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Stereoselective Total Syntheses and Reassignment of Stereochemistry of the Freshwater Cyanobacterial Hepatotoxins Cylindrospermopsin and 7-Epicylindrospermopsin

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Abstract: A stereoselective total synthesis of the structure **1** proposed for the freshwater cyanobacterial heptatotoxin cylindrospermopsin has been accomplished in approximately 30 operations starting from commercially available 4-methoxypyridine. Utilizing methodology developed by Comins, the tetrasubstituted piperidine A-ring unit of the hepatotoxin was efficiently constructed. The two remaining stereocenters in the natural product were then set by a stereospecific intramolecular *N*-sulfinylurea Diels−Alder cyclization/Grignard ring opening/allylic sulfoxide [2,3]-sigmatropic rearrangement sequence previously developed in these laboratories, leading to key intermediate **29**. The stereochemical assignment of alcohol **29**, which contains all six of the stereogenic centers of the natural product, was confirmed by an X-ray crystal structure determination of a derivative. Installation of the D-ring uracil moiety was effected by using our new methodology developed for this purpose, and construction of the C-ring guanidine completed the total synthesis of racemic structure **1**. However, the ^{1}H NMR data for this compound do not match that of cylindrospermopsin, but instead agree with the data reported for 7-epicylindrospermopsin, a minor toxic metabolite that co-occurs with cylindrospermopsin. Therefore, we propose a revision of the stereochemical assignments of these natural products such that cylindrospermopsin is now represented as structure **2** and 7-epicylindrospermopsin is **1**. This reassignment was further confirmed by Mitsunobu inversion of the C-7 alcohol **51** to epimer **52**, and conversion of this compound to tetracyclic diol **57**, which has previously been transformed to cylindrospermopsin (**2**).

Introduction

Freshwater blue-green algae (cyanobacteria) have long been known to produce a diverse group of secondary metabolites which have biological activity as hepatotoxins, cytotoxins, and neurotoxins.[1] A number of reliable reports of livestock mortality due to ingestion of cyanobacterial-contaminated water have appeared over the years but fewer cases of human poisoning have been firmly documented. One such instance occurred in 1979 on Palm Island, in northeast tropical Australia, which led to the hospitalization of about 150 people, the large majority children, with hepatitus-like symptoms. The cause of the illness was eventually traced to the cyanobacterium *Cylindrospermopsis raciborskii*, which had contaminated a reservoir (Solomon Dam) supplying drinking water.[2] This poisoning event occurred shortly after treatment of the reservoir with copper sulfate to control a severe algal bloom, thereby causing lysis of the cyanobacterial cells and presumably the release of a toxin into the water. Examination of the aqueous extract from laboratory cultured *C. raciborskii* revealed the presence of a compound that causes severe hepatotoxicity in mice and which is strongly implicated as the causitive agent of the Palm Island outbreak of hepatoenteritis in humans.

Studies by Moore and co-workers led to the isolation of the toxin that was named cylindrospermopsin, and was assigned the novel tetracyclic structure and relative stereochemistry shown in **1** primarily based upon a series of sophisticated NMR experiments.[3,4] It should be noted that an important but rather speculative premise in the assignment of the stereochemistry at C-7 originally proposed for cylindrospermopsin was that the molecule exists in the conformation indicated in **1a**, enforced by a hydrogen bond between an unusual enolic uracil D-ring tautomer and the guanidine C-ring, which was used to rationalize

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(1) For good overviews see: (a) Falconer, I. R. *Environ. Toxicol.* **1999**, *14*, 5. (b) Moore, R. E.; Ohtani, I.; Moore, B. S.; DeKoning, C. B.; Yoshida, W. Y.; Runnegar, M. T. C.; Carmichael, W. W. *Gazz. Chim. Ital.* **1993**, *123*, 329. (c) Burja, A. M.; Banaigs, B.; Abou-Mansour, E.; Burgess, J. G.; Wright, P. C. *Tetrahedron* **2001**, *57*, 9347. (d) Gerwick, W. H.; Tan, L. T.; Sitachitta, N. Nitrogen-Containing Metabolites from Marine Cyanobacteria. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: San Diego, 2001; Vol. 57, p 75.

(2) Hawkins, P. R.; Runnegar, M. T. C.; Jackson, A. R. B.; Falconer, I. R. *Appl. Environ. Microbiol.* **1985**, *50*, 1292.

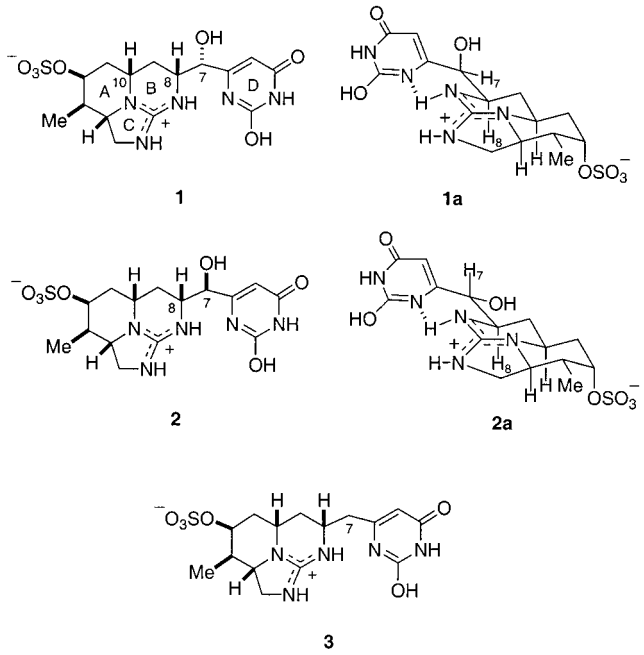
(3) Ohtani, I.; Moore, R. E.; Runnegar, M. T. C. *J. Am. Chem. Soc.* **1992**, *114*, 7941.

(4) For studies on the biosynthesis of cylindrospermopsin see: Burgoyne, D. L.; Hemscheidt, T. K.; Moore, R. E.; Runnegar, M. T. C. *J. Org. Chem.* **2000**, *65*, 152.

10.1021/ja020032h CCC: $22.00

the 4.0 Hz C-7,8 proton−proton coupling constant. The absolute configuration for this metabolite was not determined at the time on the natural material but was recently established to be as shown via enantioselective total synthesis by White and Hansen.[5]

It has become clear that the occurrence of cylindrospermopsin-producing cyanobacteria is widespread in temperate as well as tropical and subtropical areas. For example, in 1994, Harada et al. isolated cylindrospermopsin from the alga *Umezakia natans* collected in Lake Mikata (Fukui, Japan).[6] More recently, this same toxin was isolated from another cyanobacterium, *Aphanizomenon ovalisporum* (Forti), found in Lake Kinneret (Sea of Galilee), a major source of fresh drinking water in Israel.[7] *A. ovalisporum* strains which produce cylindrospermopsin have also been identified in Australia. In addition, *C. raciborskii* has been found in Europe, Brazil, and the United States.[8] Further investigation of the Israel cyanobacterium led to the isolation of a second, minor metabolite with toxicity similar to that of cylindrospermopsin.[9] This compound was assigned the 7-epicylindrospermopsin structure **2**, once again mainly based upon NMR evidence. The C-7,8 coupling constant of 6.8 Hz for this compound was rationalized by a hydrogen-bonded conformation **2a**, as was proposed for cylindrospermopsin. Finally, a third metabolite, 7-deoxycylindrospermopsin (**3**), has been isolated from *C. raciborskii* and interestingly was found to be nontoxic to mice.[10,11]



It has been postulated that cylindrospermopsin probably exerts its toxic effects by inhibiting biosynthesis of cell-reduced glutathione[11b,12] and also by inhibition of protein synthesis.[13] Production of these toxins by cyanobacteria in drinking water is clearly a serious public health problem, particularly in tropical areas, and has recently been traced to the deaths of livestock in Australia.[14] Thus, considerable work continues to appear on development of analytical methods for measuring cylindrospermopsin levels in water[13,15] as well as methodology for potentially destroying the compounds in situ.[16]

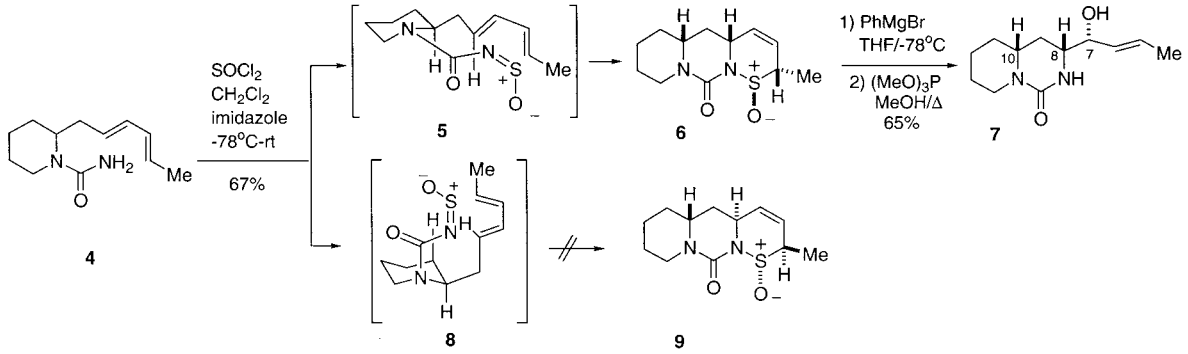
Due to the fascinating and unusual structures of **1** and **2**, as well as their significance in public health, we[17] and others[6,18,19] have undertaken studies on the synthesis of these hepatotoxins. Snider has recently reported a total synthesis of racemic cylindrospermopsin.[18c] Although the Snider group's synthetic material in fact corresponded to natural cylindrospermopsin, it was not possible to conclusively establish the C-7 stereochemistry of any of their intermediates, and therefore the work did not independently confirm the original assignment for this center. In 2001 we described in preliminary form a total synthesis that completely controls all six stereogenic centers of the putative cylindrospermopsin structure **1**, and which proves conclusively that the stereochemical assignments at C-7 in fact have been reversed in cylindrospermopsin and the 7-epi compound.[17a] In this paper we now provide the full details of this work.

Synthetic Plan

Several years ago we devised a strategy for synthesis of cylindrospermopsin based upon a novel variation of *N*-sulfinyl dienophile Diels−Alder methodology that we had previously developed.[20] We recognized that the C-7,8 relationship in structure **1** is that of a *syn*-vicinal amino alcohol derivative of the type we could access from a stereospecific *N*-sulfinyl Diels−Alder reaction of an (*E,E*)-diene to form a dihydrothiazine oxide, followed by a stereospecific Grignard ring opening/[2,3]-

(5) White, J. D.; Hansen, J. D. Submitted for publication.
(6) (a) Harada, K.; Ohtani, I.; Iwamoto, K.; Suzuki, M.; Watanabe, M. F.; Watanabe, M.; Terav, K. *Toxicon* **1994**, *32*, 73. (b) Terav, K.; Ohmori, S.; Igarashi, K.; Ohtani, I.; Watanabe, M. F.; Harada, K. I.; Ito, E.; Watanabe, M. *Toxicon* **1994**, *32*, 833 and references cited therein.
(7) Banker, R.; Carmeli, S.; Hadas, O.; Teltsch, B.; Porat, R.; Sukenik, A. *J. Phycol*. **1997**, *33*, 613.
(8) For lead references see: Schembri, M. A.; Neilan, B. A.; Saint, C. P. *Environ. Toxicol.* **2001**, *16*, 413.
(9) Banker, R.; Teltsch, B.; Sukenik, A.; Carmeli, S. *J. Nat. Prod*. **2000**, *63*, 387.
(10) Norris, R. L.; Eaglesham, G. K.; Pierens, G.; Shaw, G. R.; Smith, M. J.; Chiswell, R. K.; Seawright, A. A.; Moore, M. R. *Environ. Toxicol.* **1999**, *14*, 163.
(11) There have been some limited structure−activity relationship studies in this area: (a) Banker, R.; Carmeli, S.; Werman, M.; Telsch, B.; Porat, R.; Sukenik, A. *J. Toxicol. Environ. Health* **2001**, *62*, 281. (b) Runnegar, M. T.; Xie, C.; Snider, B. B.; Wallace, G. A.; Weinreb, S. M.; Kuhlenkamp, J. *Toxicol. Sci*. **2002**, in press.
(12) Runnegar, M. T.; Kong, S.-M.; Zhong, Y.-Z.; Ge, J.-L.; Lu, S. C. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 235. Runnegar, M. T.; Kong, S.-M.; Zhong, Y.-Z.; Lu, S. C. *Biochem. Pharmacol.* **1995**, *49*, 219.
(13) Froscio, S. M.; Humpage, A. R.; Burcham, P. C.; Falconer, I. R. *Environ. Toxicol.* **2001**, *16*, 408 and references cited therein.
(14) Saker, M. L.; Thomas, A. D.; Norton, J. H. *Environ. Toxicol.* **1999**, *14*, 179.
(15) See for example: Eaglesham, G. K.; Norris, R. L. G.; Shaw, G. R.; Smith, M. J.; Chiswell, R. K.; Davis, B. C.; Neville, G. R.; Seawright, A. A.; Moore, M. R. *Environ. Toxicol.* **1999**, *14*, 151. Norris, R. L. G.; Eaglesham, G. K.; Shaw, G. R.; Senogles, P.; Chiswell, R. K.; Smith, M. J.; Davis, B. C.; Seawright, A. A.; Moore, M. R. *Environ. Toxicol.* **2001**, *16*, 391.
(16) Chiswell, R. K.; Shaw, G. R.; Eaglesham, G.; Smith, M. J.; Norris, R. L.; Seawright, A. A.; Moore, M. R. *Environ. Toxicol.* **1999**, *14*, 155. Senogles, P.; Shaw, G.; Smith, M.; Norris, R.; Chiswell, R.; Mueller, J.; Sadler, R.; Eaglesham, G. *Toxicon* **2000**, *38*, 1203.
(17) For a preliminary account of portions of this work see: (a) Heintzelman, G. R.; Fang, W.-K.; Keen, S. P.; Wallace, G. A.; Weinreb, S. M. *J. Am. Chem. Soc*. **2001**, *123*, 8851. See also: (b) Heintzelman, G. R.; Parvez, M.; Weinreb, S. M. *Synlett* **1993**, 551. (c) Heintzelman, G. R.; Weinreb, S. M.; Parvez, M. *J. Org. Chem.* **1996**, *61*, 4594. (d) Keen, S. P.; Weinreb, S. M. *Tetrahedron Lett.* **2000**, *41*, 4307.
(18) (a) Snider, B. B.; Harvey, T. C. *Tetrahedron Lett.* **1995**, *36*, 4587. (b) Snider, B. B.; Xie, C. *Tetrahedron Lett.* **1998**, *39*, 7021. (c) Xie, C.; Runnegar, M. T. C.; Snider, B. B. *J. Am. Chem. Soc*. **2000**, *122*, 5017. (d) McAlpine, I. J.; Armstrong, R. W. *Tetrahedron Lett.* **2000**, *41*, 1849. (e) White, J. D.; Hansen, J. D. *Abstracts of Papers*; 219th National Meeting of the American Chemical Society, San Francisco, CA; American Chemical SOciety: Washington, DC, 2000; ORGN 812. (f) Djung, J. F.; Hart, D. J.; Young, E. R. R. *J. Org. Chem*. **2000**, *65*, 5668. (g) Looper, R. E.; Williams, R. M. *Tetrahedron Lett.* **2001**, *42*, 769.
(19) Synthetic work in this area has recently been reviewed: Murphy, P. J.; Thomas, C. W. *Chem. Soc. Rev*. **2001**, *30*, 303.

Scheme 1

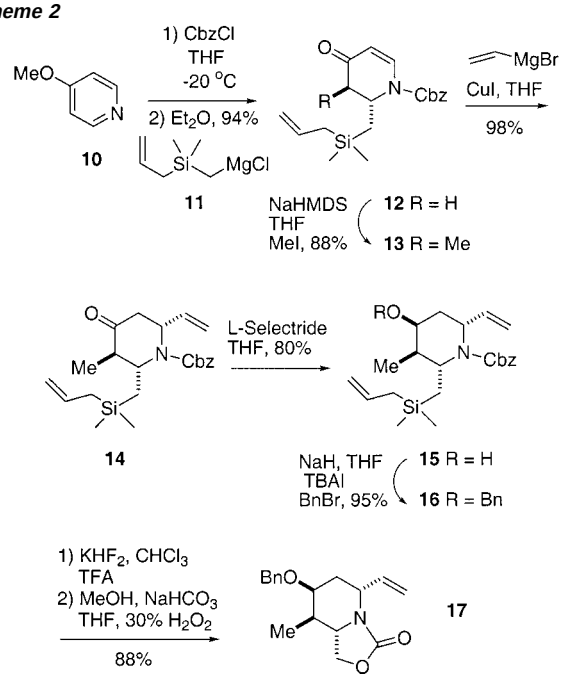


sigmatropic rearrangement.[20,21] The plan was to establish the backbone C-7,8,10 stereogenic centers and the attendant functionality of the natural product employing an intramolecular cycloaddition of an *N*-sulfinyl urea, a type of *N*-sulfinyl dienophile not previously known. This approach was tested in a simple model system as outlined in Scheme 1.[17b] Thus, urea (*E*,*E*)-diene **4** was converted in situ to the *N*-sulfinyl urea, which was found to undergo facile cycloaddition to generate a single Diels−Alder adduct **6** having the correct relative configuration at the C-8,10 centers for toxins **1** and **2**. This result can be rationalized by assuming that cycloaddition of a *Z*-*N*-sulfinyl urea[22] occurs via the conformation shown in **5**. The alternative conformation **8**, which would produce the isomeric adduct **9**, would appear from models to be destabilized vs **5** due to subtle nonbonded interactions. It is difficult to fully rationalize this result since no information is available as to the structure of an *N*-sulfinyl urea, nor is much known regarding the nature of the transition state for an *N*-sulfinyl dienophile Diels−Alder reaction.[23] Suffice it to say we were pleased to find that *N*-sulfinyl ureas are reactive hetero dienophiles, and that remote stereochemistry is controllable in cycloadditions of such a system. Using our methodology,[21] cycloadduct **6** was subsequently treated with phenylmagnesium bromide, followed by trimethyl phosphite in methanol, to produce the cyclindrospermopsin model **7** having the desired C-7,8,10 stereogenicity and functionality.

Results and Discussion

Having successfully tested the key *N*-sulfinyl Diels−Alder chemistry, we next turned to construction of a suitably substituted piperidine A-ring system equivalent to model urea diene **4**. Although we have previously reported some success in utilizing an imino dienophile-based Diels−Alder route to such an intermediate,[17c] we decided to explore a potentially more efficient and direct alternative based upon the dihydropyridinone chemistry of Comins.[24] Thus, treatment of 4-methoxypyridine

Scheme 2



(**10**) with benzyl chloroformate, followed by a hydroxymethyl anion equivalent in the form of Grignard reagent **11**,[25] led to dihydropyridinone **12** (Scheme 2). Enone **12** could be deprotonated and cleanly alkylated with methyl iodide to stereoselectively afford the desired trans product **13**.[26] As anticipated based upon the work of Comins[26] and our own earlier studies,[17c] conjugate addition of vinyl cuprate to enone **13** was stereoselective and led exclusively to the requisite adduct **14**. L-Selectride reduction of ketone **14** was also stereoselective and produced the desired alcohol **15** having the four correct A-ring stereogenic centers of the natural products.[17c] This alcohol was protected as the benzyl ether **16**, and subsequent Tamao oxidation of the silane led directly to the cyclic carbamate **17** in very high overall yield from pyridine **10**.[25]

To elaborate the required diene chain, alkene **17** was first hydroborated to afford primary alcohol **18**, which was then transformed to the corresponding aldehyde by a Swern oxidation (Scheme 3). Coupling of this aldehyde with phosphonate **19**[27] cleanly afforded the desired (*E*,*E*)-diene ester **20**. The ester group

(20) For reviews of *N*-sulfinyl dienophile Diels−Alder-based methodology, see: (a) Weinreb, S. M. *Acc. Chem. Res.* **1988**, *21*, 313. (b) Boger, D. L.; Weinreb, S. M. *Hetero Diels−Alder Methodology in Organic Synthesis*; Academic Press: San Diego, 1987; Chapter 1. (c) Weinreb, S. M. Heterodienophile Additions to Dienes. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, 1991; Vol. 5, p 401.

(21) Garigipati, R. S.; Freyer, A. J.; Whittle, R. R.; Weinreb, S. M. *J. Am. Chem. Soc.* **1984**, *106*, 7861.

(22) For comprehensive reviews of *N*-sulfinyl compounds, see: Bussas, R.; Kresze, G.; Munsterer, H.; Schwobel, A. *Sulfur Rep.* **1983**, *2*, 215. Schubart, R. In *Houben-Weyl*, 4th ed.; Klamann, D., Ed.; Thieme, Stuttgart, 1985; Vol. E11, p 122. The ground-state configuration of *N*-sulfinyl compounds is usually *Z* although in some systems an *E*/*Z* equilibrium has been observed.

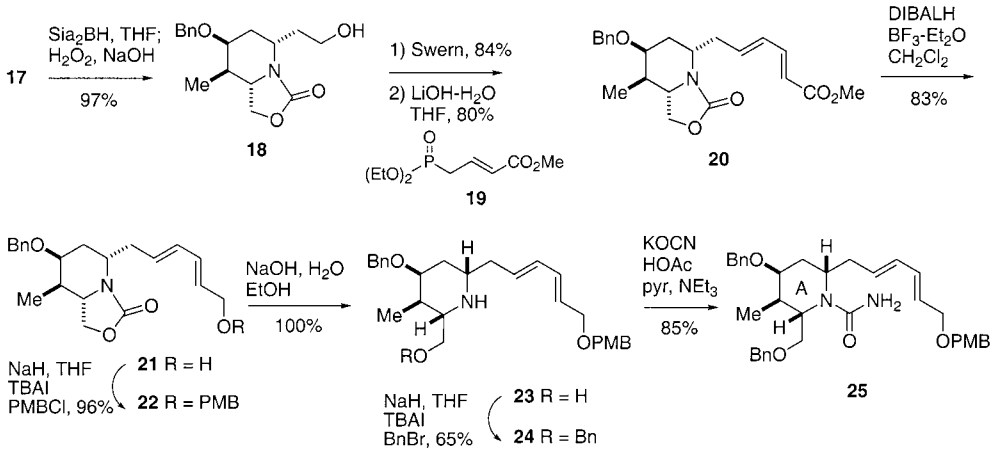
(23) For theoretical studies of the mechanism of *N*-sulfinyl Diels−Alder reactions see: Park, Y. S.; Kim, W. K.; Kim, Y. B.; Lee, I. *J. Org. Chem.* **2000**, *65*, 3997.

(24) For a review see: Comins, D. L. *J. Heterocycl. Chem.* **1999**, *36*, 1491.

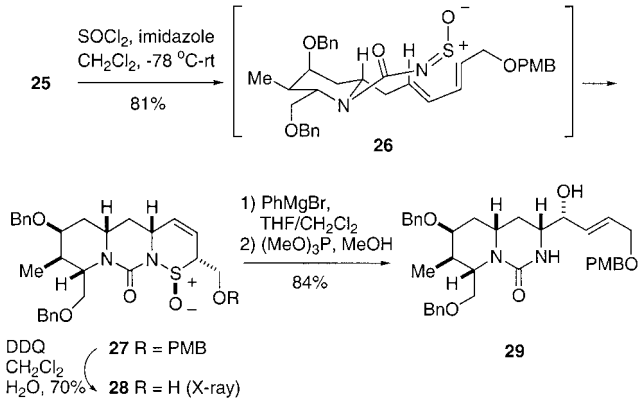
(25) Tamao, K.; Ishida, N. *Tetrahedron Lett.* **1984**, *25*, 4249. The Grignard reagent **11** was prepared from the corresponding chloride: Connolly, J. W.; Fryer, P. F. *J. Organomet. Chem.* **1971**, *30*, 315.

(26) See for example: Comins, D. L.; LaMunyon, D. H.; Chen, X. *J. Org. Chem.* **1997**, *62*, 8182.

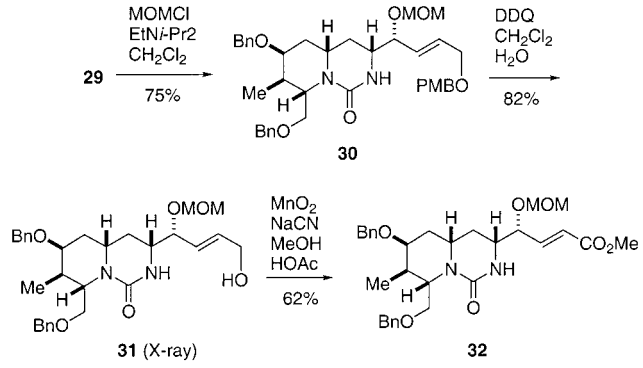
Scheme 3



Scheme 4



Scheme 5



of **20** was next reduced with DIBALH to provide the allylic alcohol **21**, which was then protected as the *p*-methoxybenzyl ether **22**. Basic hydrolysis of the cyclic carbamate functionality of **22** subsequently provided the amino alcohol **23**. Since the expectation was that we would be able to differentiate between a primary and secondary alcohol in the latter stages of the synthesis (vide infra), we decided to protect the alcohol group of **23** as the benzyl ether, leading to the dibenzyl compound **24**. The amino group of compound **24** appears to be rather hindered and thus conversion of this compound to the corresponding urea **25** proved problematic. However, after some effort it was found that a modification of the method of Magnus et al. worked reproducibly to generate the (*E*,*E*)-diene urea **25**.[28]

With intermediate **25** now in hand, we investigated the pivotal intramolecular hetero Diels−Alder reaction. We were gratified to find that simply treating the compound with thionyl chloride/imidazole in methylene chloride initally at −78 °C and warming slowly to room temperature led to an excellent yield of a single tricyclic adduct **27** (Scheme 4). The PMB group of this compound was removed with DDQ to afford the alcohol **28** whose structure and stereochemistry were firmly established by X-ray crystallography.[29] The formation of dihydrothiazine oxide **27** is fully in accord with our model studies (cf. Scheme 1) and the cycloaddition most likely occurs via the *N*-sulfinyl urea/diene conformation shown in **26**. Using our methodology, treatment of cycloadduct **27** with phenylmagnesium bromide, followed by trimethyl phosphite in methanol, cleanly led to a single stereoisomeric allylic alcohol **29**. This compound now possesses all six stereogenic centers of the cyclindrospermopsin structure **1** proposed by Moore.[3]

The next stage of the project was to be construction of the D-ring of the toxin utilizing our efficient, newly developed three-step method for uracil synthesis starting from an α,*β*-unsaturated ester.[17d] We originally opted to first protect the allylic alcohol group of intermediate **29** as the MOM ether **30** (Scheme 5). Removal of the PMB group of **30** led to a crystalline alcohol **31** whose structure was determined by X-ray analysis, thereby fully confirming the stereochemical assignment of key intermediate **29**.[30] Using the procedure of Corey,[31] allylic alcohol **31** was then directly converted to the desired α,*β*-unsaturated methyl ester **32**. Unfortunately, despite the structural similarity between this compound and the model unsaturated ester employed in our developmental studies,[17d] all attempts to effect the conjugate addition of a nitrogen nucleophile to **32** failed.

It seemed to us that one possible cause for this problem was that the bulky allylic MOM group was impeding the conjugate addition step, and we considered that perhaps changing the protecting group as well as rigidifying the system might alleviate this difficulty. We therefore converted urea alcohol **29** into the

(27) For preparation of this phosphonate see: (a) Houllemare, D.; Outurquin, F.; Paulmier, C. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1629. (b) Bæckström, P.; Jacobsson, U.; Norin, T.; Unelius, C. R. *Tetrahedron* **1988**, *44*, 2541.

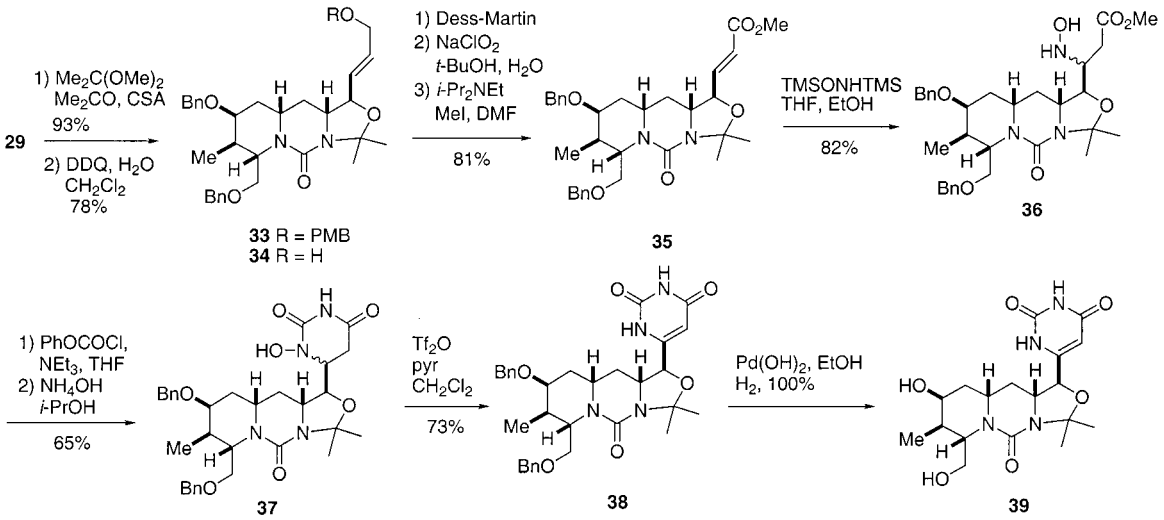
(28) Carter, P.; Fitzjohn, S.; Halazy, S.; Magnus, P. *J. Am. Chem. Soc.* **1987**, *109*, 2711.

(29) We thank Dr. Louis Todaro (Hunter College) for the X-ray determination of compounds **28**, **45**, and **54**. Data have been deposited with the Cambridge Crystallographic Data Centre (**28**: CCDC 175623; **45**: CDCC 171477; **54**: CDCC 175499).

(30) We thank Dr. D. Powell (University of Wisconsin) for the X-ray analysis of compound **31**. Data have been deposited with the Cambridge Crystallographic Data Centre (CDCC 176837).

(31) Corey, E. J.; Gilman, N. W.; Ganem, B. E. *J. Am. Chem. Soc.* **1968**, *90*, 5616.

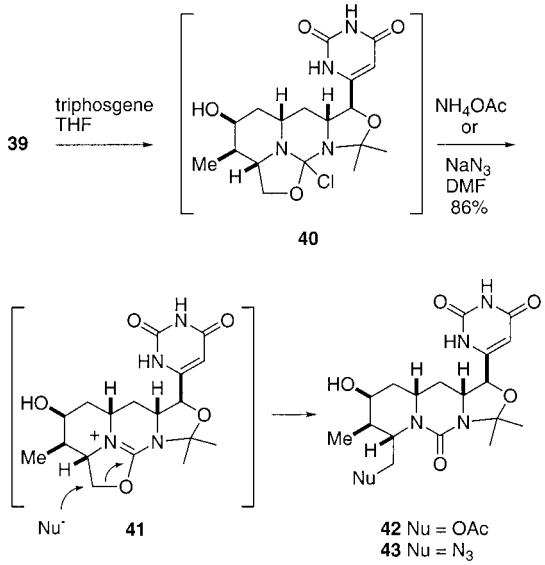
Scheme 6



cyclic acetonide **33** as has been done in a related cylindrospermopsin model system by Hart (Scheme 6).[32] Subsequent removal of the PMB group gave allylic alcohol **34**, which was then transformed by the sequence shown to the desired α,β-unsaturated methyl ester **35**. We were gratified to find that treatment of enoate **35** with *N,O*-bis-trimethylsilylhydroxylamine in the presence of ethanol in fact afforded the desired conjugate addition product **36** (a single stereoisomer of undetermined configuration).[33] Exposure of hydroxylamine **36** to phenyl chloroformate, followed by ammonium hydroxide provided the anticipated *N*-hydroxydihydrouracil **37**. Elimination of water from **37** could then be successfully effected with triflic anhydride[17d,34] to provide the fully aromatized uracil D-ring system **38**. Both benzyl groups of **38** could then be removed simultaneously by catalytic hydrogenolysis to yield diol **39**.

We next turned to construction of the remaining guanidine C-ring of the natural product. Toward this end, a number of attempts were made to activate the primary alcohol group of **39** for displacement by a nitrogen nucleophile but in general no reaction took place, probably for steric reasons. Similarly, this compound was unreactive toward Mitsunobu processes. An alternative approach that we considered was to first convert the urea functionality of **39** to the corresponding guanidine, and then to effect an intramolecular Mitsunobu closure to form the C-ring as was done in a cylindrospermopsin model study by Armstrong.[18d] Therefore, alcohol urea **39** was treated with triphosgene, followed by ammonium acetate (Scheme 7). To our surprise, however, the product of this reaction was not a guanidine but the urea monoacetate **42**. Careful examination of the reaction showed that a reasonably stable, isolable intermediate was formed upon treatment of **39** with triphosgene, and that subsequent exposure of this compound to ammonium acetate led to the observed product **42**. Although we have not been able to definitively establish the structure of this intermediate,

Scheme 7



we believe it is probably chloride **40** based upon NMR spectral data, and the fact that in the mass spectrometer it produces a molecular ion consistent with oxonium structure **41**. S_N2 displacement at carbon by acetate ion via **41** would lead to the observed product **42**. Such a ring-opening process has good precedent in related amide-derived systems analogous to **41**.[35] It was in fact possible to use this unexpected result to our advantage, since when we exposed the triphosgene product **40** to sodium azide in DMF the desired azide **43** was formed in good yield.

With azide **43** in hand, we expended a considerable amount of time and effort attempting to construct the guanidine ring directly from this intermediate. However, we eventually concluded that the uracil moiety was probably interfering with our various attempts to activate the urea functionality for ring closure. We therefore decided to investigate a variation of this strategy involving *N*-protection of the uracil prior to guanidine formation. Returning to compound **38**, it was possible to intro-

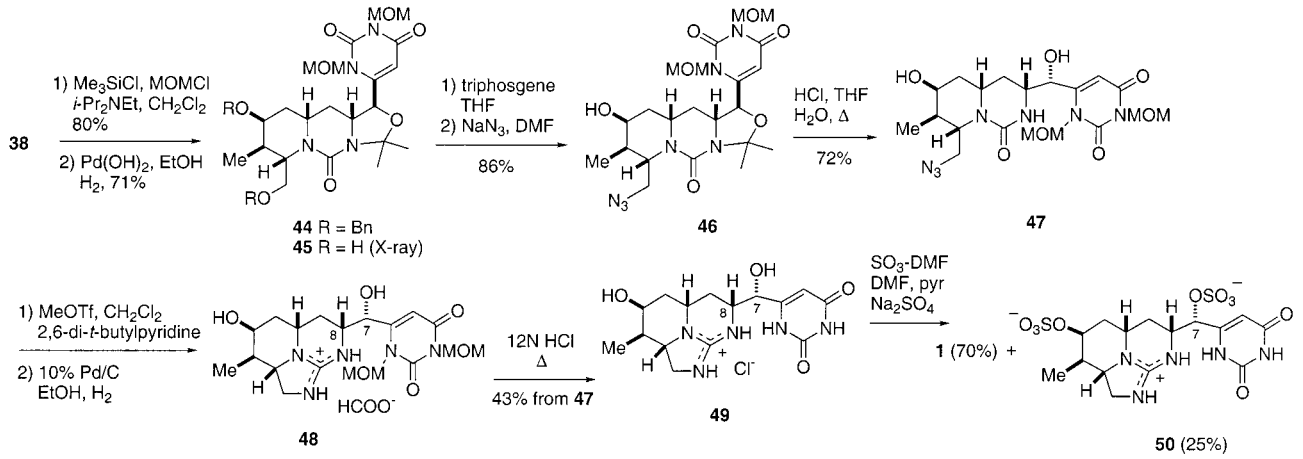
(32) We are grateful to Professor David Hart (Ohio State University) for suggesting this type of protection and also for providing experimental details.[18f]

(33) Interestingly, unlike our model system,[17d] adduct **36** did not spontaneously lactonize to produce the corresponding isoxazolidinone. Attempts to effect this cyclization under various conditions led primarily to a retro-Michael reaction.

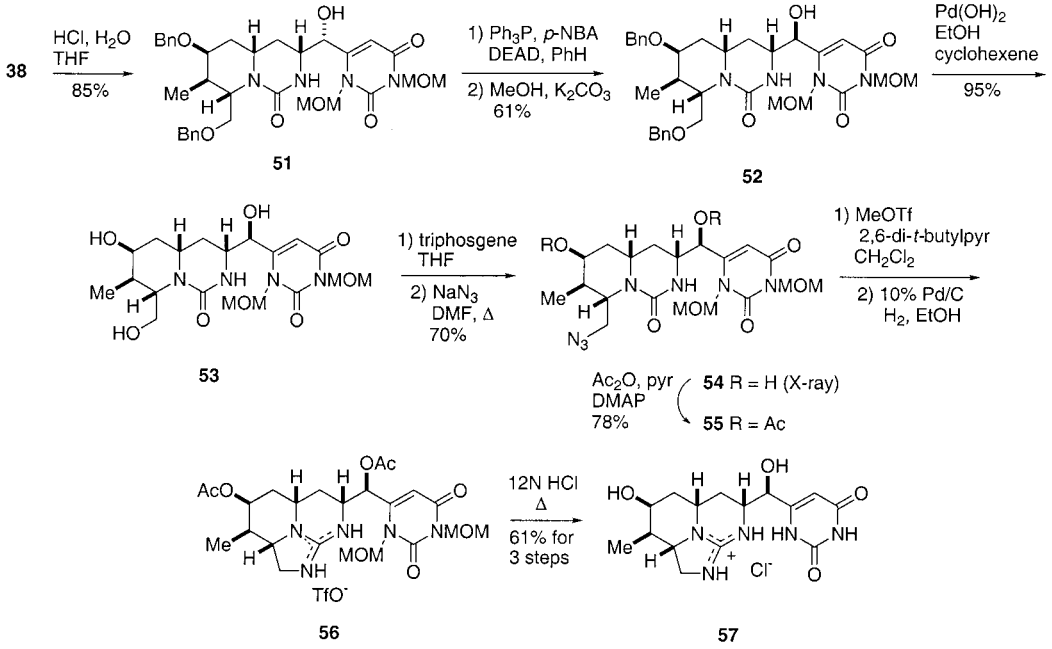
(34) Cf.: Hoffman, R. V.; Nayyar, N. K. *J. Org. Chem.* **1994**, *59*, 3530. Hoffman, R. V.; Nayyar, N. K.; Shankweiler, J. M.; Klinekole, B. W., III *Tetrahedron Lett.* **1994**, *35*, 3231.

(35) Hunig, S. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 548. Gracias, V.; Milligan, G. L.; Aube, J. *J. Org. Chem.* **1996**, *61*, 10. Meyer, F.; Uziel, J.; Papini, A. M.; Juge, S. *Tetrahedron Lett.* **2001**, *42*, 3981. We thank Professor Aube for providing these references.

Scheme 8



Scheme 9



duce MOM protecting groups onto the uracil nitrogens using the procedure of Arias and co-workers to afford **44**,[36] which was then converted to the crystalline diol **45** via catalytic hydrogenolysis (Scheme 8).[37] An X-ray analysis of **45** was conducted as further verification of the structures of these late-stage intermediates.[29] Exposure of diol **45** to triphosgene followed by sodium azide provided the desired azide **46**. Once again, formation of an intermediate like **40** could be observed in this reaction. The acetonide protecting group of **46** could then be selectively hydrolyzed with dilute HCl to produce urea diol **47**.

We were pleased to find that urea activation of **47** with MeOTf, followed by catalytic hydrogenation of the azide, now proceeded smoothly, leading directly to the tetracyclic guanidinium compound **48** (isolated as the formate salt).[38] The MOM groups of **48** could subsequently be cleaved by vigorous acidic hydrolysis[18c] to afford diol guanidinium chloride **49**. Surprisingly, diol **49** was found to be different when it was directly compared to the corresponding intermediate produced in Snider's total synthesis of cylindrospermopsin.[18c,39] In particular, our compound had δ_{C7} 4.50 ($J_{7,8}$ = 6.6 Hz, in D_2O) in accord with that reported for 7-epicylindrospermopsin,[9] compared to δ_{C7} 4.70 ($J_{7,8}$ = 4.0 Hz) for both the Snider diol and cylindrospermopsin.[3] Using Snider's protocol[18c] our diol **49** was then selectively converted to the monosulfate **1** (70%) along with some of the bis-sulfate **50** (25%). Synthetic compound **1** was in fact found to have NMR spectra identical with those of natural 7-epicylindrospermopsin and substantially different from those of cylindrospermopsin.[40]

However, to further confirm this unanticipated result, we decided to also prepare the cylindrospermopsin structure **2** from one of our late synthetic intermediates. Therefore, the acetonide

(36) Arias, L.; Guzman, A.; Jaime-Figueroa, S.; Lopez, F. J.; Morgans, D. J., Jr.; Padilla, F.; Perez-Medrano, A.; Quintero, C.; Romero, M.; Sandoval, L. *Synlett* **1997**, 1233.

(37) We have also prepared the *N*-BOM and *N*-benzyl uracil protected series of compounds. In both cases it was possible to successfully construct the guanidine rings via the methodology used for the MOM series (cf. Scheme 8) but in these cases the conditions required either for hydrogenolytic removal of the protecting groups or for azide hydrogenation led to overreduction of the uracil ring.

(38) Overman, L. E.; Rabinowitz, M. H.; Renhowe, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 2657.

(39) Thanks are due to Professor Barry Snider (Brandeis University) for providing NMR spectra and a comparison sample of synthetic diol **57**, along with spectra of cylindrospermopsin.

functionality of intermediate **38** was first removed by acid hydrolysis to afford alcohol **51** (Scheme 9). This compound could be cleanly inverted by a Mitsunobu process[41] to give the desired C-7 alcohol epimer **52**. Removal of the two benzyl groups of **52** by catalytic hydrogenolysis then provided triol **53**. We were pleased to find that treatment of this triol with triphosgene (for spectral data on the intermediate see the Supporting Information) followed by sodium azide in DMF cleanly led to regioselective formation of the requisite mono azide diol **54**, whose structure and stereochemistry were verified by X-ray crystallography.[29] Prior to guanidine formation, the diol **54** was protected as the diacetate **55** since the diol itself was not sufficiently soluble in methylene chloride for use in the next step. Activation of the urea functionality of **55** with MeOTf followed by hydrogenation of the azide led to the desired guanidinium salt **56**. Finally, acidic hydrolysis of the acetyl and MOM groups afforded the tetracyclic diol **57**, which had spectra identical with those of the Snider synthetic material.[39] This compound has previously been converted to cylindrospermopsin, thus verifying that the toxin is actually represented by stereostructure **2**.[18c]

It is evident, therefore, that the original Moore assignment of C-7 stereochemistry for cylindrospermopsin is incorrect, and that the structures of the two toxins have in fact been reversed (i.e., cylindrospermopsin is now formulated as **2** and 7-epicylindrospermopsin is **1**). Also, it presently seems unlikely that the toxins exist in the hydrogen-bonded conformations represented by **1a** and **2a**, nor is it clear at this point whether the uracil actually assumes the unusual tautomeric form shown in these structures.[42]

In conclusion, we have accomplished total syntheses of the two algal hepatotoxins cylindrospermopsin (**2**) and 7-epicylindrospermopsin (**1**) in approximately 30 steps starting from 4-methoxypyridine, leading to a revision of the C-7 stereochemistry of the natural products. Our approach utilizes a novel stereospecific intramolecular [4+2]-cycloaddition of an *N*-sulfinylurea heterodienophile and application of our new uracil synthesis[17c] as pivotal steps.

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Supporting Information Available: Full experimental details and spectral data for new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA020032H

(40) We thank Professor Shmuel Carmeli (Tel Aviv University) for copies of the proton and carbon NMR spectra of natural 7-epicylindrospermopsin.
(41) Martin, S. F.; Dodge, J. A. *Tetrahedron Lett*. **1991**, *32*, 3017.
(42) For a discussion of uracil tautomers see: Kryachko, E. S.; Nguyen, M. T.; Zeegers-Huyskens, T. *J. Phys. Chem A* **2001**, *105*, 1934 and references cited therein.