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Total Synthesis of (±)-Cylindrospermopsin

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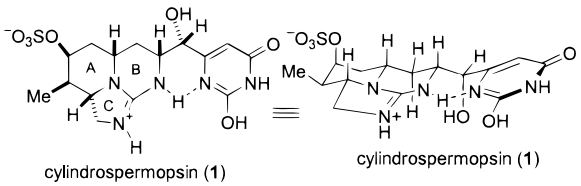
Abstract: The first total synthesis of the novel hepatotoxin (±)-cylindrospermopson (**1**) has been accomplished in 20 steps from 4-methoxy-3-methylpyridine (**12**) in 3.5% overall yield. The substituted piperidine A ring **19** was generated stereospecifically by a four-step sequence using the addition of trimethylsilylethynylmagnesium bromide to **12** to give **16** and stereospecific addition of vinylcuprate to **16** to form **17**. The reaction of diamine **26** with cyanogen bromide produced the cyclic guanidine C ring of **27**. The key step in the synthesis was bromination of ketone **31**, followed by hydrogenation to liberate the free guanidine, which underwent an intramolecular S_N2 reaction to form the tetrahydropyrimidine ring B of **32**. Further hydrogenation reduced the ketone to yield 42% of **32** containing the fully functionalized tricyclic system and protected hydroxymethyluracil side chain of cylindrospermopsin. Hydrolysis of the pyrimidine in concentrated hydrochloric acid and selective monosulfation completed the synthesis of cylindrospermopsin.

Introduction

Cylindrospermopsin (**1**) was isolated from the cyanobacterium *Cylindrospermopsis raciborskii* by Moore and shown to be the causative agent of a 1979 outbreak of hepatoenteritis in Australia.[1,2] The structure of cylindrospermopsin was determined spectroscopically. The relative stereochemistry of the ring carbons was assigned on the basis of analysis of the coupling constants.[1,2] The stereochemistry of the side chain alcohol was tentatively assigned on the basis of the coupling constants, NOEs, and the unusual behavior of the uracil carbons in the ^{13}C NMR spectrum, which suggested that the guanidinium hydrogen is hydrogen bonded to the imine tautomer of the uracil as shown. Feeding studies indicated that cylindrospermopsin is an acetogenin with guanidinoacetic acid serving as the starter unit of the polyketide chain.[3]



Cylindrospermopsin has also been isolated from *Umezakia natans* and *Aphanizomenon ovalisporum*.[4−8] Algal blooms that

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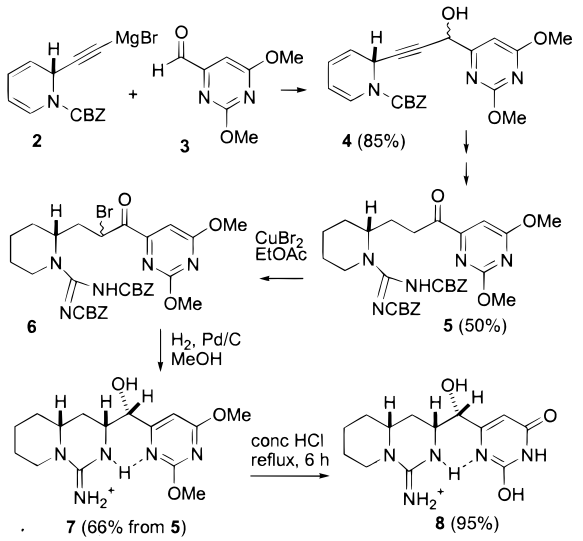
produce cylindrospermopsin are widespread in tropical waters where occurrences of gastrointestinal diseases of unknown origin are common.[4−16] The toxicological effects of cylindrospermopsin and analytical techniques for its detection in drinking water have been extensively studied.[17−28]

Cylindrospermopsin is hepatotoxic in mice and appears to function by depletion of the cellular antioxidant glutathione (GSH) in hepatocytes.[17,19] The renal toxicity of cylindrospermopsin in mice has also been demonstrated.[23] 7-Deoxycylindrospermopsin[26] and 7-epicyclindrospermopsin[27] have recently been isolated.

The novel structure of cylindrospermopsin, with a guanidine embedded in a tricyclic system, six chiral centers, and polar sulfate, uracil, and guanidine functional groups, makes its synthesis challenging. Its potent toxicity makes the synthesis of cylindrospermopsin an important problem that has been the subject of intense interest.[29−36]

We recently reported a short synthesis of model **8** that contains the AB rings and hydroxymethyluracil side chain of cylindrospermopsin (**1**).[33] Acetylide **2** was prepared by addition of trimethylsilylethynylmagnesium bromide to pyridine and benzyl chloroformate.[37] Methyl 2,6-dimethoxy-4-pyrimidinecarboxylate was prepared by the procedure of Gershon.[38] Reduction of the ester with $LiBH_4$ in THF afforded 93% of the primary alcohol, which was oxidized to 90% of aldehyde **3**[39] with Dess−Martin reagent. Addition of acetylide **2** to aldehyde **3** afforded 85% of coupled alcohol **4**. Hydrogenation, guanidinylation, and oxidation gave ketone **5** in 50% overall yield. Bromination of **5** with $CuBr_2$[40] in EtOAc for 15 min at 40 °C provided unstable bromo ketone **6**. Hydrogenolysis of crude **6** over Pd/C in MeOH liberated the free guanidine, which underwent an intramolecular S_N2 reaction to form the second ring. Under these conditions the ketone was hydrogenated[41] so that we selectively obtained 66% of the desired hydroxy guanidine **7** and 15% of the other three diastereomers. The model study was completed by hydrolysis of the pyrimidine ring in concentrated hydrochloric acid at reflux[42] for 6 h to afford **8** quantitatively.



We envisioned that cylindrospermopsin (**1**) could be prepared analogously by bromination of ketone **9**. Ketone **9** should be

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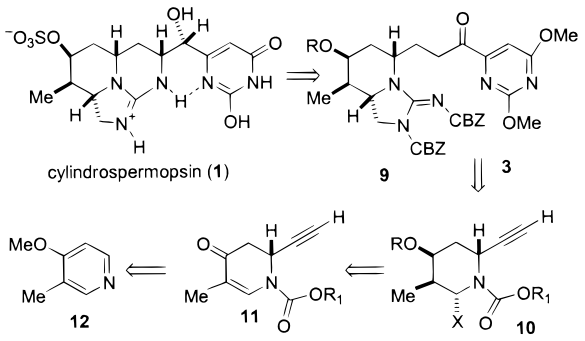
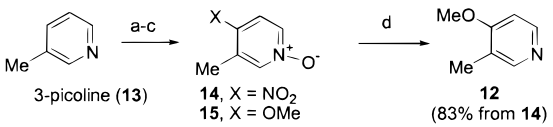
accessible by addition of acetylene **10**, where X is a latent aminomethyl group, to aldehyde **3**. It should be possible to prepare **10** by conjugate addition to vinylogous amide **11**, as extensively studied by Comins.[43] Vinylogous amide **11** should be available by addition of trimethylsilylethynylmagnesium bromide to 4-methoxy-3-methylpyridine (**12**). We report here the realization of this approach leading to the first synthesis of (±)-cylindrospermopsin in 20 steps from **12** and 3.5% overall yield.



Results and Discussion

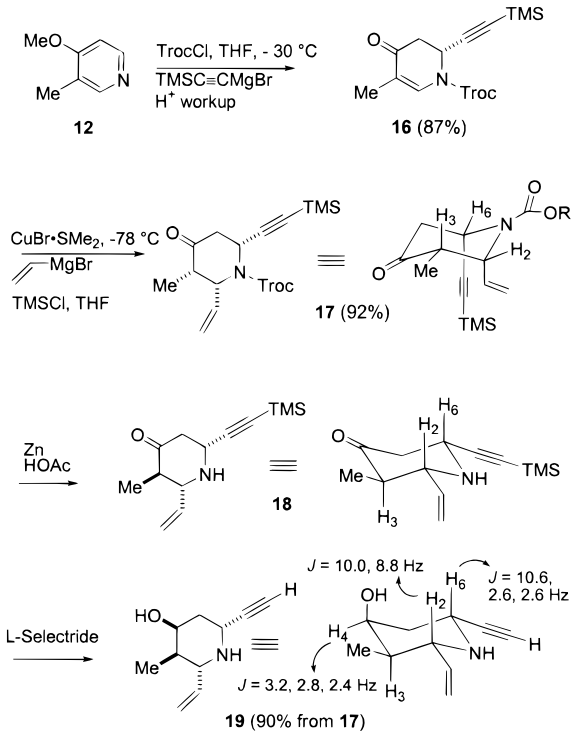
4-Methoxy-3-methylpyridine (**12**)[44,45] was prepared by modifications of the literature procedure. 3-Methyl-4-nitropyridine *N*-oxide (**14**),[46] was treated with K_2CO_3 in methanol at 70 °C to displace the nitro group providing **15**.[45] Hydrogenolysis of crude **15** over 10% Pd/C under 45 psi H_2 for a week reduced the *N*-oxide to provide **12** in 83% yield from **14**.



(a) H_2O_2, HOAc, reflux; (b) HNO_3, H_2SO_4,95 °C, 24 h; (c) K_2CO_3, MeOH, reflux, 4 h; (d)10% Pd/C, 45 psi H_2, EtOH

Addition of trimethylsilylethynylmagnesium bromide to **12** could occur at either C-2 or C-6. We anticipated that the 3-methyl group would block C-2, so that addition should occur regioselectively at C-6 even with a sterically undemanding acetylide nucleophile. Investigation of conjugate additions to **16** (see below) indicated that a vinyl group was a suitable latent aminomethyl group. Therefore, benzyl chloroformate could not be used to activate the pyridine to nucleophilic attack as in the synthesis of **2** since the Cbz group could not be removed without reduction of the vinyl group. Treatment of **12** with TrocCl and then trimethylsilylethynylmagnesium bromide at −30 °C and hydrolysis of the dihydropyridine with hydrochloric acid afforded 49% (87% based on recovered **12**) of **16** and 2−3% of the regioisomer resulting from addition at C-2. Reaction of **16** with Et_2AlCN afforded <20% of the desired 1,4-adduct. Other procedures for cyanide addition were even less successful.

We then examined the copper-catalyzed addition of vinylmagnesium bromide to **16** since Comins has shown that cuprates add to C-2 cis to a substituent at C-6 in a wide variety of *N*-carboalkoxy-5,6-dihydro-4-pyridones.[43] Piperidone **17** was



generated in 66% yield by treatment of **16** with vinylmagnesium bromide and $BF_3{\cdot}Et_2O$ in the presence of a catalytic amount of $CuBr{\cdot}Me_2S$ at −78 °C in THF. The yield of **17** was improved to 92% by utilizing TMSCl[47] instead of $BF_3{\cdot}Et_2O$ to activate the cuprate addition. Due to the strong $A^{(1,3)}$ strain between the alkynyl and *N*-acyl group of **16**, dihydropyridone **16** adopts the chair conformation with an axial alkynyl group. Stereoelectronically preferred axial attack by the organocuprate reagent at C-2 led to the desired isomer **17** with cis axial alkynyl and vinyl substituents as observed by Comins in related examples.[43] The stereochemistry of the methyl group adjacent to the carbonyl group was introduced by axial protonation of the enolate which gives the more stable equatorial methyl group. Attempted equilibration of **17** in K_2CO_3/MeOH gave only recovered starting material confirming that **17** is the thermodynamic product.

Cleavage of the Troc group of **17** with zinc dust in acetic acid for 30 min afforded the free piperidone, which flipped to give the conformer with equatorial vinyl and alkynyl substituents and an axial methyl group. The methyl group adjacent to the ketone equilibrated under the acidic reaction conditions over an additional 5 h to give the thermodynamically more stable isomer **18** with all three substituents equatorial. Reduction of **18** with L-Selectride in THF at −78 °C and basic hydrolysis afforded the axial alcohol and cleaved the alkynylsilane providing the desired piperidine **19** in 90% yield from **17** with control of all four chiral centers on the A ring. The stereochemistry of **19** was confirmed by analysis of the coupling constants. Large diaxial coupling constants, $J_{6-5ax} = 10.6$ Hz and $J_{2-3} = 10.0$ Hz, established that the methyl, ethynyl, and vinyl substituents are equatorial. The absence of large diaxial coupling constants for H_4, $J = 3.2$, 2.8, 2.4 Hz, indicated that the hydroxy group is axial.

Conversion of **19** to ketone **9** required guanidinylation, elaboration of the vinyl group to an aminomethyl group, and addition of the acetylene to aldehyde **3**. We were unable to convert the vinyl group to an aminomethyl group after introduction of the guanidine, and could not add the acetylene to aldehyde **3** after introduction of the aminomethyl group.

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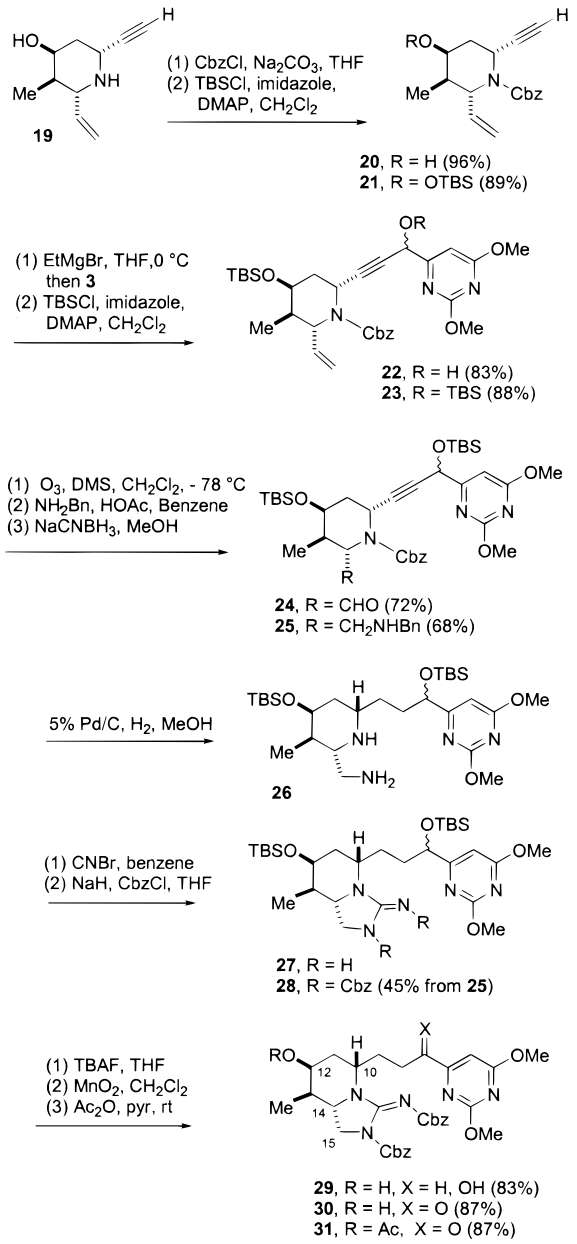
Therefore, we protected the amino and hydroxy groups of **19** to give alkyne **21**, which was coupled with aldehyde **3**, even though this generated a mixture of stereoisomers that had to be carried through several steps before they reconverged in the oxidation of alcohol **29** to ketone **30**.

Treatment of **19** with CbzCl and Na_2CO_3 in THF afforded 96% of carbamate **20**. Protection of the alcohol of **20** with TBSCl, imidazole and a catalytic amount of DMAP in CH_2Cl_2 provided 89% of **21**. Treatment of **21** with ethylmagnesium bromide formed the alkynylmagnesium bromide, which was treated with **3** to yield 83% of alcohol **22** as a 1:1 mixture of diastereomers. Protection of the alcohol of **22** with TBSCl, imidazole, and a catalytic amount of DMAP in CH_2Cl_2 gave 88% of **23**.



Ozonolysis of **23** for 20 min cleaved the double bond, providing 72% of aldehyde **24** after reductive workup with Me_2S. Condensation of aldehyde **24** with benzylamine in benzene, followed by reduction of the resulting imine with $NaCNBH_3$,[48] afforded 68% of benzylamine **25**. Hydrogenation (1 atm) of **25** over 5% Pd/C in MeOH reduced the triple bond and hydrogenolyzed the benzyl and Cbz groups to afford 65−75% of crude diamine **26**.

Conversion of the diamine of **26** to a guanidine proved very challenging. Eventually we found that slow addition of 1 equiv of cyanogen bromide[49] to **26** in dilute toluene solution gave the primary cyanamide which cyclized to form guanidine **27**. The use of excess cyanogen bromide led to the bis-cyanamide. Protection of the guanidine with CbzCl and NaH in THF at room temperature for 8 h afforded **28** in 45% overall yield from **25** and 10% of a byproduct in which one Cbz group and one benzyl group are attached to the guanidine. Presumably, reaction of chloride ion with benzyl chloroformate formed benzyl chloride.

Desilylation of **28** with TBAF in THF at room temperature overnight gave 83% of **29**, which was oxidized with MnO_2 in CH_2Cl_2 to form 87% of ketone **30**. Since the eight steps from **22** to **30** were carried out with a mixture of diastereomers, the structure and stereochemistry of ketone **30** was carefully confirmed spectroscopically. The coupling constant between H_{13} and H_{14}, $J = 11.0$ Hz, and H_{10} and H_{11ax}, $J = 11.6$ Hz, demonstrated that H_{10}, H_{13} and H_{14} are axial. H_{12}, with a very narrow $W_{1/2}$, is equatorial. The small geminal coupling constant for H_{15}, $J = 10.0$ Hz, established that the five-membered C ring had been formed.

Bromination of ketone **30** could not be accomplished. The alcohol was therefore acetylated with acetic anhydride in pyridine at room temperature, which gave 87% of a 9:1 mixture of **31** and the enol acetate. The enol acetate was the only product when DMAP was added to the reaction mixture. Basic hydrolysis of the enol acetate with $KHCO_3$ in methanol also cleaved one of the Cbz groups; thus, the 9:1 mixture was used since both the ketone and enol acetate should form the same bromo ketone.

Bromination of **31** with $CuBr_2$ in EtOAc at room temperature for 30 min gave an unstable mixture of bromo ketones and a tricyclic compound in which one of the Cbz groups had been lost and the S_N2 reaction had taken place. Immediate hydrogenation of this mixture over 5% Pd/C in methanol provided a 1:3 mixture of the desired product **32** and stereoisomer **33**. Fortunately, hydrogenation over 20% $Pd(OH)_2/C$ gave an easily separable 3:2 mixture of **32** and **33** in 71% yield from the mixture of acetoxy ketone **31** and the enol acetate. Hydrogenolysis of the Cbz groups afforded the free guanidine, which underwent an intramolecular S_N2 reaction to form the third ring. We expected that hydrogenation of the ketone should be the slowest step. Equilibration of the ketone side chain before hydrogenation should favor the desired isomer **32** with an equatorial side chain. We therefore carried out the hydrogenation in the presence of bases such as K_2CO_3, Et_3N, and $KHCO_3$ and acids such as formic acid and HCl. Unfortunately, none of the acids improved the ratio of **32** to **33**. Much lower yields were obtained in the presence of the bases.

The stereochemistry of the newly formed six-membered ring of **32** was assigned on the basis of the coupling constant, $J_{8,9ax} = 11.6$ Hz, which is very similar to that of **1** ($J_{8,9ax} = 11.4$ Hz). This established that H_8 is axial as in **1**. The absorption for H_7 in **32**, δ 4.68 (d, $J = 3.7$ Hz) corresponds closely to that

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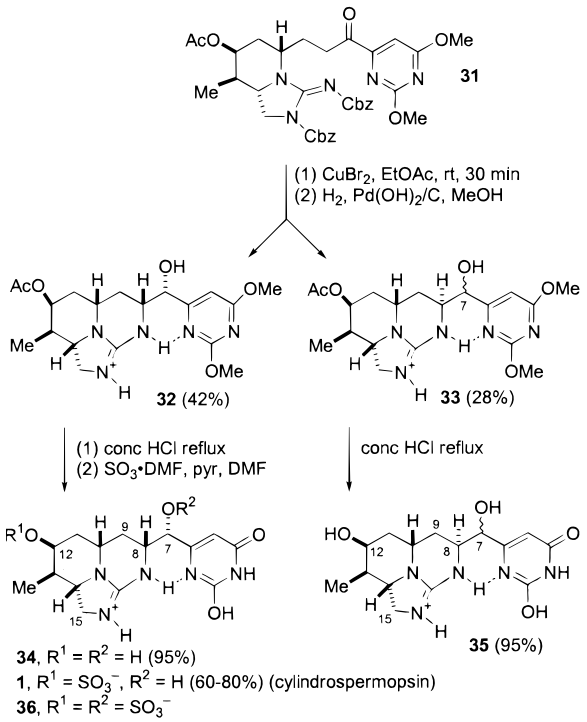

of cylindrospermopsin, δ 4.70 (d, $J = 3.9$ Hz), suggesting that hydrogenation of the ketone afforded the correct stereochemistry at C-7. Stereoisomer **33** has the opposite ring fusion stereochemistry since the coupling constant, $J_{8,9ax} = 5.5$ Hz, indicates that H_8 is equatorial. Only a single isomer was formed, but the stereochemistry at C-7 in **33** could not be assigned.

Hydrolysis of **32** in concentrated hydrochloric acid at 100 °C for 6 h afforded 95% of uracil diol **34** with ^{1}H and ^{13}C NMR spectral data virtually identical to those of cylindrospermopsin except for the protons and carbons close to C_{12}. A similar hydrolysis of **33** provided 95% of **35**.

Monosulfation[50−52] of the ring alcohol of **34** was needed to complete the synthesis. Since both alcohols are secondary, it was not obvious which would be more reactive. Reaction of **34** with 10 equiv of SO_3•DMF in anhydrous pyridine and DMF overnight and concentration in a vacuum gave a mixture of cylindrospermopsin (**1**) and the bis-sulfate ester containing some DMF and pyridine, indicating that the ring alcohol is more reactive. We were delighted to find that reverse phase chromatography on C_{18}-silica gel using D_2O as the eluent and NMR spectroscopy to monitor fractions gave pure bis-sulfate ester **36** followed by pure **1**. Sulfation of **34** with 6 equiv of SO_3•DMF gave 60−80% of cylindrospermopsin after purification. The ^{1}H and ^{13}C NMR spectral data of **1** are identical to those reported for the natural product.[1] The absorption for H_{12} shifts downfield to δ 4.60 in **1** from δ 4.03 in **32**, while the absorption for H_7 at δ 4.72 in **1** is virtually identical to that of **32** at δ 4.78. H_{12} of bis-sulfate ester **36** also absorbs at δ 4.61, while H_7 is now also shifted downfield to δ 5.20, and H_8 is shifted downfield from δ 3.87 in **1** to δ 3.99 in **36**.

The toxicities of **8**, diol **34**, and synthetic cylindrospermopsin ((±)-**1**) were compared to that of natura (−)-**1**[3] in the in vitro hepatocyte assay previously described.[17] Natural **1** has been shown to cause depletion of the cellular antioxidant glutathione (GSH) in heptatocytes. This loss always precedes cell death. Cell GSH levels of rat hepatocytes incubated for 19 h with natural (−)-**1**, synthetic (±)-**1**, and diol **34** at the indicated concentrations are shown in Table 1. Model guanidine **8** showed no reduction of GSH levels at 100−500 μM. These results indicate that synthetic (±)-**1** is active, confirming the chemical identity of the natural and synthetic material. Racemic diol **34** is more potent than racemic **1** and as least as potent as the natural toxin, clearly demonstrating that the sulfate group is not necessary for either biological activity or entry into the cell.

Table 1. Cell Glutathione (GSH) as Percentage of Control in Rat Hepatocytes Incubated with Natural (−)-**1**, Synthetic (±)-**1**, and Diol **34**

	concentration (μM)					
compound	0.32	0.63	1.25	2.50	7.50	10.0
natural (−)-**1**	109	91	54	15	12	12
synthetic (±)-**1**	--	93	109	104	36	25
diol **34**	110	61	30	19	14	9

In conclusion, the first total synthesis of the novel hepatotoxin (±)-cylindrospermopsin (**1**) has been accomplished in 20 steps from 4-methoxy-3-methylpyridine (**12**) in 3.5% overall yield. The substituted piperidine A ring **19** was generated stereospecifically by a four-step sequence using the addition of trimethylsilylethynylmagnesium bromide to **12** to give **16** and stereospecific addition of vinylcuprate **16** to form **17**. The reaction of diamine **26** with cyanogen bromide produced the cyclic guanidine C ring of **27**. The key step in the synthesis was bromination of ketone **31**, followed by hydrogenation to liberate the free guanidine, which underwent an intramolecular S_N2 reaction to form the tetrahydropyrimidine ring B. Further hydrogenation reduced the ketone to yield 42% of **32** containing the fully functionalized tricyclic system and protected hydroxymethyluracil side chain of cylindrospermopsin. Hydrolysis of the pyrimidine in concentrated hydrochloric acid and selective monosulfation completed the synthesis of cylindrospermopsin.

Experimental Section

General. NMR spectra were recorded at 400 MHz in $CDCl_3$ unless otherwise indicated. Chemical shifts are reported in δ and coupling constants in Hz. IR spectra are reported in cm^{-1}.

Formation of Dihydropyridine 16. A solution of trimethylsilylethynylmagnesium bromide in THF was prepared from ethynyltrimethylsilane (3.6 mL, 25.1 mmol) and EtMgBr (3 M in diethyl ether, 8.4 mL, 25.1 mmol) under nitrogen. TrocCl (3.9 mL, 20.9 mmol) was added dropwise at −30 °C to a solution of 4-methoxy-3-methylpyridine (**12**) (2.57 g, 20.9 mmol) in 50 mL of THF. The resulting white suspension was stirred for 30 min at −30 °C, and the above trimethylsilylethynylmagnesium bromide solution was transferred by cannula to the suspension. The mixture was stirred at −30 °C for 2 h. Hydrochloric acid (1 M, 20 mL) was then added, and the mixture was stirred at room temperature for 30 min and diluted with H_2O (60 mL). The mixture was extracted with EtOAc (2 × 80 mL). The combined organic layers were washed with water (50 mL) and brine (80 mL), dried (Na_2SO_4), and concentrated. Flash chromatography of the residue on silica gel (25:1 hexane/EtOAc, 5:1 hexane/EtOAc) gave 520 mg (7%) of a 1:2 mixture of **16** and the regioisomer resulting from addition to the carbon adjacent to the methyl group, followed by 3.99 g (49%, 87% based on recovered **12**) of **16** as a white crystalline solid. The combined aqueous layers after workup were treated with 2 N NaOH solution (70 mL), and extracted with EtOAc (2 × 60 mL). The EtOAc layers were washed with brine (70 mL), dried (Na_2SO_4), and concentrated to provide 900 mg (35%) of recovered **12**.

The data for **16**: mp 119.8−120.2 °C; ^{1}H NMR 7.66−7.58 (br, 1), 5.41 (ddd, 1, $J = 6.8, 1.5, 1.5$), 5.00−4.80 (br, 2), 2.87 (dd, 1, $J = 16.4, 6.8$), 2.70 (dd, 1, $J = 16.4, 1.5$), 1.83 (d, 1, $J = 1.2$), 0.11 (s, 9);

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^{13}C NMR (25 °C) 191.3, 150.9 (br), 137.0 and 135.7 (br), 116.6 (br), 100.3, 94.4, 89.7, 75.8, 46.1, 41.3, 13.0, −0.4 (3 C) (at 35 °C the peaks at 150.9 and 116.6 became sharper, and the two peaks at 137.0 and 135.7 collapsed to a broad peak at 136.6); IR (KBr) 3062, 2175, 1754, 1660. Anal. Calcd for $C_{14}H_{18}NO_3SiCl_3$: C, 43.93; H, 4.74; N, 3.66. Found: C, 43.76; H, 4.74; N, 3.58.

The data for the regioisomer of **16** were determined from the mixture: ^{1}H NMR 7.72 (br d, 1, J = 8), 5.52−5.48 (br, 1), 5.26−5.22 (br, 1), 5.00−4.58 (br, 2), 2.81 (dq, 1, J = 6.4, 6.4), 1.26 (d, 3, J = 6.4), 0.12 (s, 9).

Formation of 17. To a solution of CuBr·SMe_2 (486 mg, 2.36 mmol) in 80 mL of THF was added vinylmagnesium bromide (1 M in THF, 17.7 mL, 17.7 mmol), and then a solution of **16** (4.51 g, 11.8 mmol) and TMSCl (7.5 mL, 59.0 mmol) in 40 mL of THF at −78 °C. The mixture was stirred at −78 °C for 2 h, quenched by 10 mL of 1 N HCl solution, diluted with H_2O (40 mL), and extracted with EtOAc (3 × 80 mL). The combined organic layers were washed with water (120 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated. Flash chromatography of the residue on silica gel (10:1 hexane/EtOAc) gave 4.47 g (92%) of **17** as a white crystalline solid: mp 91.4−91.8 °C; ^{1}H NMR 6.15−6.05 (br, 1), 5.53 (br s, 1), 5.33 (br d, 1, J = 17.2), 5.18 (dd, 1, J = 10.0, 1.6), 5.13−5.05 (br, 1), 4.85−4.79 (br, 2), 2.90−2.81 (m, 2), 2.62 (dd, 1, J = 14.0, 2.4), 0.97 (d, 3, J = 6.8), 0.10 (s, 9); ^{13}C NMR 205.9, 153.0, 132.6, 120.3, 103.8, 95.1, 91.1, 75.4, 63.2, 46.4, 45.6, 45.1 (br), 10.9, −0.6 (3 C); IR (KBr) 3081, 2174, 1724, 1642, 1456. Anal. Calcd for $C_{16}H_{22}NO_3SiCl_3$: C, 46.78; H, 5.40; N, 3.41. Found: C, 46.72; H, 5.42; N, 3.32.

Formation of 18. A suspension of **17** (4.47 g, 10.9 mmol) and zinc dust (5.0 g) in CH_2Cl_2/HOAc (1/3, 40 mL) was stirred vigorously at room temperature for 5 h. The mixture was filtered, and the filtrate was concentrated, neutralized with Na_2CO_3 solution, and extracted with EtOAc (3 × 70 mL). The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4), and concentrated. Flash chromatography of the residue on silica gel (3:1 hexane/EtOAc) gave 2.48 g (96%) of **18**: ^{1}H NMR 5.74 (ddd, 1, J = 16.8, 10.0, 8.0), 5.16 (d, 1, J = 16.8), 5.13 (dd, 1, J = 10.0, 1.2), 3.70 (dd, 1, J = 9.6, 5.2), 2.88 (dd, 1, J = 10.4, 8.0), 2.58−2.49 (m, 2), 2.20 (dq, 1, J = 10.4, 6.8), 2.01 (br s, NH), 0.87 (d, 3, J = 6.8), −0.075 (s, 9); ^{13}C NMR 207.2, 138.1, 118.1, 104.3, 88.5, 66.1, 49.4, 48.2, 47.7, 9.9, −0.3 (3 C); IR (neat) 3316, 3079, 2178, 1715; HRMS (GC/MS,EI 20 eV) calcd for $C_{13}H_{22}NOSi$ (MH^+) 234.1314, found 234.1316.

Reduction of 18 to 19. L-Selectride (21.0 mL, 1.0 M in THF, 21.0 mmol) was added to a solution of **18** (2.48 g, 10.5 mmol) in 100 mL of THF at −78 °C. The solution was stirred 2 h, and the reaction was quenched with saturated aqueous $NaHCO_3$ (50 mL). The mixture was extracted with EtOAc (3 × 60 mL). The combined organic layers were concentrated to provide a wet oil. The oil was then taken up in EtOH (50 mL) and 1 N NaOH solution (7 mL). The solution was refluxed for 2 h, and poured onto saturated aqueous $NaHCO_3$ solution, which was extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4), and concentrated. Flash chromatography of the residue on silica gel (3:1 hexane/EtOAc, then 1:1 hexane/EtOAc) gave 1.63 g (94%) of **19** as a white crystalline solid: mp 107.0−107.2 °C; ^{1}H NMR 5.70 (ddd, 1, J = 16.4, 10.4, 8.8), 5.19 (dd, 1, J = 16.4, 2.0), 5.11 (dd, 1, J = 10.4, 2.0), 3.96 (ddd, 1, J = 10.6, 2.6, 2.6), 3.94 (ddd, 1, J = 3.2, 2.8, 2.4), 3.18 (dd, 1, J = 10.0, 8.8), 2.24 (d, 1, J = 2.6), 2.03 (ddd, 1, J = 14.0, 2.6, 3.2), 1.84 (ddd, 1, J = 14.0, 10.6, 2.8), 1.49 (ddq, 1, J = 10.0, 2.4, 6.8), 0.89 (d, 3, J = 6.8); ^{13}C NMR 139.6, 117.1, 85.3, 70.5, 68.6, 60.0, 41.7, 40.1, 39.0, 14.5; IR (KBr) 3288, 3179, 2119; HRMS (GC/MS EI 20 eV) calcd for $C_{10}H_{16}NO$ (MH^+) 164.1075, found 164.1071.

Formation of 20. To a solution of **19** (1.63 g, 9.87 mmol) in THF (40 mL) was added solid Na_2CO_3 (3.0 g) and CbzCl (1.7 mL, 11.8 mmol). The suspension was stirred vigorously overnight at room temperature, and the Na_2CO_3 was filtered off. The filtrate was concentrated and purified on silica gel (10:1 hexane/EtOAc, then 1:1 hexane/EtOAc) to give 2.84 g (96%) of **20** as an oil: ^{1}H NMR 7.37−7.30 (m, 5), 6.37 (ddd, 1, J = 17.1, 10.4, 7.3), 5.26 (ddd, 1, J = 6.1, 2.1, 2.1), 5.21 (ddd, 1, J = 17.1, 1.4, 1.4), 5.17 (s, 2), 5.11 (ddd, 1, J = 10.4, 1.4, 1.4), 4.63 (br d, 1, J = 7.3), 4.44 (dddd, 1, J = 11.3, 4.9, 4.8, 4.2), 2.28 (d, 1, J = 2.1), 2.26−2.18 (m, 1), 1.98 (ddd, 1, J = 12.8, 11.3, 6.1), 1.89 (dddd, 1, J = 12.8, 4.2, 2.1, 1.0), 1.01 (d, 3, J = 6.8); ^{13}C NMR 156.2, 137.8, 136.6, 128.7 (2 C), 128.2, 127.9 (2 C), 116.6, 84.4, 72.0, 67.9, 64.0, 61.4, 42.5, 37.9, 32.8, 11.5; IR (neat) 3448, 3295, 2106, 1685, 1406, 1326, 1262; HRMS (GC/MS EI 20 eV) calcd for $C_{18}H_{21}NO_3$ 299.1521, found 299.1507.

Silyl Ether 21. To a solution of **20** (2.84 g, 9.47 mmol) in dry CH_2Cl_2 (20 mL) was added imidazole (2.84 g, 47.4 mmol), TBSCl (1.56 g, 10.4 mmol), and a catalytic amount of DMAP (50 mg). The mixture was stirred at room temperature overnight and added to saturated NH_4Cl solution. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 90 mL). The combined organic layers were washed with water (100 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated. Flash chromatography of the residue on silica gel (10:1 hexane/EtOAc) gave 3.49 g (89%) of **21** as an oil: ^{1}H NMR 7.38−7.28 (m, 5), 6.38 (ddd, 1, J = 17.2, 10.4, 7.0), 5.22 (ddd, 1, J = 6.2, 2.4, 2.0), 5.19 (ddd, 1, J = 17.2, 1.2, 1.2), 5.17 (s, 2), 5.11 (ddd, 1, J = 10.4, 1.2, 1.2), 4.61 (br d, 1, J = 7.0), 4.38 (ddd, 1, J = 11.4, 4.2, 4.2), 2.28 (d, 1, J = 2.4), 2.10 (ddq, 1, J = 4.2, 2.8, 7.2), 1.97 (ddd, 1, J = 13.2, 11.4, 6.2), 1.75 (ddd, 1, J = 13.2, 4.2, 2.0), 0.98 (d, 3, J = 7.2), 0.89 (s, 9), 0.07 (s, 6); ^{13}C NMR 155.8, 137.8, 136.4, 128.3 (2 C), 127.6, 127.0 (2 C), 116.0, 84.3, 71.4, 67.4, 64.4, 61.0, 42.2, 38.3, 33.2, 25.7 (3 C), 17.9, 11.4, −4.8, −4.9; IR (neat) 1702, 1404, 1109; HRMS (GC/MS CI/CH_4) calcd for $C_{24}H_{36}NO_3Si$ (MH^+) 414.2464, found 414.2453.

Formation of 22. To a solution of **21** (3.49 g, 8.44 mmol) in THF (60 mL) was added EtMgBr (1 M in THF, 9.3 mL, 9.3 mmol) at 0 °C under N_2. The reaction mixture was warmed to room temperature and stirred at room temperature for 2 h. A solution of **3** (1.56 g, 9.28 mmol) in THF (40 mL) was then added dropwise to the mixture. The reaction was stirred at 0 °C for 2 h and poured into a saturated NH_4Cl solution (60 mL), the organic layer was separated, and the water layer was extracted with EtOAc (2 × 80 mL). The combined organic layers were washed with water (70 mL) and brine (70 mL), dried (Na_2SO_4), and concentrated. Flash chromatography of the residue on silica gel (10:1 hexane/EtOAc, 3:1 hexane/EtOAc, then 1:1 hexane/EtOAc) gave 569 mg (16%) of recovered **21**, followed by 3.39 g (69%, 83% based on recovered **21**) of **22** as a 1:1 mixture of diastereomers: ^{1}H NMR 7.37−7.27 (m, 5), 6.483 (s, 1 × 0.5), 6.480 (s, 1 × 0.5), 6.22 (ddd, 1 × 0.5, J = 17.0, 10.4, 5.8), 6.20 (ddd, 1 × 0.5, J = 17.2, 10.4, 5.8), 5.24−5.27 (m, 1), 5.15 (s, 2 × 0.5), 5.14 (s, 2 × 0.5), 5.13 (br d, 1, J = 17.0), 5.02 (ddd, 1 × 0.5, J = 10.4, 1.0, 1.0), 5.00 (ddd, 1 × 0.5, J = 10.4, 1.0, 1.0), 4.58 (br s, 1, $W_{1/2}$ = 14.0), 4.30 (ddd, 1 × 0.5, J = 10.8, 4.2, 4.2), 4.26 (ddd, 1 × 0.5, J = 10.8, 4.2, 4.2), 4.00 (s, 3), 3.97 (s, 3), 3.91 (d, 1 × 0.5, J = 5.2), 3.86 (d, 1 × 0.5, J = 4.8), 2.07 (m, 1), 1.94 (ddd, 1 × 0.5, J = 13.2, 11.6, 6.4), 1.92 (ddd, 1 × 0.5, J = 13.2, 11.6, 6.4), 1.74−1.67 (m, 1), 0.95 (d, 3, J = 6.8), 0.86 (s, 9 × 0.5), 0.84 (s, 9 × 0.5), 0.02 (s, 6); ^{13}C NMR 172.3, 168.5, 165.0, 155.8 (2 C), (138.18, 138.06), 136.4, 128.4, (127.91, 127.89), (127.63, 127.61) (2 C), (115.79, 115.70), (97.90, 97.88), (86.71, 86.63), 81.1, (67.49, 67.46), (64.52, 64.48), (63.14, 63.05), (60.63, 60.59), 54.9, 54.1, (42.57, 42.52), (37.93, 37.84), (33.32, 33.24), (25.69, 25.67) (3 C), (17.97, 17.96), (11.44, 11.39), (−4.86, −4.96) (2 C); IR (neat) 3429 (br), 1701, 1596, 1571, 1356; HRMS (DCI/NH_3) calcd for $C_{31}H_{44}N_3O_6Si$ (MH^+) 582.2999, found 582.3003.

Bis-silyl Ether 23. To a solution of **22** (3.39 g, 5.83 mmol) in dry CH_2Cl_2 (20 mL) was added imidazole (1.75 g, 29.2 mmol), and TBSCl (962 mg, 6.41 mmol). The above mixture was stirred at room temperature for 5 h and poured into a saturated NH_4Cl solution. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 80 mL). The combined organic layers were washed with water (120 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated. Flash chromatography of the residue on silica gel (10:1 hexane/EtOAc) gave 3.56 g (88%) of **23** as a 1:1 mixture of diastereomers: ^{1}H NMR 7.35−7.28 (m, 5), 6.62 (s, 1), 6.28 (ddd, 1, J = 17.2, 10.4, 7.4), 5.26−5.22 (m, 2), 5.17−5.08 (m, 3), 4.98−4.95 (m, 1), 4.56 (br d, 1, J = 6.7), 4.29−4.21 (m, 1), 3.97 (s, 3), 3.95 (s, 3), 2.07−2.02 (m, 1), 1.96−1.87 (m, 1), 1.71−1.65 (m, 1), 0.949 (d, 3 × 0.5, J = 6.7), 0.945 (d, 3 × 0.5, J = 6.7), 0.92 (s, 9), 0.85 (s, 9 × 0.5), 0.84 (s, 9 × 0.5), 0.16 (s, 6 × 0.5), 0.15 (s, 6 × 0.5), 0.12 (s, 6 × 0.5), 0.11 (s, 6 × 0.5); ^{13}C NMR (172.41, 172.43), 171.1, 165.0, 155.8, 138.1, 136.5, 128.4 (2 C), 127.8, 127.6 (2 C), (115.8, 115.7), 97.3, 85.8, 81.9, 67.4, (65.33,

65.29), 64.5, (60.95, 60.89), (54.68, 54.67), 53.8, (42.54, 42.53), (38.45, 38.34), (33.35, 33.24), 25.7 (6 C), (18.2, 17.9) (2 C), (11.42, 11.40), −4.68, −4.70, −5.04, −5.18; IR (neat) 1706, 1696, 1573, 1461, 1357, HRMS (FAB) calcd for $C_{37}H_{58}N_3O_6Si_2$ (MH^+) 696.3864, found 696.3850.

Formation of Aldehyde 24. A stream of ozone was introduced into a solution of **23** (3.56 g, 5.11 mmol) in CH_2Cl_2 (200 mL) at −78 °C for about 20 min until the solution turned bluish. Nitrogen was bubbled through the solution for 10 min to purge the excess ozone. Me_2S (1.50 mL, 20.5 mmol) was added, and the mixture was then stirred at −78 °C for 20 min. The solution was concentrated, and the residue was purified by flash chromatography on silica gel (5:1 hexane/EtOAc) to provide 2.56 g (72%) of **24** as a 1:1 mixture of diastereomers: ^{1}H NMR 9.77 (s, 1 × 0.5), 9.67 (s, 1 × 0.5), 7.36−7.26 (m, 5), 6.57 (s, 1 × 0.5), 6.55 (s, 1 × 0.5), 5.35−5.13 (m, 5), 4.60−4.38 (m, 1), 3.96−3.92 (m, 6), 2.72−2.53 (m, 1), 1.95−1.52 (m, 2), 0.90 (d, 3, J = 6.8), 0.89 (s, 9), 0.83 (s, 9 × 0.5), 0.82 (s, 9 × 0.5), 0.15−0.05 (m, 12); ^{13}C NMR (201.1, 200.7), (172.5, 172.4), (170.6, 170.5), 165.1, (159.80, 159.78), (135.9, 135.8), (128.6, 128.5) (2 C), (128.3, 128.2), (127.86, 127.82) (2 C), (97.24, 97.18), (84.1, 83.9), 83.3, (68.23, 68.15), 66.7 (br), (65.14, 65.11), 64.5, (54.8, 54.7), (53.89, 53.87), (43.9, 42.8), 38.4, (32.8, 32.3), (25.67, 25.64) (6 C), (18.2, 17.9) (2 C), (10.0, 9.1), (−4.8, −5.1) (2 C), (−5.1, −5.2) (2 C); IR (neat) 1709, 1595, 1574, 1358; HRMS (DCI/NH_3) calcd for $C_{36}H_{56}N_3O_7Si_2$ (MH^+) 698.3657, found 698.3698.

Benzylamine 25. To a solution of **24** (2.56 g, 3.67 mmol) in toluene (40 mL) containing Na_2SO_4 (5 g) was added benzylamine (2.0 mL, 18.4 mmol) and HOAc (1.3 mL, 22.0 mmol) under nitrogen. The solution was stirred at room temperature for 2 h. MeOH (50 mL) and $NaCNBH_3$ (1.20 g, 18.4 mmol) were then added and the resulting mixture was stirred at room temperature for another 2 h. Saturated $NaHCO_3$ solution (60 mL) was added, and the solution was extracted with EtOAc (3 × 80 mL). The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4), and concentrated. Flash chromatography of the residue on silica gel (3:1 hexane/EtOAc) gave 1.97 g (68%) of **25** as a 1:1 mixture of diastereomers: ^{1}H NMR 7.40−7.23 (m, 10), 6.65 (s, 1 × 0.5), 6.47 (s, 1 × 0.5), 5.30−5.13 (m, 4), 4.34−4.16 (m, 2), 3.97 (s, 3), 3.94 (s, 3), 3.82−3.60 (m, 2), 3.30−3.08 (m, 1), 2.94−2.82 (m, 1), 2.20−2.07 (m, 1), 1.97−1.80 (m, 1), 1.75−1.60 (m, 1), 1.00−0.80 (m, 18), 0.20−0.00 (m, 12); ^{13}C NMR 172.4, (171.11, 171.06), (165.1, 165.0), (140.6), 139.0, (136.49, 136.46), (128.6−126.6), (10 C), 97.3, (85.7, 85.6), 81.3, 67.5, (65.31, 65.25), 64.6, (58.6, 58.5), 51.2, 51.1, 49.5, 42.5, 35.3, 33.1, 27.4, (25.71, 25.68) (6 C), (18.2, 18.0) (2 C), (11.6, 11.5), (−4.7, −4.9) (2 C), (−5.0, −5.1) (2 C); IR (neat) 3320, 1946, 1700, 1574, HRMS (FAB) calcd for $C_{43}H_{65}N_4O_6Si_2$ (MH^+) 789.4443, found 789.4475.

Bis-Cbz-Protected Guanidine 28. A suspension of **25** (1.97 g, 2.49 mmol) and 5% Pd/C (1.00 g) in MeOH (70 mL) was stirred at room temperature under H_2 (1 atm) overnight. The Pd catalyst was filtered off, and the filtrate was concentrated to provide 1.02 g of crude **26**.

To a solution of crude **26** (1.02 g) in toluene (80 mL) was added slowly a solution of CNBr (170 mg, 1.61 mmol) in toluene (40 mL) over 3 h. The mixture was stirred at room temperature for 2 h and concentrated to provide crude **27**.

To a solution of crude **27** in THF (20 mL) was added excess NaH (400 mg, 9.60 mmol) and CbzCl (914 μL, 6.40 mmol) at 0 °C. The mixture was stirred at room temperature for 8 h. Unreacted NaH was filtered off and quenched carefully with 2-propanol. The filtrate was concentrated and purified by flash chromatography on silica gel (10:1 hexane/EtOAc, then 2:1 hexane/EtOAc) to give 880 mg (45% from **25**) of **28** as a 1:1 mixture of diastereomers followed by 190 mg (10%) of a byproduct with one Cbz group and one benzyl group on the guanidine.

The data for **28**: ^{1}H NMR 7.45−7.30 (m, 10), 6.58 (s, 1 × 0.5), 6.56 (s, 1 × 0.5), 5.19−5.05 (m, 4), 4.65 (dd, 1 × 0.5, J = 7.2, 3.6), 4.64 (dd, 1 × 0.5, J = 7.2, 3.6), 3.99 (s, 3 × 0.5), 3.98 (s, 3), 3.97 (s, 3 × 0.5), 3.94−3.91 (m, 1), 3.70−3.40 (m, 4), 2.62−2.54 (m, 1), 2.40−2.30 (m, 1), 2.22−2.10 (m, 1), 2.05−1.54 (m, 4), 0.95 (s, 9), 0.94 (s, 9), 0.91 (d, 3, J = 6.8), 0.11 (s, 6 × 0.5), 0.09 (s, 6 × 0.5), 0.084 (s, 6 × 0.5), 0.075 (s, 6 × 0.5); ^{13}C NMR (175.57, 175.46), (172.00, 171.97), (164.69, 164.65), 158.8, 151.4, (149.15, 149.12), 137.1, 135.1, (125.4−127.4) (10 C), 97.2, (74.5, 74.3), (68.9, 68.8), 67.9, 67.0, 56.7, 54.5, 53.5, (51.89, 51.82), (47.77, 47.69), (39.00, 38.97), 38.2, (34.28, 34.12), (27.47, 27.37), (25.7−25.6) (6 C), (17.95, 17.85) (2 C), (13.55, 13.50), (−4.5, −5.2) (4 C); IR (neat) 1758, 1594, 1355; HRMS (FAB) calcd for $C_{45}H_{68}N_5O_8Si_2$ (MH^+) 682.4606, found 682.4603.

Data for the benzyl methylene groups of the byproduct: 5.04 (d, 1, J = 12.4), 4.98 (d, 1, J = 12.4), 4.24 (d, 1, J = 14.8), 4.19 (d, 1, J = 14.8).

Formation of Diol 29. A solution of **28** (370 mg, 0.429 mmol) and TBAF (1 M in THF, 900 μL, 0.90 mmol) in THF (10 mL) was stirred at room temperature overnight. The solution was concentrated, and the residue was purified by flash chromatography on silica gel (10:1 CH_2Cl_2/MeOH) to give 226 mg (83%) of **29** as a 1:1 mixture of diastereomers: ^{1}H NMR 7.44−7.22 (m, 10), 6.44 (s, 1 × 0.5), 6.42 (s, 1 × 0.5), 5.13 (s, 2), 5.05 (br s, 2), 4.56−4.48 (m, 1), 3.958 (s, 3 × 0.5), 3.955 (s, 3 × 0.5), 3.924 (s, 3 × 0.5), 3.920 (s, 3 × 0.5), 3.96−3.92 (m, 1), 3.77−3.67 (m, 1), 3.58−3.48 (m, 3), 2.55−2.30 (m, 1), 2.20−1.50 (m, 5), 0.94 (d, 3, J = 6.8); ^{13}C NMR (174.05, 173.94), 172.2, 164.8, 159.0, 151.5, (149.9, 149.8), 137.1, 135.1, 128.6−127.6 (10 C), (97.30, 97.29), (72.53, 72.36), (68.29, 68.31), (67.94, 67.91), 67.3, (56.4, 56.3), 54.7, 53.8, (51.9, 51.7), (48.1, 47.9), (39.1, 38.9), (38.45, 38.37), (33.7, 33.5), (27.7, 27.6), (13.00, 12.93); IR (neat) 1752, 1596, 1570, 1355; HRMS (DCI/NH_3) calcd for $C_{33}H_{40}N_5O_8$ (MH^+) 634.2877, found 634.2874.

Formation of Ketone 30. Activated MnO_2 (200 mg, 2.00 mmol) was added to a solution of **29** (160 mg, 0.253 mmol) in 6 mL of CH_2Cl_2 at room temperature. The reaction mixture was stirred at room temperature for 1 h and filtered through a plug of Celite. The filtrate was concentrated and purified on silica gel (1:1 hexane/EtOAc, EtOAc) to give 138 mg (87%) of **30** as an oil: ^{1}H NMR 7.37−7.20 (m, 10), 6.89 (s, 1), 5.13 (s, 2), 4.97 (s, 2), 3.99 (s, 3), 3.98 (s, 3), 3.99−3.97 (m, 1), 3.96−3.90 (m, 1), 3.82−3.74 (m, 1), 3.58−3.55 (m, 1), 3.55 (ddd, 1, J = 10.8, 8.0, 5.6), 3.27 (br t, 2, J = 7.0, 7.0, 1.2), 2.78−2.69 (m, 1), 2.26−2.17 (m, 1), 1.96 (ddd, 1, J = 13.0, 11.0, 3.1), 1.82 (ddd, 1, J = 13.0, 3.2, 3.2), 1.71−1.64 (m, 1), 0.95 (d, 3, J = 6.7); ^{1}H NMR (C_6D_6) 7.40−7.00 (m, 11), 5.33 (s, 2), 5.02 (d, 1, J = 12.2), 4.96 (d, 1, J = 12.2), 3.78 (s, 3), 3.50 (s, 3), 3.55−3.40 (m, 2), 3.20 (dd, 1, J = 10.0, 8.0), 3.18−3.12 (m, 1), 2.99 (dd, 1, J = 10.0, 5.6), 2.98−2.91 (m, 1), 2.84 (ddd, 1, J = 12.8, 7.2, 5.6), 2.38−2.32 (m, 2), 1.5−1.2 (m, 3), 0.34 (d, 3, J = 7.3); ^{13}C NMR 200.4, 172.9, 165.6, 161.5, 158.9, 151.5, 149.2, 137.1, 135.2, 128.6 (2 C), 128.49, 128.47, 128.1 (2 C), 127.9 (2 C), 127.4 (2 C), 99.1, 68.3, 68.1, 67.2, 56.5, 55.1, 54.3, 51.4, 47.8, 38.5, 38.1, 35.2, 25.8, 12.9; IR (neat) 1752, 1707, 1592, 1566, 1387, 1357, 1254; HRMS (FAB) calcd for $C_{33}H_{38}N_5O_8$ (MH^+) 632.2720, found 632.2718.

Acetate 31. To a solution of **30** (138 mg, 0.218 mmol) in pyridine (5 mL) was added acetic anhydride (62 μL, 0.656 mmol). The mixture was stirred at room temperature overnight and concentrated under reduced pressure. Purification of the residue on silica gel (1:1 hexane/EtOAc) provided 130 mg of a 5:1 mixture of acetate **31** and enol acetate of **31** (87%) as an oil that was used for the next step.

The data for **31** determined from the mixture: ^{1}H NMR 7.36−7.21 (m, 10), 6.90 (s, 1), 5.15 (br s, 1), 5.13 (s, 2), 4.99 (s, 2), 3.99 (s, 3), 3.98 (s, 3), 3.95−3.92 (m, 1), 3.71−3.63 (m, 1), 3.56 (dd, 1, J = 10.0, 5.5), 3.48 (ddd, 1, J = 11.0, 8.8, 6.1), 3.27 (dd, 2, J = 7.0, 7.0), 2.71 (dddd, 1, J = 13.4, 7.0, 7.0, 6.7), 2.22 (dddd, 1, J = 13.4, 8.0, 7.0, 7.0), 2.08 (s, 3), 1.96 (ddd, 1, J = 14.6, 3.7, 3.7), 1.88 (ddd, 1, J = 14.6, 11.0, 3.1), 1.80 (ddq, 1, J = 11.0, 3.7, 6.8), 0.88 (d, 3, J = 6.8); ^{13}C NMR 200.2, 172.9, 170.1, 165.6, 161.4, 158.8, 151.5, 148.8, 137.1, 135.1, 128.6−127.5 (10 C), 99.1, 70.4, 68.4, 67.2, 57.1, 55.1, 54.3, 51.9, 47.9, 37.0, 34.9, 34.8, 25.8, 21.0, 12.6; IR (neat) 1752, 1798, 1590, 1566, 1387, 1358; HRMS (DCI/NH_3) calcd for $C_{35}H_{40}N_5O_9$ (MH^+) 674.2826, found 674.2807.

The data for the enol acetate were determined from the mixture: ^{1}H NMR 7.36−7.20 (m, 10), 6.72 (dd, 1, J = 7.7, 7.3), 6.24 (s, 1), 5.15 (br s, 1), 5.14 (s, 2), 5.04 (s, 2), 4.06 (m, 1), 3.94 (s, 6), 3.67 (m, 1), 3.54−3.44 (m, 2), 3.26 (ddd, 1, J = 14.4, 7.7, 4.8), 2.84 (ddd, 1, J = 14.4, 7.3, 7.0), 2.25 (s, 3), 2.07 (s, 3), 1.94 (ddd, 1, J = 14.8, 4.0, 4.0), 1.86 (ddd, 1, J = 14.8, 10.4, 3.2), 1.78 (ddq, 1, J = 10.4, 3.1, 6.7), 0.88 (d, 3, J = 6.7); ^{13}C NMR 120.9, 96.3, 68.7, 68.1, 67.5, 56.9, 54.9, 54.3, 51.1, 48.7, 37.4, 35.0, 29.5, 21.0, 20.6, 12.7 (the carbons

were assigned from an HMQC experiment; the quaternary carbons were not observed).

Formation of Tricycles 32 and 33. A suspension of $CuBr_2$ (100 mg, 0.452 mmol) in EtOAc (30 mL) was stirred vigorously at 35 °C (oil bath temperature) for 20 min. A solution of **31** and the enol acetate mixture (65 mg, 0.089 mmol) in EtOAc (5 mL) was then added by syringe. The mixture was stirred at 35 °C for 20 min, diluted with EtOAc (10 mL), and filtered through Celite. The filtrate was washed with brine (10 mL), dried (Na_2SO_4), and concentrated to give 72 mg of crude bromination product that was used immediately for the hydrogenation step.

A solution of the crude bromo ketone (72 mg) in methanol (10 mL) was stirred over 20% $Pd(OH)_2/C$ (25 mg) at room temperature under a hydrogen-filled balloon for 8 h, and filtered through Celite. The filtrate was evaporated under reduced pressure, and the residue was purified on silica gel (80:20:1 CH_2Cl_2/MeOH/HCO_2H) to give 31 mg of a 3:2 mixture of **32** and **33**. Careful flash chromatography on silica gel (96:4:1 CH_2Cl_2/MeOH/HCO_2H) gave 15 mg (37%) of **32**, followed by 7 mg (17%) of a 1:2 mixture of **32** and **33**, and 7 mg (17%) of a 8:1 mixture of **33** and an unknown impurity.

The data for **32**: ^{1}H NMR (CD_3OD) 6.69 (s, 1), 5.12 (ddd, 1, J = 3.6, 3.6, 2.4), 4.68 (d, 1, J = 3.7), 3.99 (s, 3), 3.98 (s, 3), 3.95 (ddd, 1, J = 11.0, 3.7, 3.6), 3.85 (dd, 1, J = 8.6, 8.6), 3.80 (ddd, 1, J = 10.4, 10.4, 8.6), 3.55 (dddd, 1, J = 11.6, 11.0, 3.6, 3.6), 3.25 (dd, 1, J = 10.4, 8.6), 2.15 (ddd, 1, J = 14.0, 3.6, 3.6), 2.07 (s, 3), 1.95 (ddd, 1, J = 13.4, 3.6, 3.6), 1.85 (ddq, 1, J = 10.4, 2.4, 6.7), 1.62 (ddd, 1, J = 13.4, 11.6, 11.0), 1.50 (ddd, 1, J = 14.0, 11.6, 3.6), 0.95 (d, 3, J = 6.7); ^{13}C NMR (CD_3OD) 174.3, 172.8, 172.0, 166.7, 157.0, 99.8, 73.8, 71.9, 58.9, 55.5, 55.2, 54.8, 48.9 (obscured by CD_3OD, the assignment was based on HMQC and HMBC experiments), 46.1, 40.0, 36.6, 29.2, 20.9, 13.7; HRMS (FAB) calcd for $C_{19}H_{28}N_5O_5$ (MH^+) 406.2090, found 406.2082.

The data for **33** were determined from the mixture: ^{1}H NMR (CD_3OD) 6.67 (s, 1), 5.14 (br s, 1), 4.60 (d, 1, J = 4.9), 3.99 (s, 3), 3.98 (s, 3), 4.08−4.00 (m, 1), 3.86 (dd, 1, J = 8.5, 8.5), 3.81 (ddd, 1, J = 10.0, 10.0, 8.5), 3.80−3.70 (m, 1), 3.26 (dd, 1, J = 10.0, 8.5), 2.34−2.26 (m, 1), 2.20 (ddd, 1, J = 14.0, 3.6, 3.6), 2.13 (s, 3), 1.90−1.83 (m, 1), 1.62 (ddd, 1, J = 14.0, 10.4, 5.5), 1.48 (ddd, 1, J = 12.2, 12.2, 2.4), 0.96 (d, 3, J = 6.8); ^{13}C NMR (CD_3OD) (from an HMQC experiment) 99.8, 75.7, 71.8, 59.1, 54.9, 54.8, 53.1, 49.0, 48.1, 38.9, 36.5, 27.9, 20.7, 13.3.

Formation of 34. A solution of **32** (2.5 mg, 0.004 mmol) in concentrated HCl (1 mL) was refluxed at 100 °C for 6 h. The mixture was evaporated under reduced pressure to give 2.4 mg (95%) of **34** as the hydrochloride salt: ^{1}H NMR (D_2O) 5.83 (s, 1), 4.78 (d, 1, J = 3.6), 4.03 (br s, 1), 3.88 (ddd, 1, J = 11.6, 3.7, 3.6), 3.86 (dd, 1, J = 9.2, 8.8), 3.76 (ddd, 1, J = 11.0, 11.0, 8.8), 3.62 (dddd, 1, J = 11.8, 11.6, 3.7, 3.6), 3.27 (dd, 1, J = 11.0, 9.2), 2.15 (ddd, 1, J = 13.4, 3.7, 3.7), 2.10 (ddd, 1, J = 14.7, 3.6, 3.0), 1.73 (ddq, 1, J = 11.0, 1.9, 7.2), 1.59 (ddd, 1, J = 13.4, 11.6, 11.6), 1.54 (ddd, 1, J = 14.7, 11.8, 2.5), 0.97 (d, 3, J = 7.2); ^{13}C NMR (D_2O) 169.1 (assignment was based on HMBC), 158.0, 157.8, 153.3 (assignment was based on HMBC), 101.5, 71.8, 70.6, 59.2, 55.2, 50.0, 46.3, 41.9, 40.1, 30.2, 15.4; HRMS (FAB) calcd for $C_{15}H_{22}N_5O_4$ (MH^+) 336.1672, found 336.1687.

Formation of 35. A solution of **33** (2.0 mg, 0.032 mmol) in concentrated HCl (1 mL) was refluxed at 100 °C for 6 h. The mixture was evaporated under reduced pressure to give 1.8 mg (90%) of **35** as the hydrochloride salt: ^{1}H NMR (D_2O) 5.86 (s, 1), 4.57 (d, 1, J = 7.2), 4.07 (br s, 1), 3.87 (ddd, 1, J = 8.4, 7.2, 4.3), 3.80 (dd, 1, J = 9.2, 8.8), 3.82−3.75 (m, 1), 3.68−3.54 (m, 1), 3.32 (dd, 1, J = 9.6, 9.2), 2.44 (ddd, 1, J = 14.0, 4.3, 3.0), 2.12 (ddd, 1, J = 14.0, 3.7, 3.7), 1.98−1.90 (m, 1), 1.83−1.73 (m, 1), 1.58 (ddd, 1, J = 14.0, 11.6, 2.5), 0.97 (d, 3, J = 7.2).

Formation of Cylindrospermopsin (1). A solution of **34** (1.2 mg, 0.0036 mmol) in dry pyridine (0.15 mL) containing Na_2SO_4 (20 mg) was stirred at room temperature for 30 min. A solution of SO_3·DMF in dry DMF (0.1 M, 216 μL, 0.0216 mmol, 6 equiv) was added to the mixture, which was stirred at room temperature overnight. Pyridine and DMF were evaporated, and the residue was taken up in MeOH to remove Na_2SO_4. The methanol solution was concentrated, and the residue was purified by flash chromatography on Bakerbond C_{18} (40 μm) prep LC packing using D_2O as the eluent. Pyridinium salts eluted in the earlier fractions, followed by 1.1 mg (50−70%) of cylindrospermopsin (**1**): ^{1}H NMR (D_2O) (600 MHz) 5.83 (s, 1), 4.72 (d, 1, J = 3.7), 4.63−4.60 (m, 1), 3.86 (ddd, 1, J = 11.2, 3.7, 3.5), 3.84 (dd, 1, J = 9.2, 9.0), 3.73 (ddd, 1, J = 11.4, 10.8, 9.0), 3.65 (dddd, 1, J = 11.7, 11.7, 3.5, 3.5), 3.24 (dd, 1, J = 10.8, 9.2), 2.44 (ddd, 1, J = 14.2, 3.5, 2.8), 2.15 (ddd, 1, J = 13.2, 3.5, 3.5), 1.85 (ddq, 1, J = 11.4, 2.6, 6.8), 1.57 (ddd, 1, J = 13.2, 11.7, 11.2), 1.54 (ddd, 1, J = 14.2, 11.7, 3.0), 0.98 (d, 3, J = 6.8); ^{13}C NMR (D_2O) (600 MHz) (from an HSQC experiment) 99.6, 78.2, 70.8, 57.9, 53.7, 48.3, 45.0, 39.7, 36.3, 28.5, 13.6; HRMS (FAB) calcd for $C_{15}H_{22}N_5O_7S$ (MH^+) 416.1240, found 416.1252. The data are identical to those previously reported.[1,2]

A similar reaction with 10 equiv of SO_3·DMF gave a mixture of bis-sulfate ester **36** and **1**. Reverse phase flash chromatography as above gave **1** preceded by bis-sulfate ester **36** as the pyridinium salt: ^{1}H NMR (D_2O) (600 MHz) 5.83 (s, 1), 5.20 (d, 1, J = 4.3), 4.63−4.60 (m, 1), 3.99 (ddd, 1, J = 12.2, 4.3, 4.0), 3.85 (dd, 1, J = 9.2, 8.5), 3.73 (ddd, 1, J = 11.0, 10.8, 8.5), 3.72−3.64 (m, 1), 3.27 (dd, 1, J = 11.0, 9.2), 2.46 (ddd, 1, J = 14.7, 2.5, 2.5), 2.28 (ddd, 1, J = 13.4, 4.0, 3.5), 1.90−1.84 (m, 1), 1.64 (ddd, 1, J = 13.4, 12.2, 11.6), 1.56 (ddd, 1, J = 14.7, 12.0, 3.0), 0.99 (d, 3, J = 6.8).

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Supporting Information Available: Experimental procedures for preparation of **8** and **12** and copies of ^{1}H and ^{13}C NMR spectra of key intermediates (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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