The synthesis and biological activity of the marine metabolite cylindrospermopsin

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The marine natural product cylindrospermopsin has attracted much attention due to its high toxicity, its widespread occurrence in the environment and its complex chemical structure. This article discusses the isolation and structure determination of cylindrospermopsin, together with its impact on the environment and also covers the diverse synthetic methodologies which are currently under investigation directed towards the total synthesis of this intriguing molecule.

1 Introduction

Cylindrospermopsin **1**[1] is a unique water-soluble, potent hepatotoxic alkaloid produced by three different species of cyanobacteria: the blue–green algae *Cylindrospermopsis raciborskii* (Fig. 1),† *Aphanizomenon ovalisporum* (Fig 2),† and *Umezakia nantans*.

Patrick J. Murphy received his BSc and PhD from the University of Manchester Institute of Science and Technology, completing his doctoral thesis in 1986 under the direction of Dr John Brennan. After a period of postdoctoral research at the University of Salford under the supervision of Professor Garry Procter and Dr Mike Casey, he was appointed to the post of Lecturer in Organic Chemistry at the University of Wales, Bangor, gaining promotion to Senior Lecturer in 1999. Since gaining this post he has concentrated his research efforts on the development of new synthetic methodology and its applications in synthesis, with a major theme being the synthesis of guanidine-containing marine natural products.

Clive W. Thomas is a recent Graduate of the University of Wales, Bangor.

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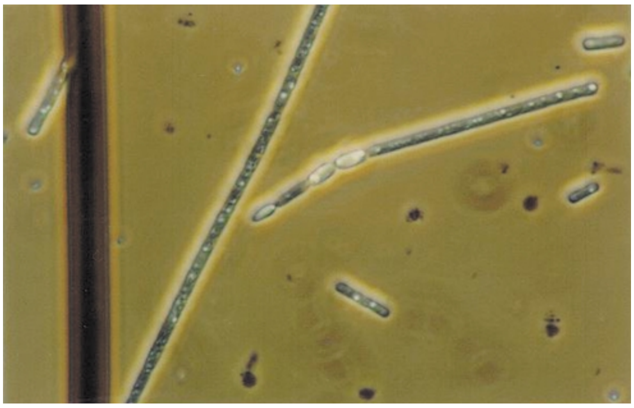
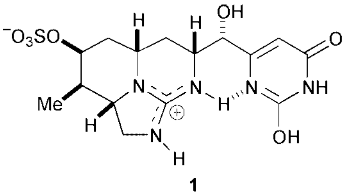


Fig. 1 *Cylindrospermopsis raciborskii.*

Fig. 2 *Aphanizomenon ovalisporum*



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These algal blooms are widespread in tropical waters, where occurrences of gastrointestinal diseases of unknown origin are common. The toxicological effects of cylindrospermopsin have been extensively studied, as have the analytical methods for its detection in drinking water. The potent toxicity of cylindrospermopsin makes its synthesis an important problem and as a consequence this has also been extensively studied.

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† For original source see: http://www.uq.edu.au/nrcet/glen.html

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2 Isolation

Cylindrospermopsin has been isolated from three different species of cyanobacteria, firstly from *Cylindrospermopsis raciborskii* by Ohtani and Moore in 1992,[1] and subsequently from the algae *Umezakia nantans* in 1994 by Harada *et al.*,[2] and twice from *Aphanizomenon ovalisporum*, in 1997 by Sukenik and co-workers,[3] and again in 1999, by Shaw *et al.*[4]

2.1 *Cylindrospermopsis raciborskii*

In November 1979, an outbreak of hepatoenteritis on Palm Island, a continental island located off the tropical northeastern coast of Queensland, Australia, was traced to *Cylindrospermopsis raciborskii*, a species that had not previously been described as toxic. The outbreak, which affected 148 people, most of whom were children and required hospitalisation, occurred a few days after treating the domestic water supply on the island with copper sulfate to control a dense algal bloom that had grown in the reservoir. The copper sulfate treatment caused lysis of the cyanobacterial cells and consequently a toxin was released into the drinking water. An epidemiological study revealed that only people that used the water from Solomon Dam had become ill. An examination of the phytoplankton in the reservoir consistently showed the presence of two blue–green algae, the non-toxic *Anabaena circinalis* and the toxic *Cylindrospermopsis raciborskii*.[5] After further studies, cylindrospermopsin was isolated from an aqueous extract of the ultrasonicated, freeze-dried *C. raciborskii* grown in culture.[1] Using a combination of NMR, mass spectrometry and UV data, the structure of the cylindrospermopsin was determined to be **1**.

In 1997, Hawkins *et al.* reported for the first time a quantitative cylindrospermopsin content for *C. raciborskii*, which was determined by high-performance liquid chromatography (HPLC).[6] The pure toxin is characterised by a distinct absorbance maximum of 262 nm due to the uracil nucleus and the measurements previously reported by Ohtani and Moore[1] were consistent with the new results.

2.2 *Umezakia nantans*

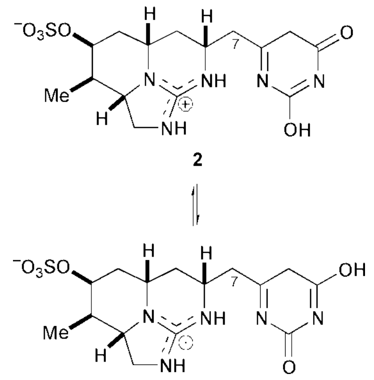
The cyanobacterium *Umezakia nantans* was first isolated from Lake Mikata, Fuki, Japan in 1987 and crude extracts from it displayed hepatotoxicity in mice. Further studies by Harada *et al.* led to the isolation of cylindrospermopsin **1** from this cyanobacterium, and its identity with **1** was confirmed by NMR spectroscopy and HPLC analysis.[2]

2.3 *Aphanizomenon ovalisporum*

In late 1994 on Lake Kinnere, Israel, *Aphanizomenon ovalisporum* was identified as the dominant bloom. It was evaluated by a commonly used mouse bioassay and was demonstrated to be toxic with an LD_{50} of 465 mg dry weight of the cyanobacterium per kg. This species had not previously been known to be toxic and when an extract was taken, it was found by mass spectrometry and NMR spectroscopy to contain cylindrospermopsin **1**.[3] Further work by Shaw *et al.* suggested that the concentration of cylindrospermopsin **1** in *A. ovalisporum* corresponds to a quarter of that present in *Cylindrospermopsis raciborskii*, but it appears that *A. ovalisporum* releases cylindrospermopsin **1** more readily into the water.[4]

2.4 Related metabolites

In addition to cylindrospermopsin **1**, two related metabolites have been isolated: the nontoxic deoxycylindrospermopsin **2**,[7] and 7-epicylindrospermopsin **3**.[8] Both cylindrospermopsin **1** and the nontoxic **2** were isolated from *Cylindrospermopsis raciborskii* by Shaw *et al.* in 1999 and apart from the obvious lack of the 7-hydroxy function in compound **2**, differences in the uracil nucleus were also apparent, in that it was found to exist in the tautomeric forms shown below.[7] 7-Epicylindrospermopsin **3** was isolated as a minor constituent of the hydrophilic extract of *Aphanizomenon ovalisporum* along with cylindrospermopsin **1** itself and was found to have similar levels of toxicity to **1**.[8] The comparative toxicity of deoxycylindrospermopsin **2** and cylindrospermopsin **1** suggests that the presence of the C-7 hydroxy group or perhaps the keto–enol status of the uracil moiety is critical for the hepatotoxic action.



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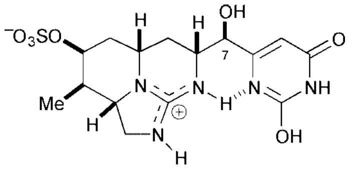
3 Toxicology studies

Following studies on animals and fish poisoned by cylindrospermopsin **1**, it has been determined that it affects primarily the liver, but also the kidney, thymus, heart and spleen.[9,10] It was also determined that there are four consecutive phases of the pathological changes in the liver. The initial phase being inhibition of protein synthesis, the second being inhibition of membrane proliferation and the third involving interference with fat-droplet accumulation leading finally to cell death.[2]

3.1 How toxic is cylindrospermopsin?

The toxicity of cylindrospermopsin **1** was estimated in 1977 by Hawkins *et al.*[6] using intraperitoneal injection to mice (20–30 g body weight) of a suspension of a freeze-dried culture sample in saline solution. The dose administered ranged from 10 to 300 mg dry weight of cyanobacterium per kg of mouse live weight, with the time of death ranging from 7.5 to 151 h. Post mortem examinations showed signs of haemorrhage within the liver, kidneys and small intestine and congestion of the lungs at high doses of *C. raciborskii* extract. The experimental toxicity (LD_{50}) of pure cylindrospermopsin from freeze-dried *C. raciborskii* is 52 mg kg^{-1} at 24 h, and 32 mg kg^{-1} at 7 days.[6] Other work[11,12,13] gave a similar result, however, studies by Falconer *et al.* in 1999 suggest that a toxin other than cylindrospermopsin **1** is present in the cyanobacteria identified above, thus increasing the overall toxicity.[14]

Most cases involving the poisoning of animals, fish and humans occur orally, and studies carried out by Seawright *et al.* in 1999 concentrated on the oral toxicity of cylindrospermopsin. They found that the median lethal dose was 4.4–6.9 mg kg^{-1} of cylindrospermopsin **1** in mice, and that the symptoms were consistent with those where **1** was administered by an intraperitoneal injection.[10]



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3.2 Why is cylindrospermopsin toxic?

3.2.1 Glutathione Glutathione is the most abundant cellular non-protein thiol and plays a key role in cellular defence against oxidative damage by participating in the detoxification of many xenobiotics. It serves as a substrate for glutathione transferase and glutathione peroxidase. The effect that cylindrospermopsin has on glutathione metabolism was examined by Runnegar and co-workers in 1994, where it was found that cylindrospermopsin toxicity was preceded by significant losses of glutathione and that depletion of glutathione results in cell death.[13,15] In a further study, they demonstrated that the fall in glutathione levels is due to an inhibition of the final common pathway of glutathione synthesis, and that this in turn contributes to cylindrospermopsin cytotoxicity as lower cell glutathione levels predispose to cylindrospermopsin toxicity.[16]

3.2.2 Cytochrome P450 A consistent feature of cylindrospermopsin toxicity in mice, whether dosed intraperitoneally or orally, was the appearance of foamy lipid vacuolation in the liver, with periacinar coagulative necrosis being also commonly observed.[11] It was found that cylindrospermopsin **1** was only rendered toxic after metabolic activation by the cytochrome P450 system in cultured rat hepatocytes, possibly because xenobiotic metabolism by the cytochrome P450 system occurs mainly in the periacinar region of the liver.[11] It was concluded that the mechanism of action involves the inhibition of protein synthesis, which may be initiated by the binding of activated metabolites to DNA or RNA.[11]

3.2.3 Carcinogenic activity Falconer *et al.* suggested that cylindrospermopsin **1** could be acting as a carcinogen, due to the presence of the uracil and the sulfonated guanidino moieties.[17] They proposed that cylindrospermopsin could cause cytogenetic damage *via* two mechanisms, firstly, at the DNA level to induce strand breaks, or secondly at the level of kinetochore/spindle function to induce loss of whole chromosomes (aneuploidy).

4 Cylindrospermopsin in the environment

As previously mentioned, the first case of cylindrospermopsin poisoning was reported in 1979, where *Cylindrospermopsis raciborskii* was found in a reservoir in Queensland, Australia.[2] Since then the occurrence of toxic blooms of cyanobacteria in eutrophicated lakes, ponds and rivers has been reported worldwide,[18] causing death to domestic animals and wildlife. Sukenik *et al.* reported an occurrence in Israel in 1998,[19] and Kabzinski *et al.* reported an occurrence in Poland in 2000.[20] In addition, in 1999, the first report of animal poisoning attributed to *C. raciborskii* was published by Saker *et al.*,[12] when three cows and ten calves were found dead near a farm dam on a cattle property at McKinlay in Northwest Queensland, Australia. It was also reported by Saker *et al.* in 1999 that cylindrospermopsin **1** was found to accumulate in the tissues of Redclaw crayfish from an aquaculture pond infested by a bloom of *C. raciborskii*.[21]

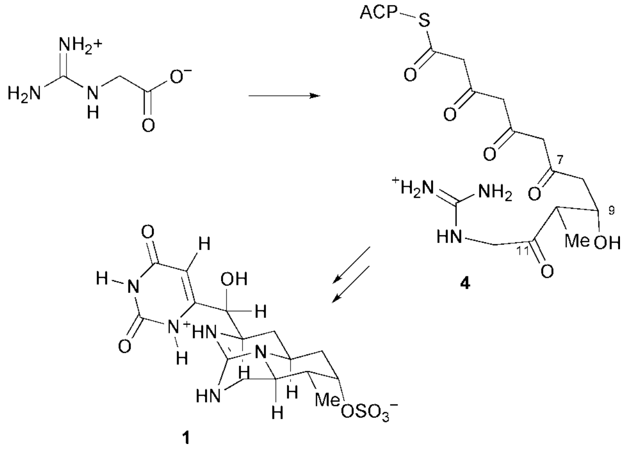
Due to the high occurrence of *C. raciborskii*, a laboratory study was carried out to determine the conditions under which *C. raciborskii* populations can develop to bloom proportions in bodies of water. It was found that two morphological forms of the bacterium existed, one of which was better suited to growth under low light conditions.[22]

4.1 Degradation

Several techniques have been proposed for the removal of cylindrospermopsin **1** from water, including physical, chemical and biological methods. Porat *et al.* have studied the effectiveness of a water purification technique used in Lake Kinneret, Israel to remove the cyanobacterium *Aphanizomenon ovalisporum*.[23] Thus, when water containing the cyanobacterium is pumped through a pipe, the increase in hydrostatic pressure causes the organism to lose its buoyancy. Chiswell *et al.* reported that cylindrospermopsin **1** decomposes slowly at temperatures between 4 and 50 °C at pH 7, and that alterations in pH do not effect its degradation.[24] After ten weeks at 50 °C, cylindrospermopsin **1** had degraded to 59% of the original concentration. They also reported that cylindrospermopsin **1** degrades rapidly when exposed to sunlight, but degrades only slowly under artificial light or in darkness.[24] Senogles and co-workers studied the effect that chlorine has on cylindrospermopsin cyanobacteria,[25] and found that for a cylindrospermopsin concentration of 130 µg L^{-1}, a minimal dose of 4 mg L^{-1} of chlorine was necessary to reduce the toxin level to below the limits of detection (0.2 µg L^{-1}). Ozonation was also found to be an effective method of destroying the cyanobacteria.[26] Porat and co-workers reported that 99.9% of the cyanobacterium *Aphanizomenon ovalisporum* biomass can be removed from the water of Lake Kinneret by a combination of the chemical and physical techniques mentioned above together with biological treatment by fish.[23] However, it is not known if toxins are released into the water during this treatment and, as cylindrospermopsin is relatively soluble and stable, it may persist for many hours.[23] In addition, Moore *et al.* reported a method of detecting and monitoring low concentrations of cylindrospermopsin in water using tandem HPLC linked to MS/MS and produced a reliable assay.[27]

4.2 Biosynthesis

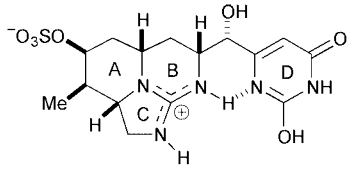
Moore speculated that cylindrospermopsin **1** originates from a polyketide precursor, and proposed two pathways for its biogenesis.[5] He performed feeding experiments that indicated that the starter unit in the biosynthesis is guanidinoacetic acid,[28] formed by amidination of glycine. The source of the amidino group in the starter unit is unknown, however, it does not appear to be derived from L-arginine. On the basis of the information obtained from several acetate feeding experiments, several plausible pathways were proposed for the biogenesis of **1**. The backbone of the polyketide is most probably assembled by either a modular (type I) or a nonmodular (type II) polyketide synthase (PKS) from guanidinoacetic acid and five acetate units. Two processing steps (C-methylation and ketoreduction) and three cyclisations involving the guanidino group either as the polyketide chain is being elongated or after it has been completed. Molecular modelling studies suggested that thioester **4** is a particularly attractive intermediate, due to strong hydrogen bonding between the guanidine group, the hydroxy group at C9 and the ketone carbonyl oxygens at C7 and C11 (Scheme 1). The modelling studies suggest that polyketide **4** readily assumes a favourable conformation for the formation of the rings A, B, and C in **1**, and thus the cyclisations may be spontaneous events that are not under enzymatic control. No evidence was obtained as to how the uracil ring is constructed.[28]



Scheme 1

5 Synthetic approaches to cylindrospermopsin

The synthesis of cylindrospermopsin represents a challenging prospect due to the complex array of functionality present within the molecule. Key structural features include the tricyclic guanidinium core, which contains five of the six stereogenic centres present in the molecule, together with a hydroxy group and polar sulfate and uracil functions. The majority of the reported synthetic approaches to **1** adopt a strategy whereby the central piperidine ring (A) is constructed first, which is followed by annelation of the B- and C-rings. Obviously, initial stereocontrol is required in the construction of the A-ring and the presence of stereogenic centres in these intermediates can then be utilised to control the formation of the stereocentres in the B- and C-rings. Both racemic and enantioselective approaches to cylindrospermopsin have been reported, with the latter drawing on 'auxiliary' and 'chiral-pool' methods for the formation of key stereogenic centres.



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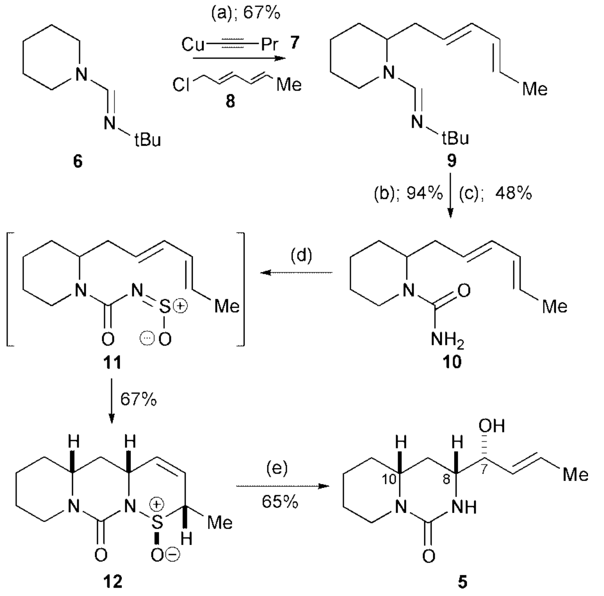
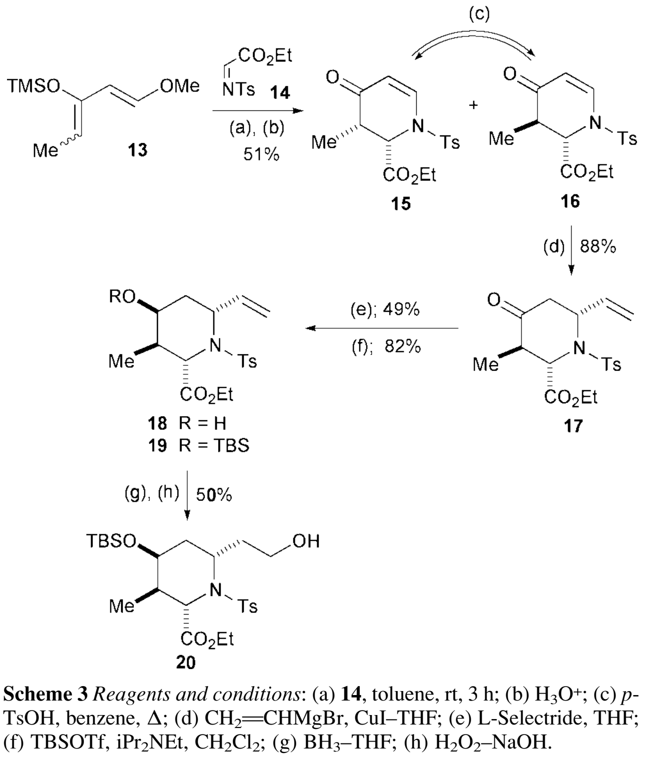
The first report of synthetic work leading to **1** was published in 1993 by Weinreb and co-workers whose general approach was to fuse the B-ring onto an A-ring precursor.[29] The method employed a hetero Diels–Alder reaction with an *N*-sulfinyl dienophile. Thus, amidine **6** was lithiated and treated with the copper acetylide **7** to give the corresponding cuprate. The cuprate was then alkylated with 1-chlorohexa-2,4-diene **8** to afford the diene **9** together with an isomeric unconjugated diene (4 : 1 ratio) as an inseparable mixture. Hydrolysis of the mixture with aqueous base followed by reaction with sodium cyanate gave the urea **10**. Subsequent reaction of this urea with thionyl chloride–imidazole generated the *N*-sulfinylurea **11** *in situ*, which cyclises to give the single Diels–Alder adduct **12** whose structure was established by X-ray crystallography. Treatment of **12** with phenylmagnesium bromide gave an allylic sulfoxide, which underwent a [2,3]-sigmatropic rearrangement to give the model compound **5**, which possesses the correct C7, C8 and C10 relative configuration for cylindrospermopsin **1** (Scheme 2).



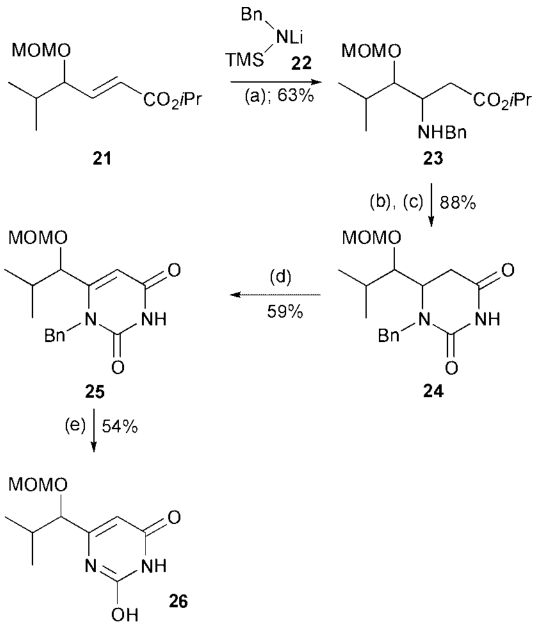
Scheme 2 *Reagents and conditions*: (a) *t*-BuLi, **7**, **8**; (b) KOH, MeOH–H$_2$O; (c) NaOCN, HCl; (d) SOCl$_2$–CH$_2$Cl$_2$, imidazole, −78 °C; (e) PhMgBr, THF, −78 °C; (f) (MeO)$_3$P, MeOH, Δ.



Scheme 3 *Reagents and conditions*: (a) **14**, toluene, rt, 3 h; (b) H$_3$O$^+$; (c) *p*-TsOH, benzene, Δ; (d) CH$_2$=CHMgBr, CuI–THF; (e) L-Selectride, THF; (f) TBSOTf, iPr$_2$NEt, CH$_2$Cl$_2$; (g) BH$_3$–THF; (h) H$_2$O$_2$–NaOH.

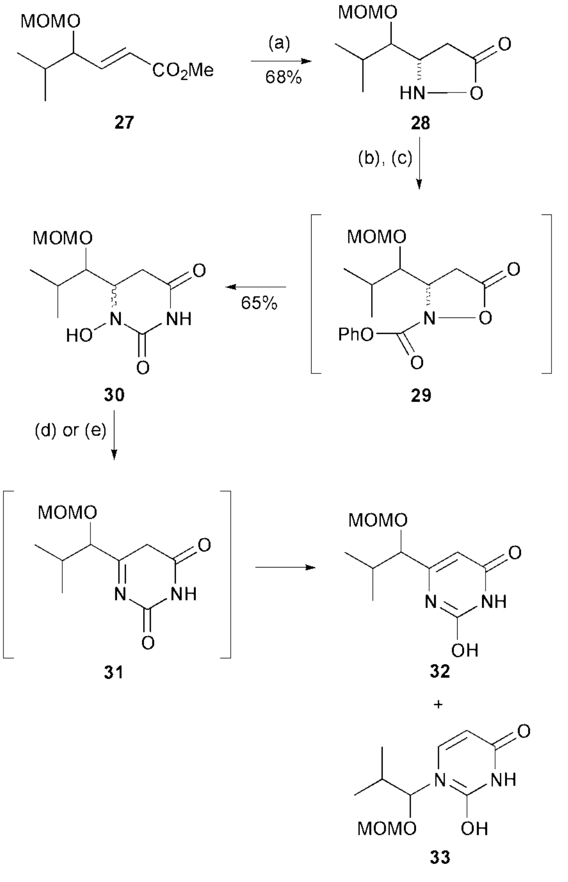
Following this initial report, Heintzelman and Weinreb described the synthesis of the piperidine A-moiety in cylindrospermopsin **1** (Scheme 3).[30] The oxygenated diene **13**, which is a 4 : 1 mixture of *Z*–*E* isomers, was treated with the glyoxylate-derived *N*-tosylimine **14** to afford, after *in situ* acidic hydrolysis, a mixture of epimeric enones **15** and **16**. The yields and ratios of these cycloadducts were dependent upon the specific reaction conditions employed. For example, with AlCl$_3$ as catalyst (PhMe, −78 °C, 3 h) a 53% yield of a 7 : 1 mixture of **15** and **16** was obtained, whilst using ZnCl$_2$ as catalyst under similar conditions, the ratio of **15** and **16** was 22 : 1 (60% total yield). The uncatalysed condensation of diene **13** with imine **14** (PhMe, rt, 3 h) led to a 4.7 : 1 mixture of **15** and **16** in 51% total yield. The major *cis* product **15** arose from the *Z*-diene *via* a transition state having the ester group of the imine **14** *endo*. Alternately, the minor *trans* adduct **16** is derived from the *E*-diene isomer. It was postulated that the variable ratios of **15** and **16** might result from *E*–*Z* isomerisation of the diene under the reaction conditions and/or by epimerisation of the adducts upon work-up and purification. In support of the latter postulate, it was found that when the major keto ester **15** was treated with *p*-TsOH in benzene at reflux, a 1 : 4 mixture of **15** and **16** was produced, from which **16** could be isolated in 57% yield. Similarly, acid-catalysed isomerisation of the minor enone **16** provides the same 1 : 4 equilibrium mixture of **15** and **16**. Enone **16** was subsequently treated with vinylmagnesium bromide to give the single vinyl ketone **17**. L-Selectride reduction of **17** yielded the alcohol **18**, which possesses the correct relative stereochemistry in the cylindrospermopsin A-ring. The alcohol functionality in **18** was protected as the silyl ether **19** and, on hydroboration, gave the primary alcohol **20** in good yield.

Weinreb also reported two novel routes to uracils related to the D-ring of cylindrospermopsin **1**, utilising the conjugate addition of *N*-nucleophiles to α,β-unsaturated esters as the key step (Schemes 4 and 5).[31] Thus, reaction between the silylated lithium amide reagent **22** and the unsaturated ester **21** (−78 °C–rt) afforded the adduct **23** which, on treatment with trichloroacetyl isocyanate, followed by basic hydrolysis produced the desired dihydrouracil **24** in high yield. It was then possible to oxidise **24** with diphenylselenic anhydride to give uracil **25**, the *N*-benzyl group being easily removed by transfer hydrogenolysis to give the desired uracil system **26**.

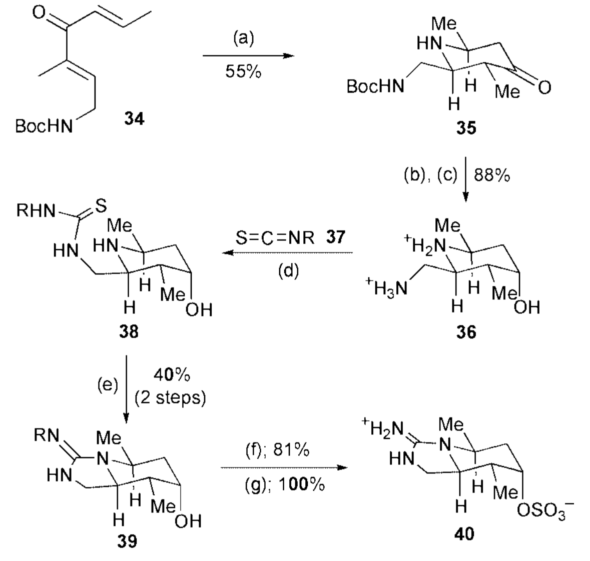


Scheme 4 *Reagents and conditions*: (a) **22**, THF, −78 °C–rt; (b) $Cl_3CCON{=}C{=}O$, CH_2Cl_2; (c) KOH, H_2O, Δ; (d) $(PhSeO)_2O$, toluene, Δ; (e) 10% Pd/C, HCO_2NH_4, MeOH, Δ.

Although this route proved successful, a shorter alternative sequence where both the oxidation step and nitrogen protection could be avoided was devised. Thus, heating the methyl ester **27** with *N*,*O*-bis(trimethylsilyl)hydroxylamine in ethanol at reflux afforded the isoxazolidin-5-one **28** as a 1 : 1 mixture of diastereomers in 68% yield. Treatment of **28** with phenyl chloroformate led to the intermediate *N*-acylation product **29**, which upon ammonolysis afforded the *N*-hydroxydihydrouracil **30**. Subsequent treatment of **30** with triflic anhydride–pyridine at room temperature led to the requisite uracil **32**, together with the unusual rearranged by-product **33**, the formation of which could be prevented by treating compound **30** with tosyl chloride–DMAP in 1,2-dichloroethane at reflux to give **32** in 81% yield.



Scheme 5 *Reagents and conditions*: (a) *N*,*O*-bis(trimethylsilyl)hydroxylamine, EtOH, Δ; (b) PhOCOCl, THF, NEt_3; (c) NH_4OH, iPrOH; (d) Tf_2O, pyridine, CH_2Cl_2, rt, **32** (54%), **33** (34%); or (e) TsCl, DMAP, $ClCH_2CH_2Cl$, reflux, 21 h, **32** (81%).



Scheme 6 *Reagents and conditions*: (a) NH_4OH, NH_4Cl, MeOH, 67 °C, 16 h; (b) L-Selectride; (c) TFA; (d) **37**; (e) $HgCl_2$, Et_3N, DMF; (f) Zn, HOAc, H_2S, HCl (aq); (g) DMF–SO_3, DMF. R = $CO_2CH_2CCl_3$ (Troc).

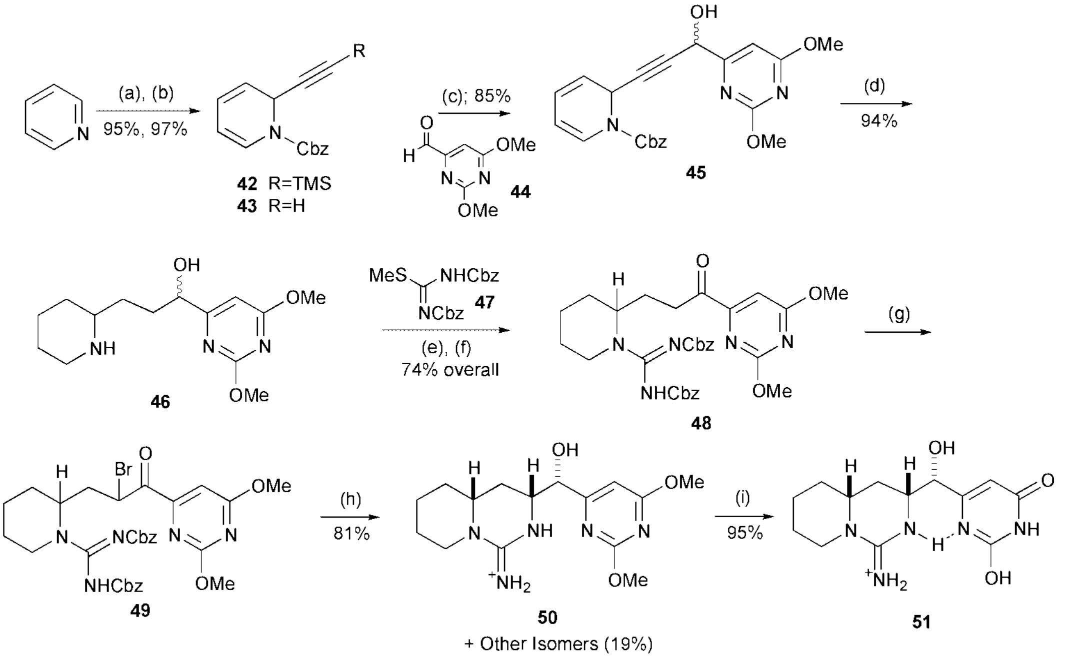
A second major contribution to the synthesis of cylindrospermopsin **1** was reported by the research group of Snider, beginning with the synthesis of the bicyclic model compound **40** (Scheme 6).[32] They reported that the addition of ammonia to dienone **34** (prepared in five steps from (*E*)-1,3-dibromobut-2-ene) gave the piperidinone **35**, which has all its large substituents in an equatorial configuration as is necessary for cylindrospermopsin **1**. To prevent a retro-Michael reaction during guanidine formation, the ketone **35** was reduced with L-Selectride which, following removal of the *t*-Boc protecting group, gave the diamino alcohol **36**. This alcohol was subsequently treated with the isothiocyanate **37** to give the protected thiourea **38**, which was reacted with $HgCl_2$ and Et_3N

in DMF to close the second ring and give the key bicyclic guanidine **39** in 40% yield from **36**. Deprotection of **39** by using zinc dust in aqueous acetic acid gave, after acidic work-up, the corresponding guanidinium chloride, which on subsequent sulfonation with one equivalent of DMF–SO_3 in DMF gave the AC model compound **40** in quantitative yield.

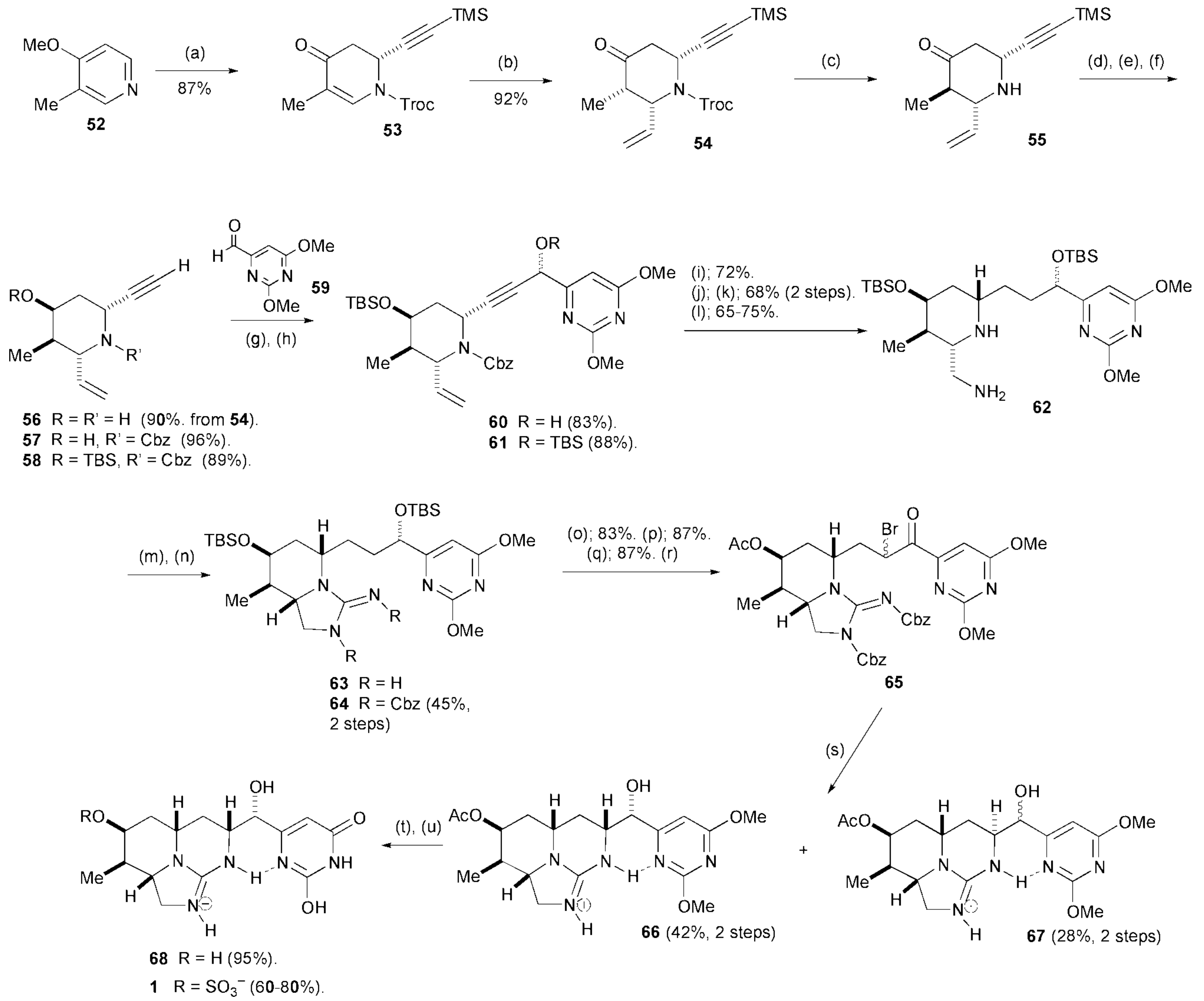
Snider next addressed the problem of constructing the B-ring and the hydroxymethyluracil side chain of cylindrospermopsin **1** with a report on the synthesis of the AB model compound **51** (Scheme 7).[33] Addition of trimethylsilylethynylmagnesium bromide **41** to pyridine mediated by benzyl chloroformate gave the silylacetylene **42**, which was desilylated with potassium carbonate in MeOH to give the terminal alkyne **43** in high yield. Treatment of **43** with EtMgBr gave the corresponding alkynyl magnesium bromide, which was reacted with the aldehyde **44** to provide the alcohol **45** as a mixture of diastereomers. Subsequent hydrogenation of **45** over 5% Pd/C reduced the dihydropyridine and alkyne, and cleaved the Cbz group affording 94% of piperidine **46** as a mixture of diastereomers. The guanidine group was introduced by reaction of **46** with *N*,*N*′-bis(benzyloxycarbonyl)-*S*-methylisothiourea **47**, Et_3N, and $HgCl_2$ in DMF, and was followed by oxidation of the alcohol with the Dess–Martin reagent to afford the ketone **48**. Bromination of **48** provided the unstable bromo ketone **49**, which on hydrogenolysis liberated the free guanidine, which, in turn, underwent cyclisation to form the B-ring of the model compound. Under these conditions, the ketone was also hydrogenated and led to an 81% yield of alcohol **50**, together with a 19% yield of three other stereoisomers. The dimethoxypyrimidine was cleaved by heating at reflux in concentrated hydrochloric acid for 6 h, with no decomposition or isomerisation occurring under these forcing conditions. It was noted that protonation of the guanidine and uracil rings shielded the alcohol from solvolysis. This process gave **51**, with ^1H and ^{13}C NMR spectral data very similar to those reported for cylindrospermopsin **1**.

Following this second approach, Snider and co-workers reported the first total synthesis of cylindrospermopsin **1** from 4-methoxy-3-methylpyridine **52** (Scheme 8).[34] Thus, treatment of **52** with TrocCl and then trimethylsilylethynylmagnesium bromide **41** at −30 °C gave a 49% (87% based on recovered **52**) yield of **53**. Piperidone **54** was then formed by Michael addition of vinylmagnesium bromide to the enone of **53** catalysed by TMSCl. Cleavage of the Troc protecting group in **54** with zinc dust in acetic acid afforded the free piperidone, in which the methyl group adjacent to the ketone has equilibrated under the acidic conditions, to give the thermodynamically stable isomer **55** with all three large substituents equatorial. Reduction of **55** with L-Selectride in THF at −78 °C followed by basic hydrolysis afforded the axial alcohol and cleaved the silyl group to give the piperidine **56** with all four stereogenic centres of the A-ring of cylindrospermopsin **1** in place. Protection of **56** with CbzCl afforded carbamate **57**, which was subsequently silyl protected to give **58**. Treatment of **58** with ethylmagnesium bromide formed the corresponding alkynylmagnesium bromide, which when reacted with aldehyde **59** gave an 83% yield of alcohol **60** as a 1 : 1 mixture of diastereomers. After silyl protection of the hydroxy group to form **61**, ozonolysis of the alkene substituent was followed by reductive amination using benzylamine, which, in turn, was followed by hydrogenation to afford a 65–75% overall yield of the crude diamine **62**. Formation of the required guanidine was effected by the slow addition of 1 equivalent of cyanogen bromide to a dilute toluene solution of **62** to give a primary cyanamide, which cyclised to form guanidine **63** in which the A- and C-rings of cylindrospermopsin have been established. Finally Cbz-protection of **63** afforded **64** in 45% overall yield from **62**. Desilylation of **64** was followed by selective oxidation of the benzylic hydroxy group using MnO_2 in CH_2Cl_2. Subsequent acetylation of the remaining alcohol group was followed by bromination to give an unstable mixture of α-bromo ketones **65**. Hydrogenation of this mixture liberated the free guanidine which, in turn, underwent cyclisation, with concomitant ketone reduction, to give an easily separable 3 : 2 mixture of the tricyclic guanidines **66** and **67** in 70% yield over two steps. Hydrolysis of **66** in concentrated hydrochloric acid at 100 °C for 6 h afforded the uracil diol **68**, which on sulfonation with 6 equivalents of SO_3–DMF gave racemic cylindrospermopsin **1** in 60–80% yield.

Shortly before the publication of Sniders total synthesis of cylindrospermopsin **1**, Armstrong and McAlpine reported an enantioselective synthesis of a guanidine tricycle similar to that found in cylindrospermopsin **1**. Their approach employed an intramolecular conjugate addition reaction as the key step to forming the piperidine A-ring, which was combined with a



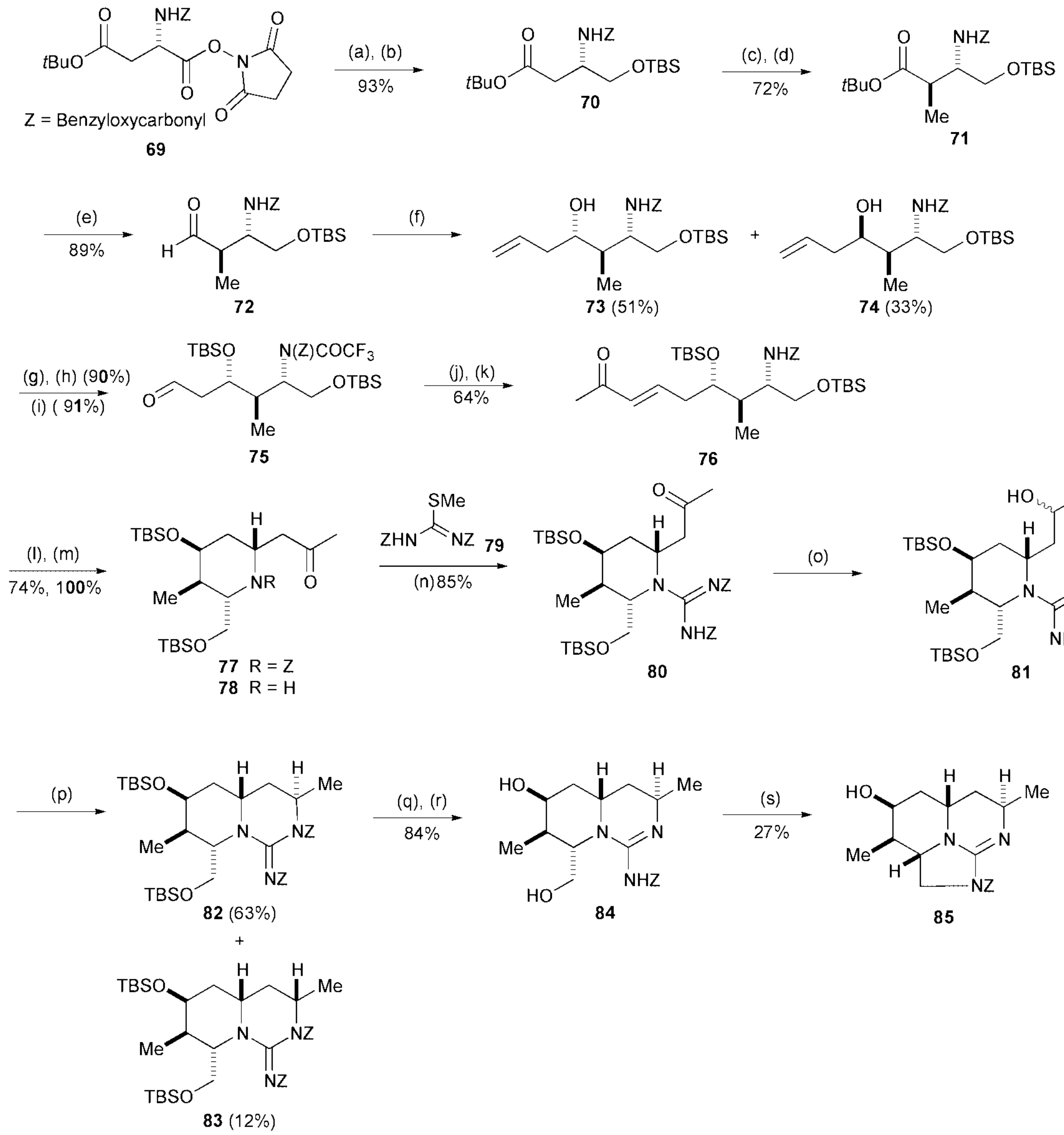
Scheme 7 *Reagents and conditions*: (a) BnOCOCl, THF, TMSC≡CMgBr **41**, 0 °C, 2 h; (b) K_2CO_3, MeOH, 25 °C, 20 min; (c) EtMgBr then **44**; (d) H_2, Pd/C, MeOH; (e) **47**, $HgCl_2$, Et_3N, DMF; (f) Dess–Martin periodinane; (g) $CuBr_2$, EtOAc, 40 °C, 15 min; (h) H_2, Pd/C, MeOH, 2 h (81% two steps); (i) conc. HCl, reflux, 6 h.



Scheme 8 *Reagents and conditions*: (a) TrocCl, THF, −30 °C, TMSC≡CMgBr **41**; (b) $CuBr{\cdot}SMe_2$, −78 °C, vinylmagnesium bromide, TMSCl, THF; (c) Zn, HOAc; (d) L-Selectride; (e) CbzCl, Na_2CO_3, THF; (f) TBSCl, imidazole, DMAP, CH_2Cl_2; (g) EtMgBr, THF, 0 °C; (h) TBSCl, imidazole, DMAP, CH_2Cl_2; (i) O_3, DMS, CH_2Cl_2, −78 °C; (j) NH_2Bn, HOAc, benzene; (k) $NaCNBH_3$, MeOH; (l) 5% Pd/C, H_2, MeOH; (m) CNBr, benzene; (n) NaH, CbzCl, THF; (o) TBAF, THF; (p) MnO_2, CH_2Cl_2; (q) Ac_2O, pyridine, rt; (r) $CuBr_2$, EtOAc, rt, 30 min; (s) H_2, $Pd(OH)_2/C$, MeOH; (t) conc. HCl reflux; (u) SO_3–DMF, pyridine, DMF (Troc = $CO_2CH_2CCl_3$).

Mitsunobu strategy for the formation of the B- and C-rings (Scheme 9).[35] The enone **76** required for this reaction was prepared in 11 steps from the commercially available activated aspartic acid derivative **69**. Sodium borohydride reduction of **69** and subsequent silyl protection gave ester **70** which was methylated (LDA–MeI) to give **71** as the major product. DIBAL-H reduction of **71** gave aldehyde **72**, which was allylated using allyl (−)-isopinocamphorborane to give alcohols **73** and **74** in a 2:1 ratio and 70% yield. An alternative approach using allyltributyltin and BF_3–OEt_2, gave a superior overall yield of 84% but with diminished diastereoselectivity for the formation of **73** and **74** (1.5:1). After protection of the alcohol and carbamate functions in **73**, ozonolysis led to the aldehyde **75**, which was olefinated under Wadsworth–Emmons conditions to give the required enone **76** in 64% yield. The key cyclisation reaction was effected by reacting enone **76** with a catalytic amount of *p*-TsOH in benzene at reflux, to give the piperidine **77** as a single diastereoisomer with all 4 stereocentres of the A-ring of cylindrospermopsin **1** in place. Hydrogenation of **77**, yielded the piperidine **78** which was subsequently guanylated with bis(benzyloxycarbonyl)methylthiopseudourea **79** to give guanidine **80** in 85% yield. Reduction of **80** under non-asymmetric conditions gave an inseparable (5:1) mixture of alcohols **81** which on cyclisation under Mitsunobu conditions gave the bicyclic guanidines **82** and **83** in a 5:1 ratio and 75% combined yield. Unfortunately the major epimer **82** was found to have the incorrect configuration at C7 with respect to cylindrospermopsin. Despite this, the feasibility of the methodology was demonstrated as, after removal of one of the Z-protecting groups and both of the silyl protecting groups, the diol **84** was cyclised under Mitsunobu conditions leading to the guanidine tricycle **85** as the only product.

A further approach towards the synthesis of cylindrospermopsin **1** was reported by Hart and co-workers who developed a synthetic methodology for the synthesis of the BD-ring system of **1** using an intramolecular conjugate addition to an alkynylpyrimidine (Scheme 10).[36] The substrate for this reaction was prepared from the carboxylic acid **86**, which was converted into urea **87** using routine chemistry and subsequently coupled with 6-bromo-2,4-dimethoxypyrimidine to afford the alkynylpyrimidine **88** in 90% yield. In the key step they found that treatment of **88** with sodium hydride in tetrahydrofuran resulted in the clean formation of the pyrimidine **89** in high yield. Subsequent treatment of **89** with dimethyldioxirane in the presence of methanol provided an unstable mixture of *N*,*O*-acetals **90**, which on careful reduction gave a 78:22 mixture of isomeric alcohols **91** and **92**. Although **91** and **92** could not be separated by chromatography or crystallisation, they could be separated after conversion to the corresponding cyclic *N*,*O*-acetals (or carbamates) and re-

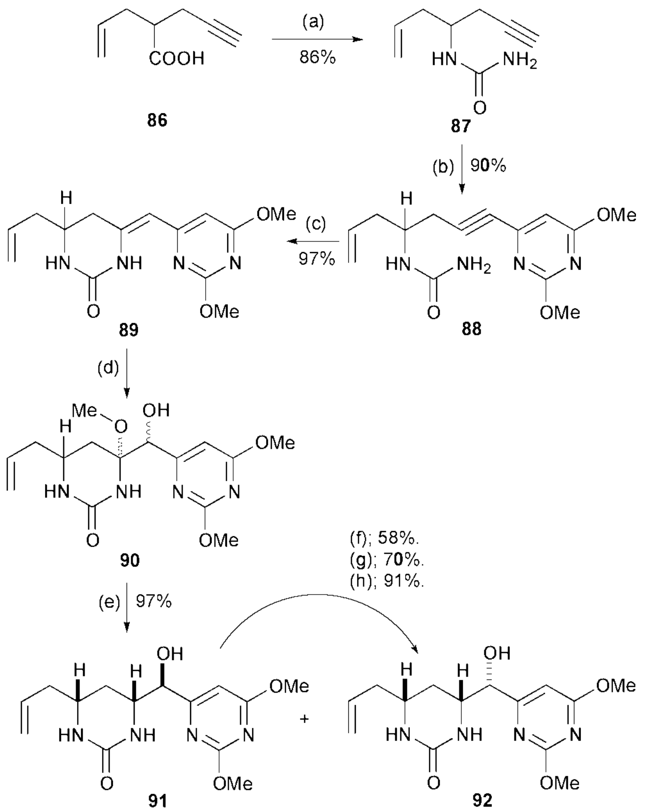


Scheme 9 *Reagents and conditions*: (a) $NaBH_4$, THF, 0 °C; (b) TBSOTf, Et_3N, 2,6-lutidine; (c) LDA, LiCl, THF, −78 °C; (d) MeI; (e) DIBAL-H, toluene, −78 °C; (f) BF_3–OEt_2, allylBu_3Sn, CH_2Cl_2, −78 °C; (g) TBSOTf, 2,6-lutidine; (h) TFAA, Et_3N; (i) O_3, CH_2Cl_2, −78 °C, then PPh_3; (j) LDA, $(MeO)_2(O)PCH_2C(O)CH_3$; (k) Na_2CO_3, MeOH; (l) PTSA, benzene; (m) H_2, Pd/C; (n) bis(Z)methylthiopseudourea **79**, $HgCl_2$, Et_3N, DMF; (o) $NaBH_4$, MeOH; (p) PPh_3, DIAD; (q) NaH, THF, MeOH; (r) TBAF, THF; (s) PPh_3, DIAD.

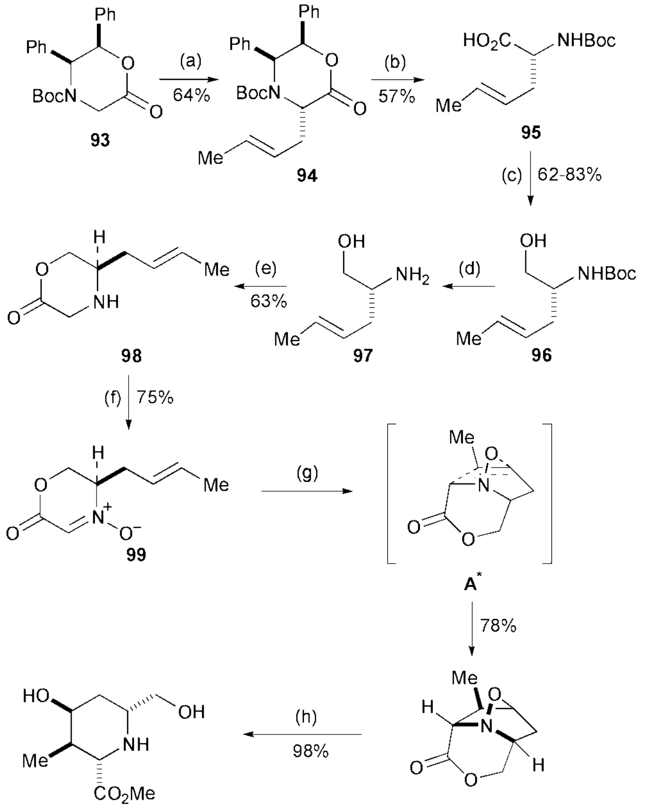
generated on acid treatment. Major isomer **91** could then be converted to the correct isomer **92** using a Mitsunobu protocol.

Recent work by Williams and Looper described an efficient asymmetric synthesis of an A-ring synthon for cylindrospermopsin **1** utilising, as the key step, an intermolecular oxazinone *N*-oxide/alkene dipolar cycloaddition (Scheme 11).[37] The required intermediate for this reaction, **99**, was prepared by crotylation of the sodium enolate of oxazinone **93** leading to **94**, which on subsequent Birch reduction afforded *N*-*t*-Boc-crotylglycine **95** in 57% yield and in >99:1 er. Reduction of **93** led to the alcohol **96**, which was subsequently deprotected to give the amino alcohol **97**. This in turn, was converted to the oxazin-2-one **98** by reaction with α-bromophenyl acetate. As **98** was prone to dimerisation, oxidation was carried out immediately after isolation using the Davis oxaziridine to give the conjugated 2-oxooxazine *N*-oxide **99** in 75% yield. In contrast to **98**, the *N*-oxide **99** was surprisingly stable and no dimerisation or spontaneous cyclisation was observed. However, exposure of **99** to elevated temperatures cleanly effected a 1,3-dipolar cycloaddition reaction to give the tricyclic isoxazolidine **100** in 87% isolated yield. It is probable that the nitrone **99** added suprafacially to the alkene predominantly through the chair like *exo*-transition state A* to give **100**. Hydrogenolysis of **100** in methanol led directly to the methyl ester **101**, which possesses the correct absolute stereochemistry required for the cylindrospermopsin A-ring.

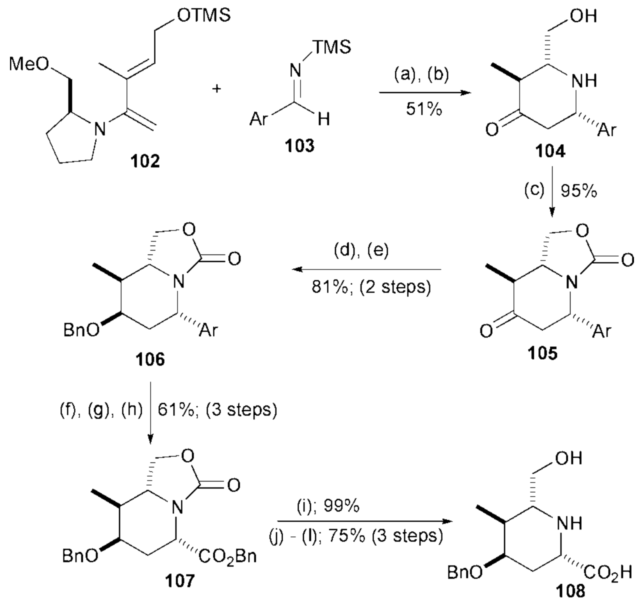
In 1998, Barluenga *et al.* described the synthesis of a similar A-ring model for cylindrospermopsin **1** (Scheme 12),[38] The approach taken was based upon their previously reported[39] methodology for the enantioselective synthesis of functionalised 4-piperidones by [4 + 2] cycloaddition of *N*-silylaldimines with chiral 2-aminobuta-1,3-dienes. For example, reaction of diene **102** with trimethylsilylaldimine **103** in the presence of $ZnCl_2$ gave 4-piperidone **104**. This was converted into the cyclic carbamate **105** on treatment with triphosgene and, in order to avoid undesired epimerisations at the stereocentre bearing the methyl group, the carbonyl functionality was stereoselectively reduced and benzyl protected to give **106** in high overall yield. Oxidative degradation of the furan ring was effected using O_3 and H_2O_2, and the crude carboxylic acid formed was converted to the corresponding benzyl ester **107** to aid isolation and purification. Hydrogenation of the benzyl ester and basic cleavage of the carbamate group gave the (hydroxymethyl)pipecolic acid **108** in 75% yield, as a single stereoisomer with the identical relative stereochemistry to that found



Scheme 10 *Reagents and conditions*: (a) i) $(COCl)_2$, ii) NaN_3, Δ, iii) NH_3; (b) $(Ph_3P)_2PdCl_2$, CuI, Et_3N, 6-bromo-2,4-dimethoxypyrimidine; (c) NaH, THF; (d) DMD, MeOH–CH_2Cl_2, acetone; (e) $NaBH_3CN$, MeOH, H_2O, pH 4; (f) $(CH_3)_2C(OMe)_2$, TsOH; (g) HCl (aq); (h) i) p-$NO_2C_6H_4COOH$, DEAD, PPh_3, ii) K_2CO_3–MeOH.



Scheme 11 *Reagents and conditions*: (a) NaHMDS, THF, crotyl chloride; (b) Li, NH_3(l), 57%; (c) $LiAlH_4$, 0 °C, THF, 62–83%; (d) BF_3–Et_2O, CH_2Cl_2, 5 eq. 1,3-dimethoxybenzene; (e) $C_6H_5OCOCH_2Br$, iPr_2NEt, MeCN, 63% (2 steps); (f) $PhCH(O)(NSO_2Ph)$, THF, 0 °C, 75%; (g) toluene, 200 °C, sealed tube; (h) H_2, Pd/C, MeOH, 98%.



Scheme 12 *Reagents and conditions*: (a) $ZnCl_2$; (b) SiO_2; (c) triphosgene, NEt_3, THF; (d) K-Selectride, THF, −78 °C; (e) NaH, BnBr, DMF, 120 °C; (f) O_3, CH_2Cl_2, NaOH, H_2O_2, −78 °C; (g) $(COCl_2)$, CH_2Cl_2; (h) BnOH, CH_2Cl_2; (i) H_2, Pd/C, EtOH; (j) t-BuOK, THF, reflux; (k) HCl 3 M; (l) Dowex 50 W. Ar = 3-furyl.

in the A-ring of cylindrospermopsin **1**, albeit in the opposite enantiomeric form to that proposed for the natural material.

6 Conclusion†

Cyanobacterial blooms containing the toxin cylindrospermopsin **1** are now known to be widespread in freshwater aquaculture ponds, especially in tropical regions. The consequences of its consumption are apparent and of particular worry; this consideration alone makes the study of this intriguing metabolite a worthwhile pursuit. On consideration of the current literature, it is apparent that this molecule has inspired many organic chemists to develop novel and elegant synthetic methodologies for its preparation which have culminated in the recent publication of the first total synthesis of cylindrospermopsin. We are confident that the results detailed in this review will be developed in the near future and be applied to an enantioselective total synthesis of cylindrospermopsin.

7 Acknowledgements

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† Whilst this manuscript was in preparation several further articles appeared on the isolation[40] and photocatalytic degradation[41] of cylindrospermopsin **1** as well as a study on the effect of growth conditions[42,43] on the production of **1**. Evidence has also been found for *in vivo* tumour initiation by oral administration of extracts of *Cylindrospermopsis raciborskii*.[44] In addition, it was also reported that two new derivatives of cylindrospermopsin **1** were formed when the natural material was treated with a chlorine solution.[45] These were identified as 5-chlorocylindrospermopsin and a product where the C6 of the pyrimidine ring was oxidised to a carboxylic acid (cylindrospermic acid). Interestingly, both compounds showed no toxic effects even at doses 50 times higher than the LD_{50} of cylindrospermopsin **1**, suggesting that the uracil moiety is required for the toxicity of **1**.

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