Natural Product Synthesis

A Concise Asymmetric Synthesis of the Marine Hepatotoxin 7-Epicylindrospermopsin**

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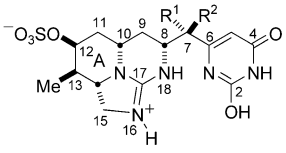
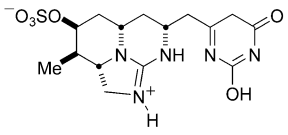
The cyanobacterial toxin cylindrospermopsin (**1**) was isolated as the principal hepatotoxin from *Cylindrospermopsis raciborskii* in 1992 after suspicion of its involvement in an outbreak of hepatoenteritis that hospitalized 150 people on Palm Island, Australia.[1] It has since been isolated in Japan from *Umezakia natans* and in Israel from *Aphazinomenon ovalisporum*.[2] Following the discovery of the parent compound, 7-epicylindrospermopsin (**2**) was isolated from *A. ovalisporum* as a toxic minor metabolite.[3] The natural congener 7-deoxycylindrospermopsin (**3**) was initially isolated from *C. raciborskii* and has recently been co-isolated with **1** in China from *Raphidiopsis curvata*.[4] Cylindrospermopsin has been shown independently to be a potent hepatotoxin ($LD_{50} \sim 0.2\ mg\,kg^{-1}$ in mice), and 7-epicylindrospermopsin is equipotent with **1**, whereas **3** is nontoxic.[1,4a,5] It is thought that the toxicity of these substances results from a general inhibition of protein synthesis, but it is unclear whether this action occurs at the ribosomal or transcriptional level by the interaction of oxidized metabolites with DNA.[6,7] 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 have prompted the National Toxicology Program (NTP) of the National Institutes of Health and the Unregulated Contaminant Monitoring Rule (UCMR) of the Environmental Protection Agency to elect **1** for toxicological and environmental evaluation.[8] The paucity of understanding of the biological functions of these agents has prompted us to develop an efficient and flexible synthetic approach that will be adapted to the production of uracil analogues to be deployed for biomechanistic evaluation.



1: R^1 = H, R^2 = OH
2: R^1 = OH, R^2 = H



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These highly polar compounds pose a significant synthetic challenge as they contain almost as many heteroatoms as carbon atoms, and their structures include a zwitterionic guanidinium sulfate, a rare tetrasubstituted tricyclic guanidine, and a uracil moiety. Not surprisingly, these natural products have attracted the attention of synthetic chemists for over ten years.[9] Snider and co-workers completed the first racemic total synthesis of cylindrospermopsin in 20 steps eight years after its discovery.[10] However, their studies failed to illuminate the misassigned stereogenic center at C7. The configuration at that center was corrected by Weinreb and coworkers in an elegant 30-step racemic, stereoselective synthesis of **2**, thus validating the illustrated structures.[11] Shortly thereafter White and Hansen completed the first asymmetric total synthesis of **2** in 28 steps and confirmed the absolute stereochemistry as 7*S*, 8*R*, 10*S*, 12*S*, 13*R*, 14*S*.[12]

We envisaged that the three contiguous stereogenic centers of the A ring of cylindrospermopsin could be constructed by a single intramolecular 1,3-dipolar cycloaddition of the nitrone **4** to yield the tricyclic compound **5** (Scheme 1). At the onset of our work, the absolute stereochemistry of these natural products was not known. Retrosynthetic analysis suggested that the nitrone **4** could be derived from a simple amino acid, namely (*R*)- or (*S*)-crotylglycine, so that **1** could be constructed from a precursor with a single stereogenic center, which would provide the inherent flexibility to produce either enantiomer of the natural product. The two remaining, remote stereogenic centers at C7 and C8 and their respective oxidation states define this family in terms of both structure and function. Considering this, it was anticipated that a nitroaldol (Henry reaction) strategy would permit access to both C7 epimers (**1** and **2**), and through reductive nitromethylation provide access to **3**.

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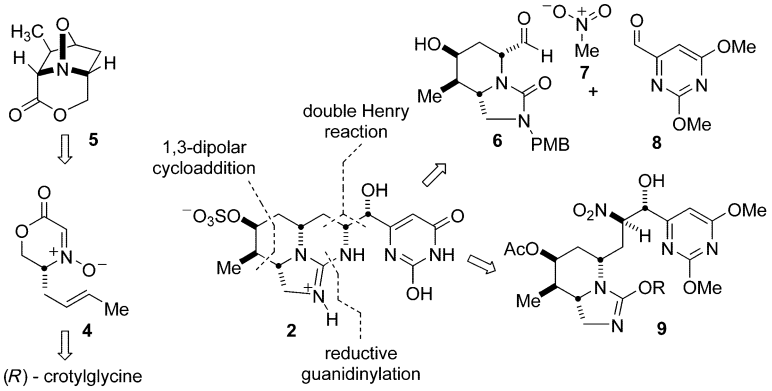
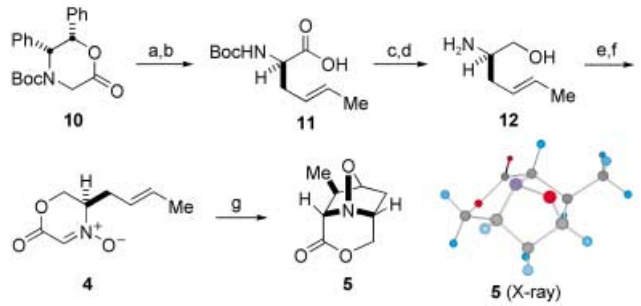


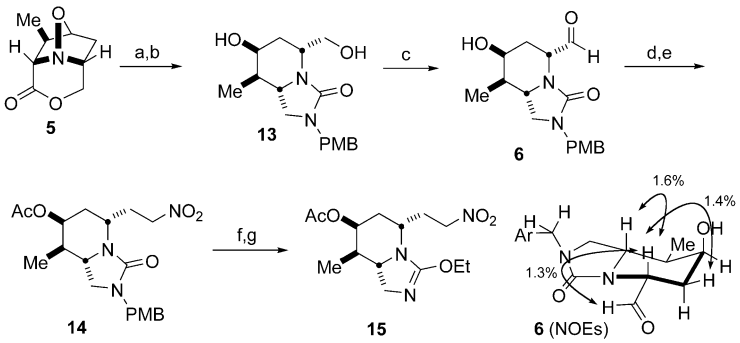
Scheme 1. Retrosynthesis of the cylindrospermopsins. PMB = *p*-methoxybenzyl.

To this end, the protected crotylglycine derivative **11** was prepared from our oxazinone template **10** (Scheme 2).[13] Thus, alkylation of **10** with (*E*)-crotyl iodide gave the corresponding crotylated lactone in 92% yield with >99% *ee*.[14] Reductive removal of the chiral auxiliary with lithium in ammonia gave **11** in moderate to good yield. Acidic removal of the Boc group with concomitant methyl ester formation followed by reduction with lithium aluminum hydride gave the optically pure alcohol **12**. This substance was then transformed into the free morpholinone in a one-pot procedure by treatment with bromophenyl acetate.[15] Oxidation of the secondary amine was most conveniently effected by treatment with purified MCPBA in dichloromethane to give the oxazinone *N*-oxide **4** in 84% yield.[16] Exposure of **4** to elevated temperatures gave the tricyclic isoxazolidine **5** in 78% yield as a 10:1 mixture with the minor product assumed to arise from an *endo* approach of the alkene to the nitrone.[9i,17] We also observed that the treatment of **4** with scandium triflate at room temperature for up to three days generated **5** in an improved 12:1 mixture. The relative stereochemistry of **5** was confirmed by X-ray crystallography.[9i]



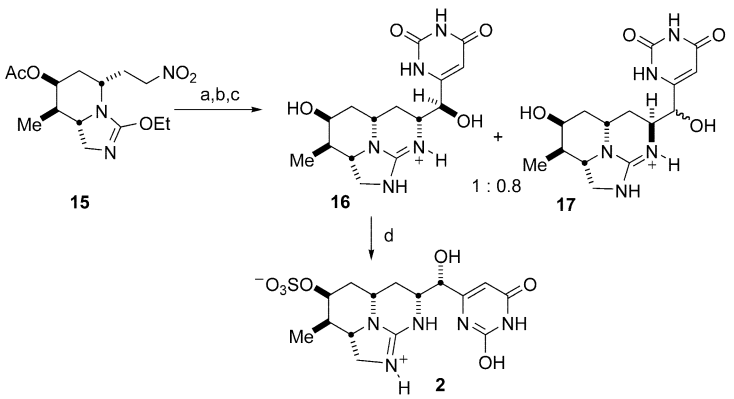
Scheme 2. a) KHMDS, (*E*)-crotyl iodide, THF, −78°C, 92%, >99% *ee*; b) Li, NH_3, THF, EtOH, 68–87%; c) AcCl, MeOH, 0°C→RT; d) $LiAlH_4$, THF, 65% (2 steps); e) $BrCH_2CO_2Ph$, *i*Pr_2NEt, MeCN, 63–80%; f) MCPBA, Na_2HPO_4, CH_2Cl_2, −78°C, 84%; g) PhMe, 200°C (sealed tube), 78%. KHMDS = potassium hexamethyldisilazide, MCPBA = 3-chloroperoxybenzoic acid.

The installation of N16 proved difficult as it necessitated a simultaneous oxidation-state change at C15. It was eventually found that a two-step protocol in which the lactone carbonyl group was first reduced to the corresponding lactol with DIBAL-H fulfilled this requirement (Scheme 3). Tandem reductive amination/N–O bond cleavage gave a free diamine which was more readily handled as the urea **13** after in situ treatment with bis(*p*-nitrophenyl)carbonate.[18] Selective oxidation of the primary alcohol with TEMPO gave the sensitive aldehyde **6** in 75% yield.[19] Most common oxidation protocols showed selectivity for the secondary hydroxy group or led to epimerization of the sensitive α-ureidoaldehyde. Eventually it was found that the addition of methanesulfonic acid (1 mol%) accelerated the reaction to give **6** without attendant epimerization, as evidenced by ^{1}H NMR NOE experiments. The aldehyde **6** underwent nitromethylation upon treatment with the lithium salt of nitromethane in THF. The resulting nitroalcohol was peracetylated with acetic anhydride, and the nitroalkene was then reduced to afford the nitroalkane **14** in 67% yield. The PMB group was removed cleanly in TFA at reflux to give an 80% yield of the urea poised for alkylative activation.[20] Attempts to methylate the oxygen atom of the urea gave an unstable compound that decomposed. Fortunately, *O*-ethylation with triethyloxonium tetrafluoroborate gave the satisfactorily stable isourea **15** (78% yield), which could be subjected to subsequent reactions.[21]



Scheme 3. a) DIBAL-H, CH_2Cl_2, −78°C, 87%; b) $PMBNH_2$, H_2/Pd/C, EtOAc, then $(p\text{-}NO_2PhO)_2CO$, MeCN, 81%; c) TEMPO, $PhI(OAc)_2$, MsOH (1 mol%), $CDCl_3$, 75%; d) $MeNO_2$, *n*BuLi, THF, room temperature, 84%; e) Ac_2O, DMAP, CH_2Cl_2, then $NaBH_4$, EtOH, 67%; f) TFA (neat), reflux, 80%; g) Et_3OBF_4, Cs_2CO_3, CH_2Cl_2, 78%. DIBAL-H = diisobutylaluminum hydride, DMAP = 4-dimethylaminopyridine, MsOH = methanesulfonic acid, TEMPO = 2,2,6,6-tetramethylpiperidine *N*-oxide, TFA = trifluoroacetic acid.

Initial attempts to effect the nitroaldol reaction yielded essentially equimolar amounts of all four C7/C8 diastereomers.[22,23] The treatment of **15** and 2,6-dimethoxypyrimidine-4-carbaldehyde with 2 equivalents of tetra-*n*-butylammonium fluoride for short reaction times, followed by reductive guanidinylation of the nitro group, gave the best selectivities (Scheme 4).[24,25] These conditions afforded a kinetic 1:0.8 mixture in favor of the diastereomer required for the syn-



Scheme 4. a) 2,6-Dimethoxypyrimidine-4-carbaldehyde, TBAF (2.0 equiv), −15 °C, THF; b) $Pd(OH)_2$, H_2, 5% AcOH/MeOH; c) conc. HCl, reflux, 32% for **16**, 29% for **17** (3 steps); d) SO_3·pyridine, DMF, MS (3 Å), 59%. DMF = *N*,*N*-dimethylformamide, TBAF = tetra-*n*-butylammonium fluoride.

thesis of **2**. The other diastereomer in the mixture led to **17**, which is epimeric at C7 with a diastereomer described by Snider and co-workers (relative configuration not assigned).[10] It was found that the nitroaldol reaction must be quenched with acetic acid and the products immediately subjected to reduction conditions. Isolation of the products after treatment with TBAF provided ~1:1:1:1 mixtures of the nitroalcohols, thus indicating that the reaction is indeed highly reversible. At this stage the diastereomeric dimethoxypyrimidines were inseparable. Acidic hydrolysis of the pyrimidines gave a separable mixture of **16** (32% yield from **15**) and **17** (29%).[10,12] In our hands, the sulfonation of the C12 hydroxy group proved capricious under the conditions reported in the literature.[10–12] The use of sulfur trioxide/pyridine complex in DMF with 3-Å molecular sieves gave **2** reproducibly in 59% yield,[26] along with the corresponding bis(sulfate) (2:1 ratio by HPLC), as previously observed by others.[11,12,27]

The asymmetric synthesis of **2** detailed herein represents the shortest successful route toward this family of natural products. 7-Epicylindrospermopsin has thus been obtained in only eighteen steps with few protecting-group manipulations from commercially available **10**. Investigations directed at controlling the diastereoselectivity of the nitroaldol process to afford cylindrospermopsin (**1**) are ongoing and will be reported in due course. The uracil moiety in **1** and **2** has been deemed essential for their hepatotoxicity.[28] We believe that the incorporation of the uracil synthon at a late stage in the synthesis should render our strategy amenable to the production of uracil analogues for evaluation of their biological and hepatotoxic activity.

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[26] Synthetic **2** had spectroscopic properties identical to those reported: $[\alpha]_D^{25} = -12.5$ ($c = 0.04$, H_2O); lit.:[3] $[\alpha]_D^{24} = -20.5°$ ($c = 0.04$, H_2O).
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