# *Communications to the Editor*

## Stereoselective Total Synthesis of the Cyanobacterial Hepatotoxin 7-Epicylindrospermopsin: Revision of the Stereochemistry of Cylindrospermopsin

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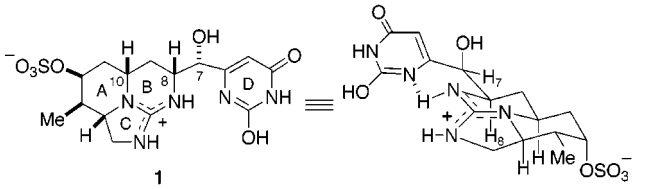
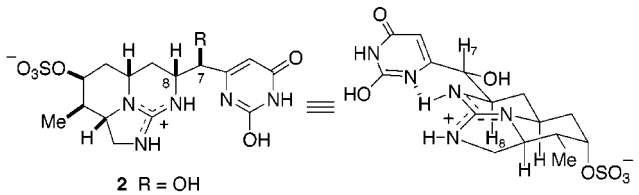
A serious outbreak of hepatoenteritis in 1979 on Palm Island (Queensland, Australia) requiring the hospitalization of about 100 people was found to be due to drinking water in which the cyanobacterium (blue-green alga) *Cylindrospermopsin raciborskii* was growing.[1] It was discovered that this freshwater alga produces a toxic substance causing hepatotoxicity symptoms in mice identical to those that afflicted the human victims. In 1992, Moore and co-workers[2] described the isolation of the toxin, which was named cylindrospermopsin, and using extensive NMR evidence proposed the tetracyclic structure and stereochemistry shown in **1** for this metabolite. More recently, the same hepatotoxin was isolated from the alga *Umezakia natans* collected in Lake Mikata (Fukui, Japan)[3] and from *Aphanizomenon ovalisporum* found in Lake Kinneret in Israel.[4] The latter cyanobacterium was also found to coproduce a minor metabolite 7-epicylindrospermopsin, formulated as **2**, which was reported to be as toxic as **1**.[5] A key premise in the assignment of stereochemistry at C-7 for **1** and **2** is that the molecules exist in the rigid conformations shown, enforced by a hydrogen bond between an enolic uracil D-ring tautomer and the guanidine C-ring. Such a conformation was used to rationalize the observed C-7,8 proton coupling constants in the two isomers. A third metabolite in the series, 7-deoxycylindrospermopsin (**3**), was also recently isolated from *C. raciborskii*.[6] Interestingly, this latter compound proved to be nontoxic. Cylindrospermopsin continues to be a serious public health problem, particularly in tropical areas, and has recently been traced to the deaths of livestock in Australia.[7] On the basis of work reported by Runnegar and co-workers it appears that cylindrospermopsin exerts its toxic effects by inhibiting biosynthesis of cell-reduced glutathione.[8]



**1**



**2** R = OH
**3** R = H

We[9] and others[10] have described studies on the synthesis of cylindrospermopsin, and the Snider group has recently reported a total synthesis of this structurally unique natural product.[10c] In this paper we disclose a synthesis which completely controls the six stereogenic centers of the proposed cylindrospermopsin structure **1** and which now proves that the stereochemical assignments at C-7 in fact have been reversed in cylindrospermopsin and the 7-epi compound. Thus, cylindrospermopsin has the constitution shown in **2** and 7-epicylindrospermopsin is **1** (vide infra). Our approach utilizes a novel stereospecific intramolecular [4 + 2]-cycloaddition of an *N*-sulfinylurea heterodienophile[11] and application of our new efficient uracil synthesis[9c] as key strategic steps.

Construction of the requisite Diels−Alder precursor **12** with the attendant four stereogenic centers contained in the piperidine A-ring was effected as outlined in Scheme 1. Using the methodology of Comins,[12] an efficient high-yield sequence was developed for preparation of vinylogous urethane **4** involving *N*-acylation of 4-methoxypyridine with benzyl chloroformate, followed by addition of (allyldimethylsilyl)methylmagnesium bromide,[13] and subsequent *trans*-selective enolate methylation of the resulting enone product. Conjugate addition of a vinyl goup to enone **4** cleanly and stereospecifically[9b,12] afforded the desired ketone **5**, which could be cleanly reduced with L-Selectride, and the resulting alcohol protected to produce benzyl ether **6**. Tamao oxidation[13] of silane **6**, followed by in situ cyclization of the resulting alcohol provided carbamate **7**, and subsequent hydroboration then yielded alcohol **8**. Swern oxidation of **8** to the

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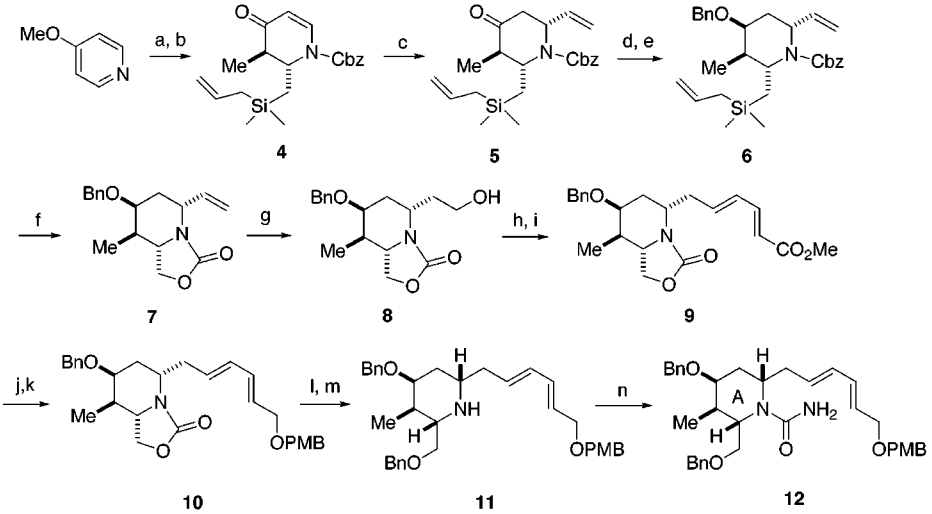
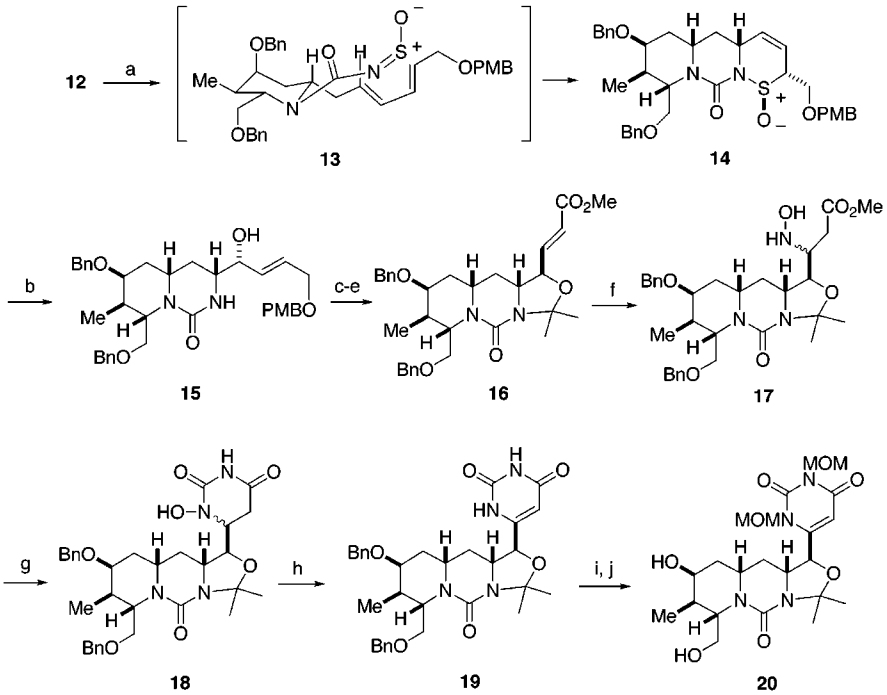
**Scheme 1**[a]



[a] Reagents: (a) i. BNOCOCl, THF, −20 °C; ii. $CH_2$═$CHCH_2Si(Me_2)CH_2MgBr$, $Et_2O$, −20 °C; iii. 5% HCl, rt, 94%; (b) NaHMDS, MeI, THF, −78 °C, 88%; (c) $CH_2$═CHMgBr, CuI, THF, −78 to −20 °C, 98%; (d) L-Selectride, THF, −78 °C, 80%; (e) BnBr, NaH, THF; TBAI, reflux, 95%; (f) i. $KHF_2$, $CHCl_3$, TFA; ii. MeOH, $NaHCO_3$, THF, 30% $H_2O_2$, reflux, 88%; (g) $Sia_2BH$, THF 0 °C; $H_2O_2$, NaOH, −20 °C−rt, 97%; (h) $(COCL)_2$, DMSO, $CH_2Cl_2$, $NEt_3$, −55 °C−rt, 84%; (i) $(EtO)_2POCH_2CH$═$CHCO_2Me$, LiOH−$H_2O$, 4 A MS, THF, reflux, 80%; (j) DlBALH, $BF_3$-$Et_2O$, $CH_2Cl_2$, −78 °C, 83%; (k) NaH, THF, PMBCl, TBAI, reflux, 96%; (l) NaOH, $H_2O$, EtOH, reflux, 100%; (m) NaH, BnBr, TBAI, THF, 0 °C-rt, 65%; (n) KOCN, HOAc, pyr, $NEt_3$, rt, 85%.

**Scheme 2**[a]



[a] Reagents: (a) $SOCL_2$, imidazole, $CH_2Cl_2$, −78 °C−rt, 81%; (b) PhMgBr, THF/$CH_2Cl_2$, −55 °C; $(MeO)_3P$, MeOH, 50 °C, 83%; (c) $Me_2C(OMe)_2$, $Me_2CO$, CSA, reflux, 70%; (d) DDQ, $H_2O$ $CH_2Cl_2$, 82%; (e) i. Dess−Martin, $CH_2Cl_2$; ii. $NaClO_2$, *t*-BuOH, $H_2O$.; iii. *i*-$Pr_2NEt$, MeI, DMF, 81%; (f) TMSONHTMS, THF, EtOH, 82%; (g) PhOCOCl, $NEt_3$, THF; $NH_4OH$, *i*-PrOH, 65%; (h) $Tf_2O$, pyr, $CH_2Cl_2$, 73%; (i) $Me_3SiCl$, MOMCl, *i*-$Pr_2NEt$, $CH_2Cl$, 80%; (j) $Pd(OH)_2$, EtOH, $H_2$, 71%.

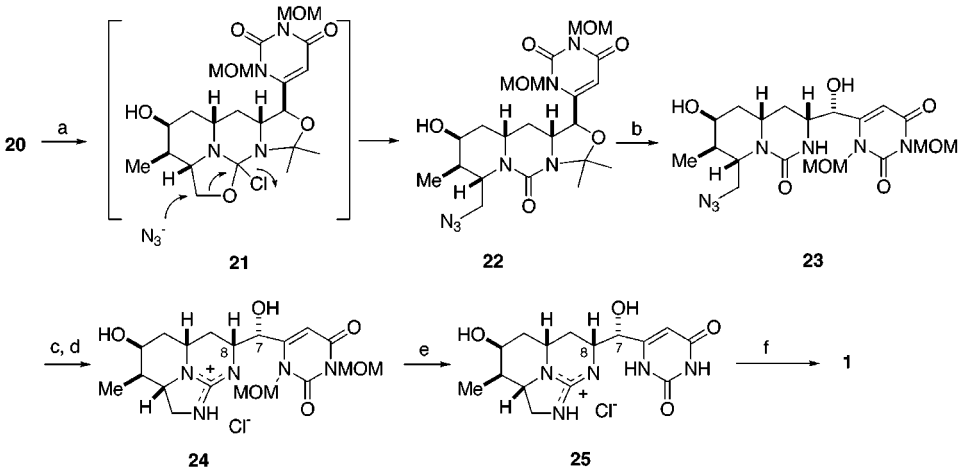
aldehyde, followed by Wadsworth−Emmons−Horner reaction gave the required *E*,*E*-diene ester **9**. The ester group was reduced and the alcohol protected as the PMB ether **10**, which was converted in two steps to dibenzyl ether amine **11**. Finally, conversion of **11** to the corresponding urea[14] provided Diels−Alder substrate **12**.

We were pleased to find that treatment of **12** with thionyl chloride/imidazole led to a single stereoisomeric cycloadduct **14** in high yield (Scheme 2).[9a,11,15] We believe this tricyclic dihydrothiazine oxide is derived from a transient *N*-sulfinylurea **13**, which cyclizes via the conformation shown. Applying methodology previously developed in these laboratories for preparation of vicinal amino alcohol derivatives,[11,16] cycloadduct **14** underwent

(14) A modification of the following procedure was used: Carter, P.; Fitzjohn, S.; Halazy, S.; Magnus, P. *J. Am. Chem. Soc.* **1987**, *109*, 2711.

(15) Compund **14** was converted to the corresponding alcohol by PMB group cleavage, thereby giving a crystalline compound whose structure was established by X-ray crystallography. We thank Dr. Louis Todaro (Hunter College) for this determination and also for the analysis of compound **20**. The X-ray structure of **20** shows an internal hydrogen bond between the alcohol and urea carbonyl.

**Scheme 3**[a]




[a] Reagents: (a) i. triphosgene THF, rt; ii. $NaN_3$, DMF, 65 °C, 86%; (b) HCl, THF, $H_2O$, 85 °C, 72%; (c) MeOTf, 2,6-di-*tert*-butylpyridine, $Ch_2Cl$, −78 °C−rt; (d) 10% Pd/C, EtOH, $H_2$; (e) 12 N HCl, 95 °C, 43% from **23**; (f) $SO_3$−DMF complex, DMF, pyr, $Na_2SO_4$, rt, 70% (+25% bis-sulfate).

a stereospecific ring-opening/[2,3]-sigmatropic rearrangement to afford bicyclic urea allylic alcohol **15** having all six of the stereogenic centers of the natural product in place.[17]

To elaborate the uracil moiety, it was found best to protect the allylic alcohol functionality of **15** as a cyclic acetonide,[18] and after a short sequence involving removal of the PMB group followed by oxidation of the resulting allylic alcohol, α,β-unsaturated ester **16** was obtained. This compound was next subjected to our newly developed three-step uracil construction methodology.[9c] Thus, conjugate addition of hydroxylamine to unsaturated ester **16** led to adduct **17**, which upon treatment with phenyl chloroformate and then ammonium hydroxide gave *N*-hydroxydihydrouracil **18**. It was then possible to dehydrate **18** with triflic anhydride to give the desired D-ring uracil **19**.[19]

The remaining challenge of the synthesis was to construct the guanidine C-ring of the natural product. On the basis of extensive experimentation, it was eventually concluded that it was not possible to activate the urea functionality for effecting ring closure to the guanidine unless the uracil was first protected. Therefore, compound **19** was converted to the bis-*N*-MOM derivative,[20] and both *O*-benzyl groups could subsequently be removed by hydrogenolysis with Pearlman's catalyst to yield diol **20**.[15] However, we were unable to effect any standard activation of the primary alcohol group of intermediate **20** which proved to be unreactive, perhaps for steric reasons or internal hydrogen bonding.[15] Interestingly, it was found that treatment of **20** with triphosgene produced a relatively stable isolable intermediate tentatively assigned structure **21** (Scheme 3), which upon exposure to sodium azide afforded the desired azido compound **22**, perhaps by the $S_N2$ process shown.[21] Acidic hydrolysis of the acetonide group then afforded tricycle **23**. The urea moiety of **23** could be successfully transformed to the corresponding imidate with methyl triflate, and reduction of the azide by catalytic hydrogenation led directly to the desired guanidine **24**.[22]

To our surprise, removal of the MOM-protecting groups of **24** by acidic hydrolysis resulted in a diol guanidinium hydrochloride **25** which was different from the corresponding intermediate produced in the Snider total synthesis of cylindrospermopsin.[10c,23] In particular, our compound had $\delta_{C7}$ 4.50 ppm with $J_{7,8} = 6.6$ Hz, in line with that reported for 7-epicylindrospermopsin,[5] versus $\delta_{C7}$ 4.70 ppm, $J_{7,8} = 4.0$ Hz found for the Snider diol and for cylindrospermopsin.[2] Moreover, using the Snider protocol[10c] diol **25** could be selectively converted to the monosulfate **1** which has NMR spectral data superimposable with those of natural 7-epicylindrospermopsin and significantly different from those of cylindrospermopsin (see Supporting Information).[5,23]

Since we have firmly established the stereochemistry of our synthetic material by X-ray analyses of three intermediates,[15,17] it appears, therefore, that the structure of cylindrospermopsin should be represented as **2** and that 7-epicylindrospermopsin is actually **1**. In addition, the conformations of the molecules and the tautomeric structure of the uracil need to be reexamined in light of these results.

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**Supporting Information Available:** Experimental details and spectral data for all new compounds and copies of the NMR spectra of diol **25** and synthetic 7-epicylindrospermopsin (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Compund **15** was *O*-protected as the MOM derivative and the PMB group was cleaved to give a compound whose structure was determined by X-ray crystallography. We thank Dr. D. Powell (University of Wisconsin) for this analysis.

(18) We thank Professor David Hart (Ohio State University) for suggesting this type of protection and for kindly providing experimental details for preparing a related system.[10f]

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(23) We are grateful to Professor Barry Snider (Brandeis University) for providing NMR spectra and a sample of the synthetic C-7 epimer of the diol depicted in **25**, and spectra of cylindrospermopsin. We also thank Professor S. Carmeli (Tel Aviv University) for copies of the proton and carbon NMR spectra of 7-epicylindrospermopsin.