectively.[78] This result was corroborated by Williams *et al.*, who determined that the two toxins inhibit protein synthesis within one order of magnitude and each also has a similar inhibitory effect on cell GSH.[71]

It is thus uncertain whether the uracil moiety of cylindrospermopsin **1** is implicated only in transport, or is a key feature for *in vitro* activity as well. The finding that the orientation or absence of the hydroxyl group at C-7 results in no appreciable loss of biological activity is also somewhat surprising, as is the fact that the sulfate group appears to play no role in the biological activity and is not implicated in transport across cell membranes.

Aims and previous work

With the report of the biosynthesis of cylindrospermopsin **1**, it was proposed by Evans and Murphy[38] that it might be possible to prepare these alkaloids in a biomimetic manner. A retrosynthetic analysis leads to the conclusion that the molecule might be split into LHS **65** and RHS **66** synthons with the stereochemistry in the LHS C-ring being defined and used to control the stereochemistry of the tricyclic ring system during the synthesis (Figure 11).[64,66,67,70,72]

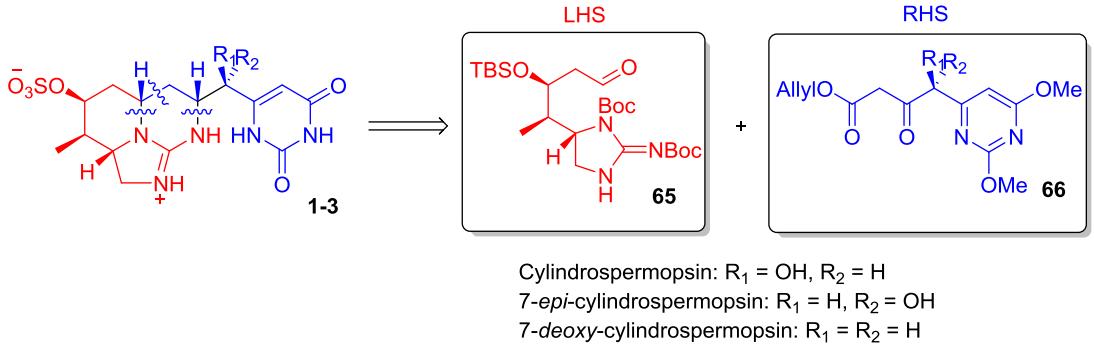
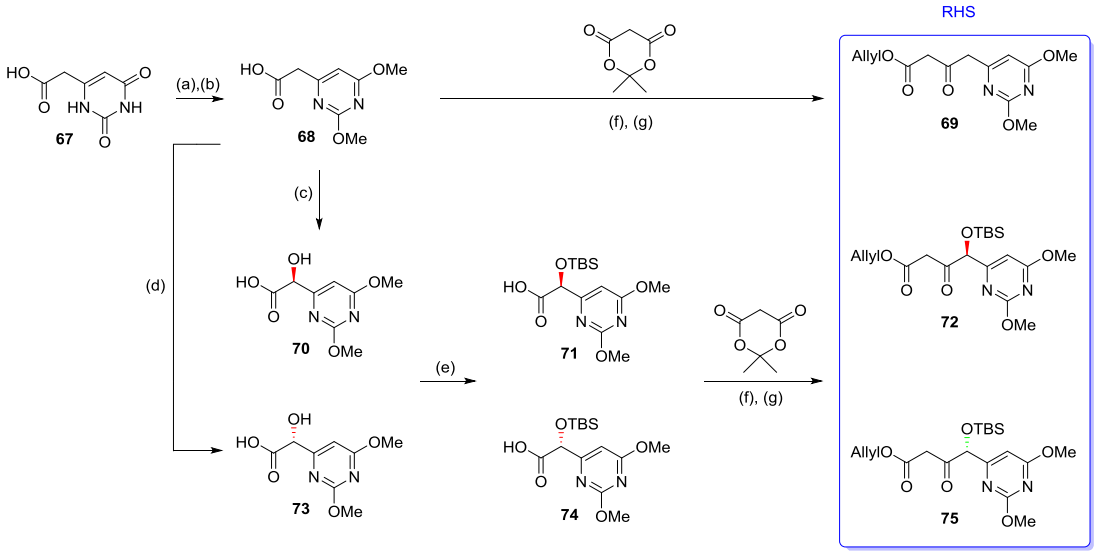


Figure 11. Retrosynthetic analysis of the cylindrospermopsin alkaloids

The aims of this work are to prepare suitable compounds which will act as the RHS synthon and to prepare models of the cylindrospermopsin alkaloids which contain the uracil and guanidine functionality.

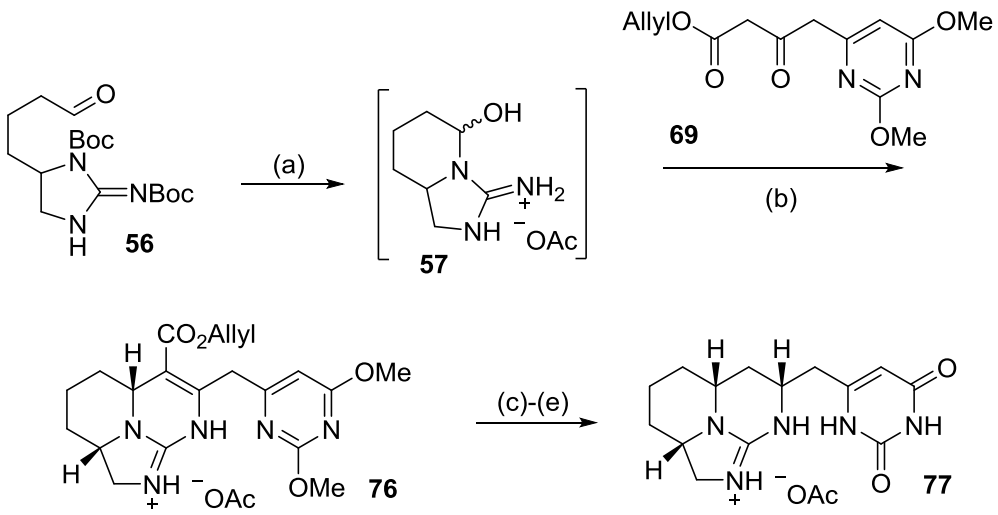
An initial hypothesis (Scheme 9) is that the right hand sub-units might be prepared from the known pyrimidine **67**.[79] It was envisaged that reaction of **67** with phosphorus oxychloride, followed by sodium methoxide should give the protected pyrimidine **68**.[80] Subsequent activation of **71** with DCC followed by treatment with Meldrum's acid, then reaction with allyl alcohol will lead to the α-ketoester precursor **69**, which is required for the synthesis of 7-*deoxy*-cylindrospermopsin **3**.[81] Introduction of the oxygen function at the 7-position in cylindrospermopsin **1** and 7-*epi*-cylindrospermopsin **2** may be possible by oxidation of an intermediate in the 7-*deoxy*-cylindrospermopsin synthesis. However a more elegant solution could be to prepare the enolate of acid **68** and oxidise this under Davis's conditions leading to the β-hydroxy acid **70**[82] Protection and coupling with Meldrum's acid and allyl alcohol

should give **72**. A similar sequence using the Davis reagent B will furnish **73** (Scheme 9).



Scheme 9. Synthetic approachs to the RHS synthons of the cylindrospemopsin alkaloids: a) $POCl_3$; (b) NaOMe, MeOH; (c) Davis oxidation A; (d) Davis oxidation B; (e) TBSCl, imid, DMF; (f) Meldrum's acid, DCC, DMAP; (g) allyl alcohol.

With suitable intermediates in hand the next stage in the synthesis is to prepare a model of the natural product; for example, incorporation of the intermediate **69** into the known model synthesis could result in the synthesis of **77** a model of 7-*deoxy*-cylindrospermopsin. (Scheme 10).



Scheme 10. Proposed synthesis of a model of 7-*deoxy*-cylindrospermopsin: (a) acetic acid; (b) morpholine acetate, **69,** Na_2SO_4, CF_3CH_2OH, Δ; (c) $Pd(PPh_3)_4$, pyrrolidine, THF/MeOH; (d) $NaBH_3CN$, AcOH/MeOH, 16h; (e) HCl, Δ.

Our interest in preparing analogues of the cylindrospermopsin alkaloids stems from a desire to investigate the minimum requirement for activity. It is apparent that the uracil and the guanidine portions of the metabolite appear to be the key factors contributing to the biological activity.[77] We propose that the removal of the ABC ring structures to give "stripped down" analogues **78** will leave the activity largely intact and the synthesis of these will be investigated (Figure 12).

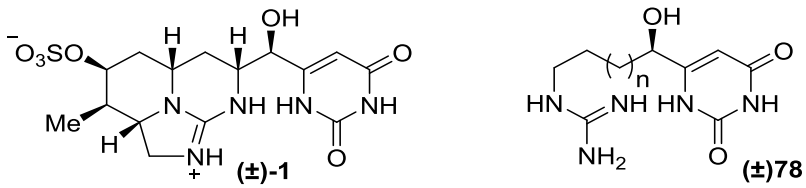
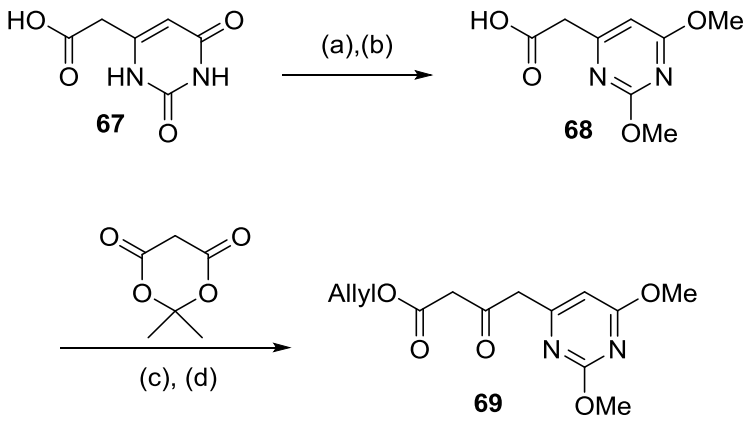


Figure 12. Synthetic analogues of the cylindrospermopsin alkaloids.

Results and Discussion

Synthesis of the uracil D-ring precursor of the cylindrospermopsin alkaloids

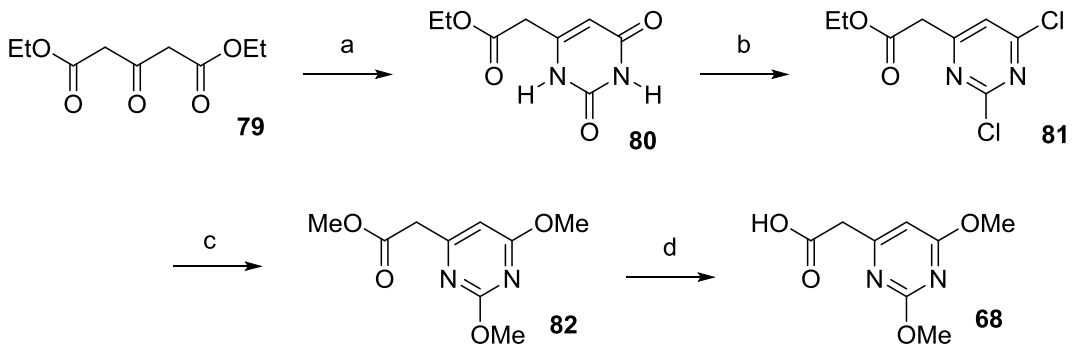
As discussed in the introduction, the first major goal of this work was the preparation of a uracil containing D-ring precursor for the total synthesis of the cylindrospermopsin alkaloids. From our retrosynthesis the synthon **69**[83] was identified as a potential candidate and a synthesis from the compound **67** was proposed (Scheme 11).



Scheme 11. Proposed synthesis of **69**.

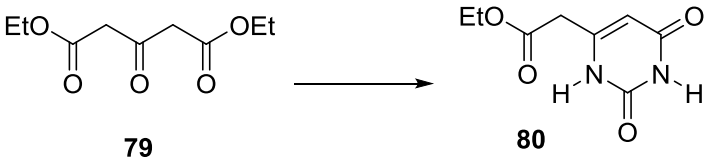
(a) $POCl_3$; (b) NaOMe, MeOH; (c) Meldrum's acid, DCC, DMAP; (g) allyl alcohol, Δ.

On investigation of the literature, compound **80** was reported[84] to be easily prepared from diethyl 1,3-acetonedicarboxylate **79** and we envisaged that the chlorinated pyrimidine could be formed by[85] treatment with $POCl_3$ in the presence of base.[85] We then envisaged that treatment of **81** with sodium methoxide would give the trans-esterified pyrimidine **82** and that hydrolysis of this under basic conditions would yield the acid **68** (Scheme 12).



Scheme 12. Proposed synthesis of of **68**: (a) urea, H_2SO_4, C_6H_6, EtOH; (b) $POCl_3$, *i*-$PrNEt_2$, Tol. reflux, 3 h; (c) NaOMe, MeOH, 1 day; (d) i) NaOH, MeOH, ii) 2M HCl.

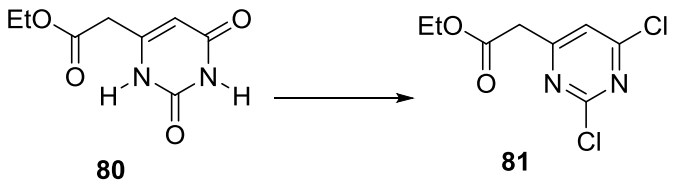
On attempting the first reaction it was found that reaction of diethyl 1,3-acetonedicarboxylate with urea in anhydrous benzene-ethanol (2:1), in the presence of a catalytic amount of sulfuric acid under reflux for 10 days, gave **80** as a white powder in 80% yield. The ^{1}H NMR of **80** displayed 6 resonances at δ_H 1.20 (3H, t, J = 7.1 Hz, Me), 3.48 (2H, s, CH_2), 4.11 (2H, q, J = 7.1, CH_2), 5.46 (1H, s, CH), and 10.41-11.27 (2H, br s, 2 x NH) ppm, together with 8 separate carbon environments in the ^{13}C NMR at δ_C 168.1 (C), 164.1 (C), 151.4 (C), 149.0 (C), 100.8 (CH), 60.9 (CH_2), 37.2 (CH_2), and 14.0 (CH_3) ppm. This data suggested a successful reaction and on determination of the melting point of **80**, we obtained a value of 188 °C, which is in good agreement with the literature[5] value of 186–189 °C (Scheme 13).



Scheme 13. Synthesis of **80**: urea, H_2SO_4, C_6H_6, EtOH,10 days, 80%.

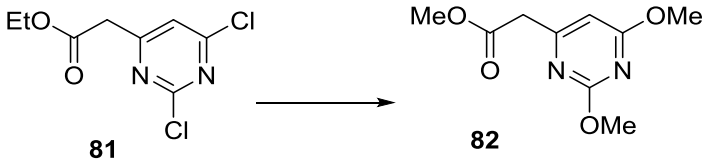
With **80** in hand, it was treated with $POCl_3$ in the presence of *N*-ethyldiisopropylamine at reflux for 3 h.[6] After a hydrolytic work up, the dichloride **81** was obtained as red oil in 76% yield. The ^{1}H NMR of **81** displayed 4 resonances at δ_H 1.26 (3H, t, J = 7.2 Hz, CH_3), 3.79 (2H, s, CH_2), 4.19 (2H, q, J = 7.2 Hz, CH_2), and

7.38 (1H, s, CH) ppm, whilst the carbon spectrum contained 8 resonances at 168.0 (C), 167.0 (C), 162.7 (C), 160.4 (C), 120.1 (CH), 61.8 (CH_2), 42.5 (CH_2), and 14.0 (CH_3) ppm, thus indicating a successful reaction. Analysis of the MS data gave the distinctive pattern for a di-chlorinated molecule with masses at 235.0 (100%, [M+H$^+$]), 237.0 (64%, [M+2+H$^+$]) and 239.0 (10%, [M+4+H$^+$]) Daltons, for the three possible isotopic combinations. In addition, high resolution MS gave a mass of 235.0038 Daltons which is in good agreement with the required mass 235.0036 for $C_8H_9{}^{35}Cl_2N_2O_2$ ([M+H$^+$]) (Scheme 14).



Scheme 14. Synthesis of 81: $POCl_3$, *i*-$PrNEt_2$, Toluene. Δ, 3 h, 76%.

Reaction of **81** with sodium methoxide in methanol for 24 h gave after column chromatography[86,87] compound **82** as a red oil in 40-57% yield over 2 attempts. The ^{1}H NMR of **82** displayed 5 resonances at δ_H 3.57 (2H, s, CH_2), 3.64 (3H, s, Me), 3.88 (3H, s, Me), 3.89 (3H, s, Me), and 6.28 (1H, s, CH) ppm, indicating that the ethyl ester had undergone trans-esterification during the course of the reaction. The ^{13}C NMR displayed 9 resonances at δ_C 171.9 (C), 169.7 (C), 165.1 (C), 164.2 (C), 100.9 (CH), 54.5 (CH_3), 53.6 (CH_3), 52.0 (CH_3), and 42.5 (CH_2) ppm which corresponds to the required environments (Scheme 15).



Scheme 15. Synthesis of **82**: NaOMe, MeOH, 3 h, 40-57%.

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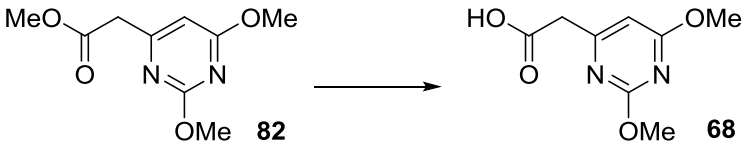
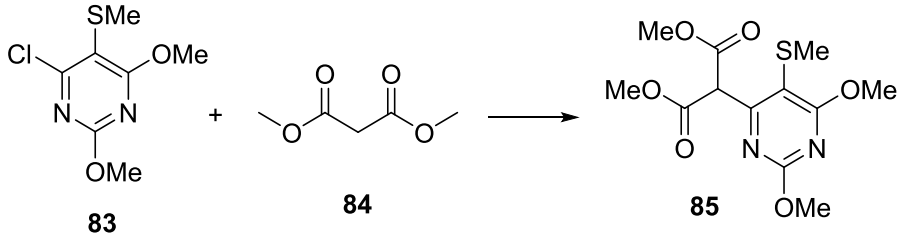
Hydrolysis of the methyl ester of **82** was easily achieved by reaction with sodium hydroxide in methanol over 16 h followed by acidification with hydrochloric acid to give **68**[88] in 96% yield. Compound **68** gave a simple proton spectrum with five resonances at δ_H 3.61 (1H, s, CH_2), 3.88 (3H, s, CH_3), 3.89 (3H, s, CH_3), 6.54 (1H, s, CH) and 5.90-8.20 (1H, br s, OH) ppm, together with eight resonances in the carbon spectrum at δ_C 171.5 (C), 170.6 (C), 165.5 (C), 164.6 (C), 101.1 (CH), 54.4 (CH_3), 53.8 (CH_3) and 42.4 (CH_2) ppm. High resolution mass spectrometry gave a mass of 199.0713 Daltons corresponding exactly to the required mass for the $[M+H]^+$ ion (Scheme 16).



Scheme 16. Synthesis of carboxylic acid **68**: i) NaOH, MeOH, 16 h. ii) 1M HCl, 96%.

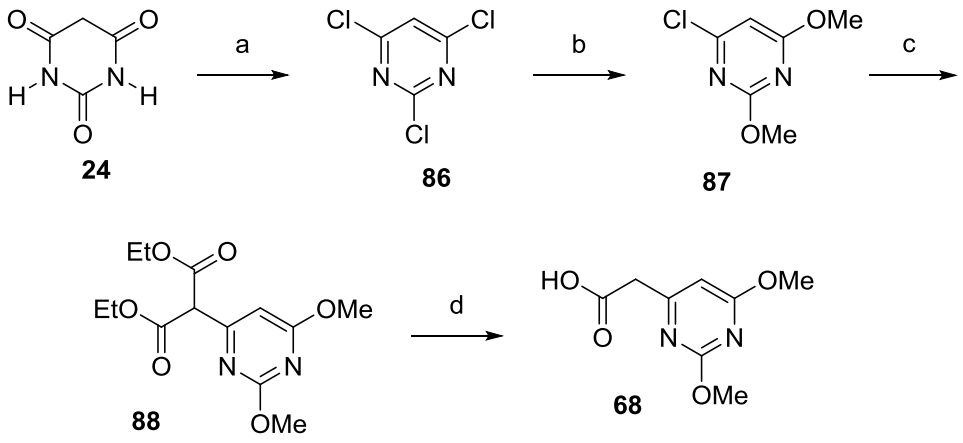
In parallel with the above study, an alternate route to **68** was investigated as some of the previous steps were low yielding. On investigation of the literature, we found that a patent[89] reported that the chlorinated pyrimidine **83** on treatment with an excess of dimethyl malonate in the presence of NaH gave the substitution product **85** in 55% yield (Scheme 17).



Scheme 17. Synthesis of malonate **85**: sodium hydride, DMF, 18h at 100 °C, 55%.

This led us to propose the Scheme 18 shown below in which barbituric acid **24** is converted[90] into the trichloropyrimide **86** and then substituted firstly by methoxide

to give **87**[91] and then by malonate to give **88**, Finally, hydrolysis and decarboxylation should lead to the previously prepared acid **68**.

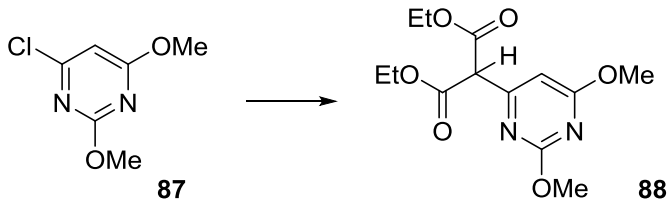


Scheme 18. Alternate synthesis of acid **68**: (a) $POCl_3$, *N*,*N*-dimethylaniline, 0 ˚C, reflux 45 min; (b) NaOMe, MeOH, 3 h, 0 ˚C; (c) diethyl malonate, NaH, DMF, 100 ˚C; (d) NaOH, then HCl**.**

We thus prepared the trichlorinated pyrimidine **87** by reaction of barbituric acid **24** with phosphorous oxychloride to give **86**[91] in 83% yield. Identification of **86** was determined by the single signal at δ 7.4 (1H, s, CH) ppm in the ^{1}H NMR spectrum along with 3 resonances for the ^{13}C NMR at δ 163.3 (C), 160.2 (C), and 120.2 (CH) ppm. Reaction of **86** with 2 equivalents of sodium methoxide in methanol at 0 ºC followed by stirring overnight gave the dimethoxypyrimidine **87** in 67% yield. The proton NMR gave 3 resonances at δ_H 3.96 (3H, s, Me), 3.99 (3H, s, Me) and 6.38 (1H, s, CH) ppm and six resonances in the carbon NMR at δ_C 172.2 (C), 164.9 (C), 161.2 (C), 100.9 (CH), 55.3 (CH_3) and 54.4 (CH_3) ppm. The recorded melting point for **87** was 68-69 ˚C which compared well with the literature value of 71-73 ˚C[91].

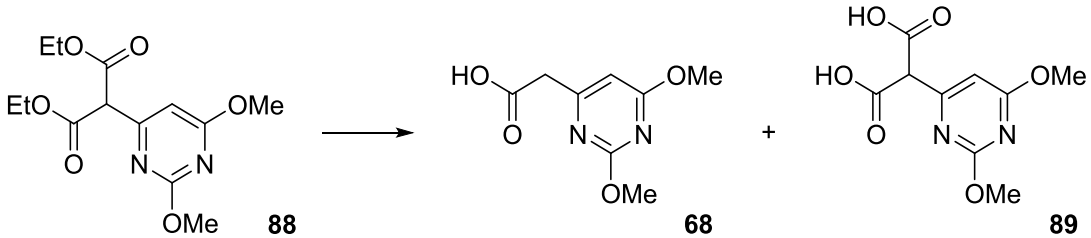
With **87** in hand the key reaction with diethyl malonate was attempted. Thus diethyl malonate was added drop wise to a stirred mixture of the pyrimidine **87** and sodium hydride in DMF at 0 ˚C which was followed by heating the mixture at 100 ˚C for 16 h. After work up and chromatography the malonate **88** was obtained as an oil in 67% yield. The proton NMR of **88** gave major resonances at δ_H 1.25 (6H, t, *J* = 7.1 Hz, 2 × Me) and 3.93 (6H, s, 2 × Me) ppm for the 6 methyl resonances and at 4.10-4.26 (4H, m, 2 × CH_2) ppm for the methylene protons of the ethyl esters. The two

methyl resonances at δ_H 4.67 (1H, s, CH) and 6.45 (1H, s, CH) ppm confirmed the formation of **88**. The required 10 resonances were present in the carbon spectrum and high resolution mass spectrometry gave a mass of 299.1237 Daltons which was in close agreement with the calculated mass of 299.1238 required for $C_{13}H_{19}N_2O_6$ ($[M+H]^+$). In addition to the major resonances in the spectrum, it was apparent that several smaller resonances were present corresponding to enolic forms of **88** (Scheme 19).



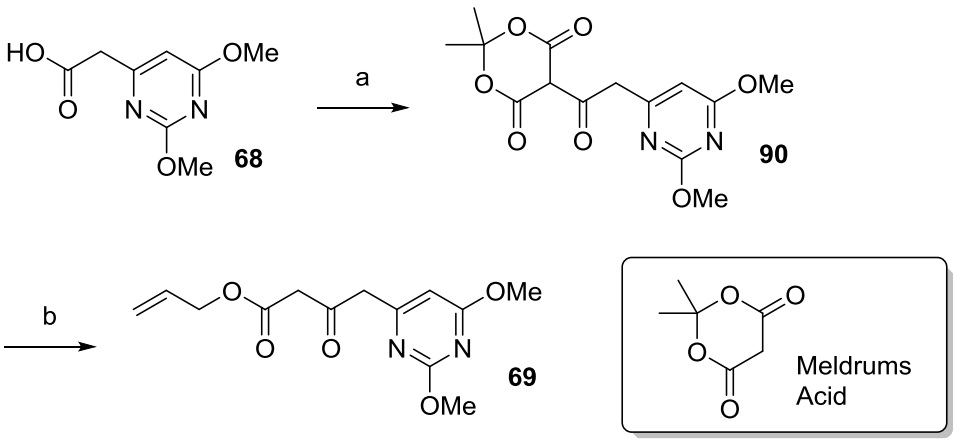
Scheme 19. Synthesis of malonate **88**: Diethyl malonate, NaH, DMF, 100 ˚C, 16h, 54-66%.

Hydrolysis of the two ethyl ester of **88** was easily achieved by reaction with sodium hydroxide in methanol over 16 h followed by acidification with hydrochloric acid to effect decarboxylation. Initial observation of the proton NMR spectrum of the crude product indicated that two products were present as there were four methoxy-environments observed. We theorized that the dicarboxylic **89** acid which is initially formed in the reaction was not undergoing complete decarboxylation It was found however, that when this mixture was treated with 3M hydrochloric acid for 6 hours conversion to the previously prepared acid prepared **68** was observed in quantitative yield which gave identical spectroscopic data to that reported above (Scheme 20).



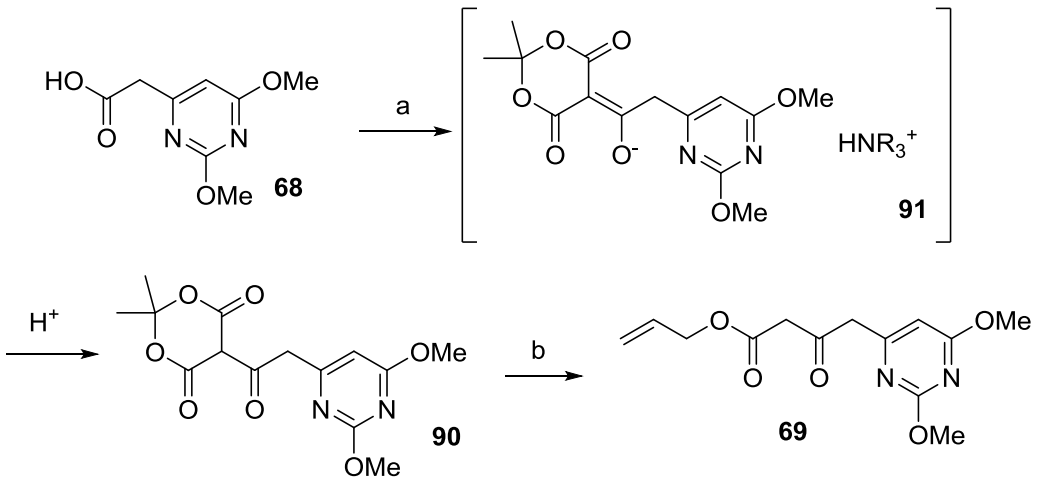
Scheme 20. Synthesis of carboxylic acid **68**: i) NaOH, MeOH, 16h. ii) 1 M HCl, 100%.

With the acid **68** in hand we moved onto the key reaction with Meldrum's acid which has been reported[83] to give acylated derivatives similar to **90** which on heating with alcohols can be converted to the desired β-ketoester **69** (Scheme 21).



Scheme 21. Proposed synthesis of β-ketoester **69**: (a) DCC, DMAP, Meldrum's acid; (b) Allyl alcohol, PhH, Δ.

The reaction of **68** was attempted using DCC as an activating agent and an excess of DMAP as a basic catalyst, which leads to the acylated intermediate **90**. Following completion of the reaction, the literature method[83] used an acidic work up to remove the DMAP and to protonate the enolate salt **91** which is an intermediate in the reaction. On applying this work up to our reaction we were unable to obtain any product which was identifiable as **90**. We repeated the reaction and on completion, we evaporated the solvent and inspected the crude product proton NMR which gave no clear evidence of the presence of **90** and it was apparent that the mixture contained large amounts of DCCU and DMAP as contaminants. Mild acidic work up of this mixture then gave a product which on proton NMR analysis, did not suggest the formation of **90**. This led us to theorize that the compound **90** might be zwitterionic in nature and might be highly soluble in water. With this in mind, we attempted to recover the putative compound **90** from the aqueous layer by careful neutralisation, however no significant material was isolated. We also attempted to convert the mixture from this reaction directly to the ester **69** by treating it with allyl alcohol in benzene at reflux. This again led to a complex mixture of products and acidic work up led to the loss of the majority of the material. Attempted chromatography of the crude reaction mixture was unsuccessful as it was difficult to remove the DMAP and DEAD by-products from the reaction mixture (Scheme 22).

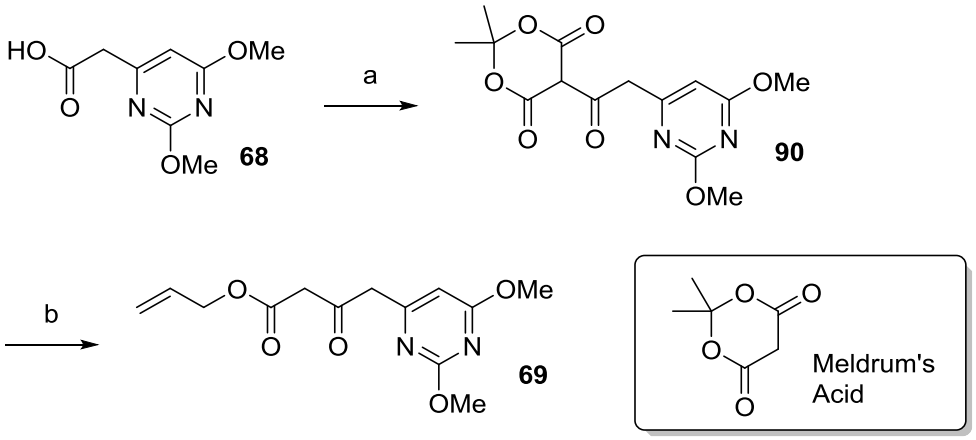


Scheme 22. Attempted preparation of β-ketoester **69**: (a) i) DCC, DMAP ii) Meldrum's acid, CH_2Cl_2, 5 h at rt; (b) allyl alcohol, reflux benzene.

On inspection of the literature an alternative method[92] was found which utilized carbodiimidazole (CDI) as the coupling reagent. This method has the advantage over the previous method of only producing imidazole as the by-product, which should be easier to remove. Thus heating the acid **68** with CDI in THF overnight was attempted which unfortunately led to considerable decomposition, which was associated with the low solubility of the acid in THF and associated charring because of this. We repeated the reaction using DMF and were pleased to observe the formation of the intermediate adduct **90** as evidenced by proton NMR resonances at δ_H 1.64 (6H, s, 2 × CH_3) ppm for the methyl groups of the cyclic diester acid and at δ_H 3.99 (1H, s, CH) ppm for the methyne proton. It was also apparent that an enol was present as a signal at δ_H 11.65 ppm was observed. This product was dissolved in allyl alcohol (7 mL) and heated at 90 °C for 16 h to effect conversion to the β-ketoester **69**. However, after evaporation and column chromatography a complex mixture of products was obtained, none of which was indicative of the presence of the required product. The reasons for the failure of this reaction were unclear and it was proposed that impurities in the crude product were the cause. It was also observed that DMF was a major contaminant and we attempted to remove this impurity by washing with saturated LiBr solution, which is known to remove DMF impurities. On attempting this process, it was observed that the product **69** passed into the aqueous phase, which supported our theory of high

water solubility in the initial reactions. We were able to get the majority of the product back by acidification and re-extraction, however the purity by proton NMR looked to have diminished and there seemed to be more contamination. We also tied to purify this material by column chromatography and again found a loss of most of the material, which suggested the decomposition of the intermediate on silica gel. It was also attempted to purify the compound on triethylamine washed silica gel, however the only product obtained was the triethylamine enolate salt **91** (R = Et) in a very low 3% yield.

Part of the problem with this reaction was assumed to be the presence of an excess of the solvent DMF in the initial step and the use of neat allyl alcohol in the final step. With this in mind, the reaction was repeated with a small volume of DMF in the initial stage of the reaction and after the isolation of the intermediate **90**, the allylation step was performed in benzene solution with five equivalents of allyl alcohol. Column chromatography of the crude compound obtained gave the desired β-ketoester **69** in 28% yield (Scheme 23).

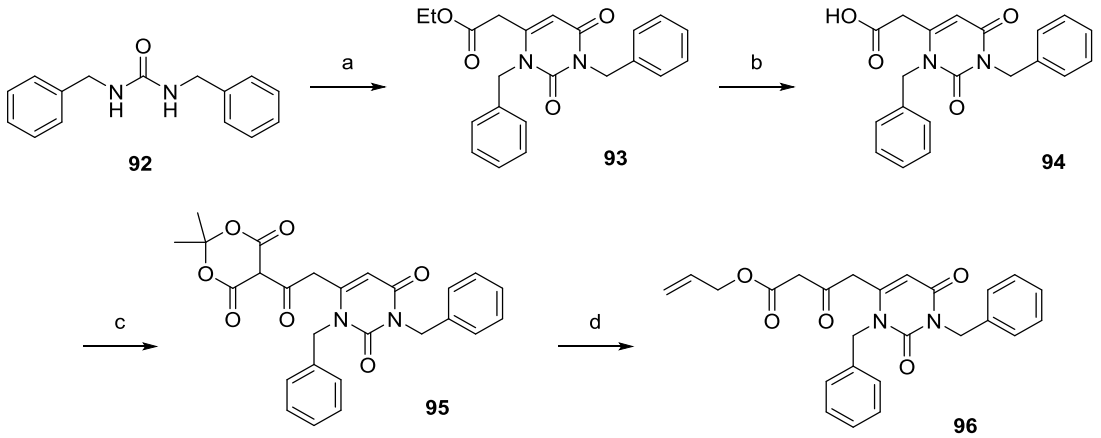


Scheme 23. Synthesis of carboxylic acid **69**:
(a) Meldrum's acid, CDI, DMF 50 ˚C, 5 h; (b) allyl alcohol, Δ, PhH, 28%.

Analysis of the proton NMR of **69** gave a complex spectrum which was thought to be due to the presence of enol tautomers of **69**. The major resonances were present at δ_H (DMSO) 3.78 (2H, s, CH_2), 3.86 (3H, s, CH_3), 3.88 (3H, s, CH_3) and 3.91 (2H, s, CH_2) ppm for the two methoxy and methylene resonances whilst the allyl ester resonances were present at δ_H 4.55-4.58 (2H, m, CH_2), 5.20-5.24 (1H, m, CH), 5.28-

5.35 (1H, m, CH) and 5.83-5.97 (1H, m, CH) ppm. The uracil CH proton was found at δ_H 6.47 (1H, s, CH) ppm and several enolic resonances were observed at δ_H 5.53 (CH), 6.25 (CH) and 13.71 (OH) ppm. The carbon spectrum gave 13 major resonances corresponding to the required number for **69**, whilst high resolution MS gave a mass of 281.1134 Daltons which corresponds closely to the desired mass of 281.1132 Daltons for $C_{13}H_{17}N_2O_5$ ($[M+H]^+$).

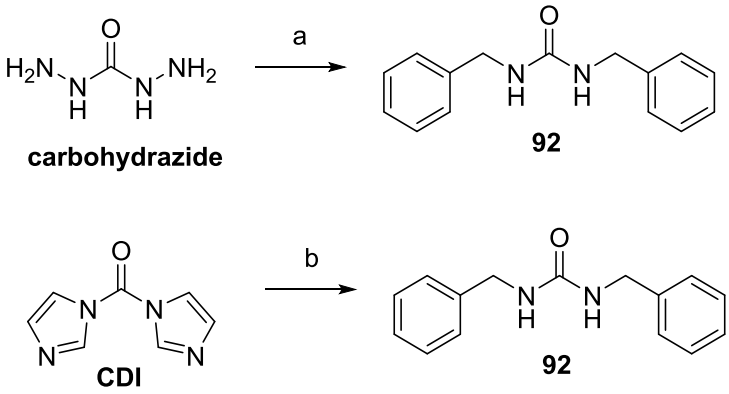
Whilst the synthesis of **69** was a satisfactory achievement the various problems with the synthesis, which stem from the coupling stage and the basicity of the pyrimidine ring, led to low yields and problems with purification. Therefore, it was proposed that an alternative approach be adopted in which we protect the pyrimidine in its benzyl amide form and the following synthesis was proposed (Scheme 24). In this synthesis the protecting groups are introduced in the first step of the synthesis which is the condensation of diethyl 1,3-acetonedicarboxylate and dibenzylurea. Hydrolysis of this ester followed by coupling and allyl ester foamation should yield the RHS analogue **96.**



Scheme 24. Proposed synthesis of the RHS analogue **96**:
(a) diethyl 1,3-acetonedicarboxylate, PhH, *p*-TsOH, Δ, 4 days; (b) NaOH, EtOH, 0°C-rt; (c) Meldrum's acid, CDI, THF, at 50 ˚C, 16 h (d) allyl alcohol, 110 ˚C, 16 h.

Dibenzylurea **92** was prepared using the method described by Wolman and Gallop,[93] in which a vigorously stirred suspension of finely powdered carbohydrazide and benzylamine was treated with I_2 to give **92**[93] as a white solid in 48-64% yield over

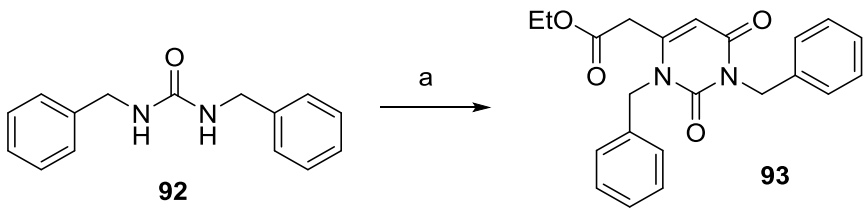
four attempts. The preparation of the urea was also realised by the reaction of benzylamine with carbodiimidazole (CDI) in refluxing THF which gave **92** in 68% yield. The proton NMR spectrum for **92** gave three diagnostic resonances at δ_H 4.23 (4H, d, J = 6.0 Hz, 2 × CH_2), 6.43 (2H, t, J = 6.0 Hz, 2 × NH) and 7.20-7.33 (10H, m, 2 × Ph) ppm with 6 resonances in the carbon spectrum at δ_C 43.0 (2 × CH_2), 126.5 (CH), 127.0 (CH), 128.2 (CH), 140.9 (C), and 158.1 (C=O) ppm. Analysis by high resolution MS gave a value of 241.1333 Daltons which corresponded well with the expected mass of 241.1335 Daltons and a recorded melting point of 165-168 °C corresponded well with the literature[93] value of 168-169 °C (Scheme 25).



Scheme 25. Two syntheses of dibenzylurea **92**.[93]

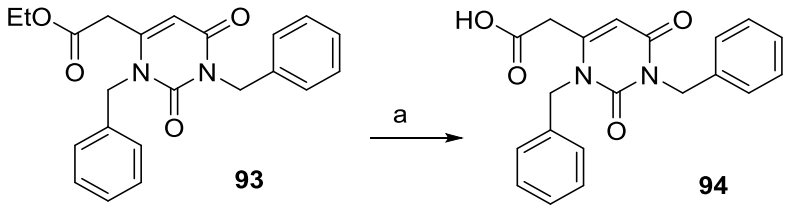
(a) I_2, DMF, benzylamine, 1h, 64%.; (b) benzylamine, THF, Δ, 16 h, 68%.

With the urea **92** in hand the preparation of the pyrimidine **93** was investigated. Reaction of **92** with 2 equivalents of diethyl 1,3-acetonedicarboxylate **92** in refluxing benzene with the removal of water under soxhlet extraction with molecular sieves (4 Å) gave **93** as a pale yellow solid in 67% yield. Diagnostic NMR resonances were at δ_H 1.26 (3H, t, J = 7.2 Hz, CH_3) and 4.13 (2H, q, J = 7.2 Hz, CH_2) ppm for the ethyl ester group and at δ_H 3.42 (s, 2H) ppm for the methylene group. The vinylic proton was also present at δ_H 5.76 (1H, s, CH) ppm with signal for the 2 benzyl groups being observed at δ_H 5.13 (2H, s, CH_2), 5.17 (2H, s, CH_2), and 7.11-7.50 (10H, m, 2 × Ph) ppm. Diagnostic quaternary were also observed at δ_C 152.3 (C=O), 161.6 (C=O) and 167.2 (C=O) for the urea carbon and carbonyl groups. High resolution mass spectroscopy gave a mass of 379.1650 ($[M+H]^+$) Daltons which is in excellent agreement with the calculated value of 379.1652 (Scheme 26).



Scheme 26. Synthesis of pyrimidine **93**:
(a) diethyl 1,3-acetonedicarboxylate, PhH, *p*-TsOH, Δ, 4 days, 90%.

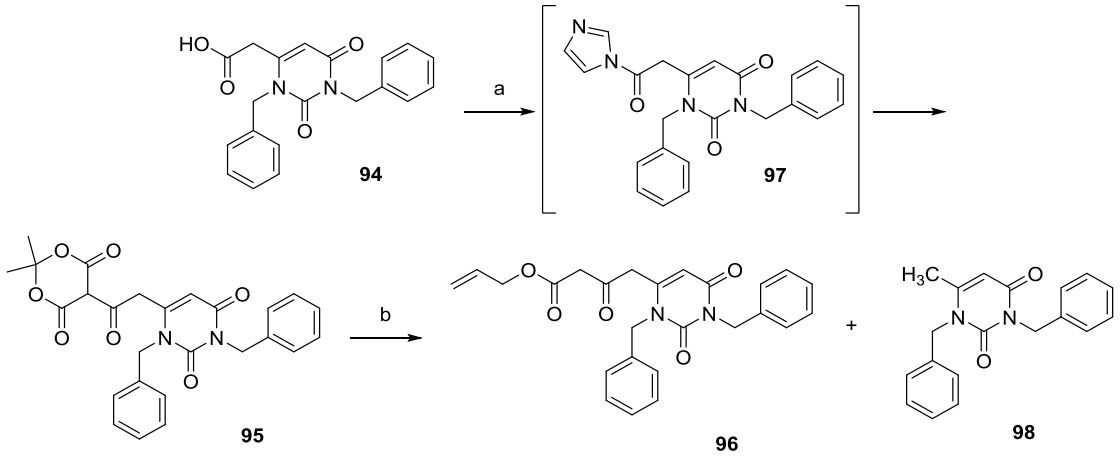
The ester **93** was then hydrolyzed in an uneventful manner with ethanolic sodium hydroxide over 5 days and after purification the acid **94** was obtained as an off white solid in 74% yield. The signal for the alkene proton was observed at δ_H 5.89 (1H, s, CH) ppm whilst the carboxylic acid proton was observed at δ_H 13.09 (1H, br s, OH) ppm. Diagnostic carbon resonances were observed for the alkene proton at δ_C 103.0 ppm and the acid carbonyl at 169.4 ppm. High resolution MS gave a mass of 351.1340 $[M+H]^+$ Daltons which is in good agreement with the expected value of 351.1339 Daltons (Scheme 27).



Scheme 27. Synthesis of acid **94**: (a) NaOH, EtOH, 0 °C rt 2 days, 93-96%.

Initial attempts the formation of β-ketoester **96** were based on the treatment of **94** with an excess of CDI at 50 °C for 5 hours followed by the addition of Meldrum's acid **98** and continued heating overnight. On work up the crude intermediate **95** was heated with excess allyl alcohol resulting in decarboxylation and ester formation to give **96**. After chromatography the desired product **96** was obtained, however large amounts of compound **98** were obtained which we thought might be occurring via decarboxylation of the acid **94** on heating. It was found that combining directly the pyrimidine **94**, Meldrum's acid and an excess of CDI followed by heating at 50 °C for

16 h minimized the formation of **98** and on work up gave **96** in 46-49% yield over three attempts (Scheme 28).



Scheme 28. Preparation of the pyrimidine **96**: (a) Meldrum's acid, CDI, anhydrous THF, 50 ˚C, 16 h; (b) Allyl alcohol, 110 ˚C, 16 h, 46-48%.

The spectrum of **96** was more complex than expected because of the presence of tautomers of the β-ketoester however, diagnostic resonances were observed at δ_H (DMSO) 3.77 (2H, s, CH_2), 3.97 (2H, s, CH_2) for the two chain methylene groups whilst the allyl ester methylene was observed at δ_H 4.57 (2H, dt, J = 5.5, 1.3 Hz, CH_2) ppm. The alkene protons were observed at δ_H 5.21 (1H, dq, J = 10.5, 1.3 Hz, CH), 5.31 (1H, dq, J = 17.3, 1.3 Hz, CH), and 5.87 (1H, ddt, J = 17.3, 10.5, 5.5 Hz, CH) ppm whilst the uracil methyne proton was observed at 5.79 (1H, s, CH) ppm. Final confirmation of structure was obtained by high resolution MS which gave a mass of 433.1756 Daltons which is in close agreement with the calculated mass for $[M+H]^+$ of 433.1758 Daltons.

The conclusions from this stage of the work are that the two RHS synthons for *deoxy*-cylindrospermopsin were successfully prepared. The methoxy protected **69** proved difficult to prepare and the benzyl protected **96** differs significantly from previously utilized methoxy- and benzyloxy- ether protected uracil groups found in other total syntheses. This protecting group will require a different deprotection

strategy and this will need to be addressed in the total synthesis. The failure of the Meldrum's acid/allylation strategy in the methoxy-ether series is not easy to explain and possibly given more time this might be worth reinvestigating.

Synthesis of analogues of the cylindrospermopsin alkaloids

The second goal of the project was to prepare simplified analogues of cylindrospermopsin to investigate structure activity relationships. From a previous report[17] it is apparent that the uracil group, the hydroxyl function and the guanidine are essential for activity whereas the sulfate group plays a small role in the biological properties.

Simplifying cylindrospermopsin **1** leads to a "stripped down" analogue **78**, which can be retrosynthesised to give the known aldehyde **11** and the protected Grignard reagents **100** (Figure 13).

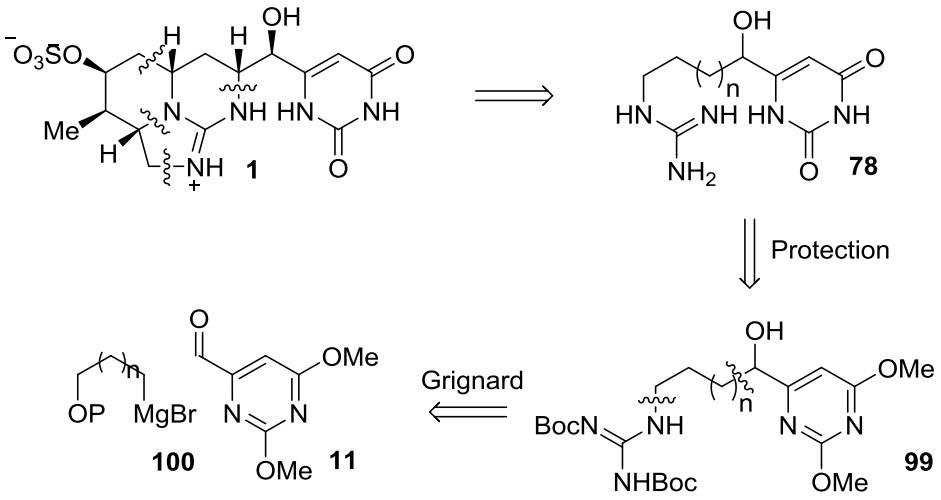
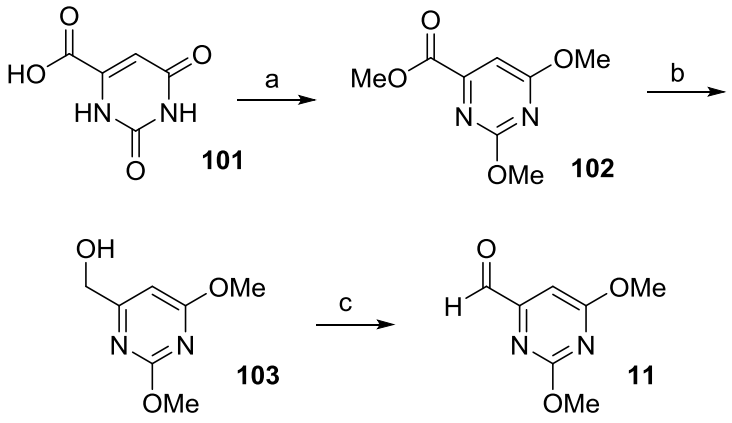


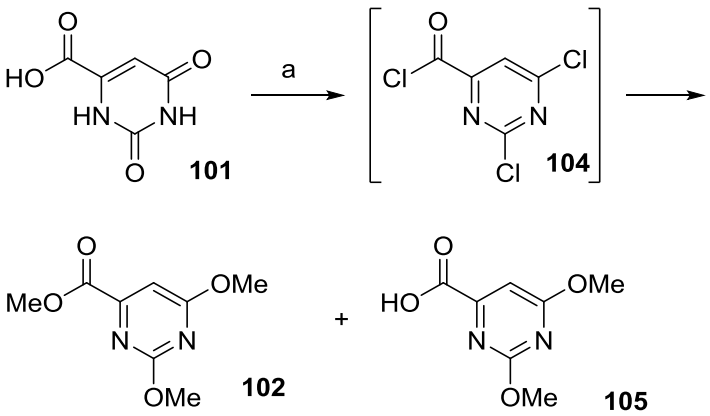
Figure 13. Proposed retrosynthesis of cylindrospermopsin 1 model compounds **78**

Aldehyde **11** was considered to be a good starting point for this synthesis as it has been employed in several syntheses of cylindrospermopsin and can be converted into the uracil by treatment with acid. Aldehyde **11** can be prepared in three steps from orotic acid as shown in (Scheme 29).[94]



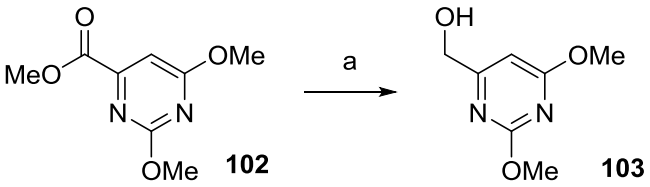
Scheme 29. Preparation of aldehyde **11**: (a) orotic acid monohydrate, $POCl_3$, PCl_5 reflux16 h, then MeOH, 24 h; (b) THF, $LiBH_4$, EtOH, rt, 2 h; (c) Dess-Martin periodinane, CH_2Cl_2, rt 2 h.

Snider reported the synthesis of the methyl ester **102** from orotic acid by heating at 120 °C overnight with $POCl_3$ and PCl_5 to giving crude 2,6-dichloropyrimidine-4-carbonyl chloride which on reaction with dry methanol gave the methyl ester **102** in 57% yield. We repeated his reported method and obtained **102** with spectroscopic data identical to that reported, however in considerably lower yields of 9-17% over three attempts. Snider also reported the isolation of the corresponding acid **105** from his reaction in 18%, however we were unable to isolate any of this by-product from the process. We were concerned that the reaction details reported in the literature were in error and that possibly sodium methoxide was required as a nucleophilic reagent to react with the intermediate acid chloride **104**. We thus repeated the reaction using this reagent but unfortunately similar yields of 9-18% were obtained (Scheme 30).



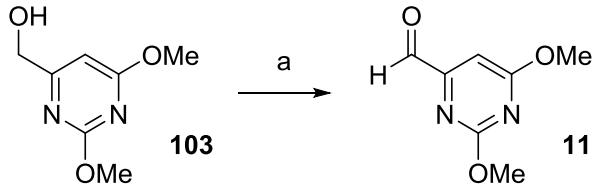
Scheme 30. Preparation of ester **102**: (a) i) orotic acid monohydrate, $POCl_3$, PCl_5 reflux,16 h; ii) MeOH, 24 h, 9-18%.

Despite the low yields obtained in the previous step, we proceeded to the reduction of the ester **102** to the aldehyde **103**. Thus a solution of ester **102** was reduced with $LiBH_4$ dissolved in EtOH/THF to give the alcohol **103** in 84%. The data for **103** was in good agreement with the literature and had diagnostic NMR resonances at δ_H 3.25 (1H, s) ppm for the alcohol group and at δ_H 4.58 (2H, s) and δ_C 63.6 ppm for the methylene protons (Scheme 31).



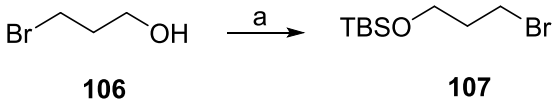
Scheme 31. Preparation of alcohol **103**: (a) $LiBH_4$, EtOH/THF, rt, 2 h, 84%.

Oxidation of **103** to the aldehyde **11** was reported to be possible using Dess-Martin periodinane in CH_2Cl_2.[94] We thus applied this method to alcohol **103** and after work up and silica gel chromatography we obtained the aldehyde **103** as a pale yellow solid in 81-96% yield over 2 attempts. Again the compound was in good agreement with the literature and had diagnostic NMR resonances for the aldehyde functional group at δ_H 9.89 (1H, s, CH) ppm and δ_C 192.1 (CH) ppm (Scheme 32).



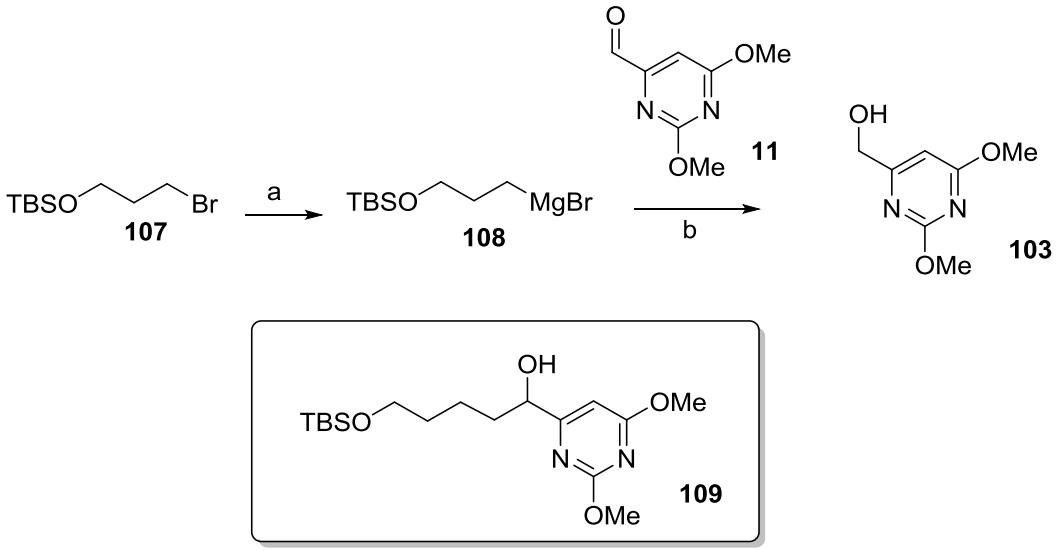
Scheme 32. Oxidation of alcohol **103** to aldehyde **11**: (a) Dess-Martin periodinane, CH_2Cl_2, rt, 2 h, 81-96%.

With the aldehyde prepared we turned to the preparation of the required Grignard precursor **109** which was easily prepared by silylation 3-bromopropan-1-ol **106** with TBSCl in the presence of imidazole and DMAP (Scheme 33),
after work up and purification the silane **107** was obtained as colourless oil in 87% yield. The data for this compound was in agreement with the literature,[94] with five resonances in the proton NMR at δ_H 0.06 (6H, s, 2 × CH_3), 0.89 (9H, s, 3 × CH_3), 2.02 (2H, tt, J = 5.7, 6.5 Hz, CH_2), 3.50 (2H, t, J = 6.5 Hz, CH_2) and 3.72 (2H, t, J = 5.7 Hz, CH_2) ppm. This together with 6 resonances in the carbon spectrum at δ_c 60.3 (CH_2), 35.5 (CH_2), 30.5 (CH_2), 25.9 (3 × CH_3), 18.2 (C) and -5.4 (2 × CH_3) ppm confirmed the structure of **11**.



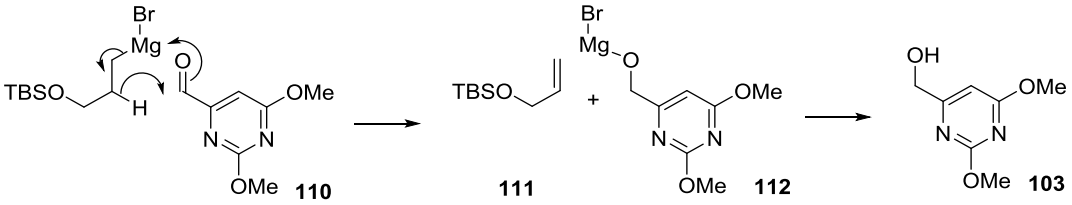
Scheme 33. Preparation of silyl ether (a) **107**: TBSCl, imidazole, DMAP, CH_2Cl_2, rt, 5 d, 90%.

Our initial attempts at forming a Grignard reagent with **108** were unsuccessful as it was slow to react with magnesium and eventually we found that we were able to form the Grignard by vigorous heating in THF for 30 min. Once formed reaction with the aldehyde **11** did not give the desired addition product but instead led to the formation of the alcohol **103** in 46% yield (Scheme 34).



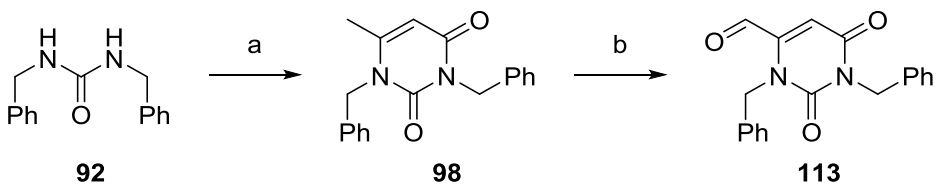
Scheme 34. Attempted preparation of alcohol **109**: (a) Mg, THF, Δ, 30 min; (b) 11, 16 h, 48%.

The rationale for the formation of **11** is that Grignard reagent are known under certain circumstances to prefer reduction as to addition and the mechanism of this is related to the β-elimination of a hydride from the Grignard leading to the formation of a reduced product and an alkene as shown in (Scheme 35).



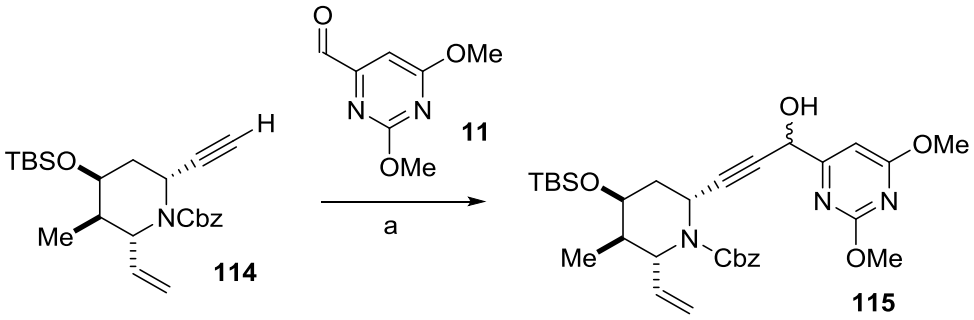
Scheme 35. Proposed mechanism for the reduction of aldehyde **103** by the Grignard reagent **108**

Our initial conclusions from this work were not promising as there were two major problems with the methodology. Firstly, we were unable to prepare the aldehyde **11** as reported by Snider in very high overall yields and secondly the addition of the Grignard reagent to the aldehyde **11** was unsuccessful. We thus required a change of strategy and on inspection of the literature[95] we found a report on the preparation of the protected aldehyde **113** in two steps for the previously prepared benzyl urea **92** in high yield (Scheme 36).



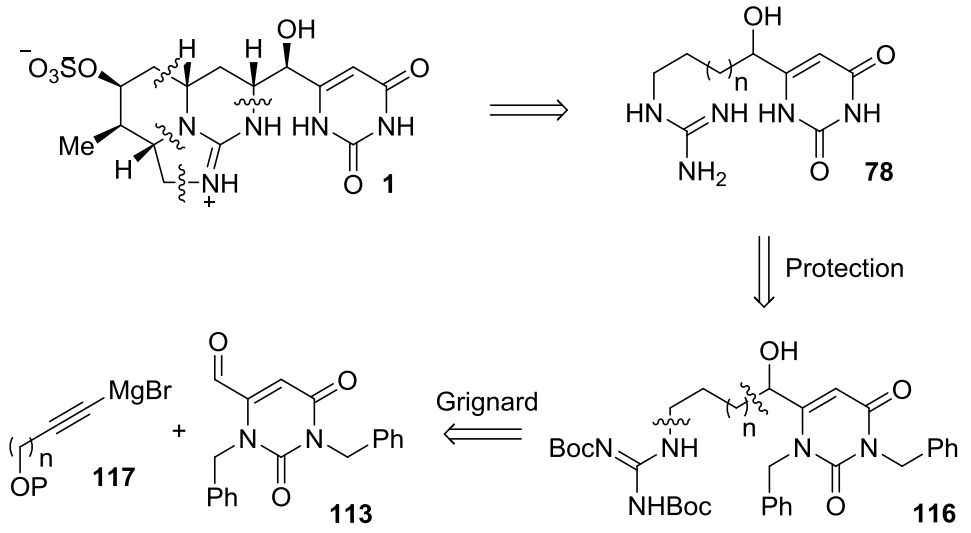
Scheme 36. Preparation of aldehyde **113**: (a) Ac_2O, DMAP, pyridine, 115 ˚C, 5 h; (b) SeO_2, THF, CH_3CO_2H, Δ, 24 h

Snider had also reported the addition of the alkyne species **114** to the aldehyde **11** in his synthetic studies towards cylindrospermopsin **1**. This reaction uses a Grignard exchange protocol and the acetylenic Grignard intermediate formed adds to the aldehyde in 83% yield [64] (scheme 37).



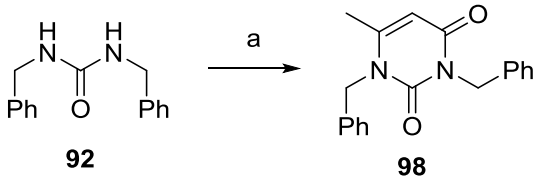
Scheme 37. Key step from Snider's synthesis[94] of **115**: (a) EtMgBr, THF, 0 ˚C

By combining these two reaction sequences we hoped to apply the modified synthetic approach shown in (Scheme 38) in which the uracil ring is protected by two *N*-benzyl groups and the alkane side chain is introduced as an alkyne and is later reduced (Scheme 38).



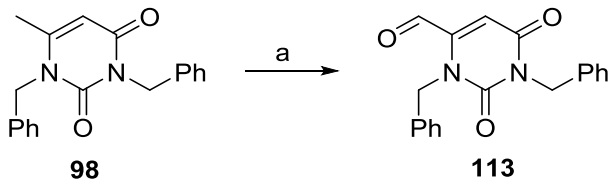
Scheme 38. Modified retrosynthesis of the cylindrospermopsin model compounds **78**

The previously prepared 1,3-dibenzylurea was next treated with an excess of acetic anhydride in the presence of DMAP in pyridine at reflux for 5h. After work up and purification by chromatography the pyrimidine **77** was obtained in a 76-78% yield over three attempts as an oil. Data was in good agreement with the literature[95] with diagnostic resonances being observed at δ_H 2.14 (3H, s, CH_3) and 5.65 (1H, s, CH) ppm together with δ_C 19.8 (CH_3) and 102.0 (CH) ppm for the methyl and alkene resonances respectively (Scheme 39).



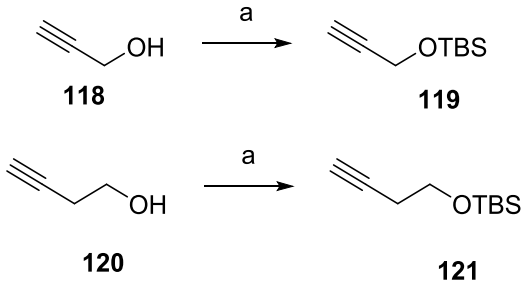
Scheme 39. Synthesis of pyrimidine **98**: (a) Ac_2O, DMAP, pyridine, Δ 115 °C 5 h, 76-78%.

The pyrimidine **98**[95] was then treated with an excess of selenium dioxide in acetic acid at reflux for 16 h and after work up the aldehyde **113**[96] was obtained as an oil in 74-77% yield over four attempts. Diagnostic resonances for the aldehyde group were observed at δ_H 9.41 (1H, s, CH) and δ_C 185.6 (CH) ppm which were in agreement with that reported in the literature[96] (Scheme 40).



Scheme 40. Preparation of aldehyde **113** by selenium dioxide oxidation: (a) SeO_2, THF, AcOH, Δ, 115 ˚C, 24 h, 74%.

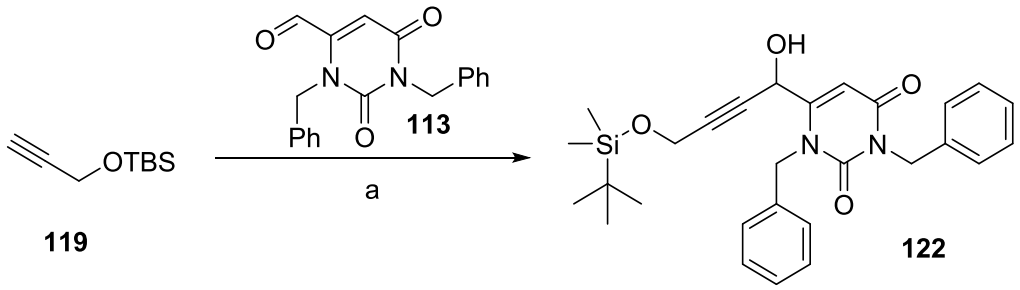
The required silylated propargyl alcohol **119** was prepared[97] in 91% yield by the addition of TBSCl to a mixture of propargyl alcohol and imidazole in dry dichloromethane. The corresponding homopropargyl silane **121** was similarly produced in 90.5% yield using the same method[98] (Scheme 41).



Scheme 41. Preparation of silanes **119**, 91% and **121**, 81%. (a) TBSCl, CH_2Cl_2, imidazole, DMAP, rt, 24 h. **119**; 91%, **121**; 81%.

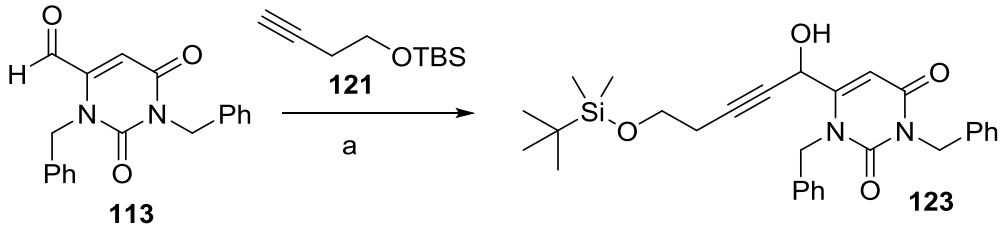
With the precursors **119** and **121** in hand, the coupling of these two was attempted. Thus a THF solution of EtMgBr was added to a cooled (-40˚C) solution of the propargylsilane **119** dissolved in dry THF and the mixture stirred to rt over 1 h. This solution was then transferred by syringe to a cooled (-78 ˚C) solution of the pyrimidine aldehyde **113** in dry THF. After 5 h, aqueous work up and chromatography gave the desired product **122** in 75-77% yield over five attempts. Confirmation of the structure of **122** was obtained on analysis of the proton NMR which gave diagnostic resonances at δ_H 3.43 (1H, d, J = 6.8 Hz, OH) ppm for the hydroxyl proton and at 5.11-5.18 (3H, m, CH, CH_2) for the methyne and methylene protons adjacent to the alcohol and silyl ether functional groups, whilst the uracil proton was found at δ_H 6.25 (1H, s,

CH) ppm. Resonances in the carbon at δ_C at 60.3 (CH) and 51.5 (CH_2) ppm confirmed the presence of these groups, whilst high resolution mass spectrometry gave a mass of 490.2350 Daltons which is in good agreement with the expected value for $C_{28}H_{35}N_2O_4Si$ ($[M+H]^+$) which requires 490.2361 Daltons (Scheme 42).



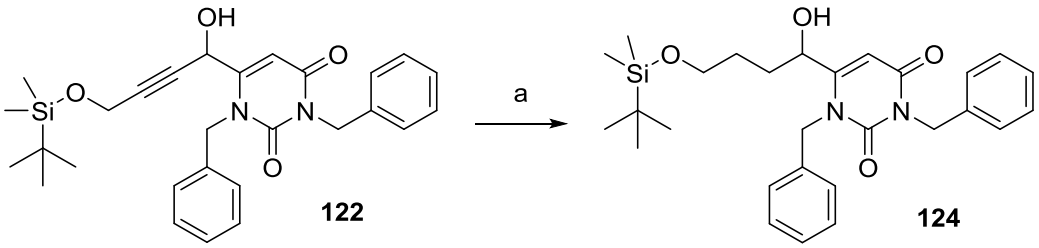
Scheme 42. Preparation of alcohol **122**: (a) i) EtMgBr in THF (1M), –40 °C; ii) **113**, –78 °C – rt, 3 h, 78%.

In a similar manner, the homopropargyl ether **121** was reacted with the aldehyde **113** to give **123** in 51-66% yield over three attempts. Confirmation of the structure of **123** was obtained on analysis of the proton NMR which gave diagnostic resonances at δ_H 2.36 (2H, dt, J = 1.8, 6.9 Hz, CH_2) and 3.64 (2H, t, J = 6.9 Hz, CH_2) ppm for the two coupled methylene protons and at δ_H 3.43 (1H, d, J = 6.8 Hz, OH) ppm for the alcohol proton. The methyne proton adjacent to the alcohol was observed at δ_H 5.07 (1H, m, CH) ppm whilst the uracil proton was found at δ_H 6.22 (1H, s, CH) ppm. Resonances in the carbon spectrum at δ_C 60.3 (CH) and 61.1 (CH_2) ppm also confirmed the presence of these functional groups. High resolution MS gave a mass of 505.2507 Daltons, which is in good agreement with the expected value for $C_{29}H_{37}N_2O_4Si$ ($[M+H]^+$) which requires 505.2517 Daltons (Scheme 43).



Scheme 43. Preparation of alcohol **123**: i) **121**, EtMgBr in THF (1M), –40 °C; ii) **113**, –78 °C – rt, 6h, 51-66%.

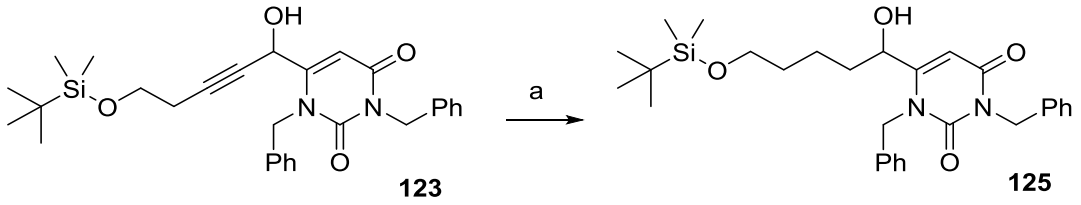
The alkyne **122** was then hydrogenated by reaction with hydrogen gas in the presence of palladium on activated carbon which gave the alcohol **124** in 73% yield after chromatography. Analysis by proton NMR confirms the disappearance of the alkyne resonances and the presence of saturated methylene resonances at δ_H 1.48-1.88 (4H, m, 2 × CH_2) and 3.55-3.67 (2H, m, CH_2) ppm. The benzylic methylene protons were still present at δ_H 5.01 (1H, d, *J* = 16.4 Hz, CH), 5.10 (1H, d, *J* = 13.8 Hz, CH), 5.15 (1H, d, *J* = 13.8 Hz, CH), and 5.33 (1H, d, *J* = 16.4 Hz, CH) whilst the uracil proton was observed at δ_H 6.04 (1H, s, CH) ppm. The carbon spectrum contained the required 21 resonances and the high resolution MS gave a mass of 494.2666 Daltons which is in good agreement with the expected value for $C_{28}H_{39}N_2O_4Si$ ($[M+H]^+$) which requires 494.2674 Daltons (Scheme 44).



Scheme 44. Hydrogenation of alkyne **122**: (a) Pd/C (10%), EtOAc, H_2, 2 h, 73%.

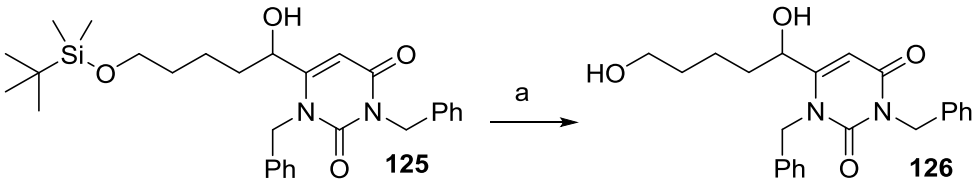
Similarly the alkyne **123** was hydrogenated by reaction with hydrogen gas in the presence of palladium on activated carbon to give the alcohol **125** in 64% yield after chromatography. Again analysis by proton NMR confirms the disappearance of the alkyne resonances and the presence of saturated methylene resonances at δ_H 1.34-1.53 (4H, m, 2 × CH_2), 1.54-1.66 (2H, m, CH_2) and 3.55-3.67 (2H, m, CH_2) ppm. The benzylic methylene protons were still present at δ_H 4.98 (1H, d, *J* = 16.3 Hz, CH), 5.10 (1H, d, *J* = 13.7 Hz, CH), 5.15 (1H, d, *J* = 13.7 Hz, CH) and 5.31 (1H, d, *J* = 16.3 Hz, CH), whilst the uracil proton was observed at δ_H 5.99 (1H, s, CH) ppm. The carbon

spectrum contained the required 22 resonances and the high resolution MS gave a mass of 509.2819 Daltons, which is in good agreement with the expected value for $C_{29}H_{45}N_2O_4Si$ ($[M+H]^+$) which requires 509.2830 Daltons (Scheme 45).



Scheme 45. Hydrogenation of alkyne **123**: (a) Pd/C (10%), EtOAc, H_2, 2 h, 64-72%.

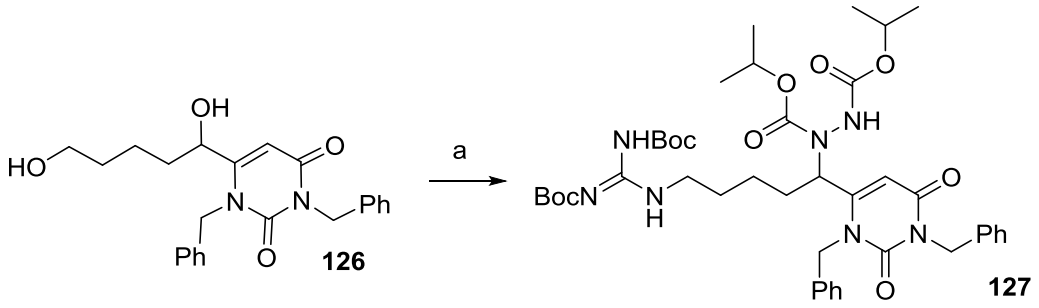
With the two alcohols **124** and **125** in hand, the desilylation of **125** was attempted and thus treatment with TBAF in THF led to the removal of the silyl protecting group and the formation of the diol **126** in 77% yield. Proton NMR confirmed the loss of the silyl protecting group resonances and the appearance of a broad signal at δ_H 3.00 (2H, br s, 2 × OH) ppm corresponding to the two hydroxyl protons. The desired 19 resonances were observed in the carbon spectrum and a broad band in the IR spectrum at 3386 cm^{-1} confirmed the presence of the 2 alcohol groups. High resolution MS also gave a mass of 396.1565 Daltons which is in exact agreement with the expected value for $C_{23}H_{27}N_2O_4$ ($[M+H]^+$) (Scheme 46).



Scheme 46. TBAF Deprotection of **125**: (a) TBAF (1M), THF, rt 2 h, 77%.

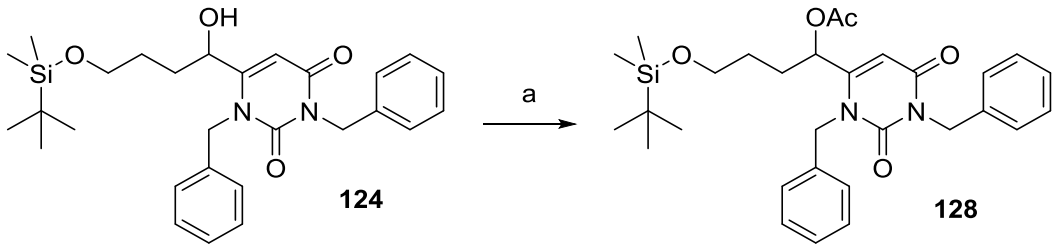
Guanylation of **126** was then attempted under Mitsunubo conditions and on treatment with *N*,*N*'-di-Boc-guanidine, triphenylphosphine and DIAD, a product was isolated which appeared to incorporate the guanidine group. However on analysis by NMR spectrometry a complex spectrum was obtained which suggested that a

compound thought to be **127** had been formed in 81% yield. This might have arisen from the incorporation of one guanidine residue and one equivalent of the by-product from the DIAD reaction and this eventuality was confirmed by the high resolution MS data which gave a mass at 822.4390 Daltons which corresponds well to the calculated mass 822.4396 Daltons for $C_{42}H_{59}N_7O_{10}$ ($[M+H]^+$) (Scheme 47).



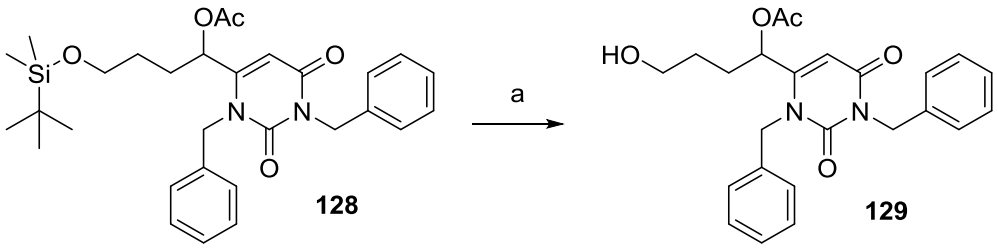
Scheme 47. Preparation of **127**: (a) DIAD, PPh_3, *N*,*N*'-di-Boc-guanidine, THF, rt 16 h, 81%.

With the problems encountered in selective guanylation, it was envisaged that a different sequence of protection/deprotection reactions was required and we thus acetylated the secondary alcohol function in **124** using acetic anhydride and pyridine in dichloromethane to give the acetate **128** in 75-98% yield over four reactions. Analysis of the proton NMR spectrum of the diagnostic resonances at δ_H 2.02 (3H, s, CH_3) ppm for the acetate methyl and at δ_H 5.49 (1H, dd, J = 9.5, 2.3 Hz, CH) ppm for the downfield shifted methyne proton adjacent to the acetate. The required 23 resonances were observed in the carbon spectrum and high resolution MS gave a mass of 537.2769 Daltons which corresponds well to the required mass of 537.2779 for $C_{30}H_{41}N_2O_5Si$ ($[M+H]^+$) (Scheme 48).



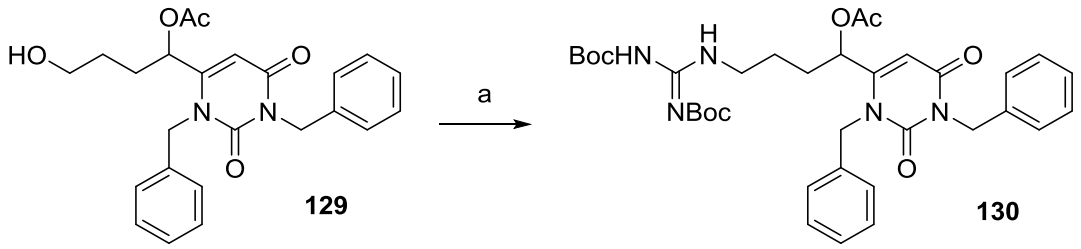
Scheme 48. Acetyl protection of alcohol **124**: (a) Ac_2O, pyridine, DMAP, CH_2Cl_2, rt, 1 h, 75-98%.

Subsequent desilylation of **128** was easily achieved using a solution of TBAF in THF to give **129** as colorless oil in 73-89% yield over 3 reactions. Analysis of the proton NMR spectrum confirmed the disappearance of the resonances for the silyl protecting group and the appearance of a signal at δ_H 3.10 (1H, br s, OH) ppm corresponding to the alcohol group. The carbon spectrum had the desired 20 resonances and the high resolution MS gave a mass of 423.1917 Daltons which corresponds well to the required mass of 423.1914 Daltons for $C_{24}H_{27}N_2O_5$ ($[M+H]^+$) (Scheme 49).



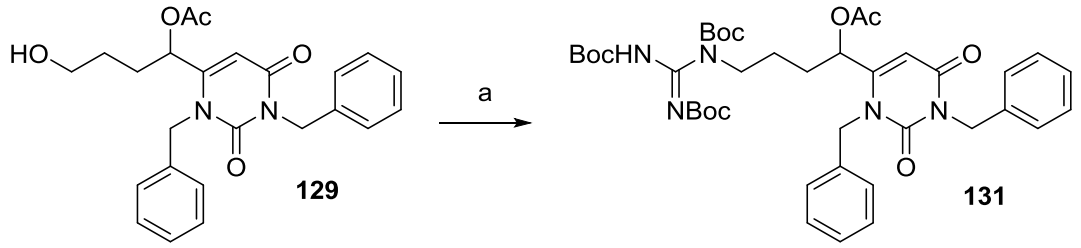
Scheme 49. TBAF Deprotection of **128**: (a) TBAF (1M), THF, rt, 2 h, 73-80%.

With alcohol **129** in hand, its guanylation was investigated. Thus alcohol **129** was coupled under Mitsunobu conditions with 2 equivalents of the *N*,*N'*-di-Boc-guanidine. After purification by column chromatography the guanidine **130** was obtained in a disappointing 19-26% yield over three attempts. Analysis of the proton NMR spectrum indicated the presence of resonances for the 2 *t*Bu groups of the Boc protecting groups at δ_H 1.37 (9H, m, 3 × Me) and 1.51 (9H, m, 3 × Me) ppm and two resonances at δ_H 8.56 (1H, br s, NH) and 8.90 (1H, br s, NH) ppm for the NH groups. The required 27 resonances were observed in the carbon spectrum and high resolution MS gave a mass of 663.3143 Daltons, which is in close agreement with the calculated mass of 663.3160 Daltons for $C_{35}H_{45}N_5O_8Na$ ($[M+Na]^+$) (Scheme 50).



Scheme 50. Guanylation of **129** using *N*,*N'*-di-Boc-guanidine: (a) DIAD, PPh_3, *N*,*N'*-di-Boc-guanidine, THF, rt 16 h, 19-26%.

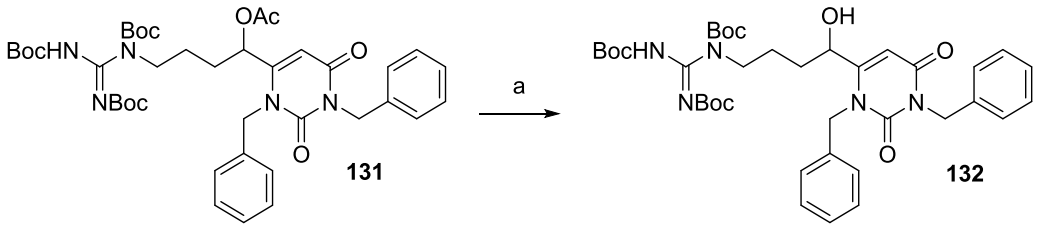
The low yield in this reaction prompted us to attempt a coupling with the *N*,*N'*,*N''*-tri-Boc-guanidine as an alternate guanylating agent. Thus reaction of **129** with 2 equivalents of the *N*,*N'*,*N''*-tri-Boc-guanidine, triphenylphosphine and DIAD on THF gave **131** in 34-39 % yield over three attempts. Again, analysis of the proton NMR spectrum indicated the presence of resonances for the 3 *t*Bu groups of the Boc protecting groups at δ_H 1.37 (9H, m, 3 × Me), 1.44 (9H, m, 3 × Me) and 1.51 (9H, m, 3 × Me) ppm and a signal at δ_H 10.40 (1H, s, NH) ppm for the NH proton. The required 29 resonances were observed in the carbon spectrum and high resolution MS gave a mass of 764.3864 Daltons, which is in close agreement with the calculated mass of 764.3865 Daltons for $C_{40}H_{54}N_5O_{10}$ ($[M+H]^+$) (Scheme 51).



Scheme 51. Guanylation of **129** using *N*,*N'*,*N''*-tri-Boc-guanidine: (a) DIAD, PPh_3, *N*,*N'*,*N''*-tri-Boc-guanidine, THF, rt 16 h, 34-39%.

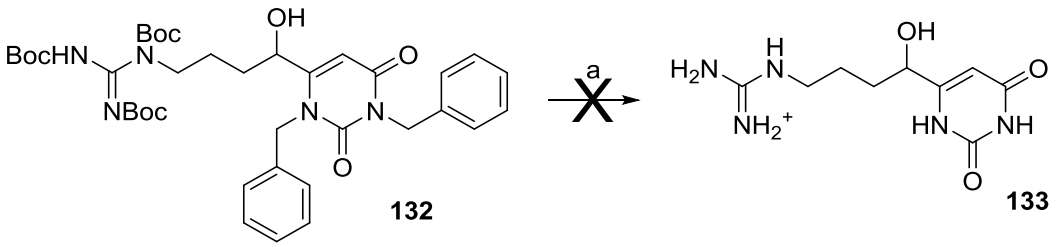
The formation of **130** and **131** represented a major achievement in the synthesis in that all the features of the required model compound are in place and all that is required is to deprotect the three required functional groups. The removal of the

acetate functional group was easily achieved by treatment of **131** with potassium carbonate in methanol rt for 1 h and after purification the alcohol **132** was obtained as a white solid in 76% yield. Analysis of the proton NMR spectrum indicated the loss of the acetate methyl and the appearance a signal at δ_H 1.94 (1H, br s, OH) ppm for the alcohol proton as well as the CH proton at δ_H 5.34 (1H, t, J = 7.4 Hz, CH) ppm. High resolution MS gave a mass of 722.3757 Daltons which is in close agreement with the required mass of 722.3760 Daltons for $C_{38}H_{52}N_5O_9$ ($[M+H]^+$) (Scheme 52).



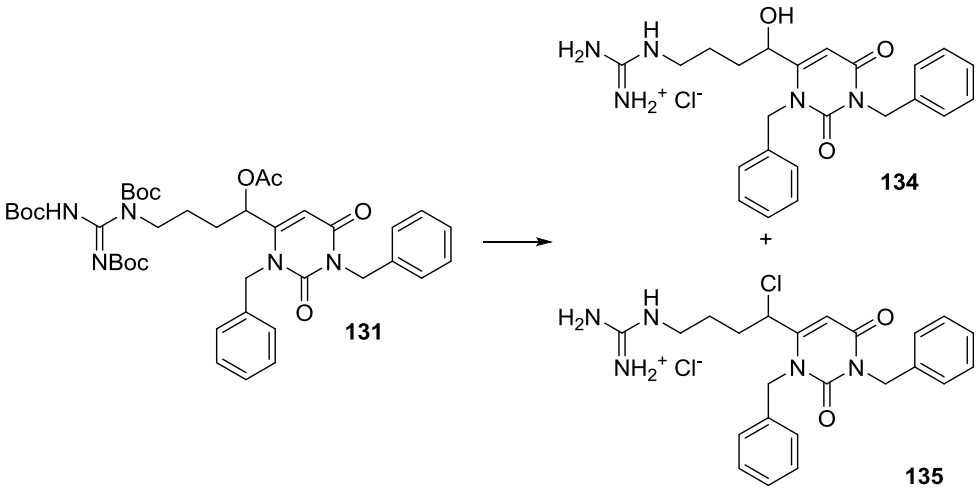
Scheme 52. Deprotection of acetate **131**: (a) K_2CO_3, MeOH, rt, 1 h, 76%.

With **132** available, we attempted to deprotect the benzyl groups and initially attempted a transfer hydrogenation reduction over Pd/C in the presence of ammonium formate as the hydrogen source. This method was reported as being effective at deprotecting similar uracils.[99] Applying this method to compound **132** and after refluxing for 16 hours, work up gave a product which on NMR appeared to be a complex mixture of compounds. The NMR spectrum lacked the required uracil CH proton signal as well as the proton adjacent to the hydroxyl group, the resonances for the Boc protecting groups had also disappeared. This suggested that under these conditions the uracil ring was possibly being destroyed and that the hydroxyl group might be undergoing hydrogenolysis. In a related hydrogenation Weinreb et al. reported[67] a similar problem in a hydrogenation of an *N,N'*-dibenzyl protected uracil. (Scheme 53).



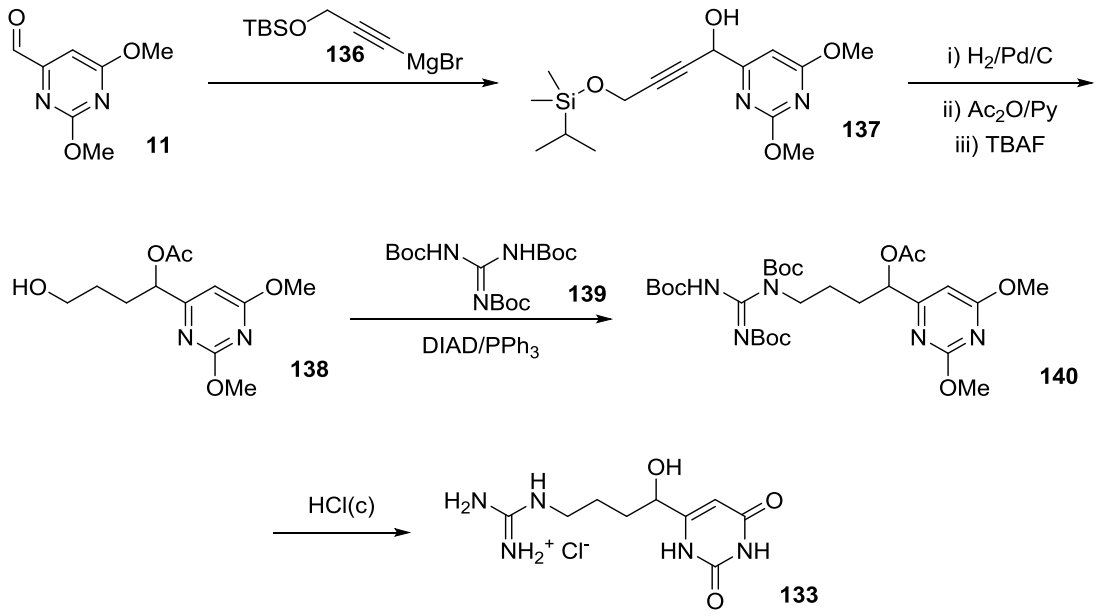
Scheme 53. Attempted deprotection of acetate **132**: (a) Pd/C, MeOH, HCO_2NH_4, Δ, 16 h.

An attempt was also made to remove all three protecting groups in one step under acidic conditions similar to those reported in several syntheses of cylindrospermopsin **1**. Whilst benzyl groups are generally resistant to acidic condition it was hoped that the strong acidic conditions might lead to a nucleophilic substitution of the benzylic groups and that the uracil group might be a good leaving group in this process. Reaction of **133** with concentrated hydrochloric acid for 16 h gave a product which on analysis by MS gave two species at 422.2 (70%, $[M+H^+]$) and 440.2 (100%, $[M+H^+]$) Daltons which did not correspond to the desired product **133**. Analysis by high resolutuon MS suggested the formation of the partially deprotected product **134** as a mass 422.2162 corresponds closely to the desired mass of 422.2187 Daltons for $C_{23}H_{28}N_5O_3$ $[M+H^+]$ Daltons. Similarly the mass at 440.1819 Daltons corresponds closely to the product **135** in which the acetate group of **131** has been replaced by a chlorine. This compound requires a mass of 440.1848 Daltons for $C_{23}H_{28}{}^{35}ClN_5O_3$ ($[M+H^+]$). It is thus apparent that the benzyl groups are resistant to the action of strong acids and that the benzylic acetyl group might be prone to substitution (Scheme 54).



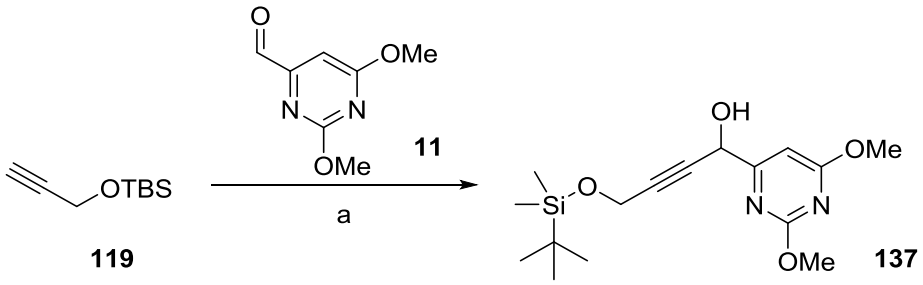
Scheme 54. Attempted deprotection of **131** under acidic conditions: (a) HCl, Δ, 16 h.

The conclusion from this work was that whilst this methodology was unsuccesful at preparing the desired analogue, it was successful in generating the methodology for joining the three required functional groups together, albeit protected. We thus wished to revert to a global deprotection strategy and proposed the synthetic route show in Scheme 55.



Scheme 55. Global deprotection approach to the cylindrospermopsin analogue **133**

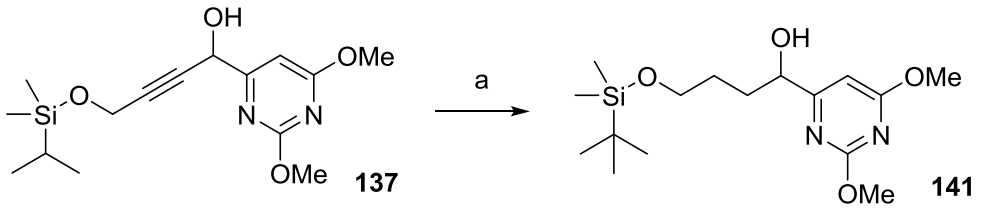
We initially investigated the coupling of the previously prepared aldehyde **11** with the silane **119**. A THF solution of EtMgBr was added to a cooled (-40˚C) solution of **119** in dry THF and the mixture stirred to rt over 1 h. This solution was then transferred by syringe to a cooled (-78 ˚C) solution of the aldehyde **11** in THF and stirred overnight. After work up and chromatography the desired product **137** was obtained in 71-75% yield over 2 attempts. Analysis of the IR spectrum of **137** gave a broad band at 3392 $cm^{-1}$for the OH group, whilst in the proton NMR resonances at δ_H 0.09 (6H, s, 2 × CH_3) and 0.87 (9H, s, 3 × CH_3) ppm confirmed the presence of the silyl protecting group. The OH signal was present at δ_H 1.95 (1H, br s, OH) whilst the two uracil methoxy groups were present at δ_H 3.97 (3H, s, CH_3) and 3.99 (3H, s, CH_3) ppm. The methylene and methyne protons were observed at δ_H 4.35 (2H, d, *J* = 1.1 Hz, CH_2) and 5.31 (1H, t, *J* = 1.1 Hz, CH) ppm respectively and shared a 1.1 Hz propargylic coupling constant, whilst the uracil proton was observed at δ_H 6.54 (1H, s, CH). The required 13 resonances were observed in the carbon spectrum whilst high resolution MS gave a mass of 338.1735 which corresponds exactly to the mass required for $C_{16}H_{27}N_2O_4Si$ ($[M+H]^+$) (Scheme 56).



Scheme 56. Preparation of alcohol **137**: (a) i) **121**, EtMgBr in THF (1M), -78 ˚C, 2 h. ii) **11**, rt 16 h, 71-75 %.

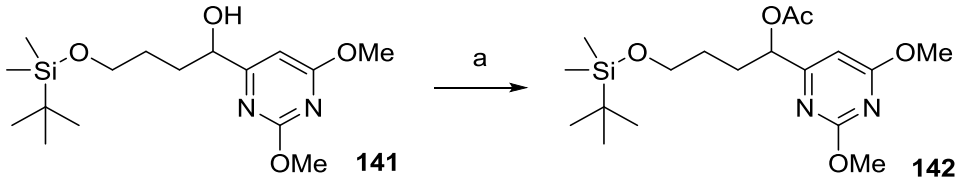
The alkyne **137** was then hydrogenated by reaction with hydrogen gas in the presence of palladium on activated carbon which gave the alcohol **141** in 79 % yield after chromatography. Analysis of the proton spectrum indicated the appearance of 2 coupled methylene groups at δ_H 1.60-1.78 (3H, m, CH, CH_2) and 1.93-2.03 (1H, m, CH) and the presence of methylene proton adjacent to oxygen at δ_H 3.65 (2H, t, *J* = 6.5 Hz, CH_2) ppm and the alcohol at δ_H 4.13 (1H, br s, OH) ppm. The carbon spectrum

contained the required 13 resonances and high resolution MS gave a mass of 343.2049 which is in very close agreement with the calculated mass of 343.2048 for $C_{16}H_{31}N_2O_4Si$ ($[M+H]^+$) (Scheme 57).



Scheme 57. Hydrogenation of alkyne **137**: Pd/C (10%), EtOAc, H_2, 2 h, 79%.

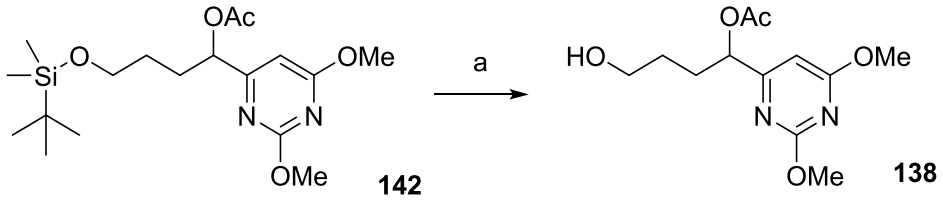
We next acetylated the secondary alcohol function in **141** using acetic anhydride and pyridine in dichloromethane to give the acetate **142** in 75% yield. Analysis of the IR spectrum indicated the absence of the OH stretch and the appearance of a carbonyl stretch at 1744 cm^{-1}, whilst the proton NMR indicated an acetate signal at δ_H 2.13 (3H, s, CH_3) ppm and the methyne proton downfield shifted to δ_H 5.61 (1H, dd, J = 4.8, 7.8 Hz, CH) ppm. The carbon NMR spectrum gave the required 11 resonances, whilst high resolution MS gave a mass at 385.2146 Daltons which is in close agreement with the calculated mass of 385.2153 for $C_{18}H_{33}N_2O_5Si$ ($[M+H]^+$) (Scheme 58).



Scheme 58. Acetyl protection of **141**: Ac_2O, pyridine, DMAP, CH_2Cl_2, rt, 1 h, 68 %.

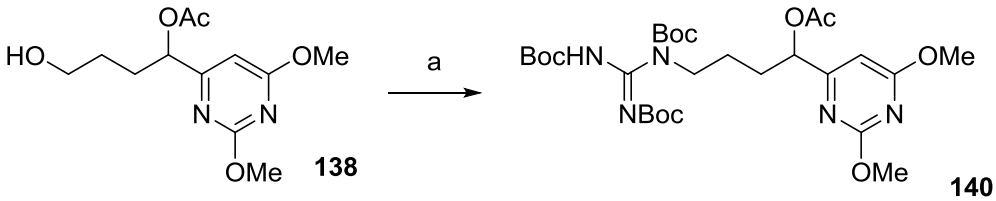
Desilylation of **142** was achieved using a solution of TBAF in THF to give **138** as colourless oil in 80% yield. Analysis of the IR spectrum of **138** indicated the presence of a hydroxyl group as evidenced by the band at 3440 cm^{-1}, whilst the carbonyl stretch of the acetate was at 1738 cm^{-1}. Analysis of the NMR spectrum

indicated the loss of the resonances associated with the silyl protecting group and the presence of the OH group at δ_H 3.59 (1H, br s, OH) ppm. The carbon NMR spectrum gave the required 12 resonances whilst high resolution MS gave a mass of 271.1287 Daltons which corresponds closely to the calculated value of 271.1288 required for $C_{12}H_{19}N_2O_5$ ($[M+H]^+$) (Scheme 59).



Scheme 59. TBAF Deprotection of **142**: TBAF (1M), THF, rt, 2 h, 80%.

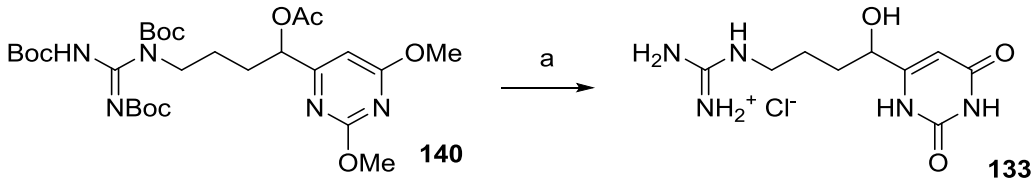
The coupling of alcohol **138** with *N,N',N''*-tri-Boc-guanidine was attempted using triphenylphosphine and DIAD in THF. After work up and chromatography the guanidine **140** was obtained in 49% yield. Analysis of the product by proton NMR indicated the presence of the three *t*Bu groups for the three Boc protecting groups at δ_H 1.32 (9H, s, 3 × CH_3), 1.49 (9H, s, 3 × CH_3) and 1.50 (9H, s, 3 × CH_3) ppm whilst the NH proton of the guanidine was observed at δ_H 10.71 (1H, s, NH) ppm. Analysis of the high resolution MS gave a mass at 612.3225 Daltons which is in close agreement with the desired mass of 612.3239 Daltons for $C_{28}H_{46}N_5O_{10}$ ($[M+H]^+$) (Scheme 60).



Scheme 60. Guanylation of **138**: (a) DIAD, PPh_3, *N,N',N''*-tri-Boc-guanidine, THF, rt 16 h, 49%.

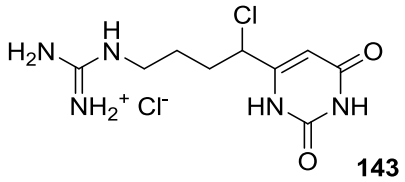
With the protected analogue prepared the global deprotection was attempted by two different methods. The first method involved heating a sample of **140** in

concentrated HCl at 100 ˚C for 6 h whilst the second method involved heating a sample under the same conditions but only at 80 ˚C but for 96 h (Scheme 61).



Scheme 61. Acetate deprotection of **140**: (a) cHCl, 100 ˚C 6 h or 80 ˚C, 96 h**.**

Visually, the longer time scale reaction seemed to give a darker product on work up but the NMR spectra of the two appeared similar; however the proton spectra were generally broad and not easy to interpret. Despite this it was possible to identify key resonances in the crude product at δ_H 1.52-2.20 (4H, m, 2 × CH_2) and 3.55-3.69 (2H, m, CH_2) for the three methylene resonances, together with broad resonances at δ_H 4.27-4.43 (1H, m, CH) and 5.56 (1H, s, CH) ppm for the methyne protons. However analysis of the carbon spectrum suggested that the product was a mixture of 2 compounds as six methylene resonances were present as pairs at δ_C 29.4/30.0, 31.8/32.5 and 44.8/61.8 ppm. Similarly two pairs of resonances were present at δ_C 53.8/53.9 and 97.9/98.1 ppm for the at two methyne protons. Analysis of the reaction product by high resolution MS indicated that the compound **133** was present and a mass of 242.1248 was measured which is an exact match to the required formula of $C_9H_{16}N_5O_3$ ($[M+H]^+$). However, closer inspection of the MS data suggested the presence of another compound which has the mass of 260.0911 Daltons which corresponds closely to the formula $C_9H_{15}{}^{35}ClN_5O_2$ ($[M+H]^+$) which has a mass of 260.0909 Daltons. This was confirmed by a peak at 262.0880 Daltons corresponding exactly to the isotopic ion $C_9H_{15}{}^{37}ClN_5O_2$ and was found in a relative ratio of 3:1 as is expected for chlorine. This would suggest the formation of the byproduct **143** in this reaction (Figure 14).



143

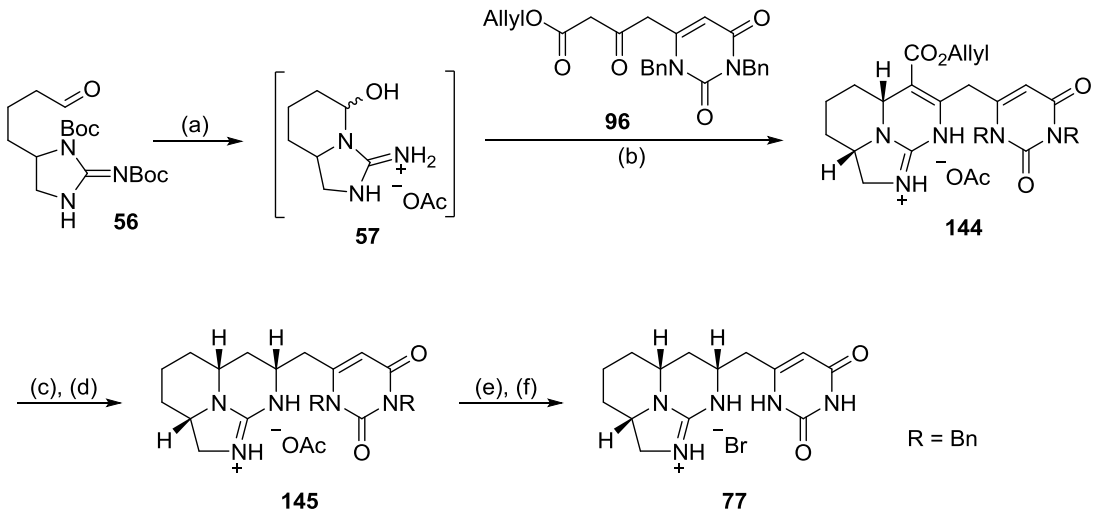
Figure 14. By-product **143**

An attempt was made to analyse the product of each reaction by LCMS and although some separation appeared to be possible, we were unable to access suitable preparative LC equipment to attempt this method of purification. Time was not available to repeat this work on a larger scale and this will remain the goal of future studies.

Conclusions and further work

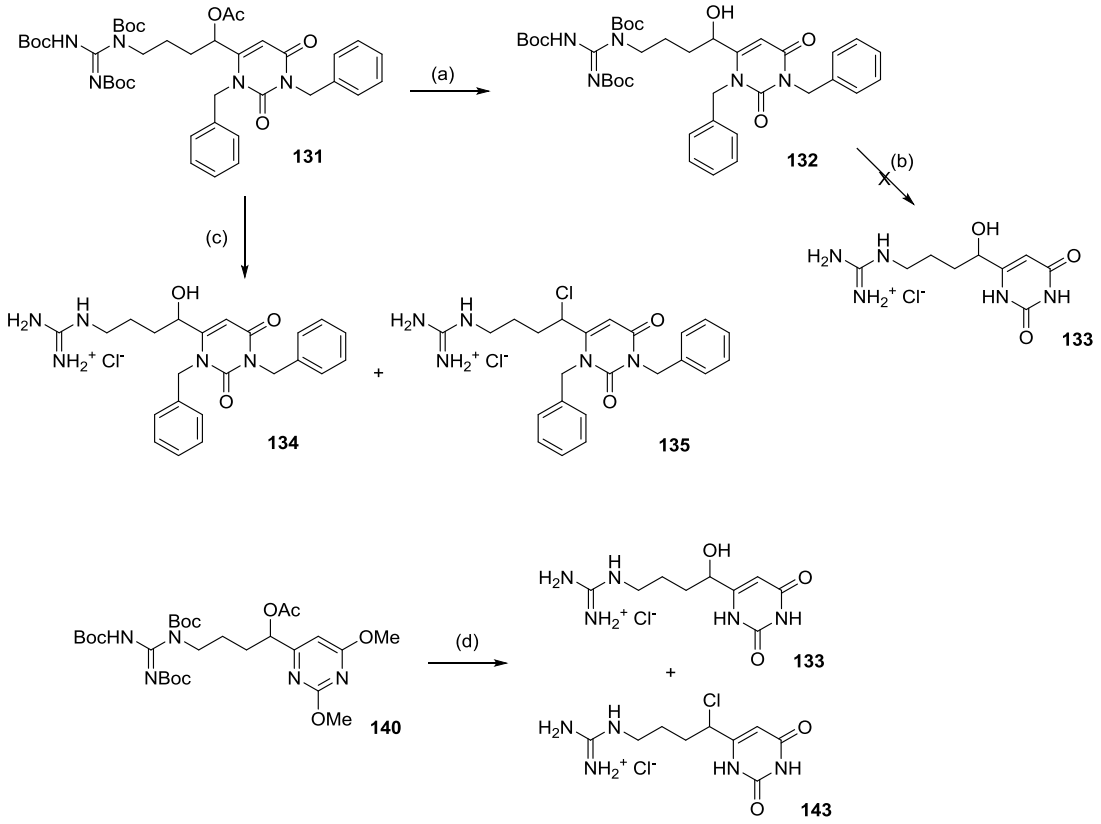
The work directed towards the synthesis of the RHS fragment of *deoxy*-cylindrospermopsin was successfully in that a benzyl**96** and methoxy-protected **69** synthon were prepared. The benzyl protected RHS fragment **96** differs significantly from the previously utilized methoxy- and benzyloxy- ether protected uracil groups used in other total syntheses and will require a different deprotection strategy which will need to be addressed in the total synthesis. The low yields in the Meldrum's acid coupling reaction in the methoxy-ether series is not easy to explain and possibly given more time this might be worth reinvestigating.

The β-Keto ester **96** was utilised by a co-worker in the tethered Biginelli reaction with aminal **57** and succesfully gave the desired tricyclic intermediate **144;** however the yield for this step was only a modest 19%. Despite this the deallylation/decarboxylation sept was undertaken using $Pd(PPh_3)_4$ followed by exposure of the resulting material to $NaBH_3CN$ in a 1:1 mixture of AcOH/MeOH which gave the guanidine **145** in 75% yield over 2 steps. As with our studies, attempts to remove the benzyl protecting groups of **145** by catalytic transfer hydrogenation was unsuccessful returning only unreacted starting material. Fortunately debenzylation could be accomplished by treatment with BBr_3 in refluxing xylenes for 16 h followed by quenching with methanol. This led to the successful preparation of a model of the biosynthetic intermediate 7-deoxy cylindrospermopsin **77** in 94% yield[100] (Scheme 62).



Scheme 62. Synthesis of the deoxy-cylindrospermopsin analogue **77**: (a) AcOH, 24h; (b) morpholinium acetate, **96**, Na_2SO_4, CF_3CH_2OH, 100 ˚C, 12 days, 19%; (c) $Pd(PPh_3)_4$, pyrrolidine**,** THF/MeOH, 16 h; (d) $NaBH_3CN$, AcOH/MeOH, 0 ˚C-rt, 16 h, 75%; (e) BBr_3, xylenes, 135 ˚C, 24h; (f) MeOH, rt, 24 h, 94%.

The attempts at preparing analogues of cylindrospermopsin proved highly informative with regard to the reactivity of this type of compound. Using one route, the desired compound (**133**) was almost cerinly prepared, albeit as a mixture with the corresponding chloride **143**. These key reactivity issues focussed on the deprotection chemistries and are summarised below The advanced fully protected intermediates **131** and **140** were successfully prepared via an acetylenic Grignard methodology, however attempts to deprotect them were not straightforward (Scheme 63). Partial deprotection of **131** was successful yielding **132**. However, attempted hydrogenation of the benzyl protecting groups was not effective and led to complete decomposition of the substrate. Similarly attempted acidic deprotection of **131** led to the loss of the Boc- and acetate protecting groups; however the benzyl groups were not removed and a byproduct **135** was also isolated in which the acetate group has been replaced by a chlorine. The fully protected analogue **140** was able to undergo deprotection under acidic condition to give **134** however, this also contained the chlorinated analogue **143** which may have arisen from the same process (Scheme 63).



Scheme 63. Attempted deprotection reactions of **131** and **140**: (a) K_2CO_3, MeOH, rt, 1 h; (b) Pd/C, MeOH, HCO_2NH_4, Δ, 16 h; (c) HCl, Δ, 16 h; (d) cHCl, 100 ˚C 6 h or 80 ˚C, 96 h.

The future of this work has two possibilities, firstly the reinvestigation of the deprotection of **131** or **132** and the work done on the synthesis of the analogue **77**[100] might offer some hope. The BBr_3 mediated deprotection of compound **145** may be applicable to compound **132**, despite the presence of the sensitive alcohol function, and this reaction will be attempted in the future. In addition, more work needs to be performed on the deprotection of compound **140**, in that the substitution of chloride for the acetate group could be suppressed by partial deprotection of **140**. Removal of the acetate group to give an alcohol followed by an acidic deprotection might now be more successful. The use of a non-nucleophilic acid such as wet trifluoroacetic could be used in the deprotection of **140** as this might suppress the substitution of the alcohol.

Section B:

Dinitrobenzamides as Pro-drugs

Dinitrobenzamides as Pro-drugs

Introduction

Cancer chemotherapy

In recent years, many different strategies have been exploited concerning the development of new chemotherapeutic drugs. The main problem with many of these strategies is a lack of tumour specificity[101] resulting in systemic toxicity and a low therapeutic index.[102] These drugs can interfere with cells by attacking some aspects of their deoxyribonucleic acid (DNA) synthesis, replication or processing.[103] Since they preferentially kill rapidly dividing cells, some normal cells are also exposed to toxic levels, resulting in severe undesirable side effects such as damage to hair follicles, the kidney, liver, bone marrow[104] and gut epithelia.[103] Despite this, chemotherapy still remains the primary systemic treatment for cancer. Consequently, there is a need to develop new and improved drugs which are more selective in their function, but still maintain their cytotoxic activity.

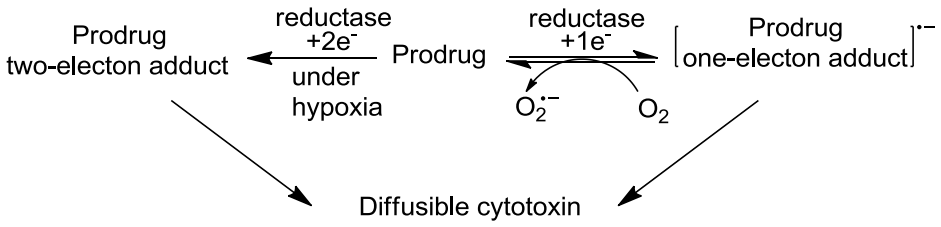
Pro-drugsOne approach first introduced by Albert[105] and Harper[106] in the late 1950's is the design of relatively non-toxic pro-drugs. These can be converted either through metabolism by endogenous enzymes or spontaneously into the pharmacologically active species and activated within the tumour mass.[107] They have been employed to improve the efficacy of anti-cancer agents by the modification, of their physiochemical[108] and/or pharmacokinetic[109] properties. More specifically this includes their (i) solubility and lipophilicity (ii) bioavailability[108] (iii) stability[110] (iv) toxicity (v) transport properties[109] (vi) site and duration of action[110,111] and (vii) first-pass metabolic effects.[112]

Classes of pro-drug

There are many different types of pro-drug which were developed to exploit various aspects of the tumour physiology, such as selective enzyme expression, hypoxia and low extracellular pH. Others are targeted towards to tumour-specific delivery techniques such as antibody- and gene-directed enzyme prodrug therapy (ADEPT and

GDEPT). The hypoxia-selective tumour-activated pro-drugs (known as hypoxia-TAP) rely on the deficiency of oxygen (hypoxia)[113] that can be classified into two broad types, the first (i) chronic or diffusion hypoxia keeps cells that are ample distance (approx 150 μm) away from the nearest functional blood capillary hypoxic in the long term. The second (ii) transient or perfusion hypoxia is due to a temporary shutdown of blood vessels keeping sections of tissue hypoxic in the short term.[107,114] Hypoxia has been shown to exist in a number of different diseases including some cancers, i.e. brain, head and neck, breast and cervix,[115] rheumatoid arthritis and diabetes.[116] It is often characterised by low oxygen tension, nutrient levels[117,118] and pH.[117,119] Hypoxic cells are commonly found in most solid tumours as the result of a poor blood supply,[114] high interstitial pressures,[114,120] enhanced metabolic rate[104] and an inefficient vasculature system.[114] They have been found to be resistant to both radiotherapy and chemotherapy.[121] Since severe hypoxia only exists in tumours, an opportunity arose to exploit this phenomenon for its potential therapeutic advantage[121] and to develop non-toxic hypoxia-activated prodrugs. By concentrating the cytotoxic anti-cancer agents inside the tumour mass, this could dramatically minimise the risk of destroying normal cells.

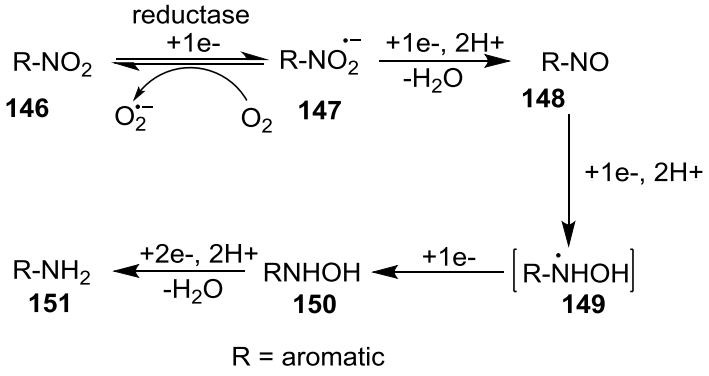
The major classes of hypoxia-TAP include nitroaromatics, heterocyclics, quinones, aromatic and aliphatic *N*-oxides and transition metal complexes. Each class can be activated under hypoxic conditions and in the presence of reductive enzymes (Scheme 64).



Scheme 64. General mechanism of prodrug activation under hypoxia[103,107].

One specific example of this is found in nitro-group containing compounds which can undergo a series of up to six one-electron reductions starting with the nitro group **146** to give the nitro anion radical **147** (Scheme 65). This intermediate can be

back-oxidised by molecular oxygen present in normoxic tissues to regenerate the parent compound **146**. Further reduction produces the nitroso **148**, radical **149**, hydroxylamine **150** and amine **151** as the subsequent products. Only in hypoxic tissue will the reduction proceed to these highly cytotoxic species.[109]



Scheme 65. Pathway of reductive activation for a nitroaromatic drug

Many nitro-aromatics prodrugs have been studied including 2-nitroimidazoles such as misonidazole **152**[123] etanidazole **153**[107] and dinitrobenzamide 5-(1-aziridinyl)-2,4-dinitrobenzamide **154**[123,124] (CB-1954) (Figure 15).

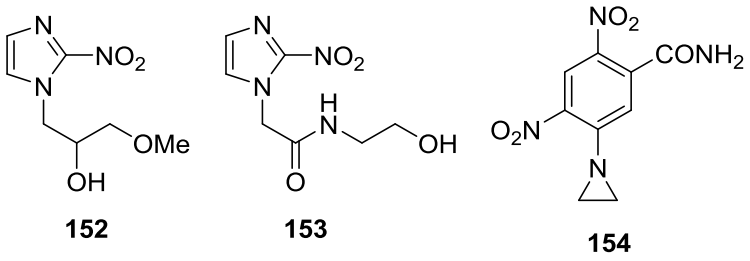
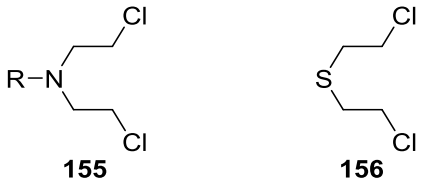


Figure 15. Structure of misonidazole **152** tanidazole **153** and 5-(1-aziridinyl)-2,4-dinitrobenzamide **154**

Nitrogen containing mustards

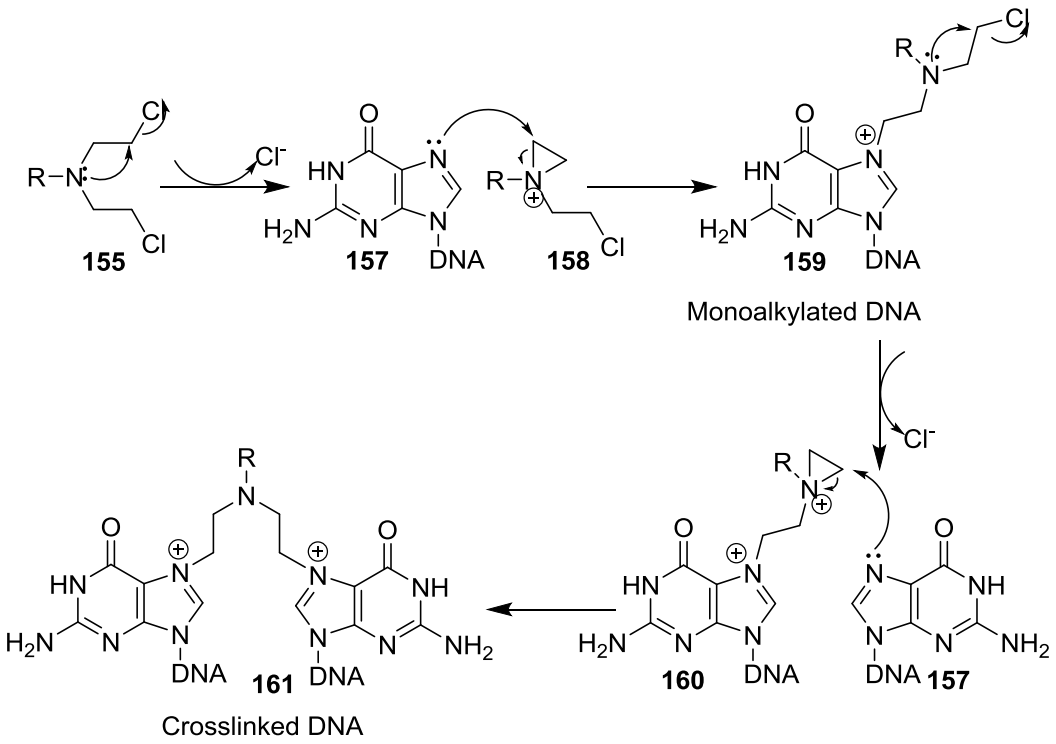
Nitrogen mustards **155** were developed during the second world war and are analogues of the sulfur mustard gas **156** which was implemented as a weapon in World War I (Figure 16).[125]



155 **156**

Figure 16. Structures of nitrogen and sulfur mustards

These mustards are DNA alkylating agents often used in chemotherapy (Scheme 66).[126,127] Since chlorine is a good leaving group this facilitates nucleophilic attack of the nitrogen in the mustard **155**[128] which cyclises to form an aziridinium ion **158**.[126] This strained three-membered ring system readily alkylates preferentially at the N^7 position of guanine **157** to form the monoalkylation adduct **159**.[128] Minor alkylations can also occur at other sites such as the N^3 position of adenine.[126,129] This process is then repeated with the cyclisation of the adduct **159** to form the aziridinium ion **160** which alkylates guanine **157** to give the crosslinked DNA **161**. These bifunctional adducts can generate an interstrand crosslink which is as much as 100 fold more cytotoxic than its monofunctional adduct.[130]



Scheme 66. Mechanism of DNA alkylation and interstrand crosslinking by nitrogen mustards

Crosslinking can occur between two complementary strands of DNA (interstrand) or on the same strand of DNA (intrastrand) (Figure 17).[128]

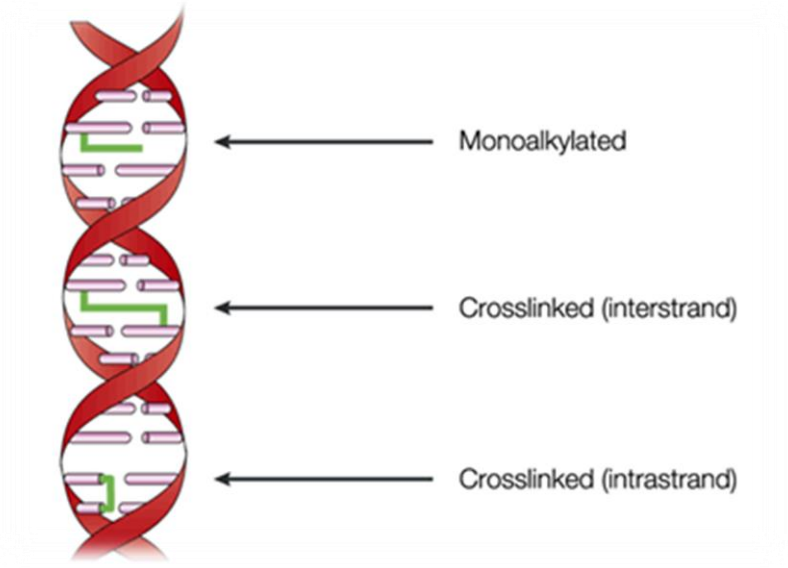


Figure 17. Types of DNA crosslinking[128]

Interstrand crosslinks are essential for maximal cell mortality[130] since they prevent two opposing strands in DNA from separating during replication or transcription. The overall result of this process is inhibition of DNA synthesis and it was found that there was a direct correlation between interstrand crosslinking and cytotoxicity.[131]

Examples of nitrogen mustards

The simplest member of the nitrogen mustard family is mechlorethamine **162** (Figure 18)[131,132] which was the first clinically useful anti-tumour substance.[131]

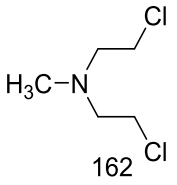
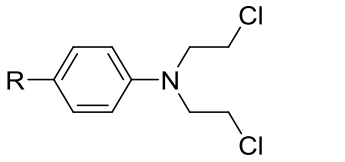


Figure 18. Structure of mechlorethamine

The problem is that mechlorethamine **162** reacts rapidly with biological material and water making it highly vesicant and chemically labile. To overcome these problems, the methyl group in **162** was replaced with an aromatic moiety (Figure 18). This lowers its reactivity, to give more time for absorption and distribution before DNA alkylation, allowing oral administration. However, the simplest aromatic mustard **163** is insoluble in water. Although its carboxylic acid analogue **164** is soluble, it was found to be inactive.[128] To overcome this solubility issue a considerable amount of research has been carried out developing more water-soluble *N*-mustards.[133,134] It was found that addition of CH_2 groups as spacers gave water-soluble active compounds such as chlorambucil **145** and melphalan **166** (Figure 19).[127]

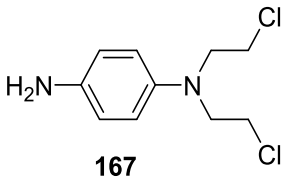


163 R = H
164 R = COOH
165 R = $(CH_2)_3COOH$
166 R = CH_2CHNH_2COOH

Figure 19. Structures of some aromatic nitrogen mustards

For over 50 years chlorambucil **165** has been used in the treatment of chronic lympocytic leukaemia.[135,136] Similarly, melphalan **166** is used in certain types of bone marrow tumours, including multiple myeloma[137] and cancers such as ovarian, breast[138] and colorectal.[139,140]

Another widely studied cytotoxic aromatic mustard is *N*^{1},*N*^{1}-*bis*(2-chloroethyl) benzene-1,4-diamine **167** (Figure 20), which can be used directly or in a pro-drug activated system.[141]



167

Figure 20. Structure of aniline mustard

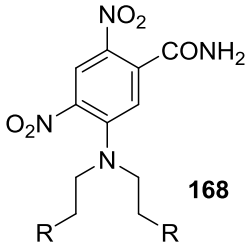
Aims of the study

Current anticancer therapies indiscriminately kill malignant cells as well as healthy cells resulting in severe side effects and the development of therapy-induced tumours. In the last decades, the search for tumour selectivity led to the development of Directed Enzyme Pro-drug Therapy (DEPT), which consists of the targeted delivery of enzymes directly to the malignant growth, where the enzymes convert an inactive pro-drug into a cytotoxic drug, normally exploiting antibodies or adenoviral vectors as vehicles for the enzymes' delivery. Two requirements are needed in order to realise this new anticancer approach: first, the pro-drug-activating enzymes must not be naturally present in human cells; second, the pro-drug must undergo a great increase in its cytotoxicity after the enzymatic activation. According to these fundamental requirements, the combination NTR/CB 1954 is currently the most investigated in human enzyme/prodrug therapy. The compound CB 1954 (5-[aziridin-1-yl]-2,4-dinitrobenzamide) **154**, is a monofunctional alkylating pro-drug, which is converted to a potent interstrand DNA-crosslinking agent upon nitro-reduction of either its 2- or 4-nitro group by *E. coli* NfsB nitroreductase (NTR).[142] However problems associated with the slow turn-over rate[143] of NfsB, for **154** and the inefficient activation of **154** *in vivo*[144] led to the evaluation of alternative enzyme/prodrug combinations for DEPT.

NfsA, the major *E. coli* oxygen-insensitive nitroreductase, has been shown to catalyse the nitroreduction of CB 1954 ~ 18-fold more efficiently than NfsB with its preferred cofactor NADPH,[144] making NfsA a suitable candidate for NTR/CB 1954 **154** combination. The crystal structure[104] shows that NfsA is a homodimeric protein, with one FMN molecule tightly bound to each subunit.[145,146] NfsA is able to reduce the 2-nitro group of CB 1954 in the presence of NADPH,[144,147,148] involving two successive two-electron transfers. These *E. coli* NTRs offer the versatility of metabolising a wide range of nitroaromatic compounds[149,150] which may be useful in the investigation of alternative pro-drugs with possibly greater therapeutic efficacy than the current NTR/CB 1954 **154** combinations.

The present study intends to investigate the activity of the alternative known pro-drugs 2,4-dinitrobenzamides **168**, which have been shown to be toxic to hypoxic rat Walker tumour cells.[154] However, to date their metabolism by NQO1 enzymes has

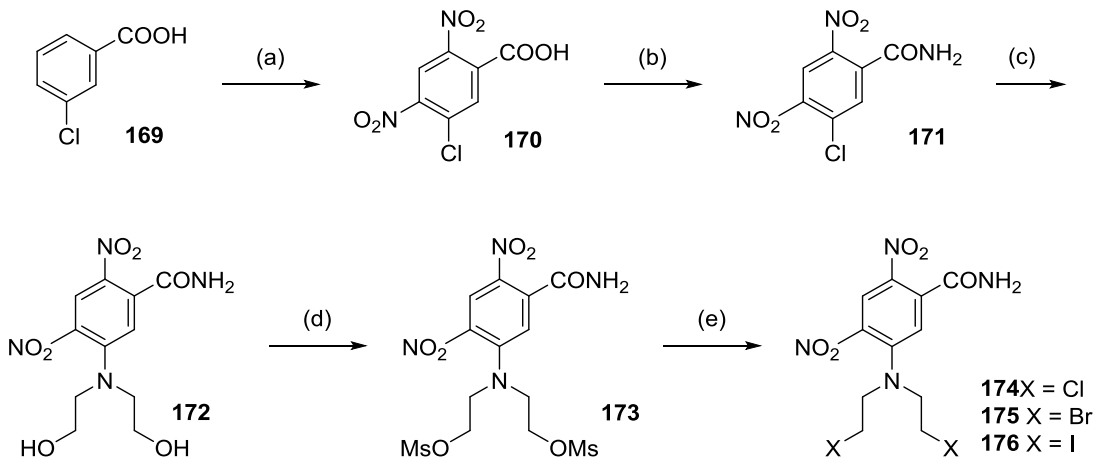
not been studied and the present work will investigate the activity of NQO1 on 2,4-dinitrobenzamide **168** (Figure 21).



168

Figure 21. Generic structure of 2,4-dinitrobenzamide; R = halogen.

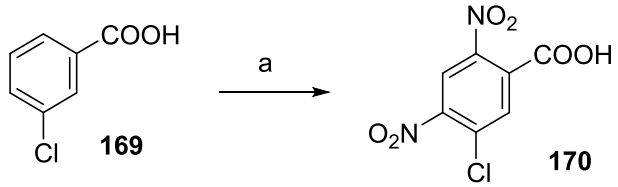
The most studied alternative prodrug to CB 1954 **154** for use in combination with NTRs is SN 23862 **174,** the nitrogen mustard analogue of CB 1954 **154**. Like CB 1954 **154**, SN 23862 **174** is a non-toxic, pro-drug which is converted to the alkylating cytotoxic derivatives by enzymatic nitroreduction.[151] *E.coli* nitroreductases can only reduce the 2-nitro group of the mustard to the corresponding 2-hydroxylamine. This is then readily reduced to the 2-amine, the principal cytotoxic metabolite, responsible for the alkylating reactivity of SN 23862 **174**.[152,153] To date, no studies have been done to evaluate the NfsA NTR capacity of SN 23862 activation. In this work, we evaluate the ability of NfsA NTR of activating the prodrug SN 23862 and its halogen analogues analogues the brominated **175** and iodinated **176**. The present study involves two stages, firstly the synthesis of a series of 2,4-dinitrobenzamide pro-drugs **174**-**176** and secondly the evaluation of these candidate molecules by electrode-binding each with *E.coli* NTR. The initial aims of this research is repeat and improve upon the synthesis of the 2,4-dinitrobenzamide **174-176** which have been reported in the literature.[155,156] The chemistry to be used is shown in Scheme 67.



Scheme 67. Synthesis of 2,4-dinitrobenzamides: (a) H_2SO_4/KNO_3, Δ; (b) i) $SOCl_2$**;** ii) NH_3.; (c) $HN(CH_2CH_2OH)_2$, Δ; (d) MsCl, pyridine.(e) DMF, MX Δ. (M = Li, Na, X = Cl, Br, I.).

Results and Discussion

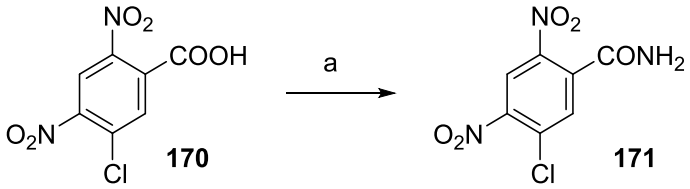
The first stage in the synthesis is the nitration of chlorobenzoic acid **169** using potassium nitrate in sulphuric acid which was reported[155] to give 5-chloro-2,4-dinitro-benzoic acid **170** in 36% yield. We repeated this literature process and obtained the desired compound **170** as a crystalline solid in an improved yield of 68% (Scheme 68). The proton NMR of **170** displayed 2 resonances at δ_H 8.75 (1H, *s*, CH) and 8.25 (1H, *s*, CH) ppm whilst the presence of 7 separate carbon environments in the carbon NMR spectrum indicated a successful reaction. The melting point of **170** was found to be 179 °C, which is in good agreement with the literature value of 180-183 °C.[155]



Scheme 68. Nitration of 169: (a) H_2SO_4/KNO_3, 100 °C, 68%.

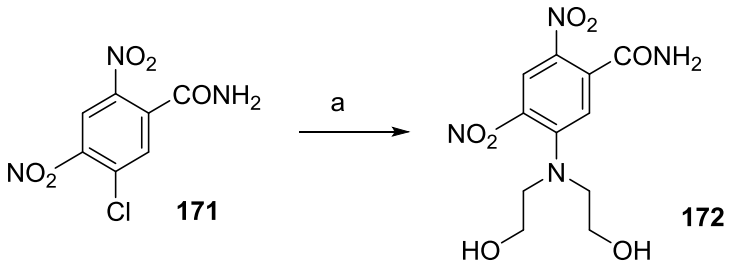
The formation of 5-chloro-2,4-dinitrobenzamide **171**[155] was reported in 84% yield by the amidation of 5-chloro-2,4-dinitro-benzoic acid **170** using thionyl chloride

and DMF followed by the addition of ammonium hydroxide. We repeated this process and obtained the desired **171** as a pale yellow solid in 87% yield (Scheme 69). The proton NMR of **170** displayed four resonances at δ_H 8.74 (1H, s, CH), 8.07 (1H, s, CH), 7.8 (1H, br s, NH) and 7.4 (1H, br s, NH) ppm, whilst the carbon spectrum displayed 7 resonances at δ_C 123.0 (CH), 132.0 (C), 133.2 (CH), 137.7 (C), 146.4 (C), 148.5 (C) and 165.3 (C) ppm. This data is indicative of the formation of **171** and the melting point of our sample was 200 °C, which is in good agreement with the literature value of 201-203 °C.[155]



Scheme 69. Preparation of amide **171**: (a) i) $SOCl_2$/DMF, Δ, 4h, ii) NH_4OH, 87%.

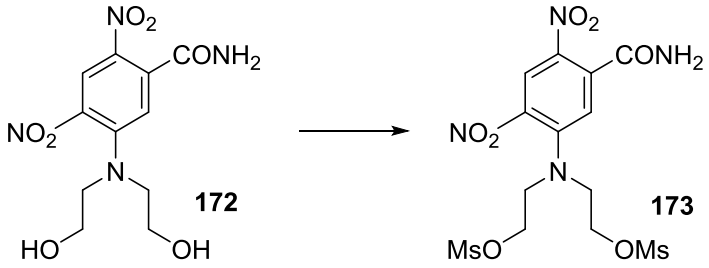
The formation of 5-[*bis*-(2-hydroxyethyl)-amino]-2,4-dinitrobenzamide **172** was reported to proceed in 45% yield by the reaction of chloride **171** with diethanolamine at 100 °C for 4 h in the absence of solvent.[156] We repeated this reaction and isolated the desired compound as an orange yellow solid in an improved yield of 80% (Scheme 70). Diagnostic proton NMR resonances were observed at δ_H 3.79 (4H, t, J = 5.4 Hz, 2 × CH_2) and 3.61 (4H, t, J = 5.4 Hz, 2 × CH_2) ppm and high resolution mass spectrometry gave a mass of 315.0939 Daltons which corresponds closely to the required mass of 315.0935 for $C_{11}H_{15}O_7N_4$ ($[M+H]^+$).



Scheme 70. $HN(CH_2CH_2OH)_2$, 100°C, 4 h, 80%.

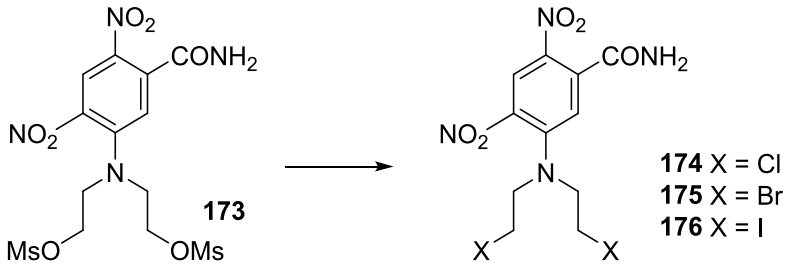
The key intermediate in the synthesis of the halogenated mustards is the mesylate **173** as this compound is able to undergo displacement by the alkali metal halides to give the desired compounds. All the preparations followed a literature

procedure,[156] and reaction of **172** with excess methanesulfonyl chloride (MsCl) in dry pyridine gave the desired *bis*-mesylate **172** in 67% yield after column chromatography. This compares well with the reported literature yield of 74%.[156] (Scheme 71) Significant nmr resonances were observed at δ_H 4.49 (4H, t, *J* = 5.3 Hz, 2 × CH_2) and 3.89 (4H, t, *J* = 5.35, Hz, 2 × CH_2) ppm for the methylene proton and at δ_H 3.09 (6H, s, 2 × CH_3) ppm for the mesylate groups. High resolution MS gave a mass of 471.0482 Daltons which corresponds closely to the required mass of 471.0486 Daltons for $C_{13}H_{19}O_{11}N_4S_2$ ($[M+H]^+$)



Scheme 71. MsCl, pyridine, 0 °C, 67%.

The final step in the synthesis of **174-176** was realized in a straightforward manner. This involved heating a solution of the mesylate **173** in dry DMF with an excess of either LiCl, NaBr, or NaI at 125 °C for 15 min, to effect nucleophilic displacement of the mesylate group.[156] Purification by column chromatography gave the chloride **174** in 75% yield, the bromide **175** in 98% yield and the iodide **176** in 81% yield respectively. NMR and mass spectrometry confirmed the displacement of the mesylate groups in each case (Scheme 72).



Scheme 72. DMF, 125 °C, MX (M = Li, Na, X = Cl; 75%. Br; 98%. I; 81%.)

Enzyme activity of the mustard pro-drugs 174-176

Concentration versus reaction rate

The rate of reduction of **174** as estimated by the rate of NADPH oxidation, was shown to increase proportionally with the enzyme concentration present in the assay, for both the NfsA wild-type and the NfsA-Cys enzymes (data not shown). For wild-type NTR, the rate of NADPH oxidation was shown to increase proportionally by adding 2.5 to 30 µg/ mL enzyme, whereas for NfsA-Cys, the rate of NADPH oxidation was found to increase proportionally by using 2.5 to 15 µg/ mL enzyme; the addition of higher enzymeconcentrations led to a decrease in the NADPH oxidation rate for both enzymes. The NADPH and CB 1954 **154** concentrations were constant during the experiment.

Substrate concentration versus reaction rate

As part of a previous study[157] the kinetic parameters K_m and V*max* were determined in 0.05 M phosphate buffer (pH 7.2) for purified NfsA-Cys by varying the substrate[154] concentration, in the presence of a constant concentration of 400 µM NADPH and of a constant enzyme concentration (10 µg/ mL). The rates of formation of the 2- and 4-hydroxylamine products of CB 1954 **154** were monitored spectrophotometrically at 420 nm, based on an equal absorption of both 2- and 4-NHOH reduction products of CB 1954 **154** at this wavelength (ε =1200 mol L^{-1} cm^{-1}). Apparent differences in K_m and V*max* between the tagged and non-tagged NfsA indicate that the incorporated cysteine residues affect the catalytic reaction: for NfsA-Cys, a decrease in the K_m value indicates that the enzyme-substrate binding is enhanced, but the enzyme-substrate complex reacts more slowly, since *Vmax* is lower, compared to NfsA wild-type. Considering the K_{cat}/K_m of the two enzymes, NfsA wild-type was shown to be ~ 5-fold more active with CB 1954 **154** than NfsA-Cys.

NfsA-Cys kinetics with SN 23862 and its analogues

The reaction rates of NfsA-Cys NTR with the pro-drug SN 23862 **174** and the bromo- **175** and iodo- **176** analogues as substrates were determined spectrophotometrically. This was achieved by observing the decrease in the NADPH UV absorbance at 340 nm, as the enzyme consumes this co-factor during the substrate

reduction and the appearance of the hydroxylamine reduction product at 420 nm. The reaction rates, i.e. the rates of NADPH oxidation, were measured by adding 2.5 to 30 µg mL^{-1} enzyme and were shown to increase proportionally with the enzyme concentration. Each concentration was tested three times, and the mean absorbance of taken as indicative of typical activity. Standard deviations of each were also calculated.

It was observed that for each of the compounds **174-176**, the relationship between concentration and activity was positive, with increasing concentration of **174-176** leading to an increased activity. In addition, this relationship was strongly linear and the standard deviations of activity at each different concentration were small. Figures 22-24 show the activity for each compound and the trend-line in each illustrated the relationship between concentration and activity. The error bars in each show the standard deviation for each concentration measurement. The figures also provide the regression equation and the amount of explained variance (R^2).

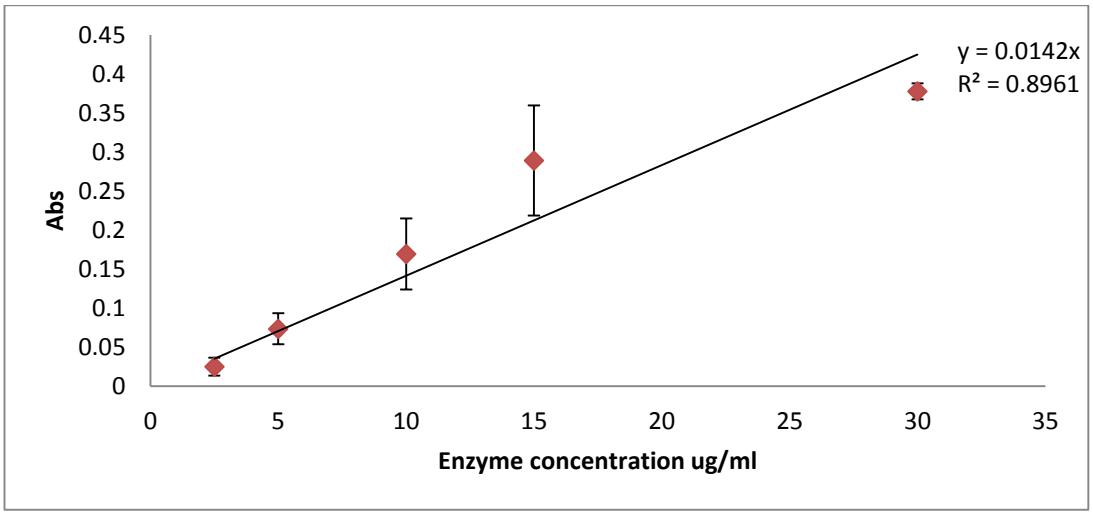


Figure 22. NfsAcys activity profile with **174**

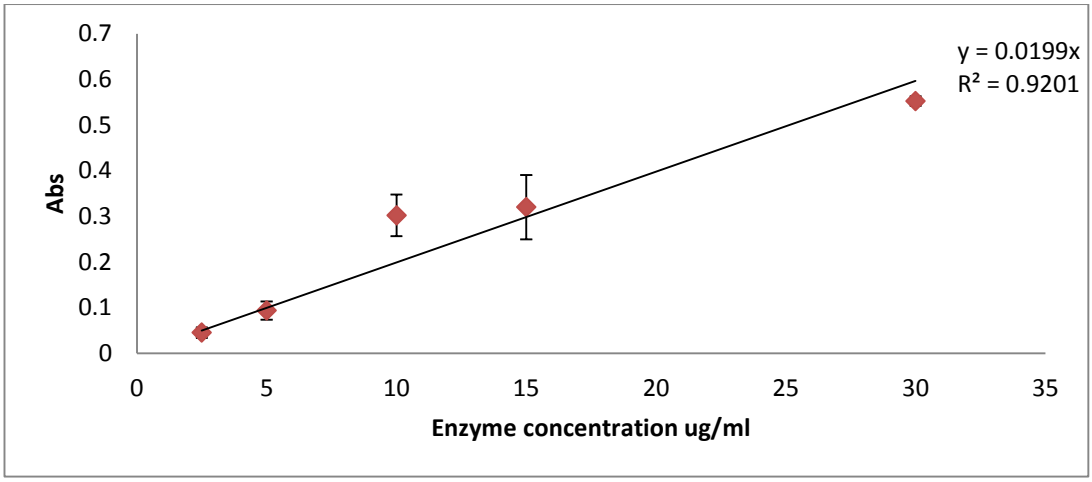


Figure 23. NfsAcys activity profile with **175**

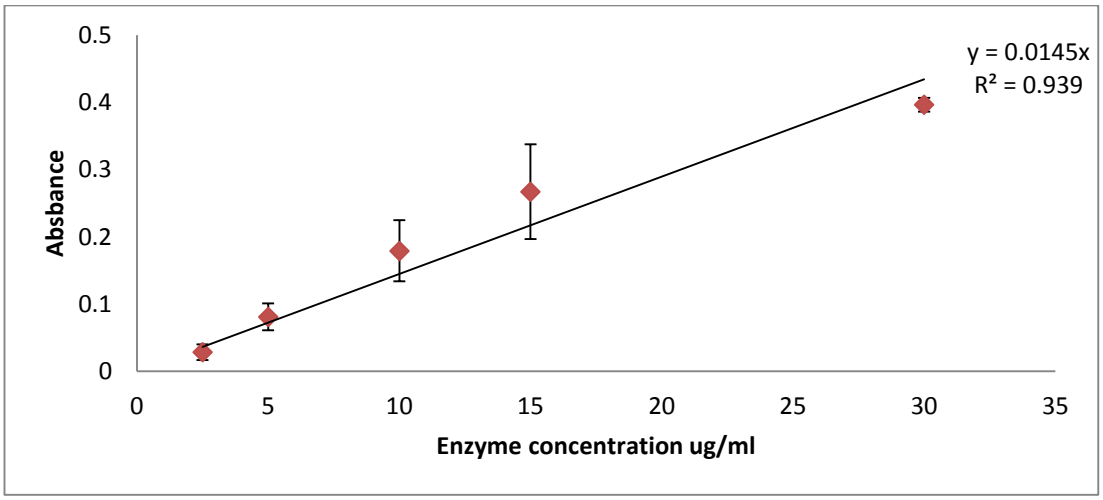


Figure 24. NfsAcys activity profile with **176**

Finally, Figure 25 compares the rates of enzymatic activation of the three prodrugs as 30 µg mL^{-1} of NfsA-Cys enzyme were added, showing the Br-substituted mustard **175** appears to be the best substrate for NfsA-Cys NTR.

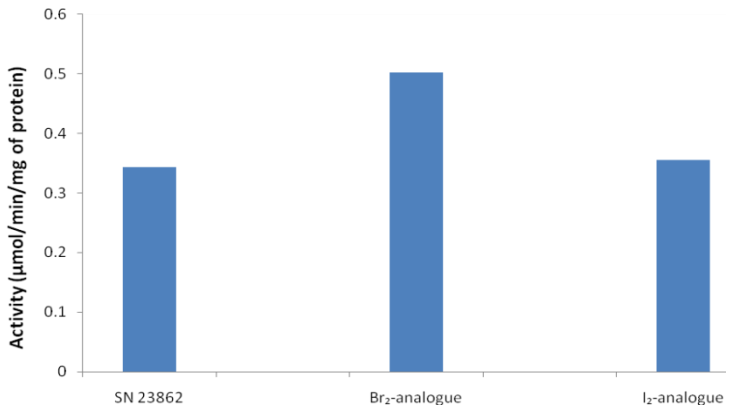


Figure 25. NfsA-Cys activity with substrates SN 23862 **174**, **175** and **176**

Conclusions

It proved relatively easy to synthesize the required 5-bis-(2-haloethyl)-amino-2,4-dinitrobenzamide **174-176**. SN 23862 **174** and its bromo- **175** and iodo- **176** analogues. These compounds proved to be good substrates for NfsA NTR, and thus can be considered as alternative pro-drugs to CB 1954 **154** in combination with NfsA for human chemotherapy. However, further studies are required to evaluate the precise rate of **174-176** bioactivation by NfsA NTR.

Experimental

General Procedures

Unless otherwise noted all reactions were stirred and monitored by TLC. plates were visualized using iodine, phosphomolybdic acid, 2,4-dinitrophenylhydrazine or under UV light. All anhydrous reactions were conducted under a static argon atmosphere using oven-dried glassware that had previously been cooled under a constant stream of nitrogen.

Materials

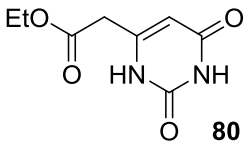
Reagents and starting materials were purchased from commercial suppliers and used without further purification unless otherwise noted. All anhydrous solvents were distilled over either sodium wire and benzophenone (THF/Et_2O) or calcium hydride (DCM); these were used immediately or stored over molecular sieves prior to use. Flash column chromatography was performed on Davisil® silica gel (35-70 microns) with the eluent specified in each case, TLC was conducted on precoated E.Merck silica gel 60 F_{254} glass plates.

Instrumentation

Melting points were determined using a Gallenkamp MF370 instrument and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 or 500 spectrometer with an internal deuterium lock at ambient temperature, with 400 or 500 MHz for proton and 100 or 125 MHz for carbon. Internal references of δ_H 7.26 and δ_C 77.0 ppm for $CDCl_3$, δ_H 3.31 and δ_C 49.0 ppm for CD_3OD and δ_H 2.54 ppm and δ_H 39.52 ppm for DMSO were used. All mass spectra were performed at the EPSRC National Mass Spectrometry Service Centre based in Swansea. Low resolution Chemical Ionisation (CI) and Electrospray Ionisation (ESI) mass spectra were recorded on a Micromass Quattro II spectrometer and high resolution mass spectra were recorded on either a Finnigan MAT 900 XLT or a Finnigan MAT 95 XP. Infrared samples were prepared by dissolving solids in dry chloroform and evaporated onto a NaCl plate, whilst oils/gums were pressed between two NaCl plates; spectra

were acquired on a Bruker Tensor 37 FT-IR machine and are reported as reciprocal wavenumbers (cm^{-1}).

Preparation of ethyl 2-(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)acetate (80).[84]

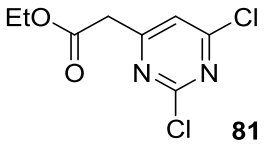


80

In a 1 L RBF, a mixture of anhydrous benzene/ethanol (68:32 v/v, 400 mL), diethyl 1,3-acetonedicarboxylate **79** (50.0 g, 0.247 mol), urea (14.8 g, 0.247 mol) and concentrated sulfuric acid (3 mL) were combined. At this point a soxhlet apparatus containing a drying thimble of 3Å powdered molecular sieves (ca. 20 g) was added and the mixture heated at reflux with stirring for 10 days with the sieves being replaced every 24 h. After 10 days the reaction was cooled and the solid precipitate removed by filtration to give **80** (39.0 g 196.8 mmol) as a white powder in 80% yield.

Mp	186-190 °C (lit. 189-191 °C[84])
R_f	0.37 (20% MeOH in $CHCl_3$)
δ_H ($(CD_3)_2SO$)	1.20 (3H, t, *J* = 7.1 Hz, Me), 3.48 (2H, s, CH_2), 4.11 (2H, q, *J* = 7.1, CH_2), 5.46 (1H, s, CH), 10.41-11.27 (2H, br s, 2 x NH)
δ_C ($(CD_3)_2SO$)	168.1 (C), 164.1 (C), 151.4 (C), 149.0 (C), 100.8 (CH), 60.9 (CH_2), 37.2 (CH_2), 14.0 (CH_3)

Preparation of ethyl 2-(2,6-dichloropyrimidin-4-yl)acetate (81).



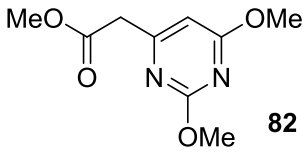
81

A mixture of *N*-ethyldiisopropylamine (12.2 g, 12.77 mL, 100.9 mmol, 2 eqv.) and compound **80** (10 g, 50.5 mmol) dissolved in toluene (150 mL) was heated to 105 °C and a solution of $POCl_3$ (23.3 g, 14.2 mL, 151.5 mmol, 3eqv.) in toluene (20 mL) was added drop wise to this mixture over 10 min. After 3 h. the reaction was cooled to rt and poured onto crushed ice and water (ca. 500 mL) with rapid stirring. After warming to rt overnight the aqueous phase was basified to pH = 9 by the addition of solid sodium carbonate then extracted with ethyl acetate (3 × 150 mL). The combined organic phases were washed with water (100 mL), dried ($MgSO_4$), filtered and evaporated in vacuo to give a dark red oil. Purification by flash chromatography on silica gel (120 g) eluting with EtOAc in petrol (gradient 0 to 35%) gave **81** (9.10 g, 38.7 mmol) in 77% yield as a red oil.

This reaction was repeated using *N*-ethyldiisopropylamine (26.09g, 35.16 mL, 201.16 mmol, 2 eqv.), **80** (20.0 g, 100.9 mmol) in toluene with $POCl_3$ (23.3 g, 14.2 mL, 151.5 mmol, 3eqv.) in toluene (150 mL) to give **81** (18.0 g, 76.6 mmol, 76%).

R_f	0.37 (20% EtOAc in petrol)
ν_{max}	3130, 3076, 2983, 1737, 1560, 1527, 1465, 1396, 1369, 1334, 1306, 1244, 1192, 1128, 1028, 867, 829, 737
δ_H ($CDCl_3$)	1.26 (3H, t, *J* = 7.2 Hz, CH_3), 3.79 (2H, s, CH_2), 4.19 (2H, q, *J* = 7.2 Hz, CH_2), 7.38 (1H, s, CH)
δ_C ($CDCl_3$)	168.0 (C), 167.0 (C), 162.7 (C), 160.4 (C), 120.1 (CH), 61.8 (CH_2), 42.5 (CH_2), 14.0 (CH_3)
m/z (CI)	235.0 (100%, $[M+H^+]$, 2 × ^{35}Cl), 237.0 (64%, $[M+H^+]$,$^{35}Cl/^{37}Cl$), 239.0 (10%, $[M+H^+]$, 2 × ^{37}Cl)
HRMS (CI)	$C_8H_9{}^{35}Cl_2N_2O_2$ $[M+H^+]$ requires 235.0036, found 235.0038

Preparation of methyl 2-(2,6-dimethoxypyrimidin-4-yl)acetate (82).



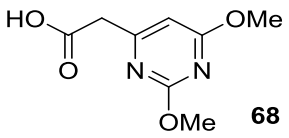
82

Sodium methoxide (prepared from sodium metal, 0.86 g, 37.4 mmol), in dry MeOH (50 mL) was added drop wise over 1 h to a cooled (0 °C) and stirred solution of **81** (4.0 g, 17.0 mmol) dissolved in dry MeOH (75 mL). After 3 h the reaction was allowed to warm to rt and stirred for a further 1 h. The reaction mixture was then concentrated in vacuo and diluted with water (100 mL) and then extracted with ethyl acetate (3 x 150 mL). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated in vacuo to give crude **7** which was purified by column chromatography (gradient elution; 5-15% EtOAc in petrol) to give **82** (1.53 g, 6.76 mmol) in 40% yield as a yellow oil.

This reaction was repeated using sodium methoxide (prepared from sodium metal, 0.62g, 27.1 mmol), in dry MeOH (30 mL) with **81** (2.90 g, 12.34 mmol) in dry MeOH (20 mL) to give **82** (1.50 g, 7.1 mmol, 57%)

R_f	0.28 (20% EtOAc in petrol)
ν_{max}	2995, 2955, 1743, 1597, 1571, 1483, 1460, 1330, 1255, 1157, 1101, 1058, 1044, 984, 835, 787, 756
δ_H ($CDCl_3$)	3.57 (2H, s, CH_2), 3.64 (3H, s, Me), 3.88 (3H, s, Me), 3.89 (3H, s, Me), 6.28 (1H, s, CH)
δ_C ($CDCl_3$)	171.9 (C), 169.7 (C), 165.1 (C), 164.2 (C), 100.9 (CH), 54.5 (CH_3), 53.6 (CH_3), 52.0 (CH_3), 42.5 (CH_2)
m/z (CI)	213.1 (100%, $[M+H]^+$)
HRMS (CI)	$C_9H_{13}N_2O_4$ ($[M+H]^+$) requires 213.0870, found 213.0866

Preparation of 2-(2,6-dimethoxypyrimidin-4-yl)acetic acid (68) from (82)

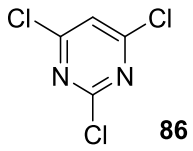


68

A solution of sodium hydroxide (175.0 mg, 4.37 mmol) in 3 mL of dry MeOH was added drop wise to a cooled (0 °C) solution of **82** (232.0 mg, 1.09 mmol) in dry MeOH (3 mL) and the mixture stirred overnight to rt (TLC indicated the complete consumption of **82**). The reaction mixture was then cooled (0 °C) and acidified to pH 3 by the careful addition of HCl (1 M). After evaporated to dryness under vacuum, the solid mass obtained was triturated with diethyl ethyl acetate (3 x 20 mL) and the triturates discarded. The remaining solid was then triturated with a 3:2 mixture of $CHCl_3$ and MeOH (2 x 10 mL) and the triturates filtered and evaporated to give **68** (207.0 mg,1.05 mmol) in 96% yield as an off white/cream coloured solid.

Mp	82-85 °C
R_f	0.45 (20% MeOH/$CHCl_3$)
δ_H (DMSO)	3.61 (2H, s, CH_2), 3.88 (3H, s, CH_3), 3.89 (3H, s, CH_3), 6.54, (1H, s, CH), 5.90-8.20 (1H, br s, OH)
δ_C (DMSO)	171.5 (C), 170.6 (C), 165.5 (C), 164.6 (C), 101.1 (CH), 54.4 (CH_3), 53.8 (CH_3), 42.4 (CH_2)
ν_{max}	3500-2500 (broad), 1727, 1648, 1614, 1574, 1496, 1455, 1396, 1359 1273, 1215, 1180, 1089, 1060, 923, 898, 838, 758
m/z (CI)	199.1 (100%, $[M+H]^+$)
HRMS (CI)	$C_8H_{11}N_2O_4$ ($[M+H]^+$) requires 199.0713, found 199.0713.

Preparation of 2,4,6-trichloropyrimidine (86).[91]

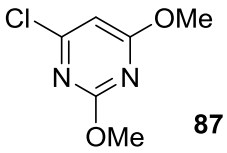


86

Barbituric acid **24** (49.85 g, 389.2 mmol, 1 eqv.) and phosphorus oxychloride (257 g, 1.67 mol, 4.3 eqv.) were combined in a 1 L RBF and the mixture stirred at 0 °C for 20 mins. *N*,*N*-Dimethylaniline (46.4 g, 311.4 mmol, 0.8 eqv.) was then added drop-wise and the mixture heated at reflux for 45 mins. After cooling (0 °C) crushed ice (400 g) was then added in small portions to the red reaction mixture which was then stirred to rt overnight. Diethyl ether (200 mL) was added and after agitation the ether layer was separated and the aqueous layer further extracted with diethyl ether (3 x 100 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure to yield **86** as a yellow oil (70.1 g 382 mmol, 98%).

R_f	0.58 (20% ether petrol)
δ_H ($CDCl_3$)	7.4 (1H, s, CH)
δ_C ($CDCl_3$)	163.0 (C), 160.2 (C), 120.2 (CH)

Preparation of 4-chloro-2,6-dimethoxypyrimidine (87).[91]



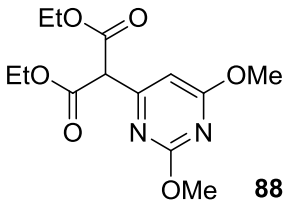
87

A solution of sodium methoxide was prepared by adding sodium metal (10.08 g, 438.3 mmol, 2 eqv.) in small portions to cooled (0 °C) anhydrous methanol (200 mL) with stirring until all the sodium was consumed. A solution of pyrimidine **86** (40.34 g, 219.95 mmol, 1 eqv.) in anhydrous methanol (200 mL) was then added drop wise over a period of 1 h. The solution was stirred to rt overnight and then concentrated in vacuo and diluted with water (200 mL). The resultant mixture was extracted with ethyl acetate (3 x 100 mL) and the combined organic extracts dried ($MgSO_4$), filtered and the solvent removed in vacuo to give a creamy white, crystalline solid. This solid was dissolved in hot ether (ca. 100 mL) and diluted with hexane (ca. 100 mL) to the cloud point. On standing, a white solid deposited which were removed by filtration to give **87** (22.63 g 129.6 mmol) in 59% yield.

This reaction was repeated using sodium metal (5.8 g, 251.9 mmol, 2.2 eqv.), methanol (150mL) and **86** (21.0 g, 114.5 mmol, 1 eqv.) in anhydrous methanol (200 mL) to give **87** (18.0 g, 103.1 mmol) in 90% yield.

Rf	0.39 (20% ether: petrol)
Mp	68-69 °C (Lit. 71-73 °C[91])
δ_H ($CDCl_3$)	3.96 (s, 3H), 3.99 (s, 3H), 6.38 (s, 1H)
δ_C ($CDCl_3$)	172.2 (C), 164.9 (C), 161.2 (C), 100.9 (CH), 55.3 (CH_3), 54.4 (CH_3)
ν_{max}	3092, 2987, 1587, 1563, 1450, 1397, 1343, 1190, 1167, 976, 937, 832, 760

Preparation of diethyl 2-(2,6-dimethoxypyrimidin-4-yl)malonate (88)



88

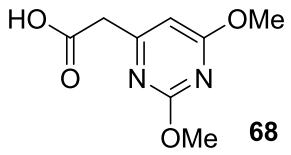
Diethylmalonate (11.0 g, 68.7 mmol, 6.0 eq) was added to a stirred suspension of sodium hydride (2.75 g of 60 percent dispersion in mineral oil, 68.7 mmol) in cooled (0 °C) dimethylformamide (50 mL). The reaction was stirred at this temperature for 1 h, whereupon pyrimidine **87** (2.0 g, 11.46 mmol) was added in small portions over 5 min and the reaction stirred at rt for 1 h, then heated to 100 °C for 18 hours. The reaction was then cooled to rt and the solvent was removed in vacuo. The residue was then dissolved in ethyl acetate (100 mL) and water (100 mL), separated and the aqueous phase extracted with further ethyl acetate (3 x 50 mL) then the combined extracts were washed with water (3 x 50 mL). After drying ($MgSO_4$) and concentration in vacuo, purification by flash chromatography on silica gel (5-15% EtOAc in petrol) gave **88** (2.27 g, 7.61 mmol) as a pale yellow oil in 66% yield.

This reaction was repeated twice: i) Diethylmalonate (55.0g, 343.7 mmol, 6.0 eq), sodium hydride (8.25g, 343.7 mmol), DMF (200 mL) and **87** (2.0 g, 11.46 mmol) to give **88** (9.5 g, 341.8 mmol, 55%); ii) Diethylmalonate (66.1 g, 412.4 mmol, 6.0 eq), sodium hydride (9.9g, 412.4 mmol), DMF (200mL) and **87** (2.0 g, 11.46 mmol) to give **88** (11.0g, 36.9mmol, 54%).

Rf	0.18 (20% EtOAc in petrol)
δ_H ($CDCl_3$)	1.25 (6H, t, *J* = 7.1 Hz, 2 × Me), 3.93 (6H, s, 2 × Me), 4.10-4.26 (4H, m, 2 × CH_2), 4.67 (1H, s, CH), 6.45 (1H, s, CH)
δ_C ($CDCl_3$)	172.2 (C), 166.5 (C), 165.2 (C), 162.8 (C), 101.4 (CH), 62.2 (CH_2), 59.7 (CH), 54.90 (CH_3), 54.1 (CH_3), 14.1 (CH_3)
ν_{max}	3456, 2986, 1737, 1627, 1598, 1571, 1483, 1462, 1386, 1358, 1307 1248, 1206, 1176, 1153, 1101, 1029, 984, 863, 788, 749

m/z (CI)	299.1 (100%, $[M+H]^+$), 227.1 (35%)
HRMS (CI)	$C_{13}H_{19}N_2O_6$ ($[M+H]^+$) requires 299.1238, found 299.1237

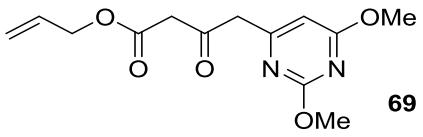
Preparation of 2-(2,6-dimethoxypyrimidin-4-yl)acetic acid (68) from (88)



68

Sodium hydroxide (1.05 g, 26.3 mmol) dissolved in dry MeOH (5 mL) was added drop wise over 5 min to a cooled (0 °C) solution of **88** (0.87 g, 2.92 mmol) in dry MeOH (10 mL). After stirring to rt overnight (tlc at this point indicated the complete consumption of **88** the solution was adjusted to pH = 1 using aqueous HCl (2 M) and then evaporated in vacuo to give a solid. This was triturated firstly with diethyl ethyl (2 x 30 mL) to remove any unreacted **88** and the remaining solid was triturated with a mixture of $CHCl_3$/MeOH (3:2, 2 x 10 mL), filtered and the filtrate evaporated to give **68** (0.58 g, 2.92 mmol) as a pale yellow solid in 100% yield. Data was as reported previously on page 88.

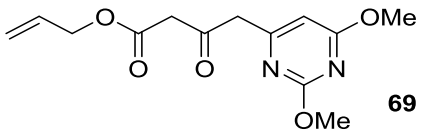
Attempted preparation of allyl 4-(2,6-dimethoxypyrimidin-4-yl)-3-oxobutanoate (69) using DCCI and Meldrum's acid



69

A mixture of pyrimidine **68** (0.50 g, 2.53 mmol, 1 eqv), DMAP (457.0 mg, 3.75 mmol 1.45 eqv.) and Meldrum's acid (364.0 mg, 2.53 mmol, 1.1 eqv) were combined in dissolved in CH_2Cl_2 (15 mL) to give a clear solution. At this point DCCI (0.60 g, 2.91 mmol, 1.15 eqv) was added and the mixture stirred at rt for 5 h. After filtration (to remove precipitated DCCU (0.67 g)) and the solid washed with a small volume of cold CH_2Cl_2 (ca 10 mL) and then evaporated to give a solid (1.50 g). This was dissolved in DCM and washed with saturated sodium sulfate solution (20 mL), water (20 mL) and brine (20 mL). After drying ($MgSO_4$) and evaporated under vacuum, a brown solid (0.40 g) **90** was obtained. On acidification of the aqueous layer and re-extraction no further material was obtained. This residue was dissolved in dry benzene (10 mL) together with allyl alcohol (1.47 g, 25.3 mmol, 1.7 mL) and heated at reflux for 3 h. Removal of solvent under reduced pressure gave a brown solid (0.42 g) which was a complex mixture of compounds as evidenced by tlc and NMR Attempts to isolate **69** from this mixture by column chromatography were unsuccessful.

Attempted preparation of allyl 4-(2,6-dimethoxypyrimidin-4-yl)-3-oxobutanoate (69) using CDI and Meldrum's acid in allyl alcohol



69

Meldrum's acid (148.0 mg, 1.03 mmol, 1.1 eqv) and CDI (227.0 g, 1.40 mmol, 1.5 eqv) were added to a stirred solution of pyrimidine **68** (185.0 mg, 0.93 mmol, 1.0 eqv.) in anhydrous DMF (2 mL) and the mixture heated at 50 °C for 16 h. On cooling to rt the reaction was diluted with ethyl acetate (50 mL) and water (50 mL) and acidified to pH 1 by the slow addition of HCl (aqueous 1 M). The organic layer separated and the aqueous phase extracted with further ethyl acetate (3 x 50 mL). The combined extracts were washed with brine (20 mL), dried ($MgSO_4$) and evaporated to give crude **90** as a red solid (0.30 g).

Partial data

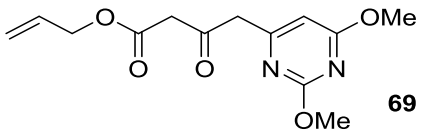
Rf	0.33 (streaks in 10% MeOH in $CHCl_3$)
δ_H ($CDCl_3$)	1.64 (6H, s, 2 × CH_3), 3.83 (3H, s, CH_3), 3.85 (3H, s, CH_3), 3.99 (1H, s, CH), 4.31 (2H, s, CH_2), 6.26 (1H, s, CH), 11.65 (enol)
δ_C ($CDCl_3$)	191.7 (C), 171.9 (C), 165.1 (C), 164.5 (C), 162.7 (C), 101.1 (CH), 54.6 (CH_3), 53.8 (CH_3), 42.7 (CH_2), 26.7 (CH_3)

This solid was dissolved in allyl alcohol (7 mL) and heated at 90 °C for 16 h. After evaporation, column chromatography gave a complex mixture of products.

This reaction was repeated using Meldrum's acid (400.0 mg, 2.78 mmol, 1.1 eqv), CDI (613.7 mg, 3.78 mmol, 1.5 eqv) and pyrimidine **68** (500.0 mg, 2.52 mmol, 1.0 eqv.) in DMF (10 mL). After work up (which included a LiBr wash and re-extraction), the crude product was purified by column chromatography to give the triethylamine salt of 90 (26.0 mg, 0.072 mmol, 3% yield) as the only identifiable product

Rf	0.33 (streaks in 10% MeOH in $CHCl_3$);
δ_H ($CDCl_3$)	1.14 (9H, t, *J* = 7.3 Hz, 3 × CH_3), 1.61 (6H, s, 2 × CH_3), 3.04 (6H, q, *J* = 7.3 Hz, 3 × CH_2), 3.88 (3H, s, CH_3), 3.89 (3H, s, CH_3), 4.25 (2H, s, CH_2), 6.26 (1H, s, CH), 10.33 (1H, br s, enol H)
δ_C ($CDCl_3$)	192.9 (C), 171.7 (C), 169.4 (C), 166.1 (C), 164.9 (C), 161.4 (C), 101.4 (CH), 89.4 (C), 54.5(CH_3), 53.7 (CH_3), 50.4 (CH_2), 45.9 (CH_2), 26.4 (CH_3), 8.4 (CH_3)

Preparation of allyl 4-(2,6-dimethoxypyrimidin-4-yl)-3-oxobutanoate (69) using CDI and Meldrum's acid in benzene



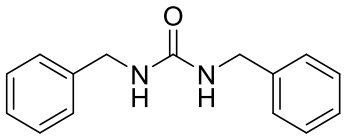
69

Meldrum's acid (160.0 mg, 1.11 mmol, 1.1 eqv) and CDI (246.0mg, 1.51 mmol, 1.5 eqv) were added to a stirred solution of pyrimidine **68** (200.0 mg, 1.01 mmol, 1.0 eqv.) in anhydrous DMF (1 mL) and the mixture heated at 50 °C for 16 h. The solvent was removed by evaporation under high vaccum (70 °C) and the residue diluted with ethyl acetate (10 mL) and water (10 mL) and acidified to pH 1 by the slow addition of HCl (aqueous 1 M). The organic layer separated and the aqueous phase extracted with further ethyl acetate (3 x 50 mL). The combined extracts were washed with brine (2 x 10 mL), dried ($MgSO_4$) and evaporated to give a red solid (0.65 g).This solid was dissolved in benzene (5 mL) and allyl alcohol (0.57 g, 0.67 mmol, 0.70 mL) was added and the mixture heated at reflux for 16 h. The reaction was evaporated onto silica and purified by column chromatography (5-20% EtOAc in petrol) gave **69** (79.7 g, 0.28 mmol) as a pale yellow oil in 28% yield.

Rf	0.31 (streaks 30% EtOAc in petrol)
δ_H (DMSO)	3.78 (2H, s, CH_2), 3.86 (3H, s, CH_3), 3.88 (3H, s, CH_3), 3.91 (2H, s, CH_2) 4.55-4.58 (2H, m, CH_2), 5.20-5.24 (1H, m, CH), 5.28-5.35 (1H, m, CH), 5.83-5.97 (1H, m, CH), 6.47, (1H, s, CH); enolic resonances at 5.53 (CH), 6.25 (CH) and 13.71 (OH)
δ_C (DMSO)	199.4 (C), 171.5 (C), 166.6 (C), 165.0 (C), 164.8 (C), 132.3 (CH), 118.0 (CH_2), 101.5 (CH), 65.0 (CH_2), 54.4 (CH_3), 53.8 (CH_3), 50.4 (CH_2), 48.8 (CH_2)
ν_{max}	3089, 3017, 2992, 2952, 1743, 1654, 1596, 1570, 1549, 1483, 1459, 1410, 1392, 1359, 1326, 1256, 1209, 1149, 1059, 1003, 991, 933, 838, 756

m/z (CI)	281.1 (100%, $[M+H]^+$), 303.1 (30%, $[M+Na]^+$)
HRMS (CI)	$C_{13}H_{17}N_2O_5$ ($[M+H]^+$), requires 281.1132, found 281.1128

Preparation of 1,3-dibenzylurea (92)[93]



92

From carbohydrazide: A vigorously stirred suspension of finely powdered carbohydrazide **97** (4.50 g, 50.0 mmol, 1 eqv) in DMF (100 mL) and benzylamine (54.5 mL, 500 mmol, 10 eqv) was cooled to 0 °C. Iodine (50.76 g, 200 mmol, 4 eqv) was then added portion-wise over 1 h until a brown colour persisted. Excess iodine was then quenched by the addition of $Na_2S_2O_3$ (Sat.) until a straw yellow solution resulted and the desired compound was precipitated from the by the addition of water (400 mL). After filtration and washing with water, the solid was dried under vacuum to give **92** as a white solid (7.65 g, 32.0 mmol, 64%)

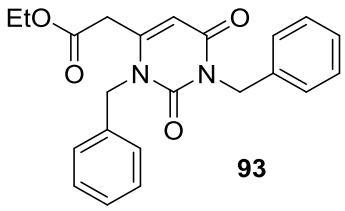
From CDI: CDI (8.25g, 50.88 mmol) and benzyl amine (16.36 g, 152.7 mmol, 16.86 mL, and 3 eqv) were dissolved in THF (250 mL) and the mixture heated at reflux for 16 h. After cooling, the reaction was evaporated and the residue dissolved in dichloromethane, washed with water (200 mL), HCl (aqueous, 1M, 100 mL) and brine (50 mL) after separation and drying ($MgSO_4$), evaporation gave a crude product which was recrystallized from hot ethyl acetate to give **92** as white crystal (8.35 g, 34.87 mmol) 68%.

This reaction was repeated on 10 occasions and gave yields of 49-68%.

Rf	0.20 (50% ethyl acetate in petrol);
Mp	165-168 °C (lit.[2] 168-169 °C)
ν_{max}	3337, 3086, 3062, 3030, 2919, 2874, 1627, 1613, 1589, 1574, 1493, 1472, 1453 and 1421
δ_H **$(CD_3)_2SO$)**	4.23 (4H, d, *J* = 6.0 Hz, 2 × CH_2), 6.43 (2H, t, *J* = 6.0 Hz, 2 × NH), 7.20-7.33 (10H, m, 2 × Ph)
δ_C **$(CD_3)_2SO$)**	43.0 (2 × CH_2), 126.5 (CH), 127.0 (CH), 128.2 (CH), 140.9 (C) and 158.1 (C=O)

m/z (CI)	503.0 (25%, $[2M+Na]^+$), 481.0 (100%, $[2M+H]^+$), 263.0 (8%, $[M+Na]^+$) and 241.1 (95%, $[M+H]^+$)
HRMS (CI)	$C_{15}H_{17}N_2O$ ($[M+H]^+$) requires 241.1335, found 241.1333

Preparation of ethyl 2-(1,3-dibenzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)acetate (93)



93

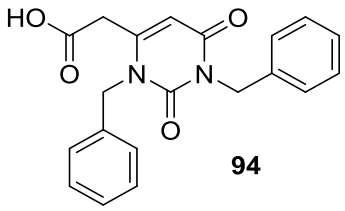
To a stirred solution of urea **92** (5 g, 20.82 mmol, 1 eqv.) and diethyl 1,3-acetonedicarboxylate (7.56 mL, 41.64 mmol, 2 eqv) in benzene (150 mL) was added p-TsOH (717.0 mg, 4.16 mmol, 0.2 eqv) and the mixture heated to reflux at 115 °C in a soxhlet extractor containing 4 Å molecular sieves in the thimble. The molecular sieves were replaced every other day and after 3 days, 1H NMR analysis indicated complete consumption of the starting material. The reaction mixture was then cooled to rt, diluted with dichloromethane (400 mL) and washed with $NaHCO_3$ (saturated 3 x 150 mL) and brine (100 mL). After drying and evaporation the resulting viscous oil was stirred/triturated with petroleum ether (300 mL). After 1 h the petroleum ether was decanted and the remaining solid precipitate triturated with Et_2O (4 x 100 mL) to give ester **93** as a pale yellow solid (7.0 g, 18.5 mmol, 89%).

This reaction was repeated twice: i) Urea **92** (12.0 g, 50.0 mmol, 1 eqv.) and diethyl 1,3-acetonedicarboxylate (11.7 mL, 70.0 mmol, 1.4 eqv) in benzene (300 mL) with *p*-TsOH (860 mg, 4.99 mmol, 0.1eqv) to **93** (16.40 g, 43.3 mmol, 87%); ii) Urea **92** (24.0 g,100.0 mmol, 1 eqv.) and diethyl 1,3-acetonedicarboxylate (33.5 mL, 200 mmol, 2 eqv) in benzene (300 mL) with *p*-TsOH (3.44 g, 20 mmol, 0.2 eqv) to give **93** (34.0 g, 89.8 mmol, 90%).

Rf	0.41 (50% ethyl acetate in petrol);
Mp	120-123 °C
ν_{max}	3090, 3061, 3023, 3008, 2979, 2935, 1738, 1702, 1693, 1654, 1662, 1617, 1584, 1495, 1451, 1423, 1396, 1367, 1333

δ_H ($CDCl_3$)	1.26 (3H, t, *J* = 7.1 Hz, CH_3), 3.42 (2H, s, CH_2), 4.13 (2H, q, *J* = 7.2 Hz, CH_2), 5.15 (2H, s, CH_2), 5.19 (2H, s, CH_2), 5.76 (1H, s, CH), 7.12-7.16 (2H, m, 2 × CH), 7.25-7.37 (6H, m, 6 × CH), and 7.47-7.52 (2H, m, 2 × CH)
δ_C ($CDCl_3$)	13.9 (CH_3), 38.3 (CH_2), 44.5 (CH_2), 47.8 (CH_2), 60.1 (CH_2), 104.0 (CH), 125.8 (2 ×CH), 127.3 (CH), 127.6 (CH), 128.1 (2 × CH), 128.6 (2 × CH), 128.8 (2 × CH), 135.6 (C), 136.5 (C), 147.6 (C), 152.3 (C=O), 161.6 (C=O) and 167.2 (C=O)
m/z (CI)	779 (6%, $[2M+Na]^+$), 757 (100%, $[2M+H]^+$), 401 (5%, $[M+Na]^+$) and 379 (95%, $[M+H]^+$)
HRMS (CI)	$C_{22}H_{23}N_2O_4$, requires 379.1652, found 379.1650

Preparation of 2-(1,3-dibenzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)acetic acid (94)



94

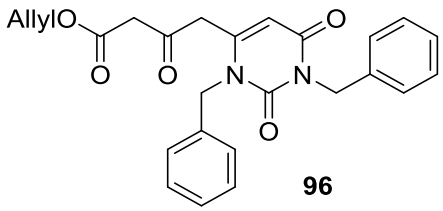
Sodium hydroxide (5.28 g, 132.13 mmol, 10 eqv,) was added to a cooled (0 °C) and stirred suspension of ester **93** (5.0 g, 13.213 mmol, 1 eqv) in ethanol (100 mL). After stirring to rt overnight. The mixture was diluted with water (600 mL) and the pH adjusted to pH 1-2 by the slow addition of HCl (aqueous, 1M) then extracted with dichloromethane (3 x 100 mL) and the combined organic extracts dried ($MgSO_4$). After evaporation the resulting light brown solid was triturated with Et_2O (3 x 40 mL) to give the acid **94** as an off white solid (4.46 g, 12.73 mmol, 96%).

This reaction was repeated three times: i) Sodium hydroxide (306.5 mg, 7.66 mmol, 2.9 eqv) and ester **93** (1.00 g, 2.64 mmol, 1 eqv) in ethanol (10 mL) gave **94** (0.90 g, 2.6 mmol, 97%); ii) Sodium hydroxide (1.90g, 47.6 mmol, 9 eqv,) and ester **93** (2.00 g, 5.29 mmol, 1 eqv) in ethanol (60 mL) gave **94** as an off white solid (1.8 g, 5.14 mmol, 97%); iii) sodium hydroxide (10.0 g, 250.0 mmol, 9 eqv,) and ester **93** (10.5g, 27.8 mmol, 1 eqv) in ethanol (150 mL) gave **94** (9.0 g, 25.7 mmol, 93%).

Rf	0.27 (20% methanol in CH_2Cl_2)
Mp	139-140 °C
ν_{max}	3442, 3096, 3063, 3034, 2921, 2793, 2695, 2576, 1722, 1705, 1693, 1642, 1598, 1496, 1472, 1454, 1423, 1399, 1367, 1351 1315
δ_H $(CD_3)_2SO$)	3.58 (2H, s, CH_2, obscured), 5.03 (2H, s, CH_2), 5.05 (2H, s, CH_2), 5.89 (1H, s, CH), 7.13-7.19 (2H, m, 2 × CH), 7.22-7.38 (8H, m, 8 × CH) and 13.09 (1H, br s, OH)

δ_C (CD_3)$_2$SO)	38.0 (CH_2), 43.8 (CH_2), 47.5 (CH_2), 103.0 (CH), 125.9 (2 × CH), 127.2 (CH), 127.4 (CH), 127.6 (2 × CH), 128.4 (2 × CH), 128.7 (2 × CH), 136.4 (C), 137.0 (C), 149.6 (C), 152.0 (C=O), 161.3 (C=O) and 169.4 (COOH)
m/z (CI)	701 (100%, $[2M+H]^+$), 373 (15%, $[M+Na]^+$) and 351 (100%, $[M+H]^+$)
HRMS (CI)	$C_{20}H_{19}N_2O_4$ ($[M+H]^+$) requires 351.1399, found 351.1340

Preparation of allyl 4-(1,3-dibenzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-3-oxobutanoate (96)



96

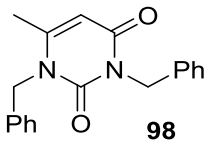
Meldrum's acid (1.23 g, 8.56 mmol, 1.1 eqv) and CDI (1.38 g, 8.56 mmol, 1.1 eqv) were added to a stirred solution of pyrimidine **94** (2.7 g, 7.71 mmol, 1.0 eqv.) in anhydrous THF (60 mL) and the mixture heated at 50 °C for 16 h. On cooling to rt the solvent was removed under reduced pressure and the residue taken in CH_2Cl_2/H_2O (80/60 mL). The pH was then adjusted to pH 2 by the slow addition of HCl (aqueous 1 M), the organic layer separated, and the organic extract washed with H_2O (100 mL) and dried ($MgSO_4$) and evaporated to give a yellow solid (3.30 g). This solid was dissolved in allyl alcohol (50 mL) and heated at 110 °C for 16 h. Excess alcohol was removed under reduced pressure and the resulting material purified by flash column chromatography on silica gel using ethyl acetate/petroleum ether (15:85 to 30:70). Fractions eluting in 25:75 ethyl acetate/petroleum ether gave **96** as viscous yellow oil (1.60 g, 3.70 mmol, 48%)

This reaction was repeated twice: i) Meldrum's acid (452.5 mg, 3.14 mmol, 1.1 eqv) CDI (509.1 mg, 3.14 mmol, 1.1 eqv) and **94** (1.00 g, 2.85 mmol, 1 eqv.) in THF (20 mL) to give **96** (0.578 g, 2.31 mmol, 47%); ii) Meldrum's acid (3.69 g, 25.62 mmol, 1.1 eqv), CDI (4.15 g, 25.29 mmol, 1.1 eqv) and **94** (8.16 g, 23.29 mmol, 1 eqv.) in THF (180mL) to give **96** (4.67 g, 10.8 mmol, 46%).

Rf	0.08 (30% ethyl acetate in petrol)
ν_{max}	3094, 3067, 3023, 3002, 2968, 2939, 1746, 1727, 1705, 1702, 1663, 1628, 1586, 1497, 1453,1396, 1347, 1328
δ_H $(CD_3)_2SO$)	3.77 (2H, s, CH_2), 3.98 (2H, s, CH_2), 4.57 (2H, dt, *J* = 5.5, 1.3 Hz, CH_2), 4.95 (2H, s, CH_2), 5.01 (2H, s,

	CH_2), 5.21 (1H, dq, *J* = 10.5, 1.3 Hz, CH), 5.31 (1H, dq, *J* = 17.3, 1.3 Hz, CH), 5.80 (1H, s, CH), 5.88 (1H, ddt, *J* = 17.3, 10.5, 5.5 Hz, CH), 7.14-7.16 (2H, m, 2 × CH); 7.23-7.36 (8H, m, 8 × CH)
δ_C $(CD_3)_2SO$)	43.8 (CH_2), 46.0 (CH_2), 47.6 (CH_2), 48.1 (CH_2), 65.1 (CH_2), 103.2 (CH), 118.2 (CH_2), 126.0 (CH), 127.2 (CH), 127.3 (CH), 127.5 (CH), 128.3 (CH), 128.7 (CH), 132.1 (CH), 136.6 (C), 137.0 (C),149.2 (C), 151.8 (C), 161.2 (C), 166.6 (C), 198.3 (C)
MS (CI)	865 (50%, $[2M+H]^+$), 455 (6%, $[M+Na]^+$) 433 (100%, $[M+H]^+$)
HRMS (CI)	$C_{25}H_{25}N_2O_5$ ($[M+H]^+$) requires 433.1758, found 433.1756

Preparation of 1,3-dibenzyl-6-methylpyrimidine-2,4 (98)[95]



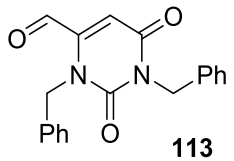
98

Acetic anhydride (15.4 g, 150.9 mmol, 3.36 eqv, 14.3 mL) was added slowly to a cooled (0 ˚C) solution of **92** (10.8 g, 44.9 mmol) in pyridine (200 mL) following which DMAP (5.49 g, 44.9 mmol, 1.0 eqv) was added in small portions and the mixture was heated (115 ˚C) for 5 hours. The reaction mixture was then cooled and the pyridine removed under reduced pressure to give a solid residue was dissolved in $CHCl_3$ (150 mL) and washed with HC1 (aqueous, 2N, 2 x 200 mL) and saturated sodium bicarbonate solution (2 x 60 mL). After drying ($MgSO_4$) and evaporation the residue was purified using column chromatography (10-50% ethyl acetate in petrol) to give **98** (10.7 g, 34.9 mmol) as a yellow oil in 78% yield.

This reaction was repeated twice: i) acetic anhydride (25.6 g, 280.0 mmol, 3.36 eqv. 26.38 mL), **92** (20.0 g, 83.23 mmol), pyridine (500 mL) and DMAP (10.17 g, 83.23 mmol, 1.0 eqv) to give **98** (19.87 g, 65 mmol, 78%): ii) acetic anhydride (7.14 g, 69.91 mmol, 3.36 eqv. 6.60 mL), **92** (5.0g, 20.81 mmol), pyridine (70 mL) and (2.54 g, 20.81 mmol, 1.0 eqv) to give **98** (4.85 g, 65.0 mmol, 76% yield).

Rf	0.25 (40% ethyl acetate in petrol);
δ_H ($CDCl_3$)	2.14 (3H, s, CH_3), 5.09 (2H, s, CH_2), 5.17 (2H, s, CH_2), 5.65 (1H, s, CH), 7.12-7.17 (2H, m, 2 × CH), 7.22-7.36 (6H, m, 6 × CH), 7.46-7.50 (2H, m, 2 × CH)..
δ_C ($CDCl_3$)	162.0 (C), 152.6 (C), 151.7 (C), 136.9 (C), 136.0 (C), 128.9 (2 × CH), 128.8 (2 × CH), 128.3 (2 × CH), 127.7 (2 × CH), 127.4 (CH), 126.0 (CH), 102.0 (CH), 47.9 (CH_2), 44.5 (CH_2), 19.8 (CH_3).
MS (CI)	307.1 (100%, $[M+H]^+$), 613.3 (20%, $[2M+H]^+$), 635.3 (10%, $[2M+Na]^+$)
HRMS (CI)	$C_{19}H_{19}N_2O_2$ ($[M+H]^+$) requires 307.1441, found 307.1441

Preparation of 1,3-dibenzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carbaldehyde (113)[96]



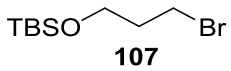
113

A mixture of **98** (10.7 g, 34.93 mmol) and SeO_2 (11.63 g, 104.8 mmol) was suspended in a mixture of THF (200 mL) and glacial acetic acid (200 mL) and heated at reflux for 24 h. After cooling the solution was filtered through a pad of Celite©, evaporated to dryness and the residue purified by silica gel chromatography (10-50% EtOAc in petrol) to give **113** (8.28 g, 35.0 mmol) as yellow oil in 74% yield.

This reaction was repeated three times: i) pyrimidine **98** (4.59 g, 15 mmol), SeO_2 (5.0 g, 45.0 mmol, 3.0 eqv), in THF (45 mL) and glacial acetic acid (45 mL) to give **116** (3.60 g, 11.2 mmol, 75%); ii) pyrimidine **113** (9.45 g, 30.85 mol), SeO_2 (10.3 g, 92.5 mmol, 3.0 eqv) in THF (90 mL) and glacial acetic acid (90 mL) to give **116** (7.60 g, 23.7 mmol, 77%); iii) pyrimidine **98** (3.0 g, 9.79 mmol), SeO_2 (3.26 g, 29.4 mmol, 3 eqv) in THF (50 mL) and glacial acetic acid (50 mL) to give **113** (2.33 g, 7.3 mmol, 74% yield)

Rf	0.23 (35% EtOAc/petrol)
δ_H ($CDCl_3$)	5.19 (2H, s, CH), 5.51 (2H, s, CH_2), 6.28 (1H, s, CH), 7.17-7.22 (2H, m, 2 × CH), 7.23-7.34 (6H, m, 6 × CH). 7.46-7.51 (2H, m, 2 × CH), 9.41 (1H, s, CH)
δ_C ($CDCl_3$)	185.6 (C), 161.6 (C), 152.0 (C), 144.6 (C), 136.4 (C), 136.4 (C), 129.1 (2 × CH), 128.8 (2 × CH), 128.6 (2 × CH), 128.0 (2 × CH), 127.9 (CH), 127.3 (CH), 114.1 (CH), 46.7 (CH_2), 45.1 (CH_2).
ν_{max}	3749, 3649, 3609, 3467, 2926, 2857, 2726, 1743, 1607, 1554, 1535, 1493, 1460, 1377, 1326, 1258, 1209, 1154, 1049, 824,
HRMS (CI)	$C_{19}H_{17}N_2O_3$ [M+H^+] requires 321.1234, found 321.1237

Preparation of (3-bromopropoxy)(*tert*-butyl)dimethylsilane (107)[94]

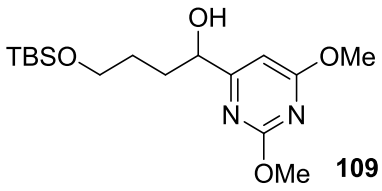


107

Alcohol, **105** (5.0 g, 36.0 mmol) was dissolved in cooled (0 °C) dry CH_2Cl_2 (70 mL) and imidazole (2.94 g, 43.2 mmol, 1.2 eqv) and DMAP (ca. 25 mg) were added and the mixture stirred. *tert*-Butyldimethylsilyl chloride (5.42 g, 36.0 mmol, 1 eqv) was then added and the mixture stirred at rt for 3 days. The mixture was then diluted with water, separated and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with water (3 × 30 mL), then dried ($MgSO_4$) and concentrated under reduced pressure to give an oil. This was dissolved in hexane and passed through a short plug of silica gel (ca. 2 cm) to give **107** (8.22 g, 32.46 mmol) as an oil in 90% yield.

δ_H ($CDCl_3$)	0.06 (6H, s, 2 × CH_3), 0.89 (9H, s, 3 × CH_3), 2.02 (2H, tt, *J* = 5.7, 6.5 Hz, CH_2), 3.50 (2H, t, *J* = 6.5 Hz, CH_2), 3.72 (2H, t, *J* = 5.7 Hz, CH_2)
δ_C ($CDCl_3$)	60.3 (CH_2), 35.5 (CH_2), 30.5 (CH_2), 25.9 (3 × CH_3), 18.2 (C), -5.4 (2 × CH_3)
ν_{max}	3749, 3749, 3648, 3470, 3098, 2985, 2960, 2906, 2875, 2670, 2583.

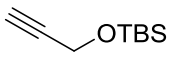
Attempted preparation of 4-((*tert*-butyldimethylsilyl)oxy)-1-(2,6-dimethoxypyrimidin-4-yl)butan-1-ol (109)



109

Bromide **108** (1.0 g, 3.95 mmol, 2.77 eqv.) was added to a suspension of magnesium turnings (0.148 g, 6.09 mmol) in dry THF (5 mL) and the mixture heated at reflux until the metal had largely reacted (ca 30 min). At this point the reaction was cooled (0 $^{\circ}$C) and the aldehyde **11** (0.24 g, 1.43 mmol) was added as a solution in dry THF (2 mL). After stirring to rt over 12 h, the reaction was diluted with ether (30 mL) and quenched with saturated NH_4Cl (100 mL). After separation, the organic layer was washed with brine (2 × 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Purification of the residue on silica gel chromatography (gradient elution; 10-100% EtOAc in petrol) gave no evidence for the formation of **109**, but gave instead the previously prepared alcohol **103** (0.116 g, 0.68 mmol) as a solid in 48% yield. Data was identical to that previously prepared (See page 46)

Preparation of *tert*-butyldimethyl(prop-2-yn-1-yloxy)silane (119).[97]



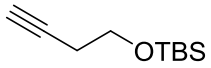
119

Alcohol **118** (8.0 g, 142.7 mmol, 1.0 eqv.) was dissolved in dry CH_2Cl_2 (50 mL) and cooled (0 °C) whereupon imidazole (19.43 g, 285.4 mmol, 2 eqv.) and *tert*-butyldimethylsilyl chloride (21.5 g, 142.7 mmol) were added and the reaction mixture stirred to rt overnight. The reaction was diluted with cold water 50 mL and filtered through a pad of celite, which was washed with further portions of CH_2Cl_2. After separation of the organic phase the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL) and the combined extracts washed with water (50 mL) and brine (50 mL) then dried ($MgSO_4$) and evaporated under reduced pressure to give and oil.This was dissolved in hexane and passed through a short plug of silica gel (ca. 2 cm) to give **119** (22.0 g, 129.2 mmol) in 91% yield as a colourless oil.

This reaction was repeated using **118** (5.0 g, 89.2 mmol, 1.0 eqv.), CH_2Cl_2 (35 mL), imidazole (12.14 g, 178.4 mmol, 2 eqv.) and *tert*-butyldimethylsilyl chloride to give **119** (13.77 g, 81.0 mmol, 91%).

δ_H ($CDCl_3$)	0.09 (6H, s, 2 × CH_3), 0.87 (9H, s, 3 × CH_3), 2.34 (1H, t, *J* = 2.4 Hz, CH), 4.26 (2H, d, *J* = 2.4 Hz, CH_2)
δ_C ($CDCl_3$)	82.3 (C), 72.8 (CH), 51.4 (CH_2), 22.7 (3 × CH_3), 18.2 (C), -5.3 (2 × CH_3).

Preparation of (but-3-yn-1-yloxy)(*tert*-butyl)dimethylsilane (121)[98]

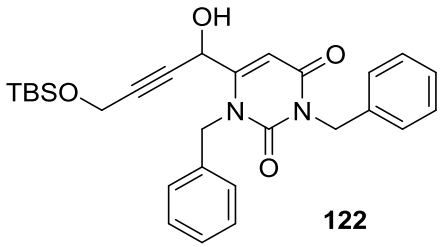


121

Following a general literature procedure:[98] Alcohol **120** (8.0 g, 114.1 mmol, 1.0 eqv.) was dissolved in dry CH_2Cl_2 (50 mL) and cooled (0 ˚C) whereupon imidazole (18.92 g, 171.2 mmol, 2 eqv.) and *tert*-butyldimethylsilyl chloride (18.92 g, 125.6 mmol 1.1 eqv.) were added and the reaction mixture stirred to rt overnight. The reaction was diluted with cold water 50 mL and filtered through a pad of celite, which was washed with further portions of CH_2Cl_2. After separation of the organic phase the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL) and the combined extracts washed with water (50 mL) and brine (50 mL) then dried ($MgSO_4$) and evaporated under reduced pressure to givean oil.This was dissolved in hexane and passed through a short plug of silica gel (ca. 2 cm) to give **121** (17.0 g, 92.21 mmol) in 81% yield as a colourless oil.

δ_H ($CDCl_3$)	0.07 (6H, s, 2 × CH_3), 0.89 (9H, s, 3 × CH_3), 1.95 (1H, t, J = 2.6 Hz, CH), 2.39 (2H, dt, J = 2.6, 7.1 Hz, CH_2), 3.73 (2H, t, J = 7.1 Hz, CH_2)
δ_C ($CDCl_3$)	81.5 (C), 69.3 (CH), 61.7 (CH_2), 25.9 (3 × CH_3), 22.8 (CH_2), 18.3 (C), -5.3 (2 × CH_3)

Preparation of 1,3-dibenzyl-6-(4-((*tert*-butyldimethylsilyl)oxy)-1-hydroxybut-2-yn-1-yl)pyrimidine-2,4(1H,3H)-dione (122)



122

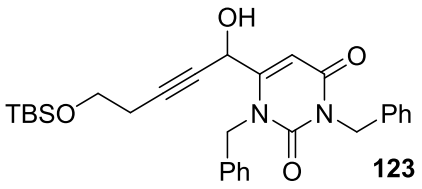
Alkyne **119** (1.35 g, 7.93 mmol, 2.0 eqv) dissolved in dry THF (1 mL) was added drop wise over 10 min to a cooled (-40 °C) solution of EtMgBr in THF (1M, 6.0 mL 6.0 mmol, 1.5 eqv) and the resulting mixture stirred for 1 h. The reaction was then cooled (– 78 °C) and a solution of aldehyde **113** (1.27 g, 3.96 mmol) in dry THF (1 mL) was then added and the mixture was stirred at this temperature for 3 h. After warming to rt overnight, NH_4Cl solution (saturated. aq. 20 mL) was added and the reaction extracted with EtOAc (3 × 50 mL), following which the combined organic extracts were washed with brine (50 mL) and water (50 mL) then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (EtOAc/petrol 30-50%) to give **122** (1.52 g, 3.10 mmol) as a colourless oil in 78% yield.

This reaction was repeated 4 times: i) alkyne **119** (1.22 g, 7.2 mmol, 2.0 eqv), EtMgBr in THF (1M, 5.4 mL 5.4 mmol) and aldehyde **113** (1.15 g, 15.61 mmol) gave **122** (1.38 g, 2.81 mmol, 78%); ii) alkyne **119** (2.96 g, 17.36 mmol, 2.0 eqv), EtMgBr in THF (1M, 16.5 mL 16.5 mmol, 19 eqv) and aldehyde **113** (2.78 g, 8.68 mmol) gave **122** (3.30 g, 6.7 mmol, 78%); iii) alkyne **119** (5.30 g, 31.2 mmol, 2.0 eqv), EtMgBr in THF (1M, 29.7 mL 29.7 mmol, 1.9 eqv) and aldehyde **113** (5.00 g, 15.6 mmol) stirring for 5 h (- 78 °C) gave **122** (5.78 g, 11.8 mmol, 75%); iv) alkyne **119** (4.25 g, 25.0 mmol, 2.0 eqv), EtMgBr in THF (1M, 23.7 mL 23.7 mmol, 1.9 eqv) and aldehyde **113** (4.00 g, 12.50 mmol) gave **122** (4.80 g, 9.80 mmol, 78%).

Rf 0.27 (30% EtOAc/petrol)

δ_H ($CDCl_3$)	0.07 (6H, s, 2 × CH_3), 0.87 (9H, s, 3 × CH_3), 3.43 (1H, d, *J* = 6.8 Hz, OH), 4.28 (2H, d, *J* = 0.6 Hz, CH_2), 5.11-5.18 (3H, m, CH, CH_2), 5.28 (1H, d, *J* = 16.6 Hz, CH), 5.35 (1H, d, *J* = 16.6 Hz, CH), 6.25 (1H, s, CH) 7.11-7.13 (2H, m, 2 × CH), 7.22-7.36 (6H, m, 6 × CH) and 7.45-7.47 (2H, m, 2 × CH)
δ_C ($CDCl_3$)	162.6 (C), 152.4 (C), 152.2 (C), 136.6 (C), 136.1 (C), 129.1 (2 x CH), 129.0 (2 x CH), 128.4 (2 × CH), 127.8 (CH), 127.7 (CH), 125.9 (2 × CH), 100.7 (CH), 87.8 (C), 79.8 (C), 60.3 (CH), 51.5 (CH_2), 47.3 (CH_2), 44.7 (CH_2), 25.7 (3 × CH_3), 18.2 (C) and -5.3 (2 × CH_3)
ν_{max}	3419, 3032, 2954, 2928, 2885, 2856, 1691, 1646, 1604, 1585, 1496, 1454, 1400, 1361, 1339, 1100, 1073, 1053, 833, 697
MS(CI)	491.2 (100%, $[M+H]^+$), 513. 2 (25%, $[M+Na]^+$) 918.5 (25%, $[2M+H]^+$)
HRMS (CI)	$C_{28}H_{35}N_2O_4Si$ ($[M+H]^+$) requires 490.2361, found 490.2350

Preparation of 1,3-dibenzyl-6-(5-((*tert*-butyldimethylsilyl)oxy)-1-hydroxypent-2-yn-1-yl)pyrimidine-2,4(1H,3H)-dione (123)



123

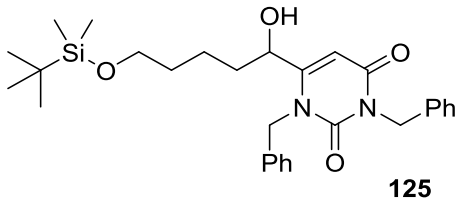
A solution of EtMgBr in THF (0.9 M, 7.71 mL 6.94 mmol, 1.9 eqv) was added over 10 min to a stirred and cooled (–40 °C) solution of **121** (1.35 g, 7.30 mmol, 2.0 eqv.) in dry THF (5 mL) and the resulting mixture was stirred for 6 hour at rt. At this point the reaction was cooled (–78° C) and a solution of aldehyde **113** (1.17 g, 3.65 mmol, 1.0 eqv) dissolved in dry THF (3 mL) was added by syringe and the mixture was stirred for 1 h. After warming to rt overnight, a solution of NH_4Cl (aq. sat. 50 mL) was added and the mixture extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine (50 mL) and water (50 mL) then dried ($MgSO_4$) and evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel (EtOAc/petrol 20-40%) to give **123** (0.935 g, 1.85 mmol) in 51% as a yellow semi-solid.

This reaction was repeated twice: i) EtMgBr in THF (0.9 M, 16.7 mL 16.7 mmol, 1.9 eqv), **121** (2.91 g 15.80 mmol, 2.0 eqv.), THF (5 mL) and aldehyde **113** (2.53 g, 7.90 mmol, 1.0 eqv) gave **123** (2.51 g, 5.00 mmol, 63%); ii) EtMgBr in THF (0.9 M, 7.71 mL 6.94 mmol, 1.9 eqv), **121** (1.35 g 7.30 mmol, 2.0 eqv.), THF (5 mL) and **113** (1.17 g, 3.65 mmol, 1.0 eqv) gave **123** (1.22g, 2.40 mmol, 66%).

Rf	0.32 (35% EtOAc in petrol)
δ_H ($CDCl_3$)	0.02 (6H, s, 6 × CH_3), 0.85 (9H, s, 9 × CH_3), 2.36 (2H, dt, *J* = 1.8, 6.9 Hz, CH_2), 3.42 (1H, d, *J* = 6.0 Hz, OH), 3.64 (2H, t, *J* = 6.9 Hz, CH_2), 5.07 (1H, m, CH), 5.12 (2H, s, CH_2), 5.31 (2H, s, CH_2), 6.22 (1H, s, CH), 7.10-7.12 (2H, m, 2 × CH), 7.20-7.35 (6H, m, 6 × CH) and 7.43-7.46 (2H, m, 2 × CH)

δ_C ($CDCl_3$)	162.7 (C), 153.0 (C), 152.4 (C), 136.6 (C), 136.2 (C), 129.0 (2 × CH), 128.9 (2 × CH), 128.4 (2 × CH), 127.7 (CH), 127.6 (CH), 125.9 (2 × CH), 100.3 (CH), 87.3 (C), 76.6 (C), 61.1 (CH_2), 60.3 (CH), 47.3 (CH_2), 44.7 (CH_2), 25.8 (3 × CH_3), 23.0 (CH_2), 18.2 (C), -5.4 (2 × CH_3)
ν_{max}	3414, 3089, 3065, 3033, 2954, 2923, 2851, 1711, 1655, 1649, 1585, 1496, 1464, 1389, 1349, 1205, 1130, 1005, 836, 779, 729, 696, 601
m/z (CI)	505.3 (100%, $[M+H]^+$), 1009.5 (25%, $[2M+H]^+$)
HRMS (CI)	$C_{29}H_{37}N_2O_4Si$ ($[M+H]^+$) requires 505.2517, found 505.2507

Preparation of 1,3-dibenzyl-6-(5-((*tert*-butyldimethylsilyl)oxy)-1-hydroxypentyl) pyrimidine-2,4(1H,3H)-dione (125)



125

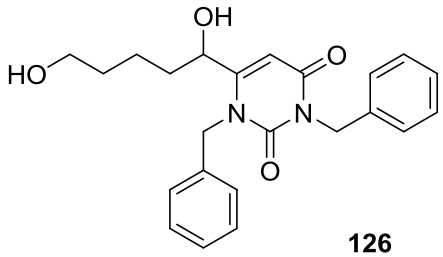
Palladium supported on activated carbon (10%, 0.66 g) was added to a solution of alkyne **123** (0.66 g, 1.22 mmol) in EtOAc (4 mL) under an argon blanket in a 25 mL RBF. The flask was purged with hydrogen gas (balloon) mixture and the mixture vigorously stirred under the hydrogen atmosphere for 2 h. The mixture was then filtered through a Celite© pad which was washed with further portions of EtOAc and the filtrate concentrated by evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (10-20% EtOAc/petrol) to give **125** (0.428 g, 0.84 mmol) as a colorless oil in 64% yield.

This reaction was repeated twice: i) Pd/C (10%, 2.0g), and alkyne **123** (2.0 g, 4.08 mmol) in EtOAc (10 mL) gave **125** (1.42 g, 2.90 mmol, 70%); ii) Pd/C (10%, 2.0 g) alkyne **123** (2.77 g, 5.64 mmol) in EtOAc (10 mL) gave **125** (2.00 g, 4.04 mmol, 72%).

Rf	0.23 (20 % EtOAc/petrol)
δ_H ($CDCl_3$)	0.03 (6H, s, 2 × CH_3), 0.88 (9H, s, 9 × CH_3), 1.34-1.53 (4H, m, 2 × CH_2), 1.54-1.66 (2H, m, CH_2), 3.13 (1H, d, *J* = 5.2 Hz, OH), 3.56 (2H, t, *J* = 5.0 Hz, CH_2), 4.38-4.46 (1H, m, CH), 4.98 (1H, d, *J* = 16.3 Hz, CH), 5.10 (1H, d, *J* = 13.7 Hz, CH), 5.15 (1H, d, *J* = 13.7 Hz, CH), 5.31 (1H, d, *J* = 16.3 Hz, CH), 5.99 (1H, s, CH), 7.08-7.10 (2H, m, 2 × CH), 7.19-7.37 (6H, m, 6 × CH) and 7.43-7.46 (2H, m, 2 × CH)
δ_C ($CDCl_3$)	162.8 (C), 157.7 (C), 152.5 (C), 136.7 (C), 136.2 (C), 129.0 (2 × CH), 128.9 (2 × CH), 128.4 (2 × CH), 127.7 (CH), 127.6 (CH), 125.8 (2 x CH), 99.1 (CH), 68.6 (CH), 62.7 (CH_2), 47.2

	(CH_2), 44.6 (CH_2), 35.8 (CH_2), 31.9 (CH_2), 25.9 (3 × CH_3), 22.3 (CH_2), 18.3 (C), -5.3 (2 × CH_3)
ν_{max}	3387, 3065, 3033, 2953, 2930, 2857, 1702, 1654, 1496, 1453, 1393, 1348, 1255, 1215, 1192, 1099, 1030, 1005, 937, 835, 776, 733, 697, 668
m/z (CI)	509.3 (100%, $[M+H]^+$), 1017.6 (20%, $[2M+H]^+$)
HRMS (CI)	$C_{29}H_{41}N_2O_4Si$ ($[M+H]^+$) requires 509.2830, found 509.2819

Preparation of 1,3-dibenzyl-6-(1,5-dihydroxypentyl)pyrimidine-2,4(1H,3H)-dione (126)

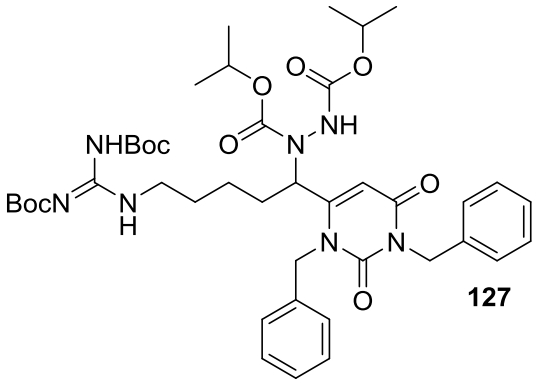


126

A solution of TBAF (1 M, 0.943 mL, 0.943 mmol, 1.5 eqv) in THF was added to a cooled (0 °C) solution of silane **125** (0.32 g, 0.629 mmol, 1.0 eqv.) in dry THF (2 mL) and the mixture stirred for 2h. After warming to rt overnight, the solvent was removed under reduced pressure the residue purified by flash chromatography on silica gel (gradient elution; 50-100% EtOAc in petrol) to give **126** (0.19 g, 0.482 mmol) as a colourless oil in 77% yield.

Rf	0.28 (EtOAc)
δ_H ($CDCl_3$)	1.33-1.62 (6H, m, 3 × CH_2), 3.00 (2 H, br s, 2 × OH), 3.46-57 (2H, m, CH_2), 4.42-4.46 (1H, m, CH), 5.01 (1H, d, *J* = 16.4 Hz, CH), 5.10 (1H, d, *J* = 13.9 Hz, CH), 5.15 (1H, d, *J* = 13.9 Hz, CH), 5.29 (1H, d, *J* = 16.4 Hz, CH), 5.99 (1H, s, CH), 7.03-7.17 (2H, m, 2 × CH), 7.18-7.36 (6H, m, 6 × CH) and 7.41-7.50 (2H, m, 2 × CH)
δ_C ($CDCl_3$)	163.1 (C), 158.1 (C), 152.5 (C), 136.6 (C), 136.2 (C), 129.0 (2 × CH), 128.9 (2 × CH), 128.4 (2 × CH), 127.7 (CH), 127.6 (CH), 125.9 (2 × CH), 98.9 (CH), 68.4 (CH), 62.1 (CH_2), 47.2, (CH_2) 44.7 (CH_2), 35.7 (CH_2), 31.5 (CH_2), 22.1 (CH_2)
ν_{max}	3386, 3032, 2940, 2878, 1702, 1652, 1496, 1453, 1396, 1216, 1073, 910, 835, 755, 732, 697
m/z (CI)	395.2 (100%, $[M+H]^+$), 789.4 (30%, $[2M+H]^+$)
HRMS (CI)	$C_{23}H_{27}N_2O_4$ ($[M+H]^+$) requires 396.1565, found 396.1565

Preparation of 1,1'-(1-(1,3-dibenzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)pentane-1,5-diyl)-di-(2,3-bis(*tert*-butoxycarbonyl))diguanidine (127)

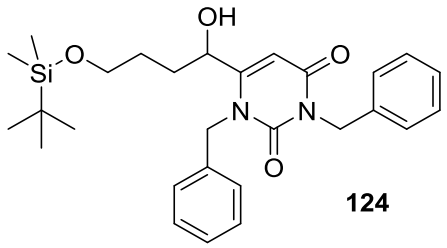


127

DIAD (67.1 mg, 0.332 mmol, 1.1 eqv.) was added over 10 min to a cooled (0 ˚C.) and stirred mixture of **126** (119.0 mg 0.302 mmol), PPh_3 (95.0 mg, 0.362 mmol, 1.2 eqv.), and *N*,*N*'-di-Boc-guanidine (38.0 mg, 0.362 mmol, 1.2 eqv.) dissolved in dry THF (2 mL). After stirring to rt overnight a small drop of water was added and the solution evaporated under reduced pressure and purified by flash chromatography on silica gel (30-40% EtOAc in petrol) to give impure **127** (201.0 mg, 0.24 mmol) as a white solid in 81% yield.

δ_H ($CDCl_3$)	0.08-1.75 (36H, m, 3 × CH_2, 10 × CH_3), 3.58-80 (2H, m, CH_2), 4.60-5.30 (7H, 3 × CH, 2 × CH_2), 5.60-5.90 (2H, m, CH, NH), 7.00-7.60 (10H, m, 10 × CH) and 9.00-9.50 (2H, m, 2 × NH)
δ_C ($CDCl_3$)	Partial (from COSY): 102.76 (CH) 55.4 (NCH), 44.6 (NCH_2)
m/z (CI)	822.4 (100%, $[M+H]^+$), 844.4 (15% $[M+Na]^+$)
HRMS (CI)	$C_{42}H_{59}N_7O_{10}$ ($[M+H]^+$) requires 822.4396, found 822.4390

Preparation of 1,3-dibenzyl-6-(4-((*tert*-butyldimethylsilyl)oxy)-1-hydroxybutyl) pyrimidine-2,4(1H,3H)-dione (124)



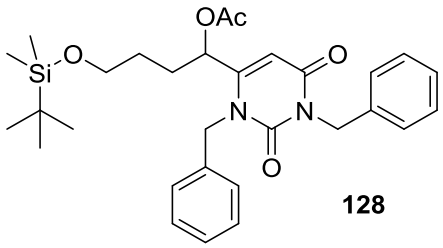
124

Palladium supported on activated carbon (10%, 1.49 g) was added to a solution of alkyne **122** (1.49 g, 3.04 mmol, 1.0 eqv.) in EtOAc (5 mL) under an argon blanket in a 50 mL RBF. The flask was then purged with hydrogen gas (balloon) and the mixture vigorously stirred under the hydrogen atmosphere for 2 h. The mixture was then filtered through a Celite© pad which was washed with further portions of EtOAc and the filtrate concentrated by evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (20-30% EtOAc/petrol) to give **124** (1.10 g, 2.22 mmol) as a white solid in 73% yield.

Rf	0.23 (30% EtOAc/petrol)
Mp	80-82 °C
δ_H ($CDCl_3$)	0.06 (3H, s, CH_3), 0.07 (3H, s, CH_3) 0.89 (9H, s, 3 × CH_3), 1.48-1.88 (4H, m, 2 × CH_2), 3.55-3.67 (2H, m, CH_2), 4.44-4.49 (1H, m, CH), 4.66 (11H, br s, OH), 5.01 (1H, d, *J* = 16.4 Hz, CH), 5.10 (1H, d, *J* = 13.8 Hz, CH), 5.15 (1H, d, *J* = 13.8 Hz, CH), 5.33 (1H, d, *J* = 16.4 Hz, CH), 6.04 (1H, s, CH), 7.10-7.12 (2H, m, 2 × CH), 7.20-7.35 (6H, m, 6 × CH) and 7.43-7.47 (2H, m, 2 × CH)
δ_C ($CDCl_3$)	162.7 (C), 157.8 (C), 152.4 (C), 136.6 (C), 136.2 (C), 128.8 (2 × CH), 128.7 (2 × CH), 128.2 (2 × CH), 127.5 (CH), 127.4 (CH), 125.7 (2 × CH), 98.9 (CH), 68.1 (CH), 62.5 (CH_2), 47.0 (CH_2), 44.4 (CH_2), 33.6 (CH_2), 28.8 (CH_2), 25.7 (3 × CH_3), 18.1 (CH), -5.6 (2×CH_3)

ν_{max}	3405, 3065, 3033, 2954, 2928, 2856, 1702, 1652 1452, 1392, 1345, 1254, 1215, 1187, 1096, 1030, 834, 776, 696.
m/z (CI)	495.3 (100%, $[M+H]^+$), 989.5 (20%, $[2M+H]^+$) 1011.5 (4%, $[2M+Na]^+$)
HRMS (CI)	$C_{28}H_{39}N_2O_4Si$ ($[M+H]^+$) requires 494.2674, found 494.2666

Preparation of 4-((*tert*-butyldimethylsilyl)oxy)-1-(1,3-dibenzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)butyl acetate (128)



128

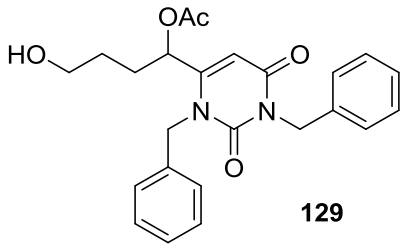
Acetic anhydride (0.303 g, 3.82 mmol, 3.7 eqv.) was added drop wise to a cooled (0˚C) and stirred mixture of alcohol **124** (0.49 g, 0.99 mmol), pyridine (311.0 mg, 3.86 mmol, 3.9 eqv.) and DMAP (12.0 mg) in dry CH_2Cl_2 (3 mL). The mixture was warmed to rt and stirred for 1 h, whereupon HCl (aqueous. 2 M, 20 mL) was added the mixture stirred for 20 min. The mixture was the extracted with CH_2Cl_2 (3 x 50 mL) and the combined extracts washed with 2M HCl (aqueous, 3 × 10 mL) and water (20mL). After drying ($MgSO_4$) and evaporated under reduced pressure, column chromatography on silica gel gave **128** (0.40 g, 0.745 mmol) as a white solid in 75% yield.

This reaction was repeated three times: i) Ac_2O (336.0 mg, 3.30 mmol, 3.7 eqv.), **124** (440.0 mg, 0.89 mmol), pyridine (274.4 mg, 3.50 mmol, 3.9 eqv.) and DMAP (12.0 mg) in CH_2Cl_2 (2 mL) gave **128** (0.451 g, 0.86 mmol, 94%). ii) Ac_2O (370.0 mg, 3.46 mmol, 3.7 eqv.), **124** (490.0 mg, 0.99 mmol), pyridine (305.6 mg, 3.86 mmol, 3.9 eqv.) and DMAP (12.0 mg) in CH_2Cl_2 (4 mL) gave **128** (0.400 g, 0.75 mmol, 75%). ii) Ac_2O (717.0 mg, 7.00 mmol, 3.7 eqv.), **124** (0.94 g, 1.90 mmol), pyridine (586.0 mg, 7.40 mmol, 3.9 eqv.) and DMAP (16 mg) in CH_2Cl_2 (4 mL) gave **128** (1.00 g, 1.86 mmol, 98%).

Rf	0.27 (20% EtOAc/petrol), 0.43 (30% EtOAc/petrol)
δ_H ($CDCl_3$)	0.00 (6H, s, 2 × CH_3), 0.86 (9H, s, 3 × CH_3), 1.21-1.36 (1H, m, CH), 1.41-1.55 (2H, m, CH_2), 1.58-1.71 (1H, m, CH), 2.02 (3H, s, CH_3), 3.42 (2H, t, *J* = 6.0 Hz, CH_2), 5.11-5.20 (3H, m,

	CH, CH_2), 5.29 (1H, d, *J* = 16.3 Hz, CH), 5.49 (1H, dd, *J* = 9.5, 2.3 Hz, CH), 5.82 (1H, s, CH), 7.20-7.51 (10H, m, 2 ×Ph)
δ_C ($CDCl_3$)	170.1 (C), 162.3 (C), 155.2 (C), 152.4 (C), 136.6 (C), 135.9 (C), 129.1 (2 × CH), 128.9 (2 × CH), 128.4 (2 × CH), 127.9 (CH), 127.6 (CH), 126.4 (2 × CH), 99.3 (CH), 69.8 (CH), 61.7 (CH_2), 47.8 (CH_2), 44.7 (CH_2), 31.0 (CH_2), 28.5 (CH_2), 25.9 (3 × CH_3), 20.6 (CH_3), 18.2 (C), -5.4 (2 × CH_3)
ν_{max}	3362, 3089, 3065, 3033, 2955, 2929, 2855, 2856, 1748, 1708, 1666, 1624, 1607, 1586, 1496, 1452, 1395, 1360, 1248, 1222, 1156, 1098, 1049, 1030, 977, 938, 834, 777, 760, 728, 697, 665
m/z (CI)	537.3 (100%, $[M+H]^+$), 1073.6 (60%, $[2M+H]^+$)
HRMS (CI)	$C_{30}H_{41}N_2O_5Si$ ($[M+H]^+$) requires 537.2779, found 537.2769,

Preparation of 1-(1,3-dibenzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-hydroxybutyl acetate (129)



129

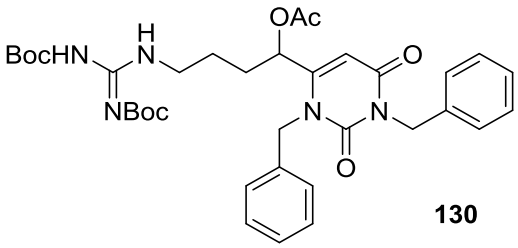
A solution of TBAF (1 M, 3.02 mL, 3.02 mmol, 1.5 eqv) in THF was added to a cooled (0 ˚C) solution of silane **128 (**1.08 g, 2.01 mmol, 1.0 eqv.) in dry THF (2 mL) and the mixture stirred for 2h. After warming to rt overnight, the solvent was removed under reduced pressure the residue purified by flash chromatography on silica gel (gradient elution, 35-40% EtOAc in petrol) to give **129** (0.62 g, 1.47 mmol) as a colorless oil in 73% yield.

This reaction was repeated twice: i) TBAF (1 M, 1.1 mL, 1.1 mmol, 1.5 eqv) and **128** (0.40 g, 0.745 mmol, 1.0 eqv.) in dry THF (7 mL) gave **129** (0.28 g, 0.66 mmol, 89%); ii) TBAF (1 M, 3.1 mL, 3.1 mmol, 1.5 eqv) and **128** (1.10 g, 2.05 mmol, 1.0 eqv.) in dry THF (7 mL) gave **129** (0.69 g, 1.63 mmol, 80%).

Rf	0.35 (60% EtOAc in petrol)
δ_H ($CDCl_3$)	1.50-1.69 (3H, m, C<u>H</u>H, CH_2), 1.70-1.83 (1H, m, C<u>H</u>H) 1.98 (3H, s, CH_3), 3.10 (1H, br s, OH), 3.99 (1H, t, *J* = 6.0 Hz, CH_2), 4.43-4.50 (1H, m, CH), 4.98 (1H, d, *J* = 16.8 Hz, CH), 5.10 (1H, d, *J* = 13.8 Hz, CH), 5.15 (1H, d, *J* = 13.8 Hz, CH), 5.30 (1H, d, *J* = 16.8 Hz, CH), 6.00 (1H, s, CH), 7.06-7.11 (2H, m, 2 × CH), 7.23-7.33 (6H, m, 6 × CH), 7.39-7.44 (2H, m, 2 × CH)
δ_C ($CDCl_3$)	171.19 (C), 162.9 (C), 157.9 (C), 152.4 (C), 136.5 (C), 136.1 (C), 129.0 (2 × CH), 128.8 (2 × CH), 128.4 (2 × CH), 127.8 (CH), 127.6 (CH), 125.8 (2 × CH), 99.0 (CH), 68.0 (CH), 63.6 (CH_2), 47.1 (CH_2), 44.6 (CH_2), 32.5 (CH_2), 24.8 (CH_2), 20.8 (CH_3)

ν_{max}	3417, 2962, 1736, 1702, 1652, 1496, 1451, 1392, 1349, 1243, 1031, 832, 731, 696
m/z (CI)	423.2 (100%, $[M+H]^+$), 845.4 (28%,$[2M+H]^+$) 867.4 (18%, $[2M+Na]^+$)
HRMS (CI)	$C_{24}H_{27}N_2O_5$ ($[M+H]^+$) requires 423.1914, found 423.1917

Preparation of 4-(2,3-bis(*tert*-butoxycarbonyl)guanidino)-1-(1,3-dibenzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)butyl acetate (130)



130

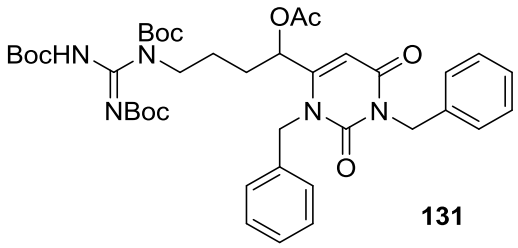
DIAD (0.19 g, 0.95 mmol, 2 eqv.) was added drop wise over 10 min to a cooled (0 ˚C) and stirred solution of alcohol **129** (0.20 g, 0.47 mmol, 1.0 eqv.), PPh_3 (0.248 g, 0.95 mmol, 2 eqv.), and *N*,*N*'-di-Boc-guanidine (0.246 g, 0.95 mmol, 2 eqv.) in dry THF (3 mL). After stirring to rt overnight, a drop of water was added and the solution was evaporated under reduced pressure. The crude product was then purified by flash chromatography on silica gel (30-40% EtOAc/petrol) to give **130** (0.06 g, 0.09 mmol) as a white solid in 19% yield.

This reaction was repeated twice: i) DIAD (0.19 g, 0.95 mmol, 2 eqv.), **129** (0.20 g, 0.47 mmol, 1.0 eqv), PPh_3 (0.25 g, 0.95 mmol, 2 eqv.) and *N*,*N*'-di-Boc-guanidine (0.246 g, 0.95 mmol, 2 eqv.) in dry THF (3 mL) gave **130** (0.06 g, 0.09 mmol, 19%); DIAD (71.8 mg, 0.355 mmol, 1.5 eqv.) **129** (100.0 mg, 0.237 mmol, 1.0 eqv.), PPh_3 (93.1mg, 0.355 mmol, 1.5 eqv.) and *N*,*N*'-di-Boc-guanidine (170.0 mg, 0.473 mmol, 2 eqv.) in dry THF (3 mL) gave **130** (40.0 mg, 0.06 mmol) as a white solid in 26% yield.

Rf	0.24 (30% EtOAc in petrol)
δ_H ($CDCl_3$)	1.37 (9H, m, 3 × Me), 1.40-1.50 (1H, m, CHH), 1.51 (9H, m, 3 × Me), 1.59-1.57 (1H, m, CHH), 1.86-2.05 (2H, m, CH_2), 2.03 (3H, s, CH_3), 4.02 (2H, t, *J* = 6.0 Hz CH_2), 5.13 (1H, d, *J* = 17.0 Hz, CH), 5.20 (2H, s, CH_2), 5.30 (1H, d, *J* = 17.0 Hz, CH), 5.91 (1H, s, CH), 6.52 (1H, t, *J* = 7.2 Hz, CH), 6.88 (1H, d, *J* = 6.5 Hz, CH), 7.15-7.31 (7H, m, 7 × CH), 7.45-7.49 (2H, m, 2 × CH), 8.56 (1H, br s, NH), 8.90 (1H, br s, NH)

δ_C ($CDCl_3$)	170.8 (C), 162.9 (C), 162.0 (C), 159.3 (C), 153.6 (C), 153.4 (C), 152.7 (C), 136.9 (C), 136.0 (C), 128.9 (2 × CH), 128.5 (2 × CH), 128.4 (2 × CH), 127.6 (CH), 126.9 (CH), 124.7 (2 × CH), 103.9 (CH), 86.0 (C), 79.2 (C), 63.6 (CH_2), 51.4 (CH), 48.4 (CH_2), 44.4 (CH_2), 28.2 (CH_2), 28.0 (3 × CH_3), 28.0 (3 × CH_3), 24.6 (CH_2), 20.9 (CH_3)
ν_{max}	3391, 2978, 1704, 1663, 1614 1444, 1367, 1308 1243, 1142, 1106, 1045
m/z (CI)	686.3 (12%, $[M+Na]^+$), 664.3 (8%, $[M+H]^+$), 464.2 (100% $[M-2Boc+H]^+$)
HRMS (CI)	$C_{35}H_{45}N_5O_8Na$ ($[M+Na]^+$) requires 663.3160, found 663.3143

Preparation of 1-(1,3-dibenzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-(1,2,3-tris(*tert*-butoxycarbonyl)guanidino)butyl acetate (131)



131

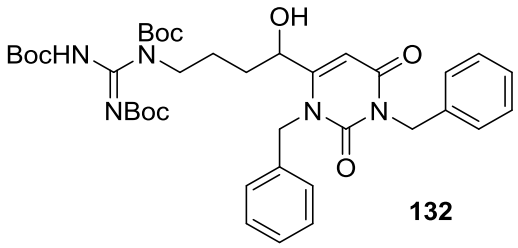
DIAD (0.15 g, 0.762 mmol, 1.5 eqv.) was added drop wise over 20 min to a cooled (0 ˚C) and stirred solution of alcohol **129** (0.21g, 0.497 mmol, 1.0 eqv.), PPh_3 (0.20 g, 0.76 mmol, 1.53 eqv.), and *N,N′,N″*-tri-Boc-guanidine (0.36 g, 1.00 mmol, 2.02 eqv.) in dry THF (3 mL). After stirring to rt overnight further PPh_3 (0.065 g, 0.248 mmol, 0.5 eqv.) and DIAD (0.050g, 0.249 mmol, 0.5 eqv.) were added and the reaction was stirred for a further 3 h. At this point a drop of water was added and the solution was evaporated under reduced pressure. The crude product was then purified by flash chromatography on silica gel (20-30% EtOAc/petrol) to give **131** (0.146 g, 0.20 mmol) as a white solid in 39% yield.

This reaction was repeated twice: i) Alcohol **129** (0.30 g, 0.734 mmol), PPh_3 (0.37g, 1.42mmol, 1.93 eqv.), *N,N',N''*-tri-Boc-guanidine (0.51 g, 1.42 mmol, 1.93 eqv.) and DIAD (0.287 g, 1.42 mmol, 2 eqv.) gave **131** (0.193 g, 0.253 mmol, 34%): ii) Alcohol **129** (0.63 g, 1.49 mmol), PPh_3 (0.78 g, 2.97, 1.99 eqv.) and *N,N',N''*-tri-Boc-guanidine (1.06 g, 2.95 mmol, 1.99 eqv.) and DIAD (0.60 g, 2.97 mmol, 1.99 eqv.) gave **131** (0.43 g, 0.563 mmol, 38%).

Rf	0.20 (20% EtOAc/petrol), 0.50 (30% EtOAc/petrol)
Mp	42- 45 ˚C
δ_H ($CDCl_3$)	1.37 (9H, m, 3 × Me), 1.44 (9H, m, 3 × Me), 1.13-1.40 (2H, m, CH_2), 1.51 (9H, m, 3 × Me), 1.85-2.01 (1H, m, CHH), 2.07-2.19 (1H, m, CHH), 1.97 (3H, s, CH_3), 3.80 (2H, t, *J* = 6.3 Hz CH_2), 5.00 (1H, d, *J* = 17.0 Hz, CH), 5.11 (1H, d, *J* = 13.8 Hz, CH), 5.23 (1H, d, *J* = 13.8 Hz, CH), 5.33 (1H, t, *J* =

	7.5 Hz, CH), 5.70 (1H, d, *J* = 17.0 Hz, CH), 5.99 (1H, s, CH), 7.21-7.34 (8H, m, 8 × CH), 7.38-7.44 (2H, m, 2 × CH), 10.40 (1H, s, NH)
δ_C ($CDCl_3$)	170.8 (C), 161.9 (C), 152.7 (C), 151.9 (C), 151.6 (C), 151.3 (C), 150.8 (C), 148.7 (C), 137.1 (C), 136.9 (C), 128.9 (2 × CH), 128.5 (2 × CH), 128.4 (2 × CH), 127.6 (CH), 127.4 (CH), 126.5 (2 × CH), 104.2 (CH), 83.9 (C), 83.05 (C), 81.95 (C), 63.3 (CH_2), 55.4 (CH), 47.3 (CH_2), 44.6 (CH_2), 29.3 (CH_3), 27.9 (3 × CH_3), 27.8 (6 × CH_3), 24.8 (CH_2), 20.8 (CH_3)
ν_{max}	3401, 2947, 2917, 1759, 1739, 1706, 1667, 1621, 1448, 1369, 1249, 1128
m/z (CI)	764.4 (75%, $[M+H]^+$), 647.3 (100%)
HRMS (CI)	$C_{40}H_{54}N_5O_{10}$ ($[M+H]^+$) requires 764.3865, found 764.3864

Preparation of 1-(1,3-dibenzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-(1,2,3-tris(*tert*-butoxycarbonyl)guanidino)butyl acetate (132)



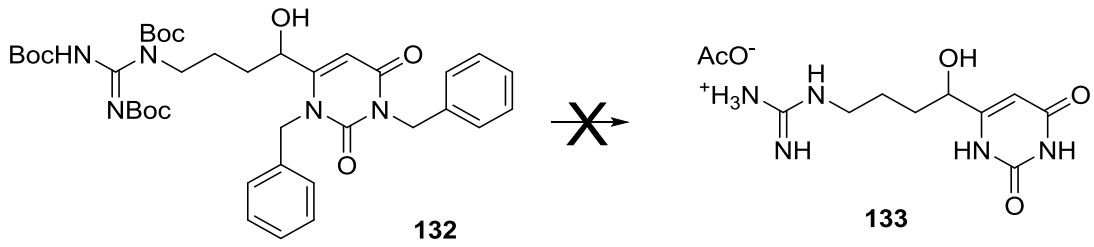
132

Potassium carbonate (36.0 mg, 0.261 mmol, 8.0 eqv.) was added to a solution of **131** (25.0 mg, 0.033 mmol) in dry methanol (5 mL) and the mixture stirred at rt for 1 h. The mixture was evaporated onto silica gel (ca. 1 g) and purified by column chromatography on silica gel (gradient elution; 30-50% EtOAc/petrol) to give **132** (18.0 mg, 0.025 mmol) in 76% yield as a white solid.

Rf	0.20 (20% EtOAc/petrol), 0.50 (30% EtOAc/petrol)
δ_H ($CDCl_3$)	0.78-0.96 (1H, m, CH), 1.16-1.30 (1H, m, CH), 1.37 (9H, s, 3 × CH_3), 1.43 (9H, s, 3 × CH_3), 1.51 (9H, s, 3 × CH_3), 1.73-1.85 (1H, m, CH), 1.94 (1H, br s, OH), 2.16-2.30 (1H, m, CH), 3.25-3.40 (2H, m, CH_2), 4.97 (1H, d, *J* = 16.3 Hz, CH), 5.11 (1H,d, *J* = 13.8 Hz, CH), 5.21 (1H, d, *J* = 13.8 Hz, CH), 5.34 (1H, t, *J* = 7.4Hz, CH), 5.71 (1H, d, *J* = 16.3 Hz, CH), 5.97 (1H, s, CH), 7.17-7.23 (2H, m, 2 × CH), 7.24-7.34 (6H, m, 6 × CH), 7.41-7.44 (2H, m, 2 × CH), 10.50 (1H, s, NH)
δ_C ($CDCl_3$)	162.2 (C), 152.9 (C), 152.4 (C), 152.2 (C), 151.6 (C), 137.2 (C), 137.1 (C), 129.0 (CH), 128.8 (CH), 128.6 (CH), 127.7 (CH), 127.6 (CH), 126.6 (CH), 103.9 (CH), 84.2 (C), 83.4 (C), 82.3 (C), 61.0 (CH_2), 55.4 (C), 47.5 (CH_2), 44.8 (CH_2), 29.9 (C), 28.8 (CH_2), 28.4 (CH_2), 28.1 (3 × CH_3) 28.0 (6 × CH_3)
ν_{max}	3448, 3066, 3034, 2980, 2932, 2251, 1759, 1705 1663, 1619, 1496, 1451, 1395, 1370, 1251, 1142, 1061, 1030, 911, 839, 732

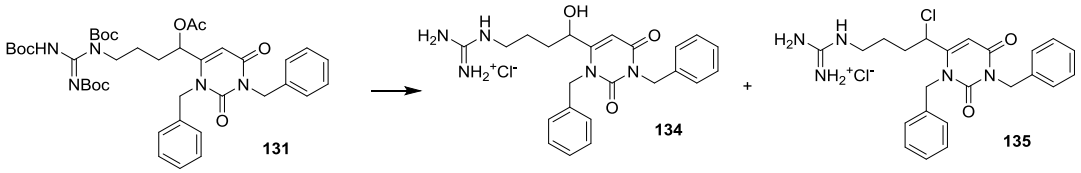
m/z (CI)	722.4 (100%, $[M+H]^+$), 744.4 (35%, $[M+Na]^+$)
HRMS (CI)	$C_{38}H_{52}N_5O_9$ ($[M+H]^+$), requires 722.3760, found 722.3757

Attempted deprotection of 1-(1,3-dibenzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-(1,2,3-tris(*tert*-butoxycarbonyl) guanidino)butyl acetate (133).



A mixture of the dibenzyluracil **132** (170.0 mg, 0.236 mmol), ammonium formate solution (40% in methanol, 1 mL) and 10% Pd/C (0.24 g) was refluxed for 24 h. The mixture was filtered through Celite©, and the solid residue washed with MeOH and $CHCl_3$ (ca. 50 mL). After evaporation, a solid (56.0 mg) was obtained which on analysis by proton NMR and TLC was shown to be a complex mixture of products.

Preparation of 1-(4-(1,3-dibenzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-hydroxybutyl)guanidine (134) and 1-(4-chloro-4-(1,3-dibenzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)butyl)guanidine (135).

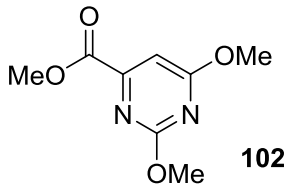


Guanidine **131** (40.0 mg, 0.052 mmol) and HCl (concentrated, 2 mL) were combined in a 5 mL round bottom flask and the mixture was heated (100˚C) for 16 h. After cooling to rt the mixture was evaporated and dried under vacuum (P_2O_5) to give a crude mixture of **134** and **135** (12.8 mg) as a solid which were identified solely by mass spectrometry.

m/z (CI) **134**; 422.2 (70%, $[M+H]^+$); **135**; 440.2 (100%, $[M+H]^+$, ^{35}Cl), 442.2 (35%, $[M+H]^+$, ^{37}Cl)

HRMS (CI) **134** $C_{23}H_{28}N_5O_3$ ($[M+H]^+$) requires 422.2187, found 422.2162
135 $C_{23}H_{28}{}^{35}ClN_5O_3$ ($[M+H]^+$) requires 440.1848, found 440.1819; $C_{23}H_{28}{}^{37}ClN_5O_3$ ($[M+H]^+$) requires 442.1800, found 440.1789

Preparation of methyl 2,6-dimethoxypyrimidine-4-carboxylate (102)[94]

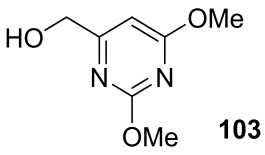


102

Orotic acid monohydrate **101** (10.2 g. 58.6 mmol) was suspended in $POCl_3$ (80 mL) and the solution stirred and heated (120 °C) overnight. After cooling to rt, PCl_5 (25.5 g, 117.0 mmol) was added to the red solution and the mixture refluxed overnight. At this point excess solvent was removed by distillation to give crude 2,6-dichloropyrimidine-4-carbonyl chloride as an oily residue. This oil was cooled (0 °C) and dry methanol (50 mL) was added drop-wise over 2h (**CAUTION!** exothermic reaction). After stirring to rt overnight, the mixture was evaporated onto silica gel (100 g) and the silica placed into a sintered glass filter and flushed with ethyl acetate (3 x 100 mL). Evaporation of the filtrate gave the crude product which was purified by column chromatography on silica gel (25% EtOAc in petrol) to give **102** (2.0 g, 10.1 mmol) as a white crystalline solid in 17% yield. This reaction was repeated twice on an identical scale to give 9% on both occasions. Two repeats were also performed where sodium methoxide (0.234 mol; prepared from sodium (5.39 g, 0.234 mol) and dry methanol (100 mL) was added to the intermediate crude 2,6-dichloropyrimidine-4-carbonyl chloride after the addition of methanol and after column chromatography yields of 9% and 18% were obtained.

Rf	0.22 (40% EtOAc in petrol)
Mp	102-104 °C, (Lit.108-109 °C[94])
δ_H ($CDCl_3$)	3.98 (3H, s, CH_3), 4.03 (3H, s, CH_3), 4.07 (3H, s, CH_3), 7.06 (s, 1H)
δ_C ($CDCl_3$)	172.5 (C), 165.5 (C), 164.2 (C), 156.5 (C), 102.9 (CH), 54.9 (CH_2), 54.1 (CH_3), 52.7 (CH_3)
ν_{max}	2925, 1609, 1567, 1458, 1363, 1207, 1109, 1039, 830, 784

Preparation of (2,6-dimethoxypyrimidin-4-yl)methanol (103)[94]

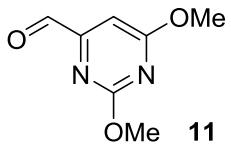


103

Ethanol (60 mL) was added to a stirred mixture of solid $LiBH_4$ (0.33 g, 15.15 mmol, 1.20 eqv) and ester **102** (2.50 g, 12.57 mmol, 1 eqv) in THF (40 mL) and the mixture stirred at rt for 2 h where TLC indicated the complete consumption of the starting material. A solution of HCl (aqueous 1 M, ca. 8 mL) was then added drop wise until the solution became clear and at this point the reaction was evaporated to reduce the volume of the mixture to ca 20 mL. Water (50 mL) was added, the mixture extracted with EtOAc (3 × 40 mL) and the combined extracts washed with cold water (30 mL) and brine (30 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was dissolved in ether (30 mL) and the solution passed through a small plug of silica (1 cm) and evaporated to give **103** as a white solid (1.80 g, 10.58 mmol) in 84% yield.

Rf	0.18 (40% EtOAc in petrol)
Mp	103-105 °C (Lit.104.9 - 106.1 °C[94])
δ_H (CDCl3)	3.25 (1H, s, OH), 3.95 (3H, s, Me), 3.97 (3H, s, Me), 4.58 (2H, s, CH_2), 6.36 (1H, s, CH)
δ_C ($CDCl_3$)	172.1 (C), 170.6 (C), 165.0 (C), 97.2 (CH), 63.6 (CH_2), 54.7 (CH_3), 53.9 (CH_3)
ν_{max}	3391, 3019, 2952, 1602, 1571, 1463, 1359, 1215, 1055, 1039, 831, 755, 668

Preparation of 2,6-dimethoxypyrimidine-4-carboxaldehyde (11)[94]



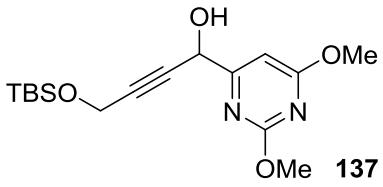
11

Dess-Martin periodinane (1.25 g, 2.94 mmol) was added to a solution of alcohol **103** (0.5 g, 2.94 mmol) in CH_2Cl_2 (20 mL) and the mixture stirred at rt for 1 h. The reaction was then washed with $Na_2S_2O_3$ (aqueous, 10%, 30 mL) and $NaHCO_3$ (aqueous, saturated, 30 mL). The combined aqueous washings were back extracted with CH_2Cl_2 (30 mL), and the combined organic layers washed in turn with water (30 mL), brine (30 mL) then dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (gradient elution; 15-20% EtOAc in petrol) to give **11** (0.40 g, 2.38 mmol) as a pale yellow solid in 81% yield.

A repeat of this reaction with **103** (1.0 g, 5.88 mmol) and Dess-Martin periodinane (2.50 g, 5.89 mmol) gave **11** (0.95 g, 5.65 mmol) in 96% yield.

Mp	104-107 °C (Lit.105-106.2 °C [94])
Rf	0.36 (20% EtOAc in petrol)
δ_H ($CDCl_3$)	4.04 (3H, s, Me), 4.09 (3H, s, Me), 6.90 (1H, s, CH), 9.89 (1H, s, CH)
δ_C ($CDCl_3$)	2.1 (CH), 172.8 (C), 166.3 (C), 160.2 (C), 99.5 (CH), 55.2 (CH_3), 54.5
ν_{max}	3023, 2990, 2949, 2886, 1608, 1582, 1567, 1471, 1361, 1207, 1110, 1085, 1039, 830, 783

Preparation of 1-(2,6-dimethoxypyrimidin-4-yl)-4-((isopropyldimethylsilyl)oxy)-but-2-yn-1-ol (137)



137

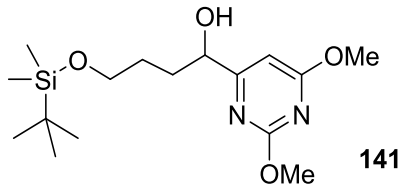
A solution of EtMgBr in THF (1M, 2.5 mL, 2.5 mmol, 1.9 eqv.) was added over 15 min to a stirred and cooled (0 °C) solution of **119** (405.2 mg, 2.38 mmol, 2 eqv.) in dry THF (5 mL) and the resulting mixture was stirred for 1 h. This solution was then added to a cooled (-78 °C) solution of aldehyde **11** (0.20 g, 1.19 mmol, 1.0 eqv) in dry THF (5 mL) via cannula and the mixture stirred for 2 h. After warming to rt overnight NH_4Cl solution (aqueous, saturated, 20 mL), the mixture extracted with EtOAc (3 × 25 mL) and the combined organic extracts washed with brine (25 mL) and water (25 mL), dried ($MgSO_4$|) and evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel (EtOAc/petrol 5-30%) to give **137** (0.304 g, 0.937 mmol) in 75% yield as a clear liquid which solidifies slowly at rt.

A repeat of this reaction with **119** (1.013 g, 6.0 mmol, 2 eqv), EtMgBr (1 M, 5.65 mL, 5.65 mmol, 1.9 Eqv) and **11** (500.0 mg, 2.97 mmol, 1.0 eqv.) in dry THF (10 mL) gave **137** (720.0 mg, 2.13 mmol) as a yellow oil in 71% yield.

Rf	0.32 (25% EtOAc/petrol)
δ_H ($CDCl_3$)	0.09 (6H, s, 2 × CH_3), 0.87 (9H, s, 3 × CH_3), 1.95 (1H, br s, OH), 3.97 (3H, s, CH_3), 3.99 (3H, s, CH_3), 4.35 (2H, d, J = 1.1 Hz, CH_2), 5.31 (1H, t, J = 1.1 Hz, CH), 6.54 (1H, s, CH).
δ_C ($CDCl_3$)	172.36 (C), 168.51 (C), 165.04 (C), 98.05 (CH), 84.96 (C), 82.66 (C), 63.17 (CH), 54.94 (CH_3), 54.13 (CH_3), 51.68 (CH_2), 25.72 (3 × CH_3), 18.21 (C), -5.23 (2 × CH_3)
ν_{max}	3392, 2955, 2930, 2885, 2858, 1597, 1572, 1484, 1463, 1382, 1358, 1255, 1203, 1129, 1059, 815, 779

m/z (CI)	339.2 (100%, $[M+H]^+$), 577.2 (90%, $[2M+H]^+$)
HRMS (CI)	$C_{16}H_{27}N_2O_4Si$ ($[M+H]^+$) requires 338.1735, found 338.1735

Preparation of 4-((*tert*-butyldimethylsilyl)oxy)-1-(2,6-dimethoxypyrimidin-4-yl)butan-1-ol (141)

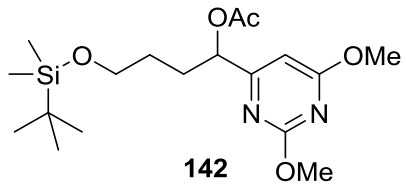


141

Palladium on activated carbon (10%, 0.20 g) was added to a solution of alkyne **137** (0.163 g, 0.476 mmol, 1.0 eqv.) in EtOAc (2 mL) under an argon blanket in a 25 mL RBF. The flask was purged with hydrogen gas (balloon) mixture and the mixture vigorously stirred under the hydrogen atmosphere for 2 h. The mixture was then filtered through a Celite© pad which was washed with further portions of EtOAc and the filtrate concentrated by evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (10-40% EtOAc/petrol) to give **141** (130.0 mg, 0.380 mmol) as a colourless oil in 79% yield.

Rf	0.25 (20% EtOAc/petrol)
δ_H ($CDCl_3$)	0.05 (6H, s, 2 × CH_3), 0.89 (9H, s, 3 × CH_3), 1.60-1.78 (3H, m, CHH, CH_2), 1.93-2.03 (1H, m, CHH), 3.65 (2H, t, *J* = 6.5 Hz, CH_2), 3.95 (3H, s, Me), 3.97 (3H, s, Me), 4.13 (1H, br s, OH), 4.56 (1H, m, CH), 6.44 (1H, s, CH)
δ_C ($CDCl_3$)	174.0 (C), 172.2 (C), 164.9 (C), 97.3 (CH), 72.5 (CH), 63.3 (CH_2), 54.7 (CH_3), 53.8 (CH_3), 34.6 (CH_2), 28.5 (CH_2), 25.9 (3 × CH_3), 18.3 (C), -5.4 (2 × CH_3)
ν_{max}	3402, 2955, 2930, 2885, 2857, 1598, 1570, 1481, 1462, 1382, 1355, 1255, 1203, 1153, 1097, 1006, 836, 776
m/z (CI)	343.2 (100%, $[M+H]^+$), 365.2 (15%, $[M+Na]^+$), 707.4 (30%, $[2M+Na]^+$)
HRMS (CI)	$C_{16}H_{31}N_2O_4Si$ ($[M+H]^+$) requires 343.2048, found 343.2049

Preparation of 1-(2,6-dimethoxypyrimidin-4-yl)-4-((tert-butydimethylsilyl)oxy)butyl acetate (142)

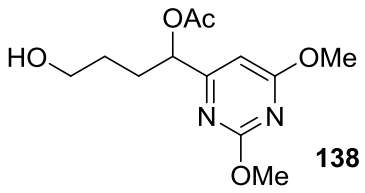


142

Acetic anhydride (0.12 g, 0.11 mL, 1.19 mmol, 3.7 eqv) was added drop wise to a cooled (0˚C) and stirred mixture of alcohol **141** (0.11 g, 0.32 mmol, 1.0 eqv), pyridine (0.10 g, 0.10 mL, 1.25 mmol, 3.9 eqv) and DMAP (4.0 mg, 0.032 mmol, 0.1 eqv.) in dry CH_2Cl_2 (3 mL). The mixture was warmed to rt and stirred for 1 h, whereupon HCl (aqueous. 2 M, 20 mL) was added the mixture stirred for 30 min. The mixture was the extracted with CH_2Cl_2 (3 x 10 mL) and the combined extracts washed with HCl (aqueous, 2M, 3 × 5 mL) and water (20 mL). After drying ($MgSO_4$) and evaporated under reduced pressure, column chromatography on silica gel (10-30% EtOAc/petrol) gave **142** (84.0 g, 0.218 mmol) as a white solid in 68% yield.

Rf	0.28 (20% EtOAc/petrol)
δ_H ($CDCl_3$)	0.02 (6H, s, 2 × CH_3), 0.87 (9H, s, 3 × CH_2), 1.51-1.62 (2H, m, CH_2), 1.84-1.94 (1H, m, CHH), 1.96-2.04 (1H, m, CHH), 2.13 (3H, s, CH_3), 3.60 (2H, t, *J* = 6.3 Hz, CH_2), 3.94 (3H, s, Me), 3.96 (3H, s, Me), 5.61 (1H, dd, *J* = 4.8, 7.8 Hz, CH), 6.30 (1H, s, CH)
δ_C ($CDCl_3$)	172.1 (C), 170.4 (C), 170.2 (C), 165.2 (C), 97.6 (CH), 75.1 (CH), 62.5 (CH_2), 54.7 (CH_3), 53.8 (CH_3), 30.4 (CH_2), 28.2 (CH_2), 25.9 (3 × CH_3), 21.0 (CH_3), 18.3 (C), -5.4 (2 × CH_3)
ν_{max}	2956, 2930, 2898, 2858, 2253, 1744, 1598, 1572, 1482, 1463, 1388, 1358, 1242, 1206, 1162, 1104, 1059, 964, 910 and 836
m/z (CI)	385.2 (100%, $[M+H]^+$), 791.4 (15%, $[2M+H]^+$)
HRMS (CI)	$C_{18}H_{33}N_2O_5Si$ ($[M+H]^+$) requires 385.2153, found 385.2146

Preparation of 1-(2,6-dimethoxypyrimidin-4-yl)-4-hydroxybutyl acetate(138)

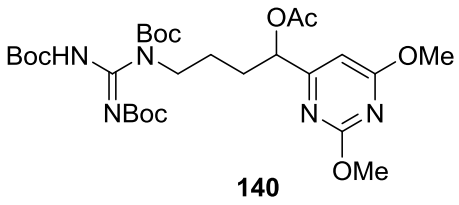


138

A solution of TBAF (0.52 mL, 0.52 mmol, 1.5 eqv) in THF was added to a cooled (0˚C) solution of **142** (0.134 g, 0.348 mmol, 1.0 eqv.) in dry THF (3 mL) and the resulting mixture stirred for 2 h. At this point the reaction was evaporated under reduced pressure and purified by flash chromatography on silica gel (gradient elution; 30-40% EtOAc/petrol) to give **138** (0.076 g, 0.281 mmol) as colorless oil in 80% yield.

Rf	0.26 (40% EtOAc in petrol)
δ_H ($CDCl_3$)	1.63-1.89 (4H, m, 2 × CH_2), 2.01 (3H, s, CH_3), 3.59 (1H, br s, OH), 3.94 (3H, s, CH_3), 3.97 (3H, s, CH_3), 4.02-4.17 (2H, m, CH_2), 4.55 (1H, dd, *J* = 2.6, 6.3 Hz, CH), 6.33 (1H, s, CH)
δ_C ($CDCl_3$)	172.9 (C), 172.1 (C), 171.1 (C), 154.9 (C), 97.2 (CH), 71.8 (CH), 64.2 (CH_2), 54.7 (CH_3), 53.9 (CH_3), 33.8 (CH_2), 24.7 (CH_2), 20.9 (CH_3)
ν_{max}	3440, 3428, 2955, 2927, 1738, 1596, 1569, 1482, 1381, 1351,
m/z (CI)	271.1 (100%, $[M+H]^+$), 293.1 (40%, $[M+Na]^+$), 563.2 (20%, $[2M+Na]^+$)
HRMS (CI)	$C_{12}H_{19}N_2O_5$ ($[M+H]^+$) requires 271.1288, found 271.1287

Preparation of 1-(2,6-dimethoxypyrimidin-4-yl)-4-(1,2,3-tris(*tert*butoxycarbonyl) guanidino)butyl acetate (140)

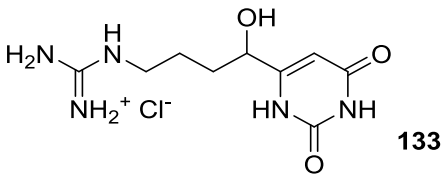


140

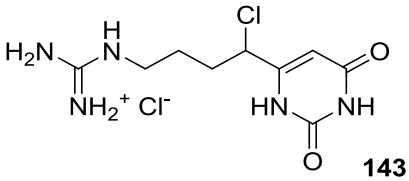
DIAD (190.8 mg, 0.94 mmol, 1.5 eqv) was added drop wise to a cooled (0 ˚C) and stirred solution of alcohol **138** (0.17 g, 0.629 mmol, 1.0 eqv.), PPh_3 (247.5 mg, 0.960 mmol, 1.53 eqv.) and *N,N′,N″*-tri-Boc-guanidine **139** (452.1 mg, 0.314 mmol, 2 eqv.) in dry THF (5 mL). After stirring to rt overnight, a drop of water was added and the solution was evaporated under reduced pressure. The crude product was then purified by flash chromatography on silica gel (5-20% EtOAc/petrol) to give **140** (0.19 g, 0.31 mmol) as a colourless oil in 49% yield.

Rf	0.26 (20% EtOAc/petrol)
δ_H ($CDCl_3$)	1.32 (9H, s, 3 × CH_3), 1.49 (9H, s, 3 × CH_3), 1.50 (9H, s, 3 × CH_3), 1.70-1.91 (2H, m, CH_2), 2.04 (3H, s, CH_3), 2.15-2.26 (2H, m, CH_2), 3.95 (3H, s, CH_3), 3.96 (3H, s, CH_3), 4.11 (2H, t, J = 6.4 Hz, CH_2), 5.35-5.65 (1H, m, CH), 6.50 (1H, s, CH), 10.71 (1H, s, NH)
δ_C ($CDCl_3$)	Partial data: 172.2 (C), 171.1 (C), 98.9 (CH, from HSQC), 83.3 (C), 64.3 (CH_2), 61.1 (CH), 54.8 (CH_3), 53.9 (CH_3), 28.0 (3 × CH_3), 28.0 (3 × CH_3), 27.8 (3 × CH_3), 25.7 (CH_2), 20.9 (CH_3), (5 × C not observed)
ν_{max}	3644, 3463, 2985, 2936, 2921, 2849, 1742, 1448, 1373, 1242, 1134, 1098, 1047, 938, 847
m/z (CI)	612.3 (100%, $[M+H]^+$), 1245.6 (90%, $[2M+Na]^+$), 312 (96%, $[M-2Boc+H]^+$)
HRMS (CI)	$C_{28}H_{46}N_5O_{10}$ ($[M+H]^+$) requires 612.3239, found 612.3225

Preparation of amino((4-(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-hydroxybutyl)-amino)methaniminium chloride (133) and amino((4-chloro-4-(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)butyl)amino)-methaniminium chloride (143)



133



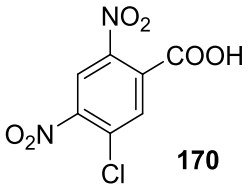
143

Protected uracil **140** (35.0 mg, 0.057 mmol) and concentrated HCl (2 mL) were heated at 80 ˚C for 96 h. After cooling to rt, evaporation under reduced pressure and drying over P_2O_5 gave a crude mixture of **133** and **143** (12.0 mg, 0.043 mmol) as a solid.

A similar reaction using **140** (36.0 g, 0.059 mmol) and concentrated HCl (2 mL) were heated at reflux for 6h. After work, up this reaction gave **143** (13.5 mg, 0.049 mmol) as a solid. The product was purified by dissolution in methanol (1 mL) and precipitation by the addition of diethyl ether to give an approximate 1:1 mixture of **133/143** (5.4 mg).

δ_H (CD_3OD)	1.52-2.20 (4H, m, 2 × CH_2), 3.55-3.69 (2H, m, CH_2), 4.27-4.43 (1H, m, CH) and 5.56 (1H, br m, CH)
δ_C (CD_3OD)	Partial data: 29.4/30.0 (CH_2), 31.8/32.5 (CH_2), 44.8/61.8 (CH_2), 53.8/53.9 (CH) and 97.9/98.1 (CH)
m/z (CI)	**133**; 242.1 (25%, $[M+H]^+$); **143**; 260.1 (86%, $[M+H]^+$, ^{35}Cl), 262.1 (28%, $[M+H]^+$, ^{37}Cl), 235 (100%)
HRMS (CI)	**133** $C_9H_{16}N_5O_3$ ($[M+H]^+$) requires 242.1248, found 242.1248 **143** $C_9H_{15}{}^{35}ClN_5O_2$ ($[M+H]^+$) requires, 260.0909, found 260.0911; $C_9H_{15}{}^{37}ClN_5O_2$ requires 262.0880, found 262.0880.

Preparation of 5-chloro-2,4-dinitro-benzoic acid (170)[155]



170

3-Chlorobenzoic acid **169** (25.0 g, 160.0 mmol) was dissolved in concentrated sulfuric acid (300 mL) and the mixture was stirred and warmed to 40 °C. Potassium nitrate (16.0 g, 158.0 mmol) was added in portions over 35 min and the reaction mixture was then warmed to 100 °C and an additional (28.0 g, 277.0 mmol) of potassium nitrate was added in portions over 25 min. The reaction was heated at 145 °C for 15 min then slowly cooled to rt and poured into ice (2 kg) which was agitated for 30 min. to precipitate **170** as a yellow solid. This precipitate was filtered under a vacuum and washed with water then added to water (1 L) stirred for 1 h at rt. The solid formed was removed by filtration and dried under vacuum to give **170** (27.0 g, 68 %) of product as a pale yellow solid

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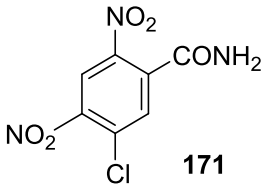
δ_H (acetone-d6):	8.75 (1H, s, CH), 8.25 (1H, s, CH)
δ_C (acetone-d6):	122.7 (CH), 131.8 (CH), 132.3 (C), 134.1 (C), 147.2 (C), 149.4 (CH), 163.7 (CH)
Mp	179 °C (lit 180-183 °C[155])
ν_{max}	3500-2500 (br), 3024, 1716, 1586, 1542, 1422, 1346, 1286, 1136, 909

Preparation of 5-chloro-2,4-dinitrobenzamide (171)[155]

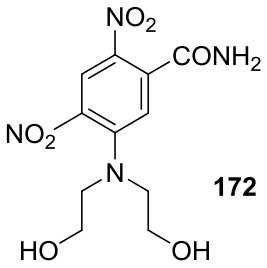


171

5-Chloro-2,4-dinitro-benzoic acid **170** (5.0 g, 20.3 mmol) was suspended in thionyl chloride (30 mL) and then DMF (3 drops) was added and the mixture was stirred under reflux for 4 h. After cooling to rt the solvent was evaporated to give a yellow liquid which was diluted with acetone (21 mL) and added to cooled (0 °C) conc. ammonium hydroxide solution (15 mL) over 20 min. The reaction mixture was cooled (0 °C) and stirred for 30 min then poured into ice (ca. 150 g) and stirred until the ice melted. The yellow precipitate formed was removed by filtration, washed with cold water and dried under high vacuum to give **171** (4.3 g, 17.5 mmol, 87% yield) as a pale yellow solid.

δ_H (acetone-d6):	8.74 (1H, s, CH), 8.07 (1H, s, CH), 7.8 (1H, br s, NH), 7.4 (1H, br s, NH).
δ_C (acetone-d6):	123.0 (CH), 132.0 (C), 133.2 (CH), 137.7 (C), 146.4 (C), 148.5 (C), 165.3 (C)
Mp	200 °C (lit 201-203 °C[155])

Preparation of 5-(bis(2-hydroxyethyl)amino)-2,4-dinitrobenzamide (172)[154,156]

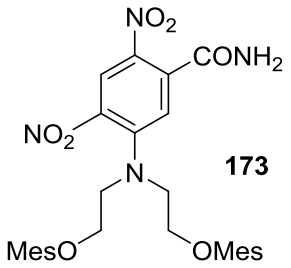


172

A mixture of 5-chloro-2,4-dinitrobenzamide **171** (3.3 g, 13.4 mmol) and diethanolamine (3.5 g, 33.3 mmol) was stirred and heated (100 °C) for 4 h. The reaction mixture was then cooled to rt and poured onto a prewashed (EtOAc) silica gel pad which was eluted with 2-8% MeOH in EtOAc. Combination of the fractions containing the product gave the diol **172** (2.30 g, 10.74 mmol, 80%) as an orange yellow solid.

δ_H (acetone-d6):	8.44 (1H, s, CH), 7.55 (1H, br s, NH), 7.50 (1H, s, CH), 7.04 (1H, br s, NH), 3.79 (4H, t, *J* = 5.4 Hz, 2 × CH_2), 3.61 (4H, t, *J* = 5.4 Hz, 2 × CH_2), 4.13 (2H, br s, 2 × OH)
δ_C (acetone-d6):	55.1 (CH_2), 59.3 (CH_2), 120.8 (CH), 124.8 (CH), 135.3 (C), 137.8 (C), 148.6 (C), 167.6 (C)
m/z (CI)	629.2 (10%, $[2M + H]^+$), 391.3, (12%), 315.1 (100%, $[M + H]^+$), 199.2 (7%).
HRMS (CI)	$C_{11}H_{15}O_7N_4$ ($[M+H]^+$) requires 315.0935, found 315.0939
Mp	180 °C (lit. 176-178 °C[154,156])
ν_{max}	3425, 3356, 2928, 1675, 1604, 1580, 1516, 1437, 1333, 1172, 970, 915

Preparation of 5-(bis(2-(mesityloxy)ethyl)amino)-2,4-dinitrobenzamide (173)[156,154]

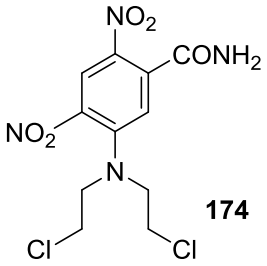


173

5-(bis(2-Hydroxyethyl)amino)-2,4-dinitrobenzamide **172** (1.00 g, 3.2 mmol) was dissolved in dry pyridine (15 mL) and cooled (0 ˚C) and MsCl (0.81 g, 7.1 mmol) was added. The mixture was then stirred to rt over 90 min and the solvent removed under reduced pressure below 40 ˚C. The residue was partitioned between EtOAc (100 mL) and water (100 mL) and the organic layer washed with deionised water (3 ×300 mL), dried ($MgSO_4$), evaporated and purified by column chromatography (EtOAc) to give **173** (1.00 g, 2.13 mmol, 67%) as a yellow solid.

δ_H (acetone-d6):	8.54 (1H, s, CH), 7.65 (1H, s, CH), 7.48 (1H, br s, NH), 7.15 (1H,br s, NH), 4.49 (4H, t, *J* = 5.3Hz, 2 × CH_2), 3.89 (4H, t, *J* = 5.35, Hz, 2 × CH_2), 3.09 (6H, s, 2 × CH_3)
δ_C (acetone-d6):	167.1 (C), 148.5 (C), 140.3 (CH), 138.5 (CH), 138.3 (C), 124.9 (CH), 122.9 (CH), 67.6 (CH_2), 51.9 (CH_2), 37.1 (CH_3)
m/z (CI)	958.1 (35%, $[2M + NH_4]^+$), 488.1 (100%, $[M + H]^+$), 454.0 (65%), 391.3 (8%)
HRMS (CI)	$C_{13}H_{19}O_{11}N_4S_2$ ($[M+H]^+$) requires 471.0486, found 471.0482
Mp	138-143 ˚C (lit. 140-144 ˚C[154,156])
ν_{max}	3410, 3292, 3020, 2938, 1671, 1579, 1509, 1346, 1328, 1174, 1214, 1174, 1002, 936, 904, 754

Preparation of 5-(bis(2-chloroethyl)amino)-2,4-dinitrobenzamide (174)[156]



174

Lithium chloride (0.21 g, 5.0 mmol) was added to a solution of **173** (0.25 g, 0.53 mmol) in dry DMF (15 mL) and the mixture stirred and warmed to 120 °C for 20 min. After cooling the mixture was concentrated to dryness under high vacuum and dissolved in a mixture of EtOAc (50 mL) and water (50 mL). After separation the organic layer was washed with saturated ammonium chloride solution (aqueous, 3 x 50 mL), dried ($MgSO_4$) and evaporated. Column chromatography of the residue on of silica gel (20% EtOAc in diethyl ether) gave **174** (0.14 g, 0.40 mmol, 75%) as a yellow solid.

δ_H (acetone-d6): 8.53 (1H, s, CH), 7.62 (1H, br s, NH), 7.58 (1H, s, CH), 7.17 (1H, br s, NH), 3.89-3,80 (8H, m, 4 × CH_2)

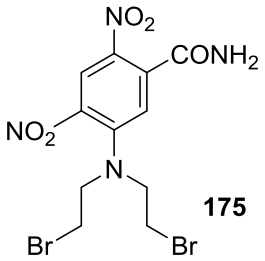
δ_C (acetone-d6): 167.2 (C), 148.2 (C), 139.9 (C), 138.6 (C), 138.0 (C), 124.9 (CH), 122.1 (CH), 54.1 (CH_2), 42.0 (CH_2)

m/z (CI) 351.0 (100%, $[M+H]^+$, 2 × ^{35}Cl), 353 (68%, $[M+H]^+$, ^{35}Cl, ^{37}Cl), 355 (10%, $[M+H]^+$, 2 × ^{37}Cl).

HRMS (CI) $C_{11}H_{13}{}^{35}Cl_2N_4O_5$ ($[M+H]^+$) requires 351.0258, found 351.0262; $C_{11}H_{13}{}^{35}Cl^{37}ClN_4O_5$ ($[M+H]^+$), requires 353.0228, found 353.0232; $C_{11}H_{13}{}^{37}Cl_2N_4O_5$ ($[M+H]^+$), requires 355.0199, found 355.0201

Mp 107-112 °C (lit. 109-111 °C[156])

Preparation of 5-(bis(2-bromoethyl)amino)-2,4-dinitrobenzamide (175)[156]

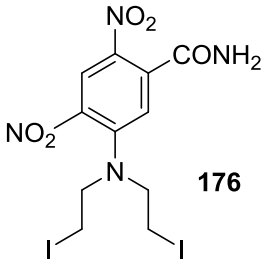


175

Sodium bromide (2.50 g, 24.7 mmol) was added to a solution of **173** (0.25 g, 0.53 mmol) in dry DMF (12.5 mL) and the mixture stirred and warmed to 125 ˚C for 20 min. After cooling the mixture was concentrated to dryness under high vacuum and dissolved in a mixture of EtOAc (50 mL) and water (50 mL). After separation the organic layer was washed with saturated ammonium chloride solution (aqueous, 3 x 50 mL), dried ($MgSO_4$) and evaporated. Column chromatography of the residue on of silica gel (50% EtOAc in diethyl ether) gave **175** (0.23 g, 0.52 mmol, 98%) as a yellow solid.

δH (acetone-d6):	8.53 (1H, s, CH), 7.61 (1H, br s, NH), 7.58 (1H, s, CH), 7.12 (1H,br s, NH), 3.89 (4H, t, *J* = 6.5 Hz, 2 × CH_2), 3.74 (4H, t, *J* = 6.5 Hz, 2 × CH_2)
δC (acetone-d6):	167.2 (C), 148.0 (C), 140.2 (C), 138.9 (C), 138.5 (C), 125.1 (CH), 122.4 (CH), 54.2 (CH_2), 30.0 (CH_2)
m/z (CI)	438.9 (51%, $[M+H]^+$, 2 × ^{79}Br), 440.9 (100%, $[M+H]^+$, ^{81}Br, ^{79}Br), 442.9 (49%, $[M+H]^+$, 2 × ^{81}Br), 897.9 (20 %, $[2M+NH_4]^+$, 2 × ^{79}Br, 100%),
HRMS (CI)	$C_{11}H_{13}{}^{79}Br_2N_4O_5$ ($[M+H]^+$) requires 438.9247, found 438.9247; $C_{11}H_{13}{}^{81}Br^{79}BrN_4O_5$ ($[M+H]^+$) requires 440.9227, found 440.9225, $C_{11}H_{13}{}^{81}Br_2N_4O_5$ ($[M+H]^+$) requires 442.9209, found 442.9204
Mp	124-130 ˚C (lit 126-128 ˚C[156])

Preparation of 5-(bis(2-iodoethyl)amino)-2,4-dinitrobenzamide (176)[156]



176

Sodium iodide (3.60 g, 24.0 mmol) was added to a solution of **173** (0.25 g, 0.53 mmol) in dry DMF (12.5 mL) and the mixture stirred and warmed to 125 °C for 20 min. After cooling to rt, the mixture was concentrated to dryness under high vacuum and dissolved in a mixture of EtOAc (50 mL) and water (50 mL). After separation the organic layer was washed with saturated ammonium chloride solution (aqueous, 3 x 50 mL), dried ($MgSO_4$) and evaporated. Column chromatography of the residue on of silica gel (30% EtOAc in diethyl ether) gave **176** (0.23 g, 0.43 mmol, 81%) as a yellow solid.

δ_H (acetone-d6):	8.52 (1H, s, CH), 7.63 (1H, br s, NH), 7.55 (1H, s, CH), 7.19 (1H, br s, NH), 3.83 (4H, t, *J* = 7.2 Hz, 2 × CH_2), 3.49 (4H, t, *J* = 7.2 Hz, 2 × CH_2)
δ_C (acetone-d6):	167.1 (C), 147.0 (C), 139.7 (C), 138.4 (C), 137.9 (C), 124.8 (CH), 122.0 (CH), 54.6 (CH_2), 1.6 (CH_2)
m/z (CI)	1085.8 (15%, $[2M+NH_4]^+$), (534.9 (100%, $[M + H]^+$), 391.3 (33%), 361.3 (23%), 313.1 (7%), 199.2 (8%)
HRMS (CI)	$C_{11}H_{13}I_2N_4O_5$ ($[M+H]^+$) requires 534.8970, found 534.8964
Mp	173-175 °C (lit 170-172 °C[156])
ν_{max}	3401, 3299, 3169, 3020, 1656, 1605, 1578, 1511, 1438, 1329, 1273, 1215, 758, 669
λ_{max}	377 nm (ε = 11 400 L mol^{-1} cm^{-1})

Assay method

All *E. coli* (DH5α), as used for genetic manipulation and cloning, was obtained from the Promega Corporation (Madison, WI, USA). All *E. coli* strain Rosetta pLysS (DE3λ), as used for protein expression, was obtained from Novagen (EMD Biosciences, Inc., affiliate of Merck KGaA, Darmstadt, Germany).

E. coli strains harbouring pET-28 plasmids were grown in Luria-Bertani (LB) liquid and solid media containing 50 µg/ mL kanamycin; all the powders were obtained from Lab M Limited (Heywood, Lancashire, UK). Plasmid and DNA purification kits were obtained from Qiagen Ltd (Manchester, UK); these were used according to manufacturer's instructions. PCR reagents were obtained from Finnzymes (Fermo Fisher Scientific Inc., Waltham, MA USA). The restriction enzymes T_4 DNA ligase and buffers were obtained from the Promega Corporation. The plasmid vector pET-28a(+), as used as cloning and expression vector, was obtained from Novagen.

Enzyme kinetics

Concentration, temperature, and pH profiles of purified enzymes were assessed spectrophotometrically as NADPH oxidation (µmol min^{-1}) by reading the absorbance at 340 nm (absorption wavelength of reduced NADPH). The candidate prodrug was the substrate, phosphate buffer 0.05 M (pH 7.2) and NADPH was the electron donor. For each assay, in order to prevent the effects of background NADPH oxidation, both blank and assay cuvettes contained NADPH and enzyme solution. The purified proteins were stored at -20 °C. All reactions were performed between 1 and 2 weeks of the initial purification; this was to prevent loss of enzyme activity.

Enzyme concentration versus reaction rate

Concentration profiles were performed in 1000 µl in cuvettes; these contained 0.05 M phosphate buffer (pH 7.2), 10 µM prodrug substrate, 30 µM NADPH, and varyious enzyme concentrations (2.5, 5, 10, 15, and 30 µg/ mL). The enzyme, PB, and NADPH were incubated at 37 °C for 5 min. The measurements were repeated three times for each enzyme concentration; the average (mean) value was then calculated.

The concentration profile of the cys-tagged enzyme was conducted using the three substrates **174-176** and CB 1954; this procedure utilised the same protocol as before, using 1000 µl curvettes and varying the enzyme concentrations.

Substrate concentration versus reaction rate

The parameters K_m and Vmax were determined spectrophotometrically at 420 nm (based on equal absorption of the 2- and 4-hydroxylamine reduction products of CB 1954 at this a wavelength of 1200 M^{-1} cm^{-1}); this was achieved using constant enzyme and NADPH concentrations under a range of different substrate concentrations. Reactions were performed in 1000 µL cuvettes; these contained 0.05 M phosphate buffer (pH 7.2), 4 mM NADPH, 10 µg/ mL enzyme, DMSO (concentration of DMSO in sample cuvettes: X µL prodrug + X µl DMSO= 50 µL; concentration of DMSO in reference = 50 µl), and increasing CB 1954 concentrations (from 0.05 to 5 mM). The enzyme, phosphate buffer and NADPH were incubated at 37 ˚C for 3 min. All measurements were repeated three times for each prodrug concentration; the average (mean) value was then calculated. Non-linear regression and Michaelis-Menten curve fitting were performed using Sigma Plot 10 (Systat Software Inc., Richmond, CA, USA). For kinetic assay, the eluted fractions containing the most pure proteins were supplemented with a 5-fold excess molar ratio of a concentrated 5.6 mM FMN solution (this was to ensure that all enzymes had bound FMN—some may have been lost during the imidazole purification and sonication step). The enzyme and FMN solution were incubated on ice for 1 h before buffer-exchange into 0.05 M PB (pH 7.2), using a PD-10 desalting column (GE Healthcare, Scientific Laboratory Supplies Ltd, Nottingham, UK).

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