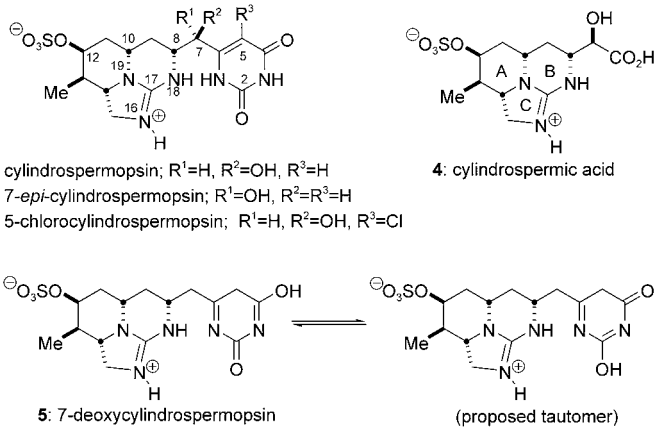

Scheme 1. Structures of the cylindrospermopsins and chlorination products.

Natural Product Synthesis

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

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

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

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

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

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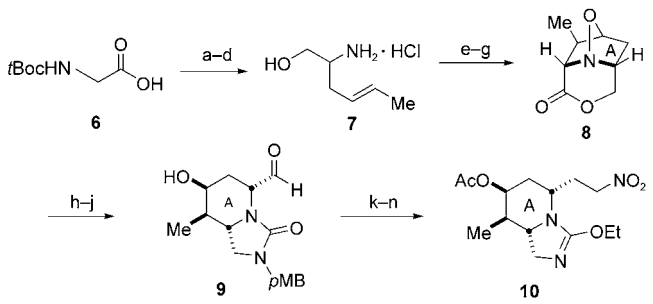


Scheme 2. a) 3-Buten-2-ol, DIC, DMAP, CH_2Cl_2 (96%); b) 2.2 equiv NaHMDS, THF, 0°C→RT (99%); c) EtOCOCl, Et_3N, THF, $NaBH_4$, H_2O; d) AcCl, MeOH (60%, 2 steps); e) $BrCH_2CO_2Ph$, iPr_2NEt, MeCN (63–80%); f) *m*CPBA, Na_2HPO_4, CH_2Cl_2, −78°C (84%); g) PhMe, 200°C, sealed tube, (78%); h) DIBAL-H,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

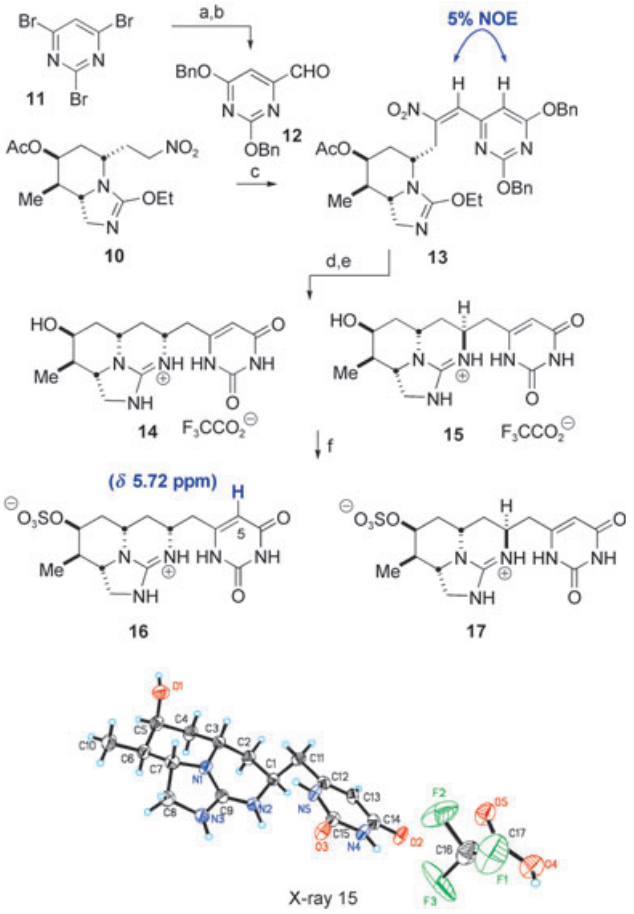
the hydroxymethyl group, thus affording good yields of the sensitive ureido aldehyde **9**.[11]

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

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

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

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



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

Table 1: Inhibition of protein synthesis in rat hepatocytes.

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

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

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

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

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[1] For an excellent review of the cylindrospermopsins, see: D. J. Griffiths, M. L. Saker, *Environ. Toxicol.* **2003**, *18*, 78; for NIH and EPA involvement, see: http://www.epa.gov/safewater/standard/ucmr/ and http://ntp-server.niehs.nih.gov/htdocs/Results_status/ResstatC/M000072.html.
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[15] Co-HPLC-injection of **16** and natural 7-deoxycylindrospermopsin (Agilent zorbax C18 column (4.6 × 150 mm) with MeOH/TFA/H_2O (0.04:0.001:1) as the elutant at 2.0 mL min^{-1} with monitoring at 262 nm) gave a single peak at 5.48 min.
[16] For an excellent review of structural reassignments by total synthesis, see: K. C. Nicolaou, S. A. Snyder, *Angew. Chem.* **2005**, *117*, 1036; *Angew. Chem. Int. Ed.* **2005**, *44*, 1012.

