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Supporting Information

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Synthesis of the Putative Structure of 7-deoxycylindrospermopsin: C7-oxygenation is not required for the inhibition of protein synthesis.

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Unless otherwise noted, materials were obtained from commercial sources and used without purification. All reactions requiring anhydrous conditions were performed under a positive pressure of argon using flame-dried glassware. 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 (J.C. 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. Alkyllithium reagents were standardized in THF with diphenylacetic acid as the acid and indicator.[1]

^{1}HNMR spectra were recorded on Varian 300, 400, or 500 MHz spectrometers as indicated. The chemical shifts (δ) of proton resonances are reported relative to $CHCl_3$, DMSO-d_5, HOD, or HD_2COD using the following format: chemical shift [multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, app = apparent), coupling constant(s) (*J* in Hz), integral].[2,3] ^{13}CNMR 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.[2]

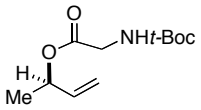
Infrared spectra were recorded on a Nicolet Avatar 320-FT IR spectrometer. All absorptions are reported in cm^{-1} relative to polystyrene (1601 cm^{-1}). Spectra that were recorded 'neat' refer to a thin film of pure liquid on NaCl plates. Spectra were also recorded as films deposited from $CDCl_3$ (dep. $CDCl_3$) or CH_2Cl_2 (dep. CH_2Cl_2) solutions on NaCl plates followed by solvent evaporation. Peaks reported in the IR spectrum are described using the following conventions: w = weak, m = medium, s = strong, vs = very strong, sh = shoulder, and br = broad.

Mass spectra were obtained at the Colorado State University CIF 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.

Experimental details for the preparation of compounds **7-10** can be found at:
http://www.wiley-vch.de/contents/jc_2002/2004/z54208_s.pdf

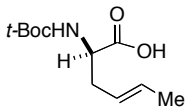
[1] Kofron, W. G.; Baclawski, L. M. *J. Org. Chem.* **1976**, 41, 1879-1880.
[2] Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**; *62*(21); 7512-7515.
[3] Hoye, T.R.; Hansen, P.R.; Vyvyan, J.R. *J. Org. Chem.* **1994**; *59*(15); 4096-4103.

tert-Butoxycarbonylamino-acetic acid 1-methyl-allyl ester.



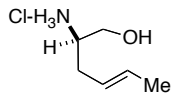
To a solution of 3-buten-2-ol (2.00 g, 27.7 mmol), 4-dimethylamino pyridine (10mol%, 346 mg, 2.77 mmol), and *N*-tert-butoxycarbonyl glycine (5.35 g, 30.5 mmol) in CH_2Cl_2 (50 mL) was added diisopropylcarbodiimide (4.78 mL, 30.5 mmol) in CH_2Cl_2 (10 mL) at 0 ℃. The mixture was stirred for 2h and filtered through Celite with CH_2Cl_2 (100 mL). The combined organics were washed with 10% HCl, sat. $NaHCO_3$, brine, and dried (Na_2SO_4). The concentrated organics were purified by flash chromatography (6:1 Hex: EtOAc) to give the ester as a colourless oil (6.12 g, 96%). ^{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 169.69, 155.84, 137.19, 116.52, 80.07, 72.39, 42.82, 28.53, 20.12. IR (Dep. $CDCl_3$): 3381 (m); 2980 (m); 1751 (s, sh); 1719 (s); 1520 (m); 1368 (m); 1168 (s).HRMS (FAB+): Calc. For $C_{11}H_{20}NO_4$ [M+H]: (*m/*z) 230.1393; Found (*m/*z) 230.1392.

rac-2-tert-Butoxycarbonylamino-hex-4-(E)-enoic acid.



To a solution of the ester (2.72 g, 11.9 mmol) in THF (30 mL) under an argon atmosphere was added a 1M solution of Sodium bis(trimethylsilyl)amide in THF (2.2 eq., 26.1 mL, 26.1 mmol) at 0°C. The mixture was allowed to warm to r.t.. After 2h the reaction was quenched with sat. NH_4Cl (5 mL) and brought to pH = 2 by the addition of 10% HCl. The mixture was extracted with Et_2O (3 x 50 mL), the combined organics were washed with brine and dried (Na_2SO_4). Concentration gave the acid as a light yellow oil (2.69g, 99%). ^{1}HNMR ($CDCl_3$, 300MHz): 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 177.22, 155.66, 130.48, 124.54, 80.45, 52.23, 35.44, 28.55, 18.27. IR (Dep. $CDCl_3$): 3330 (m, br); 2978 (m); 1716 (s, br); 1508 (m); 1165 (s). HRMS (FAB+): Calc. for $C_{11}H_{20}NO_4$ [M+H]: (*m/z)* 230.1392; Found (*m/*z) 230.1393.

2-(E)-aminohex-4-en-1-ol- hydrocloride.

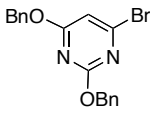


To a solution of the acid (13.5g, 58.9 mmol) was added Et_3N (9.09 mL, 64.7 mmol) in THF (100 mL) and the mixture cooled to 0°C. Ethyl chloroformate (6.16 mL, 64.7 mmol) was then added dropwise over 10 min. After stirring for 20 min the mixture was filtered to remove the Et_3NHCl. This solution was added dropwise to a slurry of $NaBH_4$ (3.34 g, 88.4 mmol) in H_2O (70 mL) over 0.5 h, after the addition another potion of $NaBH_4$ (1.0 g, 26.4 mmol) was added. The mixture was stirred for 3h at rt and quenched by the addition of AcOH. After concentration the mixture was partitioned between Et_2O and H_2O. The organics were further washed 1 x 10% HCl, 1 x sat. $NaHCO_3$, 1 x brine and dried (Na_2SO_4). *[an analytical sample was purified on silica gel eluting with 1:1 hexanes:EtOAc]* Concentration gave a light yellow oil that was added to a mixture of AcCl (6.31 mL, 88.4 mmol) in MeOH (100 mL) that had been stirred for 15 min. The combined solutions were stirred at rt for 8h and concentrated. CH_2Cl_2 (100 mL) was added and the mixture allowed to stand at 0°C until crystallization occurred. The solid was filtered off and washed with Et_2O to give the amine salt **7** as a white solid (4.82 g). The mother liquor was concentrated and diluted with CH_2Cl_2 to afford an additional crop (531 mg, 5.35 g combined, 60%).

***N-Boc-crotylglycinol*:** ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.57 (dq, *J* = 15.6, 6.3 Hz, 1H), 5.42 (dt, *J* = 15.6, 7.1 Hz, 1H), 3.66 (app t, *J* = 9 Hz, 2H), 3.60 (burried m, 1H), 2.27 (ddd, *J* = 13.2, 7.2, 1.2 Hz, 1H), 2.17 (ddd, *J* = 13.2, 6.9, 1.2 Hz, 1H), 1.71 (d, *J* = 6.3 Hz, 3H), 1.48 (s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 151.94, 124.12, 121.98, 75.17, 60.815, 48.06, 30.32, 23.95, 13.58. IR (Dep. $CDCl_3$): 3350 (br, s), 1694 (s), 1520, 1366 (both m), 1172 (s), 1056, 967 (both m). HRMS (FAB+): Calc. For $C_{11}H_{21}NO_3$ [M+H]: (*m/z)* 216.1600; Found (*m/z)* 216.1599.

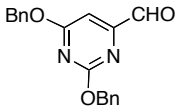
7: ^{1}H NMR (D_2O, 300 MHz): δ 5.73 (dq, *J* = 15, 6.3 Hz, 1H), 5.45 (ddd, *J* = 15, 7.5, 7.5 Hz, 1H), 3.84 (dd, *J* = 12.6, 4.2 Hz, 1H), 3.64 (dd, *J* = 12.6, 7.2 Hz, 1H), 3.38 (dddd, *J* = 7.8, 7.2, 6.6, 4.2 Hz, 1H), 2.40 (ddd, *J* = 15, 7.5, 6.6 Hz, 1H), 2.30 (ddd, *J* = 15, 7.8, 7.5 Hz, 1H), 1.70 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (D_2O, 75 MHz): δ 132.12, 124.22, 61.44, 53.41, 32.65, 17.90. HRMS (FAB+): Calc. for $C_6H_{13}NO$ [M+H]: (*m/z)* 116.1075; Found (*m/z)* 116.1080.

2,4-bis(benzyloxy)-6-bromopyrimidine.



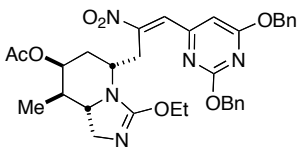
To a solution of benzyl alcohol (0.11 mL, 1.03 mmol) in THF (0.5 mL) under an argon atmosphere at 0 °C was added a 1.6 M solution of *n*BuLi in hexanes (0.62 mL, 0.99 mmol). The mixture was stirred 10 min and DMF (5 mL) added. A solution of the tribromopyrimidine in DMF (1 mL) was added and the mixture stirred at 0 °C for 3h. The reaction was quenched with sat. NH_4Cl and diluted with H_2O (10 mL). The aqueous phase was extracted with Et_2O (3 x 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%). . ^{1}H 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 171.10, 163.77, 152.26, 135.89, 135.60, 128.71, 128.58, 128.53, 128.43, 128.34, 128.25, 105.54, 70.09, 69.14. IR (Dep. $CDCl_3$): 2952 (w), 1549, 1404, 1323 (all s), 1130, 1003 (both m). HRMS (FAB+): Calc. for $C_{18}H_{16}N_2O_2{}^{81}Br_1$ [M+H]: (*m/z)* 373.0375; Found (*m/z)* 373.0363. Calc. for $C_{18}H_{16}N_2O_2Br_1$ [M+H]: (*m/z)* 371.0395; Found (*m/z)* 371.0383.

2,6-bis(benzyloxy)pyrimidine-4-carbaldehyde (12).



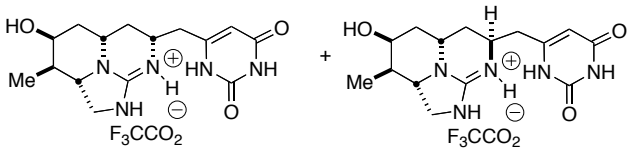
To a solution of the bromopyrimidine (491 mg, 1.32 mmol) in Et_2O (30 mL) under argon at −100°C was added a 1.6 M solution of *n*BuLi in hexanes (1.07 mL, 1.71 mmol). The mixture was stirred for 20 min. and then DMF (0.51 mL, 6.60 mmol) was added. The mixture was allowed to warm to rt over 0.5 h and then refluxed briefly with a heat gun. After cooling to rt 10% HCl (5 mL) was added and the mixture stirred vigorously for 10 min. The mixture was partitioned between 10% HCl and Et_2O and the aqueous phase extracted again with Et_2O. The combined organics were washed with brine and dried (Na_2SO_4). The crude oil was purified on silica gel eluting with 9 : 1 hexanes : EtOAc to give the aldehyde as a clear oil (308 mg, 73%) which solidified upon standing. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.90 (s, 1H), 7.53-7.35 (m, 10H), 6.96 (s, 1H), 5.51 (s, 2H), 5.46 (s, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.99, 172.29, 165.65, 160.56, 136.12, 135.54, 128.73, 128.62, 128.55, 128.34, 128.23, 99.99, 69.94, 69.33. IR (Dep. $CDCl_3$): 2953, 2836 (both w), 1721, 1590, 1566, 1401, 1337 (all s), 1248, 1097 (both m). HRMS (FAB+): Calc. for $C_{19}H_{17}N_2O_3$ [M+H]: (*m/z)* 321.1239; Found (*m/z)* 321.1238.

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 (13).



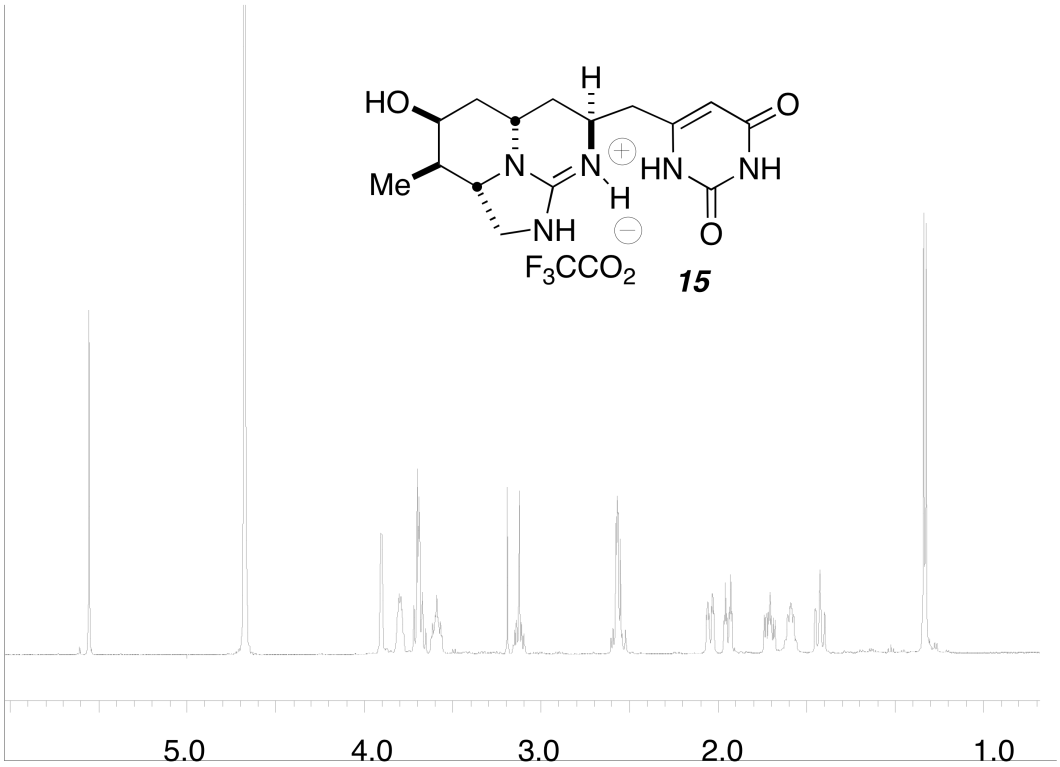
To a solution of the isourea **10** (23 mg, 73 μmol) and the pyrimidine aldehyde (26 mg, 81 μmol, 1.1 eq.) in CH_2Cl_2 (1 mL) under argon was added Ac_2O (34 μL, 0.35 mmol, 5 eq.). CsF (110 mg, 0.73 mmol) was then added as a solid in one portion. The reaction was diluted with MeCN (3 mL) and the mixture stirred for 4h. The reaction was concentratede 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.* ^{1}H 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+): Calc. for $C_{33}H_{38}N_5O_7$ [M+H]: (*m/*z) 616.2771; Found: (*m/*z) 616.2795.

7-Deoxycylindrospermopsin diol



A solution of the nitroalkene **13** (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 0.45μm Acrodisc® and concentrated. The residue was dissolved in conc. HCl and refluxed for 1h and concentrated. Purification of the uracils was achieved by HPLC using a Waters Symmetry® C-18 colum (4.6 x 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 **14** as a white solid (3.7 mg, 38%, t_R = 22.1 min) preceded by the C8 diastereomer **15** also obtained as a white crystalline solid (4 mg, 38 %, t_R = 12.6 min). A small sample of **15** (~1 mg) was recrystallized from methanol (layered with pentane) to give *X*-ray quality crystals.

15 (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+): Calc. for $C_{15}H_{22}N_5O_3$ [M+H]: (*m/*z) 320.1723; Found: (*m/*z) 320.1723. **14** (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, 1H), 1.50 (app q, *J* = 11 Hz, 2H), 0.95 (d, *J* = 6.5 Hz, 3H). HRMS (FAB+): Calc. for $C_{15}H_{22}N_5O_3$ [M+H]: (*m/z)* 320.1723; Found: 320.1712.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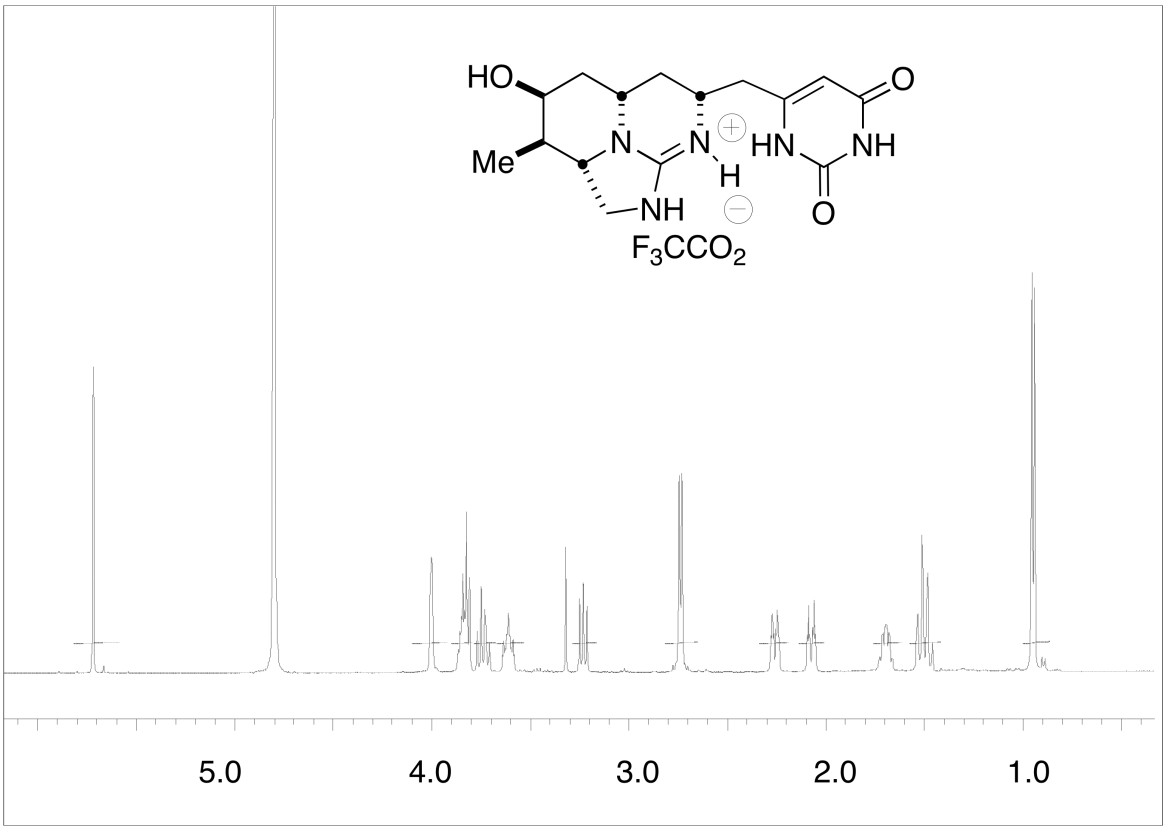
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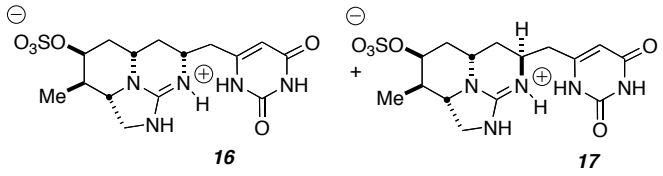
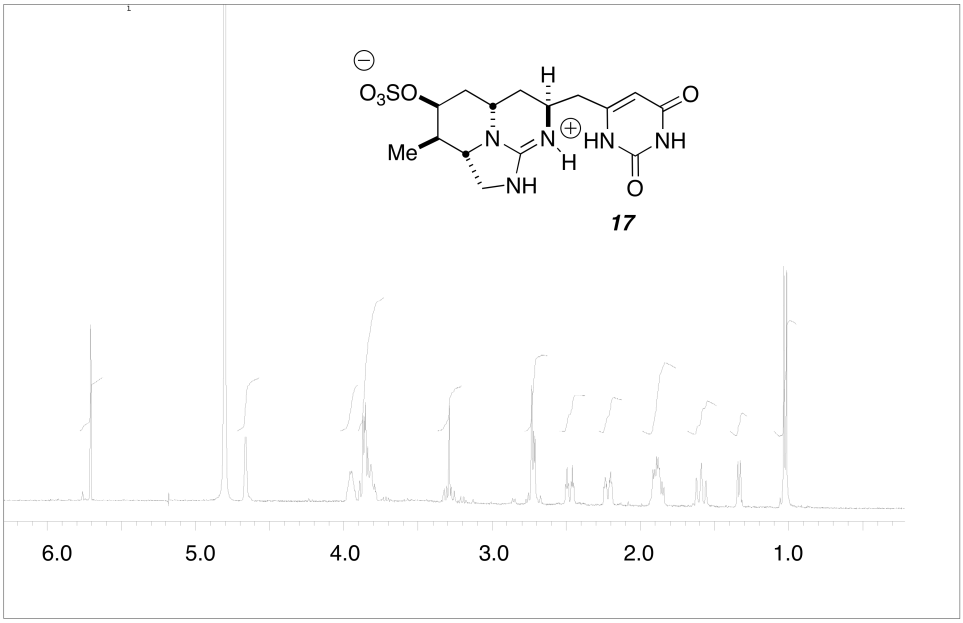
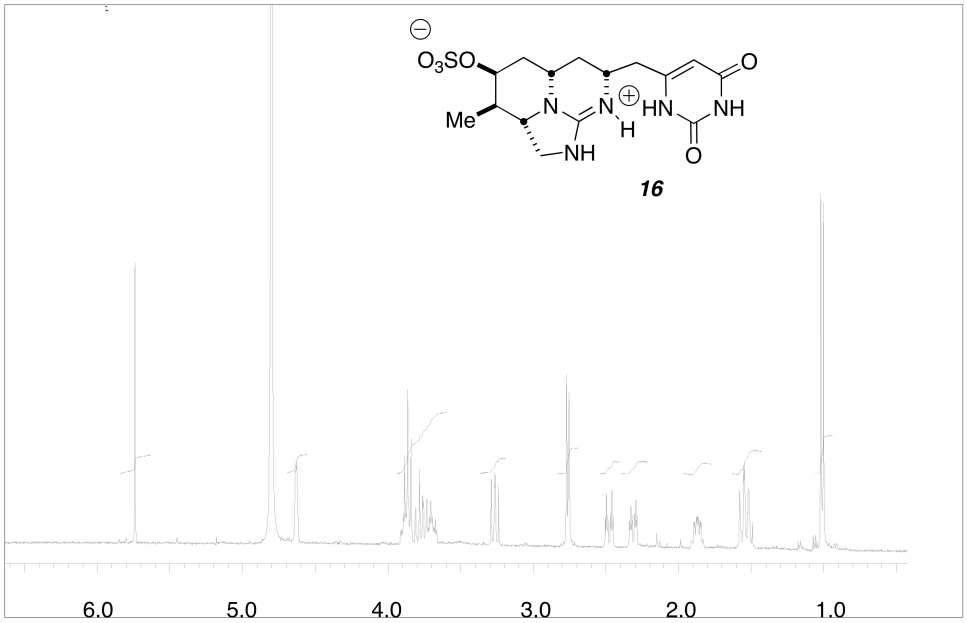
7-deoxycylindrospermopsin.



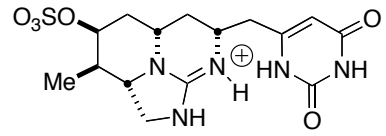
Alternatively a mixture of the C12-hydroxy uracils (3.2 mg, 7.9 μmol) can be directly sulfonated by treatment with SO_3·pyr (19 mg, 120 μmol) in DMF (300 μL). Purification of the uracils after concentration was achieved by HPLC using a Waters Symmetry® C-18 colum (4.6 x 250 mm) eluting with 8% MeOH / H_2O with 1% TFA at 1.5 mL/min, monitoring at 263 nm to give 7-deoxy-cylindrospermopsin **16** as a white solid (1 mg, 33%, t_R = 8.25 min) preceded by the C8 diastereomer **17** also obtained as a white crystalline solid (1 mg, 33 %, t_R = 4.91 min). **16**: ^{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).



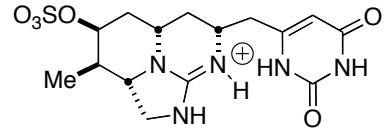


Comparison of synthetic and natural 7-deoxycylindrospermopsin. HPLC: Agilent ZORBAX® C-18 column (4.6 x 150 mm) eluting with 4% (MeOH w/ 0.1% TFA) / H_2O at 2.0 mL/min, monitoring at 262 nm.

Synthetic



Natural



Synthetic + Natural