The C2'–OH of Amphotericin B Plays an Important Role in Binding the Primary Sterol of Human But Not Yeast Cells

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Supporting Information

I. General Methods

Materials.

Commercially available materials were purchased from Sigma-Aldrich, Alfa Aesar, Strem, Avanti Polar Lipids, Fisher Scientific or Julich, and were used without further purification unless stated otherwise. Amphotericin B was a generous gift from Bristol-Myers Squibb Company. Camphorsulfonic acid was recrystallized from the ethyl acetate prior to use. All solvents were dispensed from a solvent purification system that passes solvents through packed columns according to the method of Pangborn and coworkers[1] (THF, Et_2O, CH_2Cl_2, toluene, dioxane, hexanes : dry neutral alumina; DMSO, DMF, CH_3OH : activated molecular sieves). 2,6-Lutidine and pyridine were freshly distilled under nitrogen from CaH_2. Vinyl acetate was freshly distilled under nitrogen from $CaCl_2$. EtOAc and EtOH were freshly distilled under nitrogen from activated molecular sieves. Water was doubly distilled or obtained from a Millipore MilliQ water purification system.

Reactions.

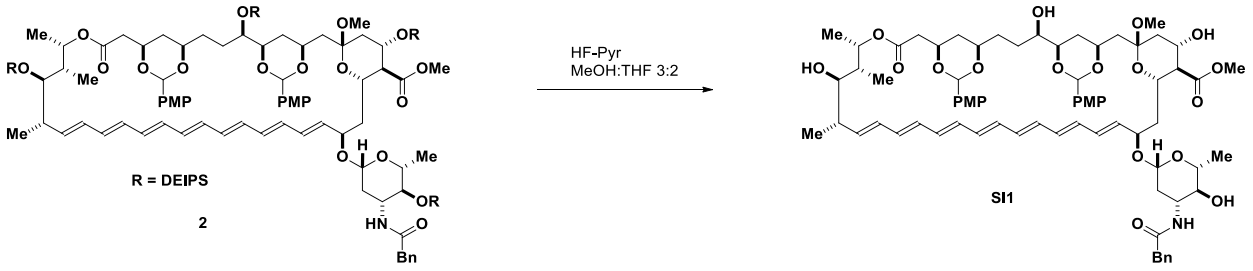
Due to the light and air sensitivity of polyenes, all manipulations of polyenes were carried out under low light conditions and compounds were stored under an argon atmosphere. All reactions were performed in oven- or flame-dried glassware under an atmosphere of argon unless otherwise indicated. Reactions were monitored by analytical thin layer chromatography performed using the indicated solvent on E. Merck silica gel 60 F_{254} plates (0.25mm). Compounds were visualized using a UV (λ_{254}) lamp or stained by a solution of *p*-anisaldehyde, $KMnO_4$, or ceric ammonium molybdate (CAM) stain. Alternatively, reactions were monitored by RP-HPLC using an Agilent 1100 series HPLC system equipped with a Symmetry® C_{18} 5 micron

4.6 x 150 mm column (Waters Corp. Milford, MA) with UV detection at 383 nm and the indicated eluent and flow rate of 1 mL/min.

Purification and Analysis.

Flash chromatography was performed as described by Still and coworkers[2] using the indicated solvent on E. Merck silica gel 60 230-400 mesh. ^{1}H NMR spectra were recorded at 23 °C on one of the following instruments: Varian Unity 400, Varian Unity 500, Varian Unity Inova 500NB. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane and referenced internally to the residual protium in the NMR solvent ($CHCl_3$, δ = 7.26, centerline; $CD_3C(O)CHD_2$, δ = 2.04, center line; $CD_3S(O)CHD_2$, δ = 2.50, center line) or to added tetramethylsilane. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd = doublet of doublets, ddd = doublet of doublet of doublets, td = triplet of doublets, m = multiplet, b = broad, app. = apparent), coupling constant (*J*) in Hertz (Hz) and integration. ^{13}C spectra were recorded at 23 °C with a Varian Unity 500. Chemical shifts (δ) are reported downfield of tetramethylsilane and are referenced to the carbon resonances in the NMR solvent ($CDCl_3$, δ = 77.0, center line; $CD_3C(O)CD_3$, δ = 29.8, center line; $CD_3S(O)CD_3$, δ = 39.5, center line) or to added tetramethylsilane. High resolution mass spectra (HRMS) were obtained at the University of Illinois mass spectrometry facility. All synthesized compounds gave HRMS within 5 ppm of calculated values.

II. Synthesis of New Compounds

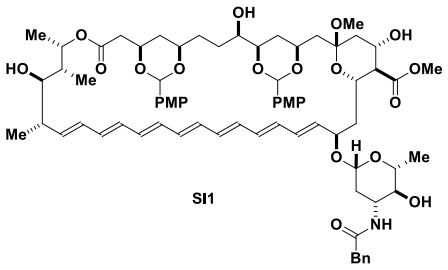


Tetraol **SI1**

2 (200 mg, 111 μmol, 1 eq) was placed in a teflon vial and azeotropically dried with toluene and placed under vacuum overnight. The vial was backfilled with argon and THF (1.5 mL) was added. A separate teflon vial was oven dried at 100 °C overnight, cooled under vacuum to room temperature, and backfilled with argon. MeOH (2.5 mL) was added to the vial. Both vials were cooled to 0 °C. HF-pyridine (425 μL) was slowly added to the vial containing the MeOH, and the vial was allowed to mix at 0 °C for 10 min. The HF-pyr-MeOH solution (2.5 mL) was added dropwise to the solution of **2** at 0 °C. The reaction was allowed to warm to room temperature and stirred for 6 hrs. The reaction was cooled to 0 °C and quenched with a stirring mixture of EtOAc and saturated sodium bicarbonate that had been cooled to 0 °C. EtOAc was used to extract the aqueous layer. The organic layer was then washed with water twice. A final

wash of saturated sodium chloride was performed, and the organic layers were dried over sodium sulfate and filtered. The solvent was removed under reduced pressure and column chromatography (SiO_2; DCM:MeOH 49:1 → 19:1) purification yielded **SI1** as a yellow-orange solid (54.4 mg, 42.2 μmol, 38 %). The product was quickly characterized by HRMS and directly taken on to the next step to minimize decomposition from residual acid left from the column chromatography.

This reaction is sensitive to water.



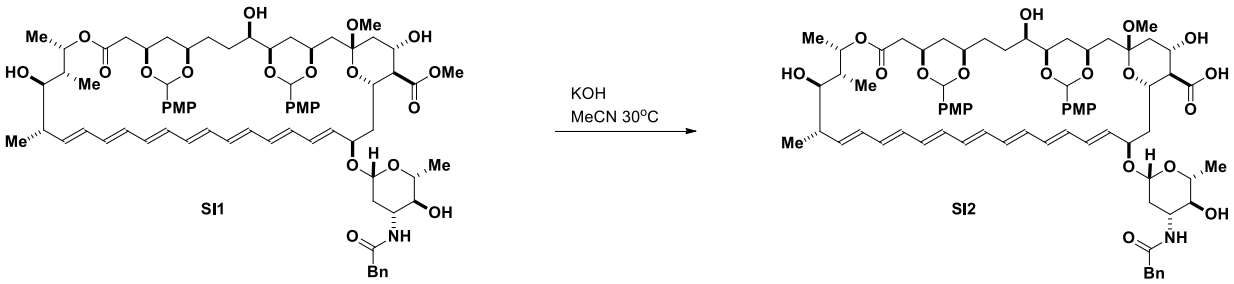
SI1

TLC (DCM:MeOH 9:1)

R_f = 0.48, stained by CAM

HRMS (ESI)

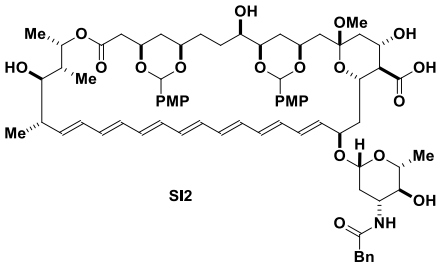
Calculated for $C_{73}H_{96}NO_{19}(M + H)^+$:	1290.6577
Found:	1290.6561



SI1 → SI2

C41-carboxylic acid **SI2**

SI1 (10 mg, 7.75 μmol, 1 eq) was suspended in MeCN:H_2O 3:1 (400 μL). KOH (4.3 mg, 77.5 μmol, 10 eq) was ground into a fine powder and added slowly while stirring. The reaction was heated to 30 °C for 6 hrs. The reaction was cooled to 0 °C and quenched with NH_4Cl (4.1 mg, 77.5 μmol, 10 eq). The solution was concentrated under reduced pressure and purification by reverse-phase silica gel column chromatography (C_{18}; H_2O:MeCN 9:1 → 1:4) yielded **SI2** as a yellow-orange solid (3.2 mg, 2.48 μmol, 32 %).



SI2

TLC (H_2O:MeCN 3:7)

R_f = 0.28, stained by CAM

^{1}HNMR (500 MHz, pyridine *d-5*:CD_3OD 1:1)

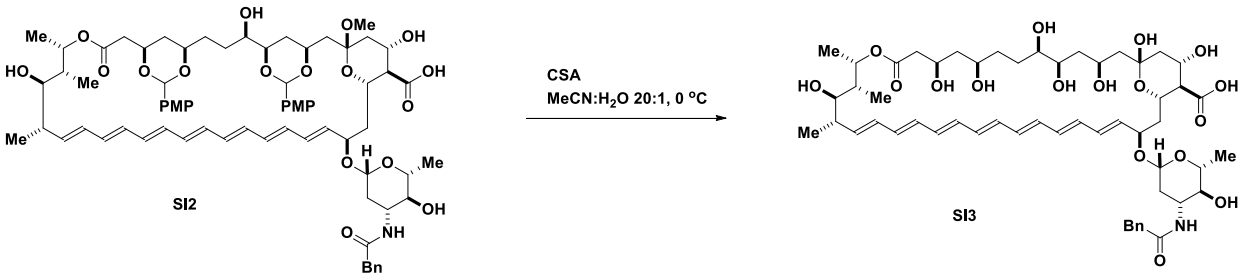
δ 7.56 (m, 6H), 7.42 (m, 2H), 7.33 (m, 2H), 6.95 (m, 2H), 6.86 (m, 2H), 6.63-6.25 (m, 11H), 6.14 (m, 1H), 5.85 (m, 1H), 5.73 (m, 1H), 5.64 (s, 1H), 5.61 (s, 1H), 5.19 (app d, *J* = 4 Hz, 1H), 5.13 (m, 1H), 4.71 (m, 1H), 4.65 (m, 1H), 4.53 (m, 1H), 4.35 (m, 1H), 4.12 (m, 2H), 4.07 (m, 1H), 3.84 (m, 2H), 3.76-3.69 (m, 8H), 3.62 (m, 1H), 3.42 (m, 1H), 3.24 (m, 1H), 3.17 (s, 3H), 2.89 (m, 2H), 2.78-2.55 (m, 5H), 2.29-1.99 (m, 7H), 1.92-1.65 (m, 6H), 1.49 (m, 1H), 1.44 (d, *J* = 6.5 Hz, 3H), 1.28 (d, *J* = 6.5 Hz, 3H), 1.11 (d, *J* = 7 Hz, 3H), 1.04 (d, *J* = 7 Hz, 3H)

^{13}CNMR (125 MHz, pyridine *d-5*:CD_3OD 1:1)

δ 172.2, 170.2, 159.9, 159.7, 138.5, 137.9, 133.8, 133.6, 133.5, 133.4, 133.3, 133.2, 132.6, 131.8, 131.7, 129.9, 129.0, 128.6, 127.7, 127.5, 126.7, 124.5, 121.2, 117.9, 114.5, 113.2, 113.1, 102.0, 100.09, 100.3, 80.9, 79.7, 77.4, 76.8, 73.3, 72.8, 72.7, 68.0, 66.8, 66.2, 54.6, 42.9, 42.4, 40.8, 37.1, 34.0, 33.9, 30.0, 29.5, 18.2, 16.4, 13.6, 11.3, 8.7.

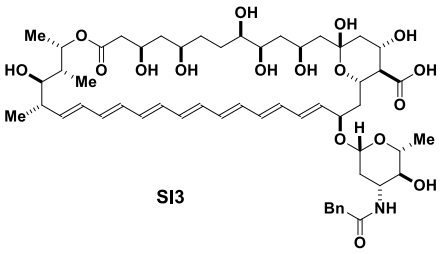
HRMS (ESI)

Calculated for $C_{72}H_{93}NO_{19}(M + Na)^+$:	1298.6240
Found:	1298.6249



Nonol **SI3**

SI2 (2 mg, 1.56 μmol, 1 eq) was suspended in MeCN:H_2O 20:1 (790 μL). The reaction was cooled to 0 °C, and recrystallized camphorsulfonic acid (28 mg, 119 μmol, 150 eq) was added. The reaction was stirred for 3 hrs. at 0 °C. The reaction was quenched with Et_3N and concentrated under reduced pressure. Purification by reverse-phase HPLC (C_{18}; 16 mM NH_4OAc in H_2O:MeCN 19:1 for 2 min. followed by 16 mM NH_4OAc in H_2O:MeCN 19:1 → 31:69 over 24 min.) yielded a mixture of product and partially-deprotected intermediates. These were resubmitted to the reaction conditions and purification again yielding **SI3** as a yellow-orange solid (241 μg, 0.235 μmol, 15 %).



HPLC

tR = 19.5 min; flow rate = 25 mL/min, gradient = 16 mM NH_4OAc in H_2O:MeCN 19:1 for 2 min. followed by 16 mM NH_4OAc in H_2O:MeCN 19:1 → 31:69 over 24 min.

^{1}HNMR (500 MHz, pyridine *d-5*:CD_3OD 1:1)

δ 7.50 (m, 2H), 7.37 (m, 2H), 7.28 (m, 1H), 6.52 (m, 1H), 6.45-6.30 (m, 12H), 5.58 (m, 1H), 5.50 (m, 1H), 5.26 (app d, *J* = 3.5 Hz, 1H), 4.70 (m, 1H), 4.49 (m, 3H), 4.27 (m, 1H), 4.15 (m, 1H), 3.91 (m, 2H), 3.76 (m, 2H), 3.62 (m, 1H), 3.55 (m, 1H), 3.48 (m, 1H), 3.42 (m, 1H), 3.19 (m, 1H), 2.82 (m, 2H), 2.59-2.39 (m, 3H), 2.26 (m, 1H), 2.06-1.65 (m, 13H), 1.58 (m, 1H), 1.38 (d, *J* = 6.0 Hz, 3H), 1.36 (d, *J* = 6.0 Hz, 3H), 1.25 (d, *J* = 6.5 Hz, 3H), 1.18 (d, *J* = 7.5 Hz, 3H)

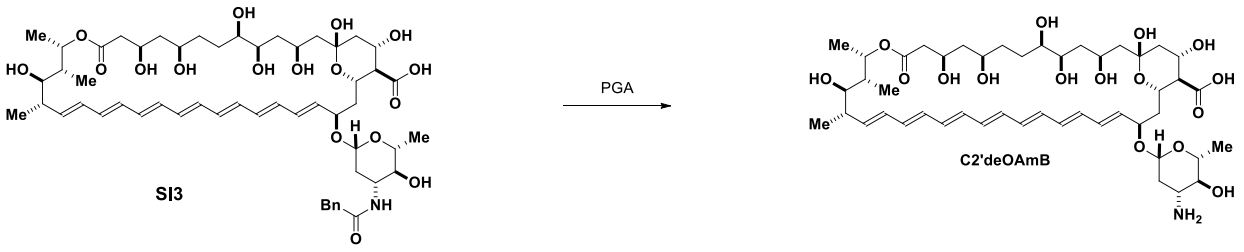
^{13}CNMR (125 MHz, pyridine *d-5*:CD_3OD 1:1)

δ 176.7, 173.3, 172.3, 135.1, 134.9, 134.4, 134.1, 133.7, 133.5, 133.4, 133.3, 133.1, 132.8, 130.1, 129.6, 127.8, 103.6, 100.0, 74.5, 74.1, 72.1, 71.2, 70.9, 69.3, 68.9, 66.9, 65.4, 45.5, 45.1, 44.7, 44.1, 43.9, 41.1, 40.7, 36.5, 34.8, 32.8, 30.5, 23.5, 19.4, 19.1, 18.8, 17.9, 14.6, 13.1.

HRMS (ESI)

Calculated for $C_{55}H_{78}NO_{16}(M\text{-}H_2O + H)^+$: 1008.5321
Found: 1008.5327

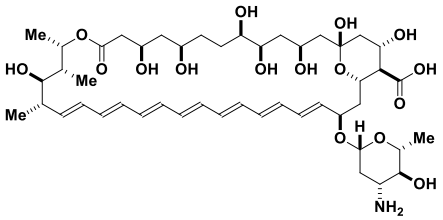
Compound **SI3** dehydrated under ESI conditions, gradient COSY NMR was performed to confirm the dehydration occurred in the process of obtaining the mass and is not present in **SI3.**



C2'deOAmB

SI3 (200 μg, 196 μmol, 1 eq) was suspended in an aqueous solution of penicillin G amidase (250 μL) prepared as described below. The reaction was heated to 37 °C for 48 hrs.

DMSO was added to dissolve the AmB derived compounds. Any precipitated protein was pelleted out by centrifugation, and the DMSO solution was purified by reverse-phase HPLC (C_{18}; 16 mM NH_4OAc in H_2O:MeCN 19:1 for 2 min. followed by 16 mM NH_4OAc in H_2O:MeCN 19:1 → 31:69 over 24 min.) yielding the desired product and undesired side products as an inseparable mixture. C2'deOAmB was characterized by HRMS.

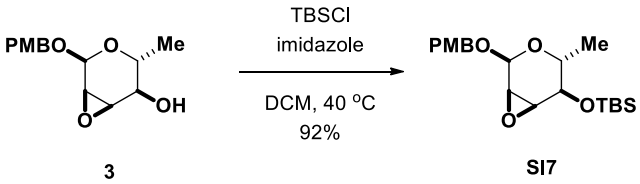


HRMS (ESI)

Calculated for $C_{47}H_{74}NO_{16}(M + H)^+$:	908.5008
Found:	908.5004
Calculated for $C_{47}H_{73}NO_{16}(M + Na)^+$:	930.4827
Found:	930.4822

Preparation of the Penicillin G Amidase (PGA) solution

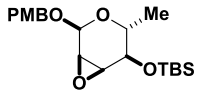
Crude PGA solution was obtained from CLEA technologies (Delft, The Netherlands). Saturated ammonium sulfate (1.67 mL) was added to the PGA solution (2.5 mL) to make a 40% solution. The precipitate was pelleted out by centrifugation, and the liquid was decanted. Additional saturated ammonium sulfate (4.2 mL) was added to the liquid and the precipitate was pelleted out by centrifugation. The liquid was decanted, and the pellet was resuspended in 3 mL buffer (1.1 M $(NH_4)_2SO_4$, 50 mM TRIS buffer pH 7.5). The PGA suspension was loaded onto a column packed with phenylsepharose resin. A gradient of 1.1 M $(NH_4)_2SO_4$ to 0.1 M $(NH_4)_2SO_4$ in 50 mM TRIS buffer pH 7.5 was run followed by water. Fractions were analyzed by SDS-page electrophoresis, and the pure PGA containing fractions were concentrated back to 2.5 mL.



3 SI7

Epoxide **SI7**

To a 1 L round bottom flask containing **3** and a stir bar under argon was added DCM (325 mL) and DMF (65 mL) and the resulting mixture was stirred to homogeneity. To the mixture was then added sequentially imidazole (31.0 g, 491 mmol) and TBSCl (51.4 g, 341 mmol). The flask was fitted with a reflux condenser and, under argon maintenance, was stirred at 40 ^{o}C for 16 h. The reaction was cooled to room temperature and diluted with Et_2O (1 L) and sat'd aq $NaHCO_3$ (1 L). The aqueous layer was separated and extracted with Et_2O (500 mL). The combined organic fractions were washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo to give a white solid. The product was purified by dry column vacuum chromatography (DCVC)[3] (Hex:EtOAc, 20:1 → 3:1) to afford **SI7** as a white solid (40.0 g 92%).



SI7

TLC (Ether:Hexane 4:1)

R_f = 0.35, stained by CAM

^{1}HNMR (500 MHz, $CDCl_3$)

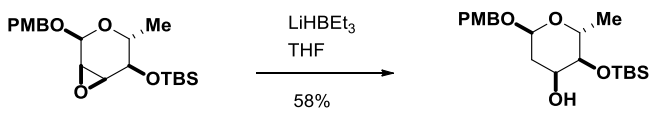
δ 7.30 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 9 Hz, 2H), 4.92 (d, *J* = 3 Hz, 1H), 4.70 (d, *J* = 12 Hz, 1H), 4.54 (d, *J* = 12 Hz, 1H), 3.85 (m, 1H), 3.81 (s, 3H), 3.61 (dd, J = 8.5, 1.5 Hz, 1H), 3.41 (app t, *J* = 4 Hz, 1J) 3.25 (dd, J = 4, 1 Hz, 1H), 1.15 (d, *J* = 6 Hz, 3H), 0.911 (s, *9*H), 0.14 (s, 3H), 0.110 (s, 3H).

^{13}CNMR (125 MHz, $CDCl_3$)

δ 159.4, 129.9, 114.0, 92.0, 72.4, 68.9, 64.9, 55.4, 55.3, 54.1, 25.9, 18.1, 17.7, -3.9, -4.6.

HRMS (ESI)

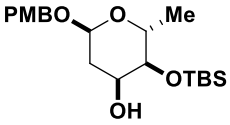
Calculated for $C_{20}H_{32}O_5Si$ $(M + Na)^+$: 403.1917
Found: 403.1918



SI7 **4**

Alcohol **4**

Epoxide intermediate **SI7** (8 g, 21 mmol, 1 eq) was dissolved in THF (263 mL). The resulting solution was cooled to 0 ^{o}C, and $LiHBEt_3$ (1M in THF) (105 mL, 105 mmol, 5eq) was added slowly. The reaction heated to 60 ^{o}C for 2.5 hrs. The reaction was cooled to 0 ^{o}C and quenched with 1M ammonium chloride. The mixture was extracted with ether. The organic layer was washed with water and saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and column chromatography (SiO_2; Ether:Hexane 1:4 → 1:3) purification yielded **4** as an oil containing 85:15 mixture of desired to undesired isomers (5.47 g, 12.1 mmol **4**, 58% + 10% regioisomer which was easily separated in the following step).



4

TLC (Ether:Hexane 3:7)

R_f = 0.38, stained by CAM

^{1}HNMR (500 MHz, $CDCl_3$)

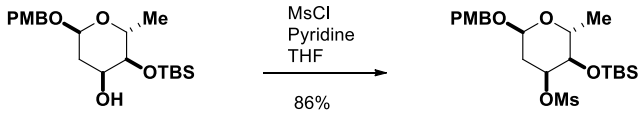
δ 7.27 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 9 Hz, 2H), 4.87 (d, *J* = 4 Hz, 1H), 4.66 (d, *J* = 12 Hz, 1H), 4.44 (d, *J* = 11.5 Hz, 1H), 4.04 (m, 1H), 3.92 (m, 1H), 3.81 (s, 3H), 3.32 (dd, *J* = 3 Hz, *J* = 9.5 Hz, 1H), 3.19 (m, 1H), 2.14 (dd, *J* = 3.5 Hz, *J* = 15 Hz, 1H), 1.89 (td, *J* = 3.5 Hz, *J* = 14.5 Hz, 1H), 1.26 (d, *J* = 6.5 Hz, 3H), 0.93 (s, 9H), 0.12 (s, 6H).

^{13}CNMR (125 MHz, $CDCl_3$)

δ 159.5, 129.9, 114.0, 95.6, 75.1, 68.9, 68.0, 63.7, 55.5, 35.7, 26.1, 18.4, -4.0, -4.4.

HRMS (ESI)

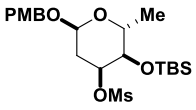
Calculated for $C_{20}H_{34}O_5Si$ $(M + Na)^+$: 405.2073
Found: 405.2078



4 **SI8**

Mesylate **SI8**

A 85:15 mixture of intermediate **4** to undesired isomers (4.83 g, 10.7 mmol **4** + 1.9 mmol undesired isomers, 1 eq) was dissolved in THF (15 mL). Pyridine (10.2 mL, 126 mmol, 10 eq) and MsCl (3.17 mL, 41 mmol, 3.25 eq) were added at 0 ^oC. The reaction was stirred overnight allowing to warm to 23 ^oC. The reaction was then quenched with saturated aqueous sodium bicarbonate and extracted with ether. The organic layer was washed with 1M ammonium chloride, water, and saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and column chromatography (SiO_2; Ether:Hexane 2:3) purification yielded **SI8** as a solid (4.24 g, 9.2 mmol, 86 %).



SI8

TLC (Ether:Hexane 2:3)

R_f = 0.27, stained by CAM

^{1}HNMR (500 MHz, $CDCl_3$)

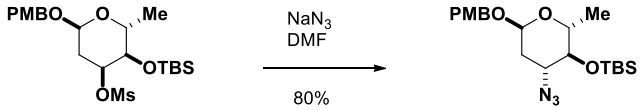
δ 7.27 (d, *J* = 9 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 4.90 (dd, *J* = 3 Hz, *J* = 8 Hz, 2H), 4.66 (d, *J* = 11.5 Hz, 1H), 4.35 (d, *J* = 11 Hz, 1H), 4.13 (m, 1H), 3.80 (s, 3H), 3.43 (dd, *J* = 3 Hz, *J* = 9 Hz, 1H), 2.91 (s, 3H), 2.41 (dd, *J* = 3 Hz, *J* = 15 Hz, 1H), 1.96 (m, 1H), 1.23 (d, *J* = 6.5 Hz, 3H), 0.93 (s, 9H), 0.14 (s, 3H), 0.11 (s, 3H).

^{13}CNMR (125 MHz, $CDCl_3$)

δ 159.4, 130.2, 129.6, 113.9, 94.8, 77.5, 72.7, 69.1, 64.3, 55.5, 39.9, 34.5, 26.0, 18.3, -3.9, -4.6.

HRMS (ESI)

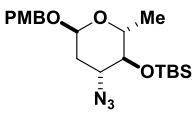
Calculated for $C_{21}H_{36}O_5SiS$ $(M + Na)^+$:	483.1849
Found:	483.1848



SI8 **SI9**

Azide **SI9**

Intermediate **SI8** (1.6 g, 3.47 mmol, 1 eq) was dissolved in DMF (15 mL). Sodium azide (1.6 g, 24.3 mmol, 7 eq) was added. The reaction heated to 160 °C for 1.5 hrs. The reaction was cooled to room temperature. The reaction was quenched with saturated aqueous sodium bicarbonate and extracted with ether. The organic layer was washed with water, and saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and column chromatography (SiO_2; Ether:Hexane 1:19) purification yielded **SI9** as a solid (1.13 g, 2.78 mmol, 80 %).



SI9

TLC (Ether:Hexane 1:19)

R_f = 0.30, stained by CAM

^{1}HNMR (500 MHz, $CDCl_3$)

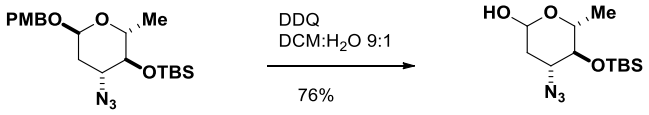
δ 7.29 (d, *J* = 9 Hz, 2H), 6.91 (d, *J* = 8.5 Hz, 2H), 4.91 (d, *J* = 3 Hz, 2H), 4.61 (d, *J* = 11.5 Hz, 1H), 4.39 (d, *J* = 11 Hz, 1H), 3.82 (s, 3H), 3.71 (m, 2H), 3.10 (t, *J* = 9 Hz, 1H), 2.19 (dd, *J* = 5 Hz, *J* = 13.5 Hz, 1H), 1.73 (td, *J* = 4 Hz, *J* = 12.5 Hz, 1H), 1.27 (d, *J* = 6 Hz, 3H), 0.94 (s, 9H), 0.22 (s, 3H), 0.13 (s, 3H).

^{13}CNMR (125 MHz, $CDCl_3$)

δ 159.6, 129.9, 129.8, 114.1, 95.4, 76.7, 68.9, 68.8, 61.8, 55.5, 35.9, 26.2, 18.7, -3.9, -4.0.

HRMS (ESI)

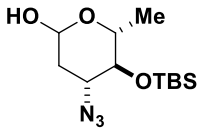
Calculated for $C_{20}H_{33}N_3O_4Si$ $(M + Na)^+$:	430.2138
Found:	430.2156



SI9 **5**

Alcohol **5**

Intermediate **SI9** (6.5 g, 15.9 mmol, 1 eq) was dissolved in DCM:H_2O 9:1 (160 mL). The solution was cooled to 0 °C, and DDQ (4.3 g, 19.1 mmol, 1.2 eq) was added. The reaction was warmed to room temperature and stirred for 2 hrs. The reaction was quenched with saturated aqueous sodium bicarbonate and extracted with ether. The organic layer was washed with water, and saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and column chromatography (SiO_2; Ether:Hexane 1:19) purification followed by (C18 SiO_2, water:MeCN 1:4) yielded **5** as a solid consisting of a 2:1 mixture of anomers (3.47 g, 12.1 mmol, 76 %).



5

TLC (EtOAc:Hexane 1:4)

R_f = 0.31, stained by CAM

(H_2O:MeCN 1:4)

R_f = 0.50, stained by CAM

^{1}HNMR (500 MHz, $CD_3C(O)CD_3$)

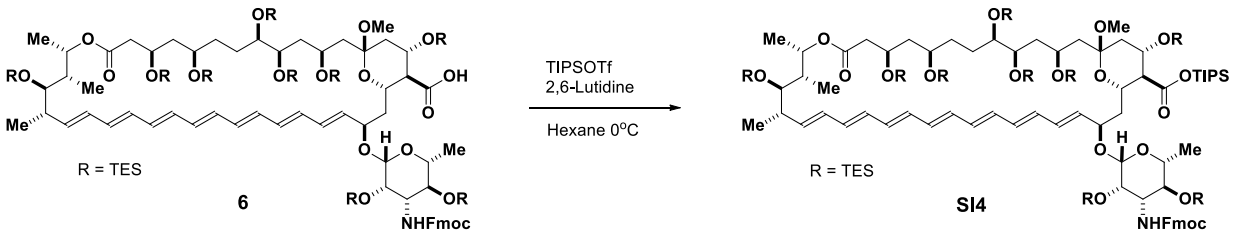
δ 5.62 (d, *J* = 6.5 Hz, 1H), 5.33 (m, 2H), 5.22 (m, 2H), 4.84 (m, 1H), 3.84 (m, 2H), 3.67 (m, 2H), 3.47 (m, 1H), 3.29 (m, 1H), 3.07 (m, 3H), 2.25 (dd, *J* = 12.5 Hz, *J* = 2 Hz, 1H), 2.11 (dd, *J* = 13 Hz, *J* = 1 Hz, 2H), 1.65 (td, *J* = 3 Hz, *J* = 12.5 Hz, 2H), 1.50 (m, 1H), 1.19 (d, *J* = 6.5 Hz, 3H), 1.14 (d, *J* = 6.5 Hz, 6H), 0.90 (m, 28H), 0.19 (m, 9H), 0.12 (s, 9H).

^{13}CNMR (125 MHz, $CD_3C(O)CD_3$)

δ 94.31, 90.96, 77.559, 76.90, 73.53, 68.49, 64.64, 62.38, 38.86, 36.80, 26.27, 18.99, 18.96, 18.64, -4.04, -4.09.

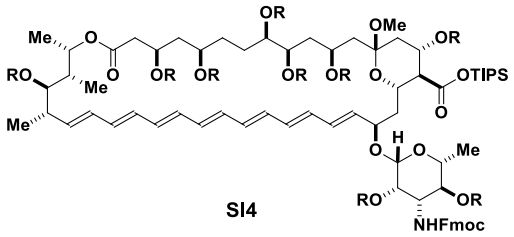
HRMS (ESI)

Calculated for $C_{12}H_{25}N_3O_3Si$ $(M + Na)^+$:	310.1563
Found:	310.1566



TIPS ester **SI4**

Intermediate **6** (15.8 g, 7.20 mmol, 1 eq) was azeotropically dried with toluene and placed under vacuum overnight. Hexane (240 mL) and 2,6-lutidine (2.9 mL, 25.2 mmol, 3.5eq) were added. The resulting solution was cooled to 0 ^{o}C and triisopropylsilyl triflate (2.9 mL, 10.8 mmol, 1.5eq) was added slowly over 15 min. The reaction was quenched after 1 hr with saturated aqueous sodium bicarbonate and extracted with ether. The organic layer was washed with copper sulfate, water, and finally saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and column chromatography (SiO_2; Ether:Hexane 5:95 → 1:4) purification yielded the **SI4** as a yellow-orange solid (15.2 g, 6.5 mmol, 90 %).



SI4

TLC (Ether:Hexane 0.1% Et_3N 3:7)

R_f = 0.72, stained by CAM

^{1}HNMR (500 MHz, $CD_3C(O)CD_3$)

δ 7.86 (d, *J* = 7.5 Hz, 2H), 7.69 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 6.53-6.05 (m, 12H), 5.51 (m, 1H), 5.34 (m, 1H), 4.65 (m, 2H), 4.47 (m, 3H), 4.34 (m, 2H), 4.24 (m, 2H), 4.13 (m, 1H), 3.98 (m, 2H), 3.90 (m, 1H), 3.83 (m, 1H), 3.66 (m, 2H), 3.45 (m, 1H), 3.27 (m, 1H), 3.15 (s, 3H), 2.56 (m, 1H), 2.42 (m, 2H), 2.10-2.01 (m, 3H), 1.94-1.59 (m, 12H), 1.50 (m, 1H), 1.38-1.30 (m, 4H), 1.23 (m, 4H), 1.16 (m, 20H), 1.07-0.89 (m, 85H), 0.78-0.55 (m, 56H).

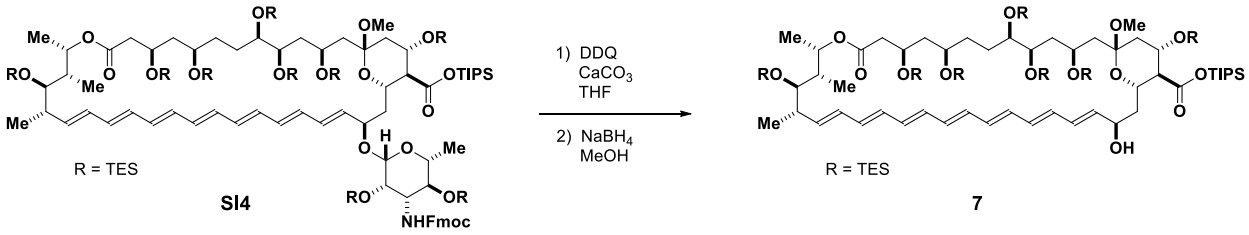
^{13}CNMR (125 MHz, $CD_3C(O)CD_3$)

δ 172.8, 170.9, 156.6, 145.5, 145.4, 142.6, 139.5, 136.1, 135.7, 135.6, 135.1, 134.2, 133.3, 133.2, 133.1, 132.9, 131.8, 131.1, 131.0, 128.8, 128.2, 128.2, 126.2, 126.1, 121.2, 101.6, 99.9, 77.1, 75.0, 74.4, 74.3, 73.6, 71.5, 69.8, 68.5, 67.9, 67.8, 67.6, 59.5, 58.6, 48.6, 48.4, 48.2, 44.7, 43.8, 42.5, 41.6, 37.6, 36.1, 27.9, 20.3, 19.6, 19.4, 18.8, 18.8, 13.3, 11.7, 8.0, 8.0,

7.9, 7.9, 7.8, 7.8, 7.8, 7.8, 7.8, 7.7, 7.70, 7.7, 7.7, 6.8, 6.6, 6.6, 6.5, 6.4, 6.4, 6.3, 6.3, 6.2, 6.2, 6.2, 6.1.

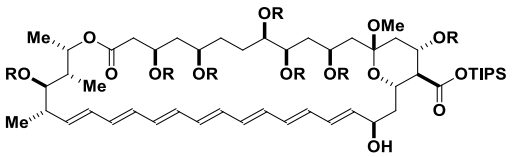
MS (ESI)

Calculated for $C_{126}H_{231}NO_{19}Si_{10}$ $(M + Na)^+$:	2365.5
Found:	2365.1



Allylic alcohol **7**

Intermediate **SI4** (12.5 g, 5.35 mmol, 1 eq) was azeotropically dried with toluene and placed under vacuum overnight. THF (100 mL) was added. The resulting solution was cooled to 0 ^oC, and DDQ (1.82 g, 8.03 mmol, 1.5eq) and $CaCO_3$ (5.3 g, 53.5 mmol, 10 eq) were added. The reaction was warmed to room temperature and quenched after 30 min with saturated aqueous sodium bicarbonate and extracted with ether. The organic layer was washed with water and then saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and purification by flash column chromatography (SiO_2; Ether:Hexane 1:4) yielded the desired heptaenone intermediate as a dark red solid [TLC (Ether:Hexane 3:17) R_f = 0.35, stained by CAM; MS (ESI) Calculated for $C_{93}H_{180}NO_{14}Si_8$ $(M + Na)^+$: 1768.1, Found: 1768.0], which was immediately subjected to the next reaction conditions. The heptaenone intermediate was azeotropically dried with toluene. THF (10 mL) and MeOH (20 mL) was added. The resulting solution was cooled to 0 ^oC, and $NaBH_4$ (1.08 g, 28.6 mmol, 5.3 eq) was added. The reaction was quenched after 30 min with 1M aqueous ammonium chloride and extracted with ether. The organic layer was washed with water and then saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and flash column chromatography (SiO_2; Ether:Hexane 1:9 → 1:4) purification yielded **7** as a yellow-orange solid. (4.5 g, 2.57 mmol, 48 % 2 steps).



7

TLC (Ether:Hexane 1:4)

R_f = 0.44, stained by CAM

^{1}HNMR (500 MHz, $CD_3C(O)CD_3$)

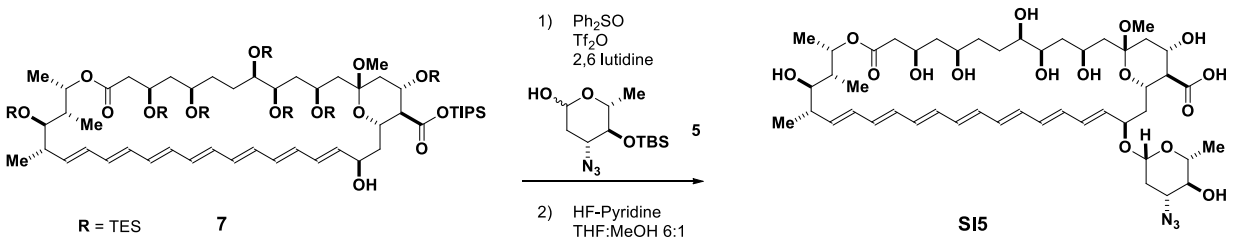
δ 6.49-6.10 (m, 13H), 5.53 (m, 1H), 4.68 (m, 1H), 4.50 (m, 2H), 4.22 (m, 1H), 4.15 (m, 1H), 4.06 (m, 1H), 4.00 (m, 1H), 3.91 (d, *J* = 4 Hz, 1H), 3.83 (m, 1H), 3.68 (m, 1H), 3.63 (m, 1H), 3.15 (s, 3H), 2.55 (m, 2H), 2.42 (m, 1H), 2.36 (m, 1H), 2.13 (m, 1H), 2.01 (m, 2H), 1.95-1.70 (m, 8H), 1.63 (m, 3H), 1.49 (m, 1H), 1.31 (m, 3H), 1.18-1.14 (m, 20H), 1.07-0.96 (m, 69H), 0.77-0.61 (m, 43H).

^{13}CNMR (125 MHz, $CD_3C(O)CD_3$)

δ 173.0, 170.9, 139.9, 139.04, 135.2, 135.1, 134.4, 133.7, 133.5, 133.5, 133.2, 133.1, 132.1, 132.0, 131.2, 128.3, 101.5, 77.1, 74.4, 73.7, 71.5, 69.8, 69.5, 67.9, 67.9, 67.7, 59.6, 48.5, 48.3, 44.8, 44.0, 42.2, 41.7, 41.1, 35.9, 31.1, 27.9, 20.3, 19.6, 18.9, 18.8, 18.9, 18.6, 13.7, 13.5, 13.3, 13.0, 11.7, 8.1, 8.1, 8.1, 8.0, 8.0, 7.9, 7.8, 7.8, 7.8, 7.8, 7.1, 6.9, 6.8, 6.6, 6.5, 6.5, 6.5, 6.5, 6.4, 6.3, 6.3, 6.3, 6.2, 6.1, 6.1, 6.0, 6.0.

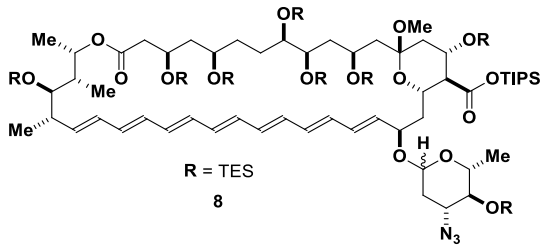
MS (ESI)

Calculated for $C_{93}H_{182}NO_{14}Si_8$ $(M + Na)^+$: 1770.2
Found: 1770.2



Octaol **SI5**

Intermediate **7** (2.5 g, 1.29 mmol, 1 eq) was azeotropically dried with toluene and placed under vacuum overnight. Hexane (80 mL) was added followed by activated 4 angstrom molecular sieves. The resulting solution was allowed to stir at room temperature while the sugar donor was prepared. The sugar donor **5** (739 mg, 2.57 mmol, 2.0 eq) was dissolved in DCM (26 mL). Diphenyl sulfoxide (911 mg, 4.50 mmol, 3.5 eq) and activated 4 angstrom molecular sieves were added. The reaction was stirred for 4 hours at room temperature. 2,6-lutidine (675 μL, 5.79 mmol, 4.5 eq) was added, and the reaction was cooled to -60 °C. Triflic anhydride (1M in DCM) (2.57 mL, 2.57 mmol, 2 eq) was added slowly. The reaction was warmed to -20 °C and stirred for 1.5 hrs. 2,6-lutidine (600 μL, 5.15 mmol, 4.0 eq) was added to the solution of **7**, and it was cooled to -30 °C. The solution containing the sugar donor was transferred to the solution containing **7** via cannula. The reaction was warmed to 0 °C and stirred for 1hr. The reaction was quenched with saturated aqueous sodium bicarbonate and extracted with ether. The organic layer was washed with copper sulfate, water, and saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and column chromatography (SiO_2; Ether:Hexane 3:47) purification yielded the glycosidated intermediate **8** as a mixture of isomers 2:1 α:β (2.12 g, 1.06 mmol, 82 %). The isomers were inseparable at this stage and thus carried forward to the next reaction.



8

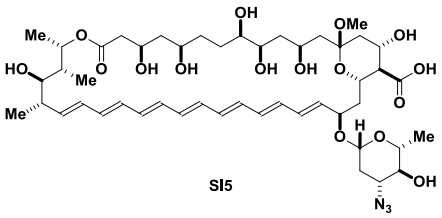
TLC (Ether:Hexane 1:19)

R_f = 0.25, stained by CAM

MS (ESI)

Calculated for $C_{105}H_{205}N_3O_{16}Si_9$ $(M + Na)^+$:	2039.3
Found:	2039.9

The glycosidated intermediate **8** (710 mg, 352 μmol, 1 eq) was azeotropically dried with toluene in a teflon vial. THF (3 mL) was added, and the solution was cooled to 0 °C. Pyridine (3 mL) in a teflon vial was cooled to 0 °C, and MeOH (0.5 mL) was added. 70% HF-pyridine was added slowly to the pyridine-MeOH solution at 0 °C. This solution was transferred slowly to the THF solution of glycosylated intermediate. The reaction was allowed to stir for 12 hours at room temperature. The reaction was quenched at 0 °C with excess MeOTMS and diluted with toluene. The solution was concentrated under reduced pressure, but not to dryness, and diluted again with toluene. This process was repeated 3 times to remove all of the pyridine. Reverse-phase HPLC purification (C18 SiO_2; MeCN:5 mM NH_4OAc in H_2O 1:19 → 19:1 over 30 minutes) provided a combined yield of 78% of the α and β isomers (86.7 mg, 91.7 μmol, 26 % β isomer and 173 mg, 183 μmol, 52 % α isomer).



SI5

HPLC (C18 SiO_2; MeCN:5 mM NH_4OAc in H_2O 1:19 → 19:1 over 30 minutes)

tR = 17.1 min, α
tR = 16.2 min, β

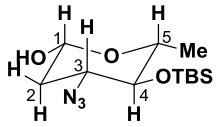
^{1}HNMR (500 MHz, $CD_3S(O)CD_3$) (β isomer)

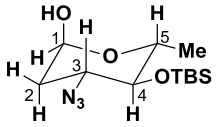
δ 6.32-6.05 (m, 12H), 5.81 (m, 1H), 5.60 (m, 1H), 4.97 (m, 1H), 4.58 (m, 1H), 4.43 (m, 1H), 3.99 (m, 1H), 3.84 (m, 1H), 3.73 (m, 2H), 3.52 (m, 2H), 3.32 (m, 1H), 3.21 (m, 1H), 3.10 (m, 1H), 3.00 (s, 3H), 2.93 (m, 2H), 2.29 (m, 1H), 2.16 (m, 2H), 2.01 (m, 2H), 1.76 (m, 1H), 1.68 (m, 1H), 1.52-1.23 (m, 14H), 1.15 (d, J = 5.5 Hz, 3H), 1.11 (d, J = 5.5 Hz, 3H), 1.03 (d, J = 6 Hz, 3H), 0.89 (d, J = 7 Hz, 3H).

HRMS (ESI) (β isomer)

Calculated for $C_{48}H_{73}N_3O_{16}$ $(M + Na)^+$:	970.4889
Found:	970.4897

Structural determination of the SI5-β anomer





	β		α	
H	δ	J^3	δ	J^3
C1	4.86 ppm	9.5 Hz	5.23 ppm	3.5 Hz
$C2_{equatorial}$	2.27 ppm	12.5 Hz, 5 Hz, 2 Hz	2.13 ppm	13 Hz, 5 Hz, 1.5 Hz
$C2_{axial}$	1.52 ppm	12.5 Hz, 9.5 Hz, 1 Hz	1.67 ppm	12.5 Hz, 4 Hz, 1 Hz
C3	3.49 ppm	m	3.69 ppm	m
C4	3.31 ppm	m	3.86 ppm	m
C5	3.07 ppm	m	3.07 ppm	m

Table S1. Chemical shifts and coupling constants for protons on C1'-C5' for α and β anomers of **5**. NMR solvent: acetone-*d6*

The structures of **SI5-β** and **SI5-α** were unambiguously determined from the following evidence:

1. Anomeric protons on glycosides have J^3 values of 7-9 Hz for axial and 2-4 Hz for equatorial.[3] In our case, **SI5-β** had an observed J^3 value of 8 Hz, while **SI5-α** had a J^3 value of 2.5 Hz. Similarly, the J^3 values of glycosylation donors **5-β** and **5-α** were 9.5 Hz and 3.5 Hz respectively (Table S1).
2. Full NMR assignments of **5-β** and **5-α** provided a general reference to assign the C2' axial and $C2'_{equatorial}$ protons in both HPLC purified compounds **SI5-β** and **SI5-α**. COSY analysis of **SI5-β** revealed a strong correlation between C1' and $C2'_{axial}$, while a weak correlation between C1' and $C2'_{equatorial}$, these results are consistent with a β configuration at the anomeric C1' position (figure SI1A).
3. NOESY spectroscopy of **SI5-β** showed strong NOEs for C1' to $C2'_{equatorial}$, C3' and C5' as predicted (figure SI1B).
4. NOESY spectroscopy of **SI5-α** showed strong NOEs for C1' to $C2'_{equatorial}$ and $C2'_{axial}$, while showing only very weak NOEs to C3', C4', and C5' as predicted (figure SI1C).

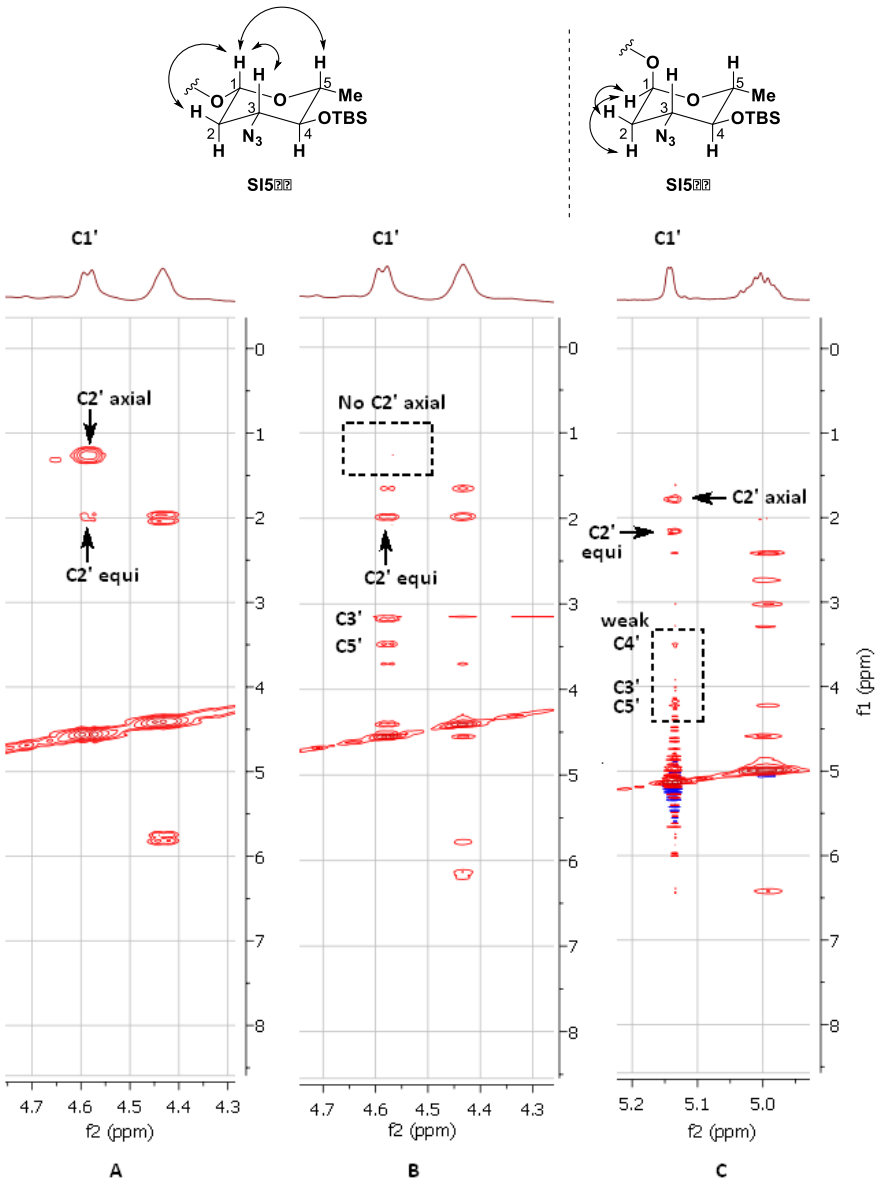
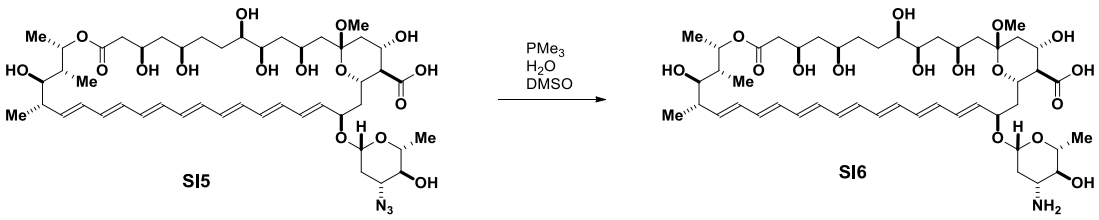


Figure SI1 A) COSY spectrum of **SI5-β** showing a strong C2'$_{axial}$ and a weak C2'$_{equatorial}$ correlation to C1' NMR solvent: DMSO-*d6*. B) NOESY spectrum of **SI5-β** showing no NOE between C1' and C2'$_{axial}$. NMR solvent: DMSO-*d6*. C) NOESY spectrum of **SI5-α** showing equal intensity NOEs between C1' and both C2'$_{axial}$ and C2'$_{equatorial}$. NMR solvent: pyridine *d-5*:CD_3OD 1:1.

^{1}HNMR (500 MHz, pyridine *d-5*:CD_3OD 1:1) (α isomer)

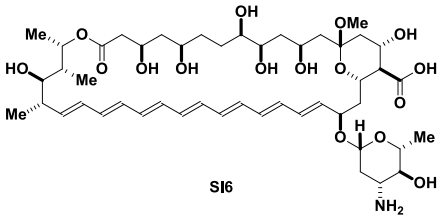
δ 6.71-6.39 (m, 12H), 6.33 (m, 1H), 5.99 (m, 1H), 5.78 (m, 1H), 5.14 (app d, J = 2.5Hz, 1H), 5.00 (m, 2H) 4.72 (m, 1H), 4.60 (m, 1H), 4.38 (m, 1H), 4.23 (m, 1H), 4.18 (m, 2H), 4.03 (m, 1H), 3.72 (m, 1H), 3.52 (m, 3H), 3.30 (s, 3H), 3.03 (t, J =10.5 Hz, 1H), 2.71 (m, 3H), 2.54 (dd, J = 16 Hz, 2.5 Hz, 1H), 2.47-2.29 (m, 4H), 2.29-2.12 (m, 5H), 2.10 (s, 1H), 2.08-1.66

(m, 14H), 1.63 (d, *J* = 5.5 Hz, 3H), 1.49 (d, *J* = 5.5 Hz, 3H), 1.33 (d, *J* = 6 Hz, 3H), 1.27 (d, *J* = 7 Hz, 3H).



Amine **SI6**

Intermediate **SI5** (19 mg, 20 μmol, 1 eq) was dissolved in DMSO (657 μL). Added water (36 μL, 200 μmol, 100 eq) and trimethyl phosphine (1M) (60 μL, 60 μmol, 3 eq). The reaction was heated to 55 °C for 3 hrs. Reverse-phase HPLC purification (C18 SiO_2; MeCN:5 mM NH_4OAc in H_2O 1:19 → 19:1 over 30 minutes) yielded **SI6** (10.5 mg, 11.4 μmol, 57 %).



HPLC (C18 SiO_2; MeCN:5 mM NH_4OAc in H_2O 1:19 → 19:1 over 30 minutes)

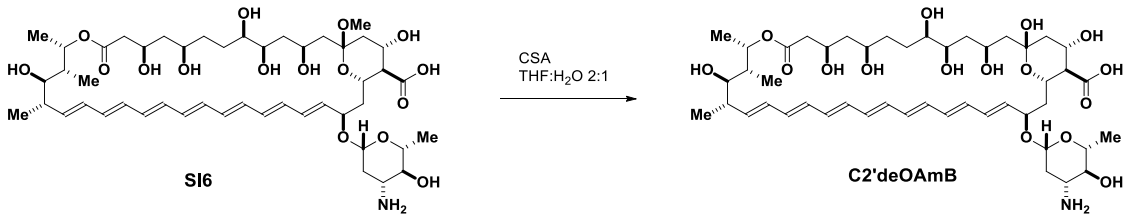
tR = 14.3 min

^{1}HNMR (500 MHz, $CD_3S(O)CD_3$)

δ 6.34-6.06 (m, 12H), 5.90 (m, 1H), 5.62 (m, 1H), 4.94 (m, 1H), 4.63 (m, 1H), 4.52 (m, 1H), 3.97 (m, 1H), 3.90 (m, 1H), 3.73 (m, 2H), 3.56 (m, 1H), 3.38 (m, 1H), 3.30 (m, 1H), 3.25 (m, 1H), 3.15 (m, 1H), 2.95 (m, 5H), 2.25 (m, 4H), 2.03 (m, 1H), 1.77 (m, 3H), 1.53-1.24 (m, 13H), 1.17 (d, *J* = 5 Hz, 3H), 1.11 (d, *J* = 6 Hz, 3H), 1.03 (d, *J* = 6 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H).

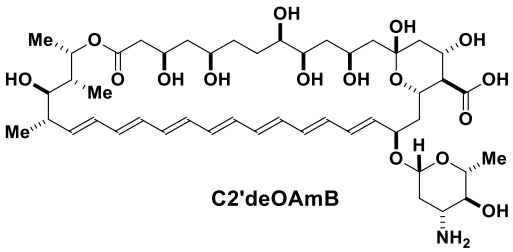
HRMS (ESI)

Calculated for $C_{48}H_{75}NO_{16}$ $(M + H)^+$: 922.5164
Found: 922.5169



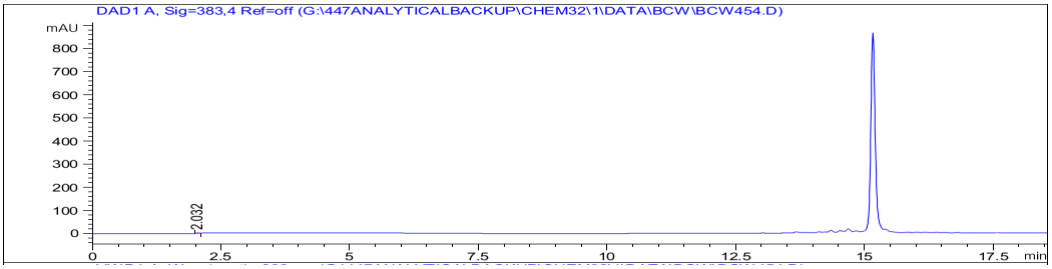
C2'deOAmB

Intermediate **SI6** (5 mg, 5.42 µmol, 1 eq) was placed in a vial. 180 µL of a 180 mM solution of CSA in 2:1 THF:H_2O was added. The reaction was stirred for 30 min. Reverse-phase HPLC purification (C18 SiO_2; MeCN:5 mM NH_4OAc in H_2O 1:19 → 19:1 over 30 minutes) yielded **C2'deOAmB** (3.9 mg, 4.34 µmol, 80 %, 94% pure by HPLC peak integration).



HPLC (C18 SiO_2; MeCN:5 mM NH_4OAc in H_2O 1:19 → 19:1 over 30 minutes)

tR = 15.1 min



^{1}HNMR (500 MHz, $CD_3S(O)CD_3$)

δ 6.47-5.94 (m, 11H), 5.73 (m, 1H), 5.42 (m, 2H), 5.23 (m, 1H), 4.77 (m, 1H), 4.61 (m, 1H), 4.38 (m, 1H), 4.26 (m, 1H), 4.15 (m, 1H), 4.06 (m, 1H), 3.99 (m, 1H), 3.70-3.20 (m, 4H), 3.09 (m, 1H), 2.92 (m, 1H), 2.36-2.16 (m, 5H), 1.99 (m, 1H), 1.83-1.72 (m, 4H), 1.56-1.51 (m, 4H), 1.39-1.23 (m, 7H), 1.15 (d, *J* = 5.5 Hz, 3H), 1.11 (d, *J* = 6 Hz, 3H), 1.03 (d, *J* = 6 Hz, 3H), 0.91 (d, *J* = 6.5 Hz, 3H).

HRMS (ESI)

Calculated for $C_{47}H_{73}NO_{16}$ $(M + H)^+$:	908.5008
Found:	908.5007

III. Extinction Coefficient Determination

General procedure.

A sample of dried compound was massed in a tared vial using a Mettler Toledo MT5 microbalance. This sample was then dissolved in DMSO to create a concentrated stock solution. A portion of this concentrated stock solution was diluted by a factor of five with DMSO to create a dilute stock solution. To achieve the final concentration for UV/Vis experiments, a volume of the dilute stock solution was diluted to 0.5 mL with MeOH. For each compound, UV/vis experiments were performed using five different final concentrations, and each concentration was prepared three times to obtain an average absorbance. The average absorbance was plotted against the concentration. The data was fitted with a linear least squares fit using Excel, and the slope of the fitted line was used as the extinction coefficient. The extinction coefficients were as follows: AmB (ε_{406} = 164,000), AmdeB (ε_{406} = 102,000), C2'deOAmB (ε_{406} = 73,000).

IV. Isothermal Titration Calorimetry

General Information.

Experiments were performed using a NanoITC isothermal titration calorimeter (TA Instruments, Wilmington, DE). Solutions of the compounds to be tested were prepared by diluting a 60.0 mM stock solution of the compound in DMSO to 600 μM with K buffer (5.0 mM HEPES/KHEPES, pH = 7.4). The final DMSO concentration in the solution was 1% v/v. POPC LUVs were prepared and phosphorus and ergosterol content was quantified as described below. The LUV solutions were diluted with buffer and DMSO to give a final phospholipid concentration of 12.0 mM in a 1% DMSO/K buffer solution. Immediately prior to use, all solutions were incubated at 37 °C for 30 minutes and degassed under vacuum at 37 °C for 10 minutes. The reference cell of the instrument (volume = 0.190 mL) was filled with a solution of 1% v/v DMSO/K buffer.

LUV Preparation.

Palmitoyl oleoyl phosphatidylcholine (POPC) was obtained as a 20 mg/mL solution in $CHCl_3$ from Avanti Polar Lipids (Alabaster, AL) and was stored at -20 °C under an atmosphere of dry argon and used within 1 month. A 4 mg/mL solution of ergosterol in $CHCl_3$ was prepared monthly and stored at 4 °C under an atmosphere of dry argon. A 4 mg/mL solution of cholesterol in $CHCl_3$ was prepared monthly and stored at 4 °C under an atmosphere of dry argon. Prior to preparing a lipid film, the solutions were warmed to ambient temperature to prevent condensation from contaminating the solutions. A 13 x 100 mm test tube was charged with 800 μL POPC and 230 μL of the ergosterol solution. For cholesterol-containing liposomes, a 13 x 100 mm test tube was charged with 800 μL POPC and 224 μL of the cholesterol solution. For sterol-free liposomes, a 13 x 100 mm test tube was charged with 800 μL POPC. The solvent was removed with a gentle stream of nitrogen and the resulting lipid film was stored under high vacuum for a minimum of eight hours prior to use. The film was then hydrated with 1 mL of K buffer and vortexed vigorously for approximately 3 minutes to form a suspension of multilamellar vesicles (MLVs). The resulting lipid suspension was pulled into a Hamilton (Reno, NV) 1 mL gastight syringe and the syringe was placed in an Avanti Polar Lipids Mini-Extruder. The lipid solution was then passed through a 0.20 μm Millipore (Billerica, MA) polycarbonate filter 21 times, the newly formed large unilamellar vesicle (LUV) suspension being collected in the syringe that did not contain the original suspension of MLVs to prevent the carryover of MLVs into the LUV solution.

Determination of Phosphorus Content.

Determination of total phosphorus was adapted from the report of Chen and coworkers.[4] The LUV solution was diluted tenfold with K buffer and three 10 μL samples of the diluted LUV suspension were added to three separate 7 mL vials. Subsequently, the solvent was removed with a stream of N_2. To each dried LUV film, and a fourth vial containing no lipids that was used as a blank, was added 450 μL of 8.9 M H_2SO_4. The four samples were incubated open to ambient atmosphere in a 225 °C aluminum heating block for 25 min and then removed to 23 °C and cooled for 5 minutes. After cooling, 150 μL of 30% w/v aqueous hydrogen peroxide was added to each sample, and the vials were returned to the 225 °C heating block for 30 minutes. The samples were then removed to 23 °C and cooled for 5 minutes before the addition of 3.9 mL water. Then 500 μL of 2.5% w/v ammonium molybdate was added to each vial and the resulting mixtures were then vortexed briefly and vigorously five times. Subsequently, 500 μL of 10% w/v ascorbic acid was added to each vial and the resulting mixtures were then vortexed briefly and vigorously five times. The vials were enclosed with a PTFE lined cap and then placed in a 100 °C aluminum heating block for 7 minutes. The samples were removed to 23 °C and cooled for approximately 15 minutes prior to analysis by UV/Vis spectroscopy. Total phosphorus was determined by observing the absorbance at 820 nm and comparing this value to a standard curve obtained through this method and a standard phosphorus solution of known concentration.

Determination of Ergosterol Content.

Ergosterol content was determined spectrophotometrically. A 50 μL portion of the LUV suspension was added to 450 μL 2:18:9 hexane:isopropanol:water (v/v/v). Three independent samples were prepared and then vortexed vigorously for approximately one minute. The solutions were then analyzed by UV/Vis spectroscopy and the concentration of ergosterol in solution was determined by the extinction coefficient of 10400 L mol^{-1} cm^{-1} at the UV_{max} of 282

nm and was compared to the concentration of phosphorus to determine the percent sterol content. The extinction coefficient was determined independently in the above ternary solvent system. LUVs prepared by this method contained between 7 and 14% ergosterol.

Titration Experiment.

Titrations were performed by injecting the LUV suspension at ambient temperature into the sample cell (volume = 0.191 mL) which contained the 600 μM solution of the compound in question at 25 °C. The volume of the first injection was 0.23 μL. Consistent with standard procedure,[5] due to the large error commonly associated with the first injection of ITC experiments, the heat of this injection was not included in the analysis of the data. Next, six 7.49 μL injections of the LUV suspension were performed. The spacing between each injection was 720 seconds to ensure that the instrument would return to a stable baseline before the next injection was made. The rate of stirring for each experiment was 300 rpm.

Data Analysis.

NanoAnalyze software (TA Instruments) was used for baseline determination and integration of the injection heats, and Microsoft Excel was used for subtraction of dilution heats and the calculation of overall heat evolved. To correct for dilution and mixing heats, the heat of the final injection from each run was subtracted from all the injection heats for that particular experiment.[6] By this method, the overall heat evolved during the experiment was calculated using the following formula:

$$\mu\text{cal}_{overall} = \sum_{i=1}^{n} (\Delta h^{i}_{injection} - \Delta h^{n}_{injection})$$

Where i = injection number, n = total number of injections, $\Delta h^{i}_{injection}$ = heat of the i^{th} injection, $\Delta h^{n}_{injection}$ = the heat of the final injection of the experiment.

V. Antifungal Assays

Growth Conditions for *S. cerevisiae*.

S. cerevisiae was maintained with yeast peptone dextrose (YPD) growth media consisting of 10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose, and 20 g/L agar for solid media. The media was sterilized by autoclaving at 250 °F for 30 min. Dextrose was subsequently added as a sterile 40% w/v solution in water (dextrose solutions were filter sterilized). Solid media was prepared by pouring sterile media containing agar (20 g/L) onto Corning (Corning, NY) 100 x 20 mm polystyrene plates. Liquid cultures were incubated at 30 °C on a rotary shaker and solid cultures were maintained at 30 °C in an incubator.

Growth Conditions for *C. albicans*.

C. albicans was cultured in a similar manner to *S. cerevisiae* except both liquid and solid cultures were incubated at 37 °C.

Broth Microdilution Minimum Inhibitory Concentration (MIC) Assay.

The protocol for the broth microdilution assay was adapted from the Clinical and Laboratory Standards Institute document M27-A2.[7] 50 mL of YPD media was inoculated and

incubated overnight at either 30 °C (*S. cerevisiae*) or 37 °C (*C. albicans*) in a shaker incubator. The cell suspension was then diluted with YPD to an OD_{600} of 0.10 (~5 x 10^5 cfu/mL) as measured by a Shimadzu (Kyoto, Japan) PharmaSpec UV-1700 UV/Vis spectrophotometer. The solution was diluted 10-fold with YPD, and 195 μL aliquots of the dilute cell suspension were added to sterile Falcon (Franklin Lakes, NJ) Microtest 96-well plates in triplicate. Compounds were prepared either as 400 μM (AmB, C2'deOAmB) or 2 mM (AmdeB) stock solutions in DMSO and serially diluted to the following concentrations with DMSO: 1600, 1200, 800, 400, 320, 240, 200, 160, 120, 80, 40, 20, 10 and 5 μM. 5 μL aliquots of each solution were added to the 96-well plate in triplicate, with each column representing a different concentration of the test compound. The concentration of DMSO in each well was 2.5% and a control well to confirm viability using only 2.5% DMSO was also performed in triplicate. This 40-fold dilution gave the following final concentrations: 50, 40, 30, 20, 10, 8, 6, 4, 1, 0.5, 0.25 and 0.125 μM. The plates were covered and incubated at 30 °C (*S. cerevisiae*) or 37 °C (*C. albicans*) for 24 hours prior to analysis. The MIC was determined to be the concentration of compound that resulted in no visible growth of the yeast. The experiments were performed in duplicate and the reported MIC represents an average of two experiments.

VI. Hemolysis Assays

Erythrocyte Preparation.

The protocol for the hemolysis assay was adapted from the report of Paquet and coworkers.[8] Whole human blood (sodium heparin) was purchased from Bioreclamation LLC (Westbury, NY) and stored at 4 °C and used within two days of receipt. To a 2.0 mL eppendorf tube, 1 mL of whole human blood was added and centrifuged at 10,000 g for 2 minutes. The supernatant was removed and the erythrocyte pellet was washed with 1 mL of sterile saline and centrifuged at 10,000 g for 2 minutes. The saline wash was repeated for a total of three washes. The erythrocyte pellet was suspended in 1 mL of RBC buffer (10 mM NaH_2PO_4, 150 mM NaCl, 1 mM $MgCl_2$, pH 7.4) to form the erythrocyte stock suspension.

Minimum Hemolysis Concentration (MHC) Assay.

Compounds were prepared as 1.03 mM (AmB) or 12.8 mM (C2'deOAmB and AmdeB) stock solutions in DMSO and serially diluted to the following concentrations with DMSO: 7689, 5126, 2563, 2050, 1538, 1025, 769, 513, 384, 256, 205, 154, 103, 77, 51, 26 μM. To a 0.2 mL PCR tube, 24 μL of RBC buffer and 1 μL of compound stock solution were added, which gave final concentrations of 500, 300, 200, 100, 80, 60, 40, 30, 20, 15, 10, 8, 6, 4, 3, 2, 1 μM. Positive and negative controls were prepared by adding 1 μL of DMSO to MilliQ water or RBC buffer, respectively to 0.2 mL PCR tube. To each PCR tube, 0.63 μL of the erythrocyte stock suspension was added and mixed by inversion. The samples were incubated at 37 °C for 2 hours. The samples were mixed by inversion and centrifuged at 10,000 g for 2 minutes. 15 μL of the supernatant from each sample was added to a 384-well place. Absorbances were read at 540 nm using a Biotek H1 Synergy Hybrid Reader (Wanooski, VT). Experiments were performed in triplicate and the reported MHC represents an average of three experiments.

Data Analysis.

Percent hemolysis was determined according to the following equation:

$$\%\ hemolysis = \frac{Abs._{sample} - Abs._{neg.}}{Abs._{pos.} - Abs._{neg.}} \times 100\%$$

Concentration vs. percent hemolysis was plotted and fitted to 4-parameter logistic (4PL)[9] dose response fit using OriginPro 8.6. The MHC was defined as the concentration to cause 90% hemolysis.

VII. WST-8 Cell Proliferation Assays

Primary Renal Proximal Tubule Epithelial Cells Preparation.

Primary human renal proximal tubule epithelial cells (RPTECs) were purchased from ATCC (Manassas, VA) and immediately cultured upon receipt. Complete growth media was prepared using renal epithelial cell basal medium (ATCC, PCS-400-030), renal epithelial cell growth kit (ATCC, PCS-400-040), and penicillin-streptomycin (10 units/mL and 10 ug/mL). Complete media was stored at 4 °C in the dark and used within 28 days. Primary RPTECs were grown in CO_2 incubator at 37 °C with an atmosphere of 95% air/5% CO_2.

WST-8 Reagent Preparation.

WST-8 cell proliferation assay kit (10010199) was purchased from Cayman Chemical Company (Ann Arbor, MI) and stored at -20 °C and used within 6 months of receipt. WST-8 reagent and electron mediator solution were thawed and mixed to prepare the WST-8 reagent solution. The solution was stored at -20 °C and used within one week.

WST-8 Assay.

A suspension of primary RPTECs in complete growth media was brought to a concentration of 1 x 10^5 cells/mL. A 96-well plate was seeded with 99 μL of the cell suspension and incubated at 37 °C with an atmosphere of 95% air/5% CO_2 for 3 hours. Positive and negative controls were prepared by seeding with 100 μL of the cell suspension or 100 μL of the complete media. Compounds were prepared as 5 mM (AmB), 20 mM (C2'deOAmB), and 50 mM (AmdeB) stock solutions in DMSO and serially diluted to the following concentrations with DMSO: 50000, 40000, 30000, 20000, 10000, 8000, 6000, 4000, 3000, 2000, 1500, 1000, 800, 600, 400, 300, 200, 100, 50, 25, 10, 5, 2.5, 1, 0.5, 0.25, and 0.1 μM. 1 μL aliquots of each solution were added to the 96-well plate in triplicate, with each column representing a different concentration of the test compound. The 96-well plate was incubated at 37 °C with an atmosphere of 95% air/5% CO_2 for 24 hours. After incubation, the media was aspirated and 100 μL of serum-free media was added and 10 μL of the WST-8 reagent solution was added to each well. The 96-well plate was mixed in a shaking incubator at 200 rpm for 1 minute and incubated at 37 °C with an atmosphere of 95% air/5% CO_2 for 2 hours. Following incubation, the 96-well plate was mixed in a shaking incubator at 200 rpm for 1 minute and absorbances were read at 450 nm using a Biotek H1 Synergy Hybrid Reader (Wanooski, VT). Experiments were performed in triplicate and the reported cytotoxicity represents an average of three experiments.

Data Analysis.

Percent hemolysis was determined according to the following equation:

$$\%\ cell\ viability = \frac{Abs._{sample} - Abs._{neg.}}{Abs._{pos.} - Abs._{neg.}} \times 100\%$$

Concentration vs. percent hemolysis was plotted and fitted to 4-parameter logistic (4PL)[8] dose response fit using OriginPro 8.6. The MTC was defined as the concentration to cause 90% loss of cell viability.

Microscopy.

Cells were imaged using an AMG (Bothell, WA) EVOS fl Microscope after treatment with DMSO (vehicle) or the compounds at the indicated concentrations for 24 hours. Images were taken using transmitted light at 10x objective.

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