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Syntheses of the cylindrospermopsin alkaloids

Ryan E. Looper,[a] Maria T. C. Runnegar[b] and Robert M. Williams[a,*]

[a]*Department of Chemistry, Colorado State University, Fort Collins, CO 80523, USA*
[b]*Research Center for Liver Diseases, University of Southern California Medical Center, Los Angeles, CA 90033, USA*

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

1. Introduction

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

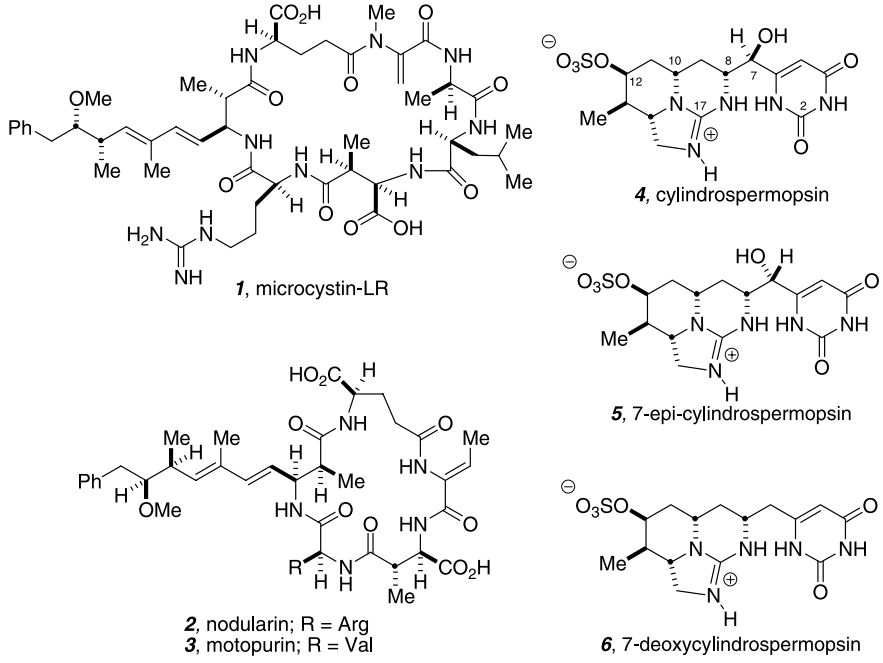

Figure 1. Hepatotoxic cyanobacterial metabolites.

Keywords: Cycloaddition; Guanidine; Alkaloid; Cyanobacteria; Hepatotoxin.
* Corresponding author. Tel.: +1 970 491 6747; fax: +1 970 491 3944; e-mail: rmw@chem.colostate.edu

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

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

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

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

2. Synthetic considerations

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

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

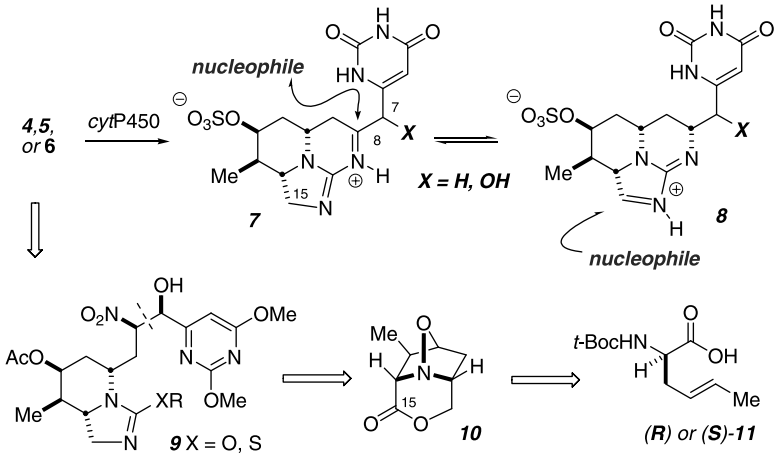


Figure 2. Synthetic strategy.

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

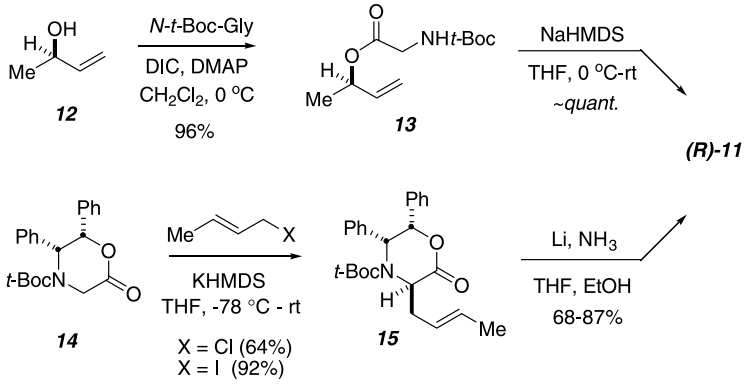
3. Results and discussion

3.1. Synthesis of a common precursor

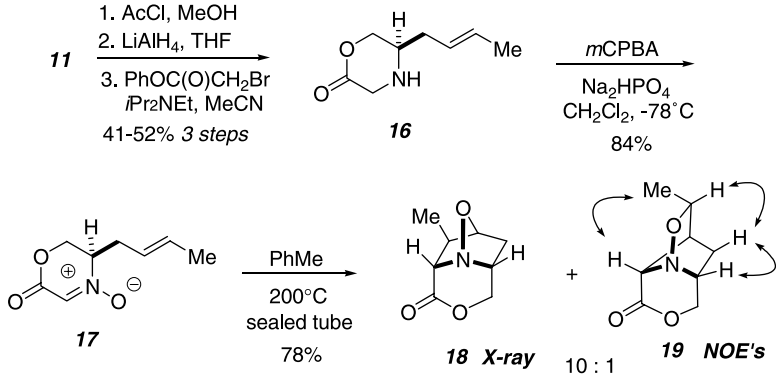
We investigated two strategies to obtain **11** (Scheme 1). The first began with *rac*- or (*R*)-3-buten-2-ol (**12**), which was coupled to *N*-Boc-Gly to give the ester **13**. Enolate-Claisen rearrangement of **13** gave good yields of **11** and was used to generate large quantities of racemic material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

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

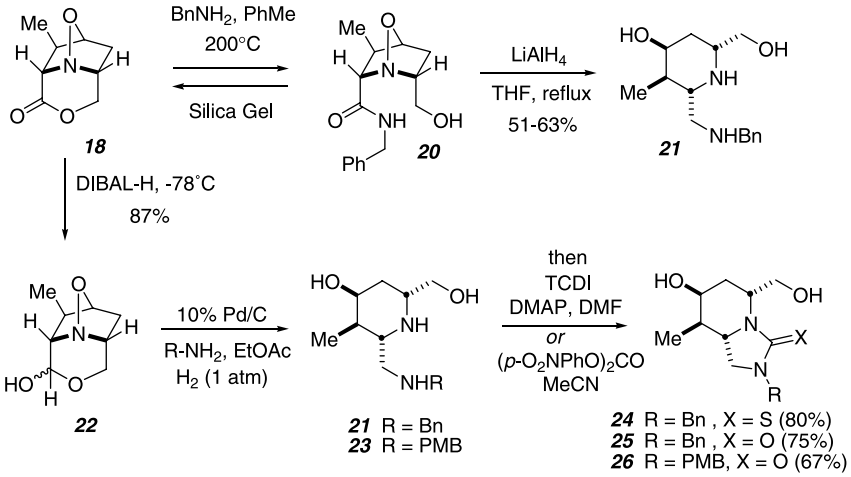
Having established the stereochemistry in the A-ring, we needed to install N16 (Scheme 3). The lactone in **18** could be opened with benzylamine to give **20**, however, purification on silica gel returned **18**. To obviate this reactivity the intermediate amide and *N*,*O*-bond could be reduced with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



Scheme 1. Preparation of crotyl glycine.



Scheme 2. Construction of the A-ring.

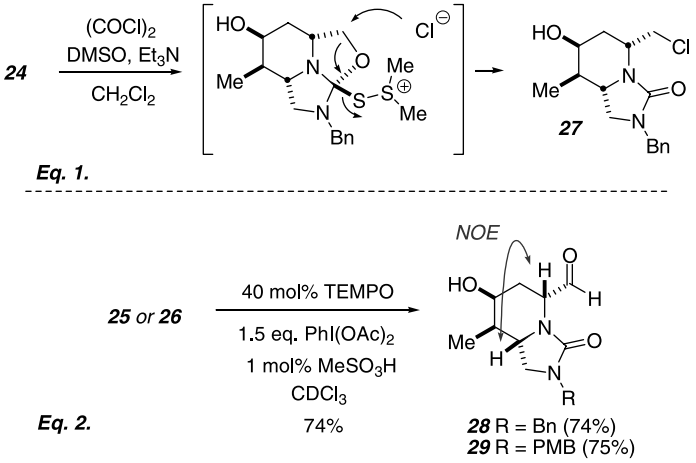


Scheme 3. N16 introduction.

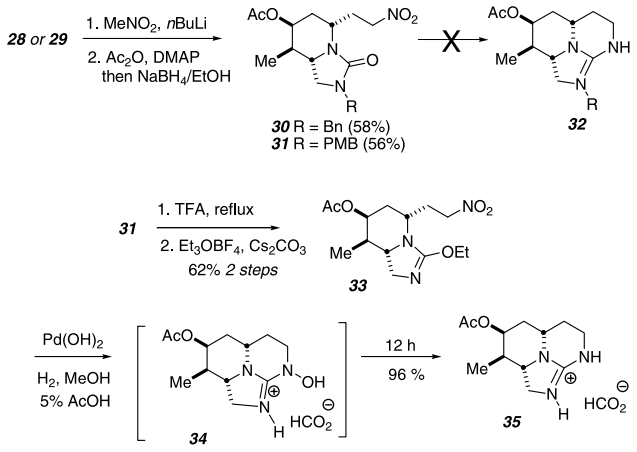
22 in 87% yield. Reductive amination of **22** with either $BnNH_2$ or $PMBNH_2$ proceeded smoothly to afford **21** or **23**, respectively. To aid purification, these diaminodiols were immediately converted to the thiourea using 1,1′-thiocarbonyldiimidazole or the urea using bis-*p*-nitrophenylcarbonate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

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

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



Scheme 4. Oxidation of the ureas.



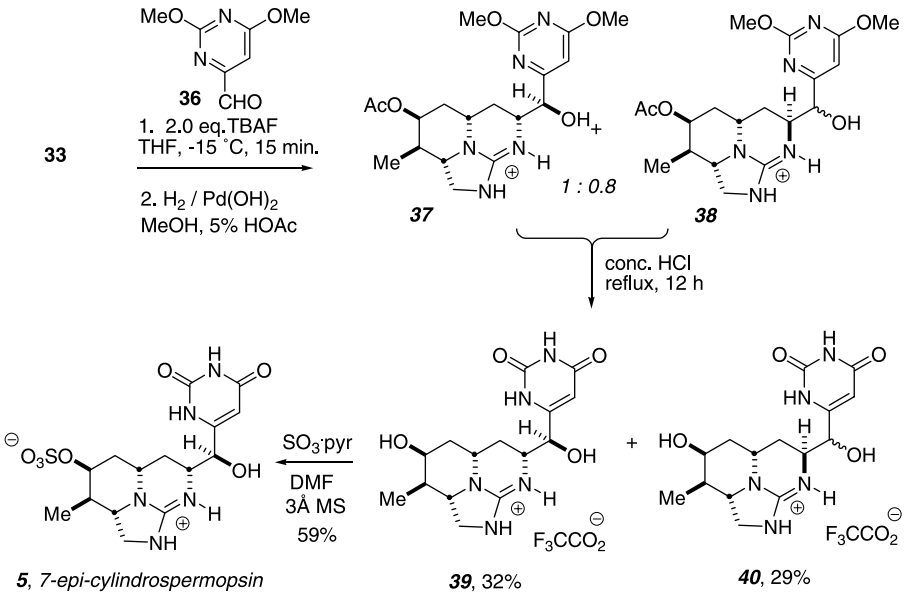
Scheme 5. Successful reductive guanidinylation.

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

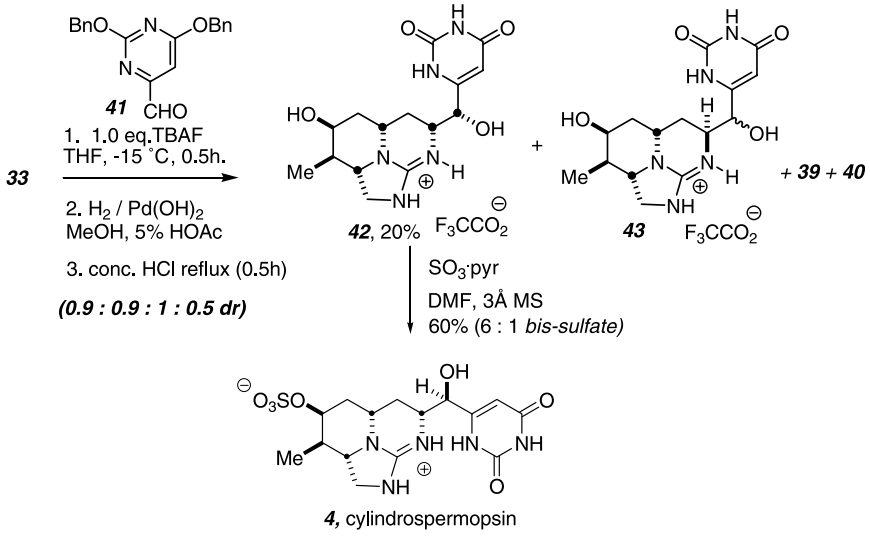
3.2. Synthesis of 7-*epi*-cylindrospermopsin

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

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



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



Scheme 7. Synthesis of cylindrospermopsin.

3.3. Synthesis of cylindrospermopsin

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

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

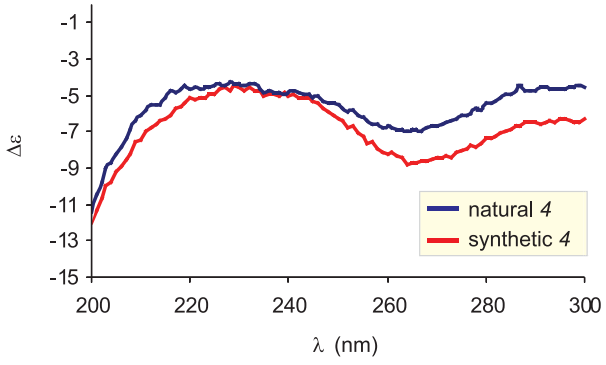
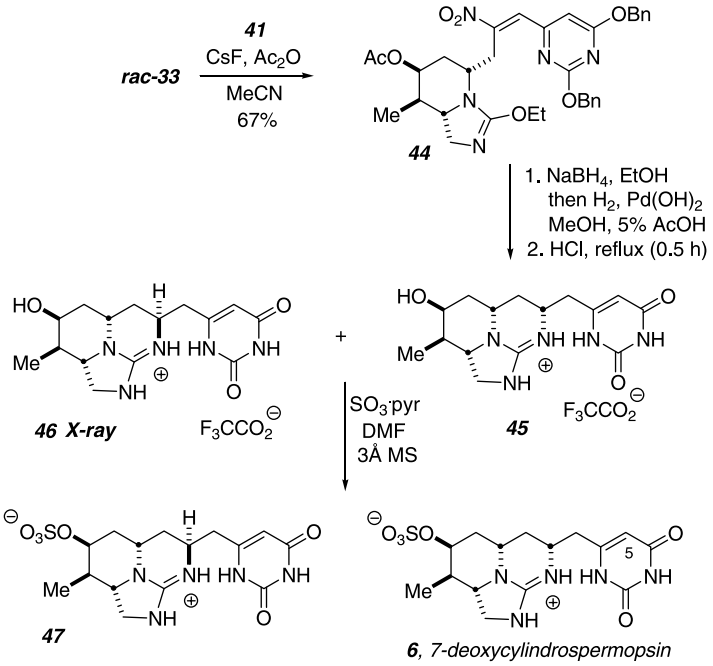


Figure 3. CD spectra of natural and synthetic **4**.

3.4. Synthesis of 7-deoxycylindrospermopsin

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

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



Scheme 8. Synthesis of 7-deoxycylindrospermopin.

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

3.5. Inhibition of protein synthesis

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

4. Conclusion

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

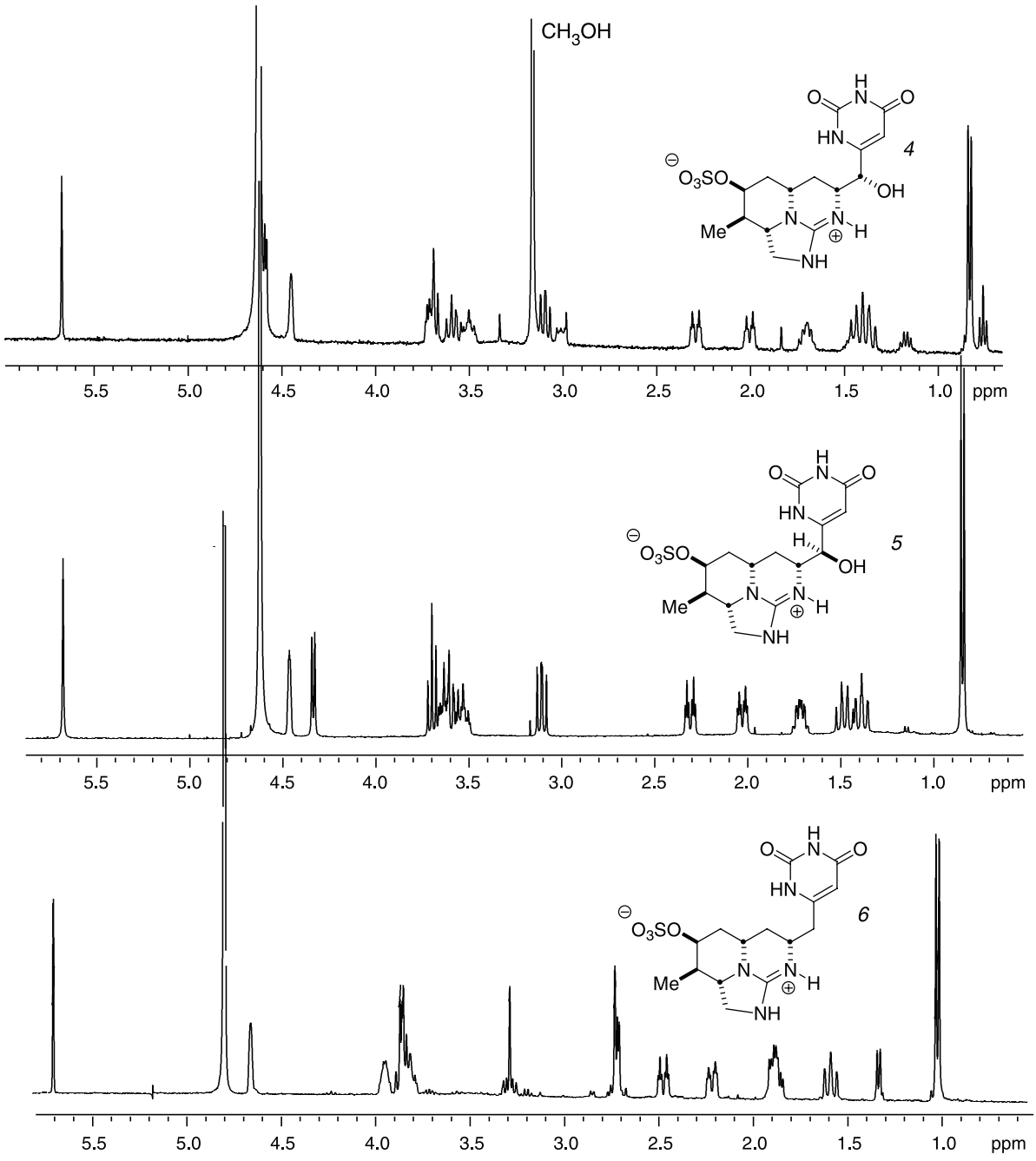


Figure 4. ^{1}H NMR of the synthetic cylindrospermopsins in D_2O (CH_3OH used as internal reference); (1) **4**, (2) **5**, (3) **6**.

5. Experimental

5.1. General

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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