A Post-PKS Oxidation of the Amphotericin B Skeleton Predicted to be Critical for Channel Formation is Not Required for Potent Antifungal Activity

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Supporting Information

Complete Versions of Truncated References

(4e) Zumbuehl, A.; Jeannerat, D.; Martin, S.E.; Sohrmann, M.; Stano, P.; Vigassy, T.; Clark, D.D.; Hussey, S.L.; Peter, M.; Peterson, B.R.; Pretsch, E.; Walde, P.; Carreira, E.M. *Angew. Chem. Int. Ed.* **2004**, *43*, 5181-5185.
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(10) (a) Chéron, M.; Cybulska, B.; Mazerski, J.; Grzybowska, J.; Czerwinski, A.; Borowski, E. *Biochem. Pharmacol.* **1988**, *37*, 827-836. (b) Matsumori, N.; Yamaji, N.; Matsuoka, S.; Oishi, T.; Murata, M. *J. Am. Chem. Soc.* **2002**, *124*, 4180-4181. (e) Umegawa, Y.; Matsumori, N.; Oishi, T.; Murata, M. *Tetrahedron Lett.* **2007**, *48*, 3393-3396.
(13) (a) Nicolaou, K.C.; Chakraborty, T.K.; Ogawa, Y.; Daines, R.A.; Simpkins, N.S.; Furst, G.T. *J. Am. Chem. Soc.* 1988, *110*, 4660-4672. (b) A degradative synthesis of **4** is described in the patent literature: Driver, M.J.; Greenless, A.R.; and MacPherson, D.T. U.S. Patent # 5,204,330, 1993. (c) MacPherson, D.T.; Corbett, D.F.; Costello, B.C.; Driver, M.J.; Greenless, A.R.; Maclachlan, W.S.; Shanks, C.T.; Taylor, A.W. Adventures in Polyene Macrolide Chemistry :The Derivatisation of Amphotericin B. In *Recent Advances in the Chemistry of Anti-Infective Agents;* Bently P.H. and Ponsford, R. Eds.; Royal Society of Chemistry : Cambridge, 1993; Special Publication No. 119, pp 205-222. (d) An impure sample of **4** was prepared via genetic engineering of the producer: Carmody, M.; Murphy, B.; Byrne, B.; Power, P.; Rai, D.; Rawlings, B.; Caffrey, P. *J. Biol. Chem.* **2005**, *280*, 34420-34426.
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I. General Methods

Materials. Commercially available materials were purchased from Aldrich Chemical Co. (Milwaukee, WI), Fisher Scientific (Hampton, NH), Lipoid (Luwigshafen, Germany) and Silicycle (Quebec, Canada) and used without further purification unless noted otherwise.

Amphotericin B was a generous gift from Bristol-Myers Squibb Company. 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 : dry neutral alumina; DMSO, DMF, CH_3OH : activated molecular sieves). Hexanes, 2,6-lutidine, triethylamine, and pyridine were freshly distilled under nitrogen from CaH_2. Camphorsulfonic acid was recrystallized from ethyl acetate. Water was doubly distilled or obtained from a Millipore (Billerica, MA) MilliQ water purification system.

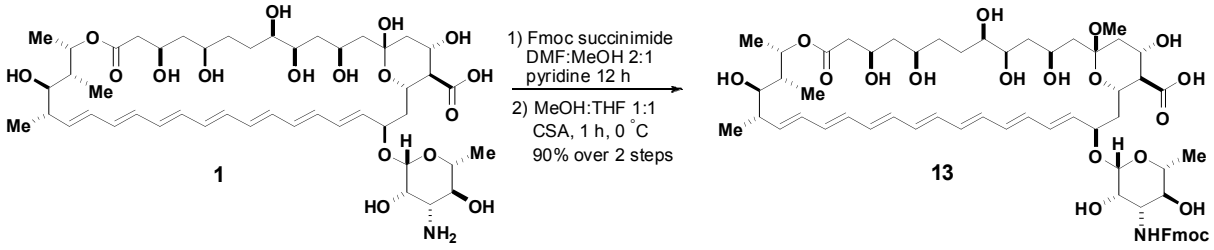
Reactions. Due to the light and air sensitivity of amphotericin B, all manipulations were carried out under low light conditions and compounds were stored under an anaerobic atmosphere. All reactions were performed in oven- or flame-dried glassware under an atmosphere of argon unless otherwise indicated. 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 an acidic solution of *p*-anisaldehyde. Alternatively, reactions were monitored by RP-HPLC using an Agilent 1100 Series HPLC system equipped with a Sunfire™ C_{18} 5 micron 10 x 250 mm column (Waters Corp. Milford, MA) with UV detection at 406 nm and the indicated eluent and flow rate.

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 or on Silicycle 17% carbon C_{18} 230-400 mesh reverse phase silica gel. ^{1}H NMR spectra were recorded at 23 °C on a Varian Unity Inova Narrow Bore spectrometer operating at a ^{1}H frequency of 500 MHz with a Varian 5 mm ^{1}H{^{13}C/^{15}N} pulsed-field gradient Z probe or a Varian Unity Inova spectrometer operating at a ^{1}H frequency of 600 MHz with a Varian 5 mm ^{1}H{^{13}C/^{15}N} pulsed-field gradient X,Y,Z probe. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane and referenced internally to the residual protium in the NMR solvent (CHD_2OD, δ = 3.31, center line, $CD_3C(O)CHD_2$, δ = 2.05, center line) or to added tetramethylsilane. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet, b = broad, app = apparent), coupling constant (*J*) in Hertz (Hz) and integration. For compounds **17** (a more soluble derivative of **1**), **2**, **21** (a more soluble derivative of **3**), and **23** (a more soluble derivative of **4**), proton and coupling constant assigments were made using a variety of two-dimensional NMR techniques including phase-sensitive COSY experiments combined with amplitude constrained multiplet evaluation (ACME)[3] (see Section III for a detailed discussion). ^{13}C spectra were recorded at 23 °C with a Varian Unity Inova spectrometer operating at a ^{13}C frequency of 125 MHz with a 5 mm Nalorac gradient {^{13}C/^{15}N}^{1}H quad probe or a Varian Unity Inova spectrometer operating at a ^{13}C frequency of 150 MHz and equipped with a Varian 5 mm 600 DB Auto X probe. Chemical shifts (δ) are reported downfield of tetramethylsilane and are referenced to the carbon resonances in the NMR solvent (CD_3OD, δ = 49.0, center line, $CD_3C(O)CD_3$, δ = 29.8, center line) or to added tetramethylsilane (δ = 0.00). MS analysis was performed with an Applied Biosystems Micromass Ultima system with ESI ionization. High resolution mass spectra (HRMS) were obtained at the University of Illinois mass spectrometry facility. All synthesized compounds (**2**-**4** and **7**-**23**)

[1] Pangborn, A.B.; Giardello, M.A.; Grubbs, R.H.; Rosen, R.K.; Timmers, F.J. *Organometallics* **1996**, *15*, 1518-1520.
[2] Still, W.C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.
[3] Delaglio, F.; Wu, Z.; Bax, A. *J. Magn. Reson.* **2001**, *149*, 276-281.

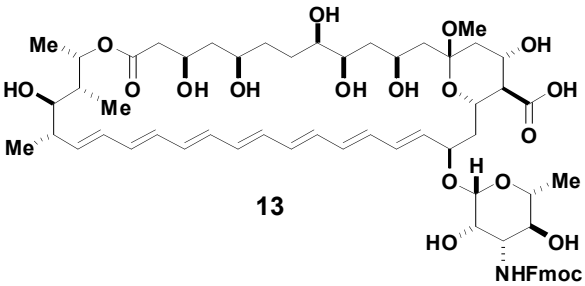
gave HRMS within 5 ppm of the calculated values. The purity of amphotericin B and its derivatives was determined by HPLC analysis using a Waters SunFire Prep C_{18} OBD 5 micron 30 x 150 mm Lot # 168I161701 column with detection at 406 nm and an eluent of acetonitrile and aqueous ammonium acetate unless otherwise indicated.

II. Synthesis of AmB derivatives



Methyl ketal 13

A round bottom flask was charged with amphotericin B (1.5 g, ~55% pure, *ca.* 0.891 mmol, 1 eq) and Fmoc-succinimide (0.840 g, 2.48 mmol, 2.8 eq) which were dissolved in a mixture of DMF:MeOH 2:1 (105 mL) at 23 °C. Pyridine (0.84 mL, 10.22 mmol, 11.5 eq) was subsequently added and the reaction was stirred for 12 hours. The reaction mixture was then poured into diethyl ether (1.8 L) stirring at 0 °C. After stirring for 30 minutes at 0 °C the resulting yellow precipitate was isolated via Büchner filtration using Whatman 50 filter paper to afford a yellow solid. The residual solvent was removed by coevaporating with acetonitrile (3 x 20 mL) and storing under vacuum for one hour. The resulting powder (1.69 g, *ca.* 0.8 mmol) was dissolved in THF:MeOH 1:1 (50 mL) and cooled to 0 °C. To this solution was added camphorsulfonic acid (0.100 g, 0.438 mmol, 0.55 eq) and the resulting mixture was stirred for 1 hour at 0 °C. The reaction was then quenched at 0 °C with triethylamine (0.06 mL, 0.438 mmol, 0.55 eq) and gravity filtered. The filtrate was concentrated *in vacuo* until a yellow solid began to precipitate. The resulting supersaturated solution was poured into hexanes:diethyl ether 1:1 (800 mL) and the yellow precipitate was collected via Büchner filtration and washed with ethyl acetate:diethyl ether 1:1 (200 mL) to yield **13** as a yellow solid (1.33 g, ~70% purity, *ca.* 0.80 mmol, *ca.* 90% over two steps). This material was carried forward without further purification.



TLC (CH_2Cl_2:MeOH 5:1)

R_f = 0.15, stained by anisaldehyde

HPLC

tR = 13.2 min; flow rate = 4 mL/min, gradient of 5 → 95% MeCN in 5 mM ammonium acetate over 15 min.

^{1}H NMR (500 MHz, pyridine *d*-5:CD_3OD 10:1)

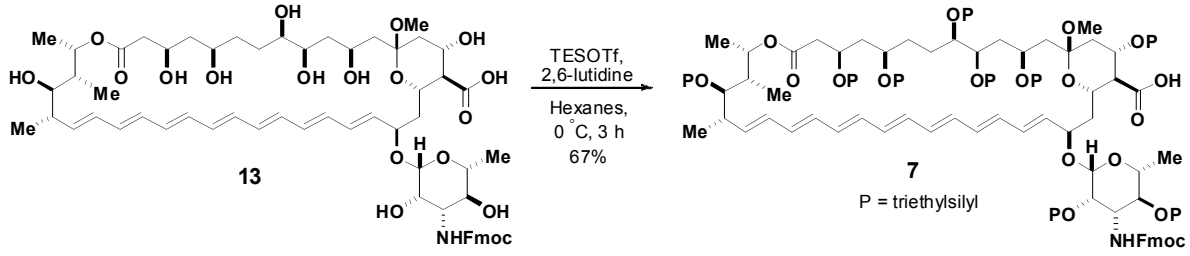
δ 7.86 (d, *J* = 7.5 Hz, 2H), 7.73 (t, *J* = 8 Hz, 2H), 7.43 (t, *J* = 7.5 Hz, 2H), 7.28 (dd, *J* = 2, 8 Hz, 2H), 6.59-6.34 (m, 12H), 6.23 (dd, *J* = 6.5, 14 Hz, 1H), 5.66 (dd, *J* = 10, 15 Hz, 1H), 4.97 (bs, 1H), 4.92 (bs, 1H), 4.69 (bs, 1H), 4.54 (app t, *J* = 9.5 Hz, 1H), 4.52-4.31 (m, 4H) 4.31 (bs, 1H), 4.26-4.20 (m, 3H), 4.07-4.01 (m, 2H), 3.87 (app dd, *J* = 7.5, 16.5 Hz, 2H), 3.67-3.63 (m, 2H), 3.58 (app d, 10.5 Hz, 1H) 3.48 (app d, *J* = 8.5 Hz, 1H), 3.28 (s, 3H), 2.77-2.54 (m, 5H), 2.45 (dd, *J* = 3.5, 16.5 Hz, 1H), 2.08-2.00 (m, 4H), 1.88-1.72 (m, 7H), 1.68-1.64 (app d, *J* = 14 Hz, 1H), 1.52 (d, *J* = 6 Hz, 3H), 1.39 (d, *J* = 6.5 Hz, 3H), 1.29 (d, *J* = 6.5 Hz, 3H), 1.20 (d, *J* = 7 Hz, 3H).

^{13}C NMR (125 MHz, pyridine *d-5*:CD_3OD 10:1)

δ 171.7, 158.0, 144.8, 144.7, 141.8, 137.3, 134.6, 134.5, 134.3, 134.0, 133.4, 133.3, 133.2, 133.1, 132.8, 132.5, 132.2, 128.2, 127.6, 125.9, 125.8, 120.4, 101.9, 99.1, 78.1, 75.4, 74.6, 74.5, 71.8, 71.6, 71.1, 70.7, 68.2, 67.7, 67.2, 66.8, 58.1, 46.3, 44.6, 43.5, 43.1, 42.7, 42.5, 41.5, 41.3, 39.3, 36.1, 34.1, 30.8, 30.5, 30.1, 29.7, 29.3, 24.9, 24.2, 23.3, 18.7, 18.4, 17.4, 14.1, 14.0, 12.2, 11.0.

HRMS (ESI)

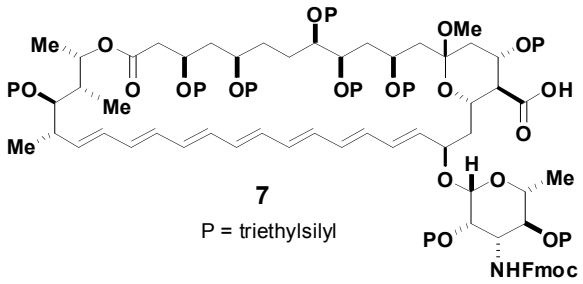
calculated for $C_{63}H_{85}NO_{19}$ $(M + Na)^+$:	1182.5614
found:	1182.5608



Nonatriethylsilyl ether 7

Prior to the reaction **13**, was azeotropically dried via coevaporation with acetonitrile (3 x 20 mL) and was left under vacuum for a minimum of eight hours. The resulting yellow powder (3.9 g, ~ 70% purity, *ca.* 2.35 mmol, 1 eq) was suspended in hexanes (110 mL). 2,6-lutidine (7.0 mL, 60.5 mmol, 26 eq) was added and the resulting suspension was then cooled to 0 °C. Triethylsilyl triflate (10.6 mL, 47.0 mmol, 20 eq) was added dropwise over 10 minutes and the resulting yellow suspension was stirred for 2 hours at 0 °C. Additional 2,6-lutidine (1.8 mL, 18.3 mmol, 6.5 eq) was then added followed by additional triethylsilyl triflate (2.5 mL, 11.1 mmol, 5 eq) dropwise over 5 minutes. After 15 minutes of stirring additional 2,6-lutidine (1.8 mL, 18.3 mmol, 6.5 eq) was added followed by additional triethylsilyl triflate (2.5 mL, 11.1 mmol, 5 eq) dropwise over 5 minutes. The mixture was stirred at 0 °C for 1 hour following completion of the final addition and was then quenched at 0 °C with saturated aqueous sodium bicarbonate (250 mL). The resulting emulsion was diluted with diethyl ether (500 mL) and the layers were separated. The organic phase was washed with saturated aqueous sodium bicarbonate (1 x 100 mL) and water (3 x 50 mL), and the combined aqueous washings were back-extracted with diethyl ether (3 x 100 mL). The combined organic phases were then washed with 1M aqueous

copper sulfate (10 x 25 mL). The combined copper sulfate layers were then back-extracted with diethyl ether (5 x 50 mL). The combined organic layers were washed with water (3 x 50 mL) and brine (1 x 50 mL). This second set of aqueous washings was back extracted with diethyl ether (3 x 50 mL) and the combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. Purification of the crude yellow oil by flash chromatography (SiO_2; hexanes:diethyl ether 20:1 → 7:1) furnished **7** as an orange solid (4.94 g, 2.26 mmol, 96%).



7

P = triethylsilyl

TLC (hexanes:diethyl ether 2:1)

R_f = 0.38, visualized by UV

^{1}H NMR (500 MHz, acetone *d*-6)

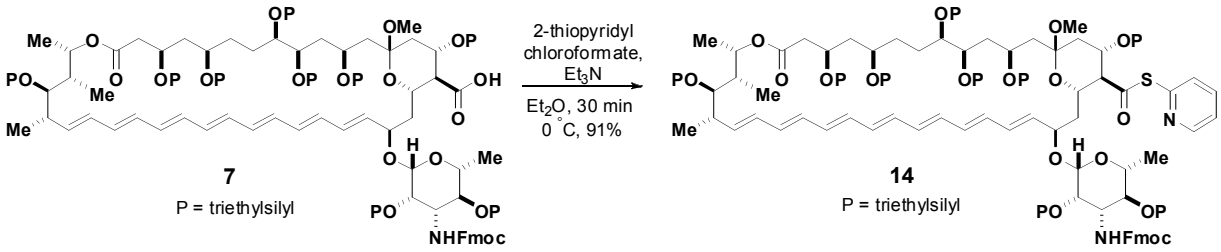
δ 7.90 (d, *J* = 7.5 Hz, 2H), 7.72 (d, *J* = 7 Hz, 2H), 7.45 (t, *J* = 7.5 Hz, 2H), 7.37 (t, *J* = 7.5 Hz, 2H) 6.63-6.56 (m, 3H), 6.49-6.14 (m, 9H) 5.98 (dd, *J* = 5.5, 15.5 Hz, 1H), 5.52 (dd, *J* = 10, 15 Hz, 1H), 5.42 (d, *J* = 10 Hz, 1H), 4.74 (bs, 1H), 4.67 (bs, 1H), 4.57 (s, 1H), 4.54-4.45 (m, 2H), 4.37 (dd, *J* = 6.5, 10 Hz, 1H), 4.28 (app t, *J* = 6 Hz, 2H), 4.17 (t, *J* = 10 Hz, 1H), 4.02 (app t, *J* = 9 Hz, 2H), 3.92 (d, *J* = 2.5 Hz, 1H), 3.89 (app d, *J* = 8.5 Hz, 1H), 3.73 (bs, 1H), 3.65-3.60 (m, 2H), 3.48 (app t, *J* = 9 Hz, 2H), 3.33 (dd, *J* = 6.5, 8.5 Hz, 1H), 3.16 (s, 4H), 2.62 (d, *J* = 7 Hz, 1H) 2.51-2.46 (m, 2H), 2.35 (t, *J* = 10.5 Hz, 1H) 2.17 (dd, *J* = 7, 15 Hz, 1H), 2.04 (dd, 7.5, 15.5 Hz, 1H), 1.97-1.62 (m, 10H), 1.56-1.52 (m, 1H) 1.27 (d, *J* = 6.5 Hz, 3H), 1.20 (d, *J* = 6 Hz, 3H), 1.11-0.92 (m, 87H), 0.82-0.59 (m, 54H).

^{13}C NMR (125 MHz, acetone *d*-6)

δ 174.3, 170.5, 158.2, 156.4, 145.0, 142.2, 139.5, 137.2, 135.8, 135.6, 135.2, 134.5, 134.2, 134.0, 132.4, 132.3, 132.1, 132.0, 131.2, 130.6, 128.5, 127.9, 127.8, 125.8, 125.7, 120.8, 120.6, 101.5, 98.0, 76.8, 75.0, 74.5, 74.1, 73.5, 73.4, 71.1, 68.9, 67.5, 67.3, 67.2, 58.1, 57.5, 48.1, 48.0, 44.3, 43.4, 42.2, 41.5, 40.7, 35.6, 27.6, 24.4, 20.0, 19.3, 18.9, 11.1, 7.65, 7.61, 7.51, 7.49, 7.34, 7.30, 7.27, 7.15, 6.37, 6.18, 5.88, 5.87, 5.83, 5.78, 5.76, 5.63.

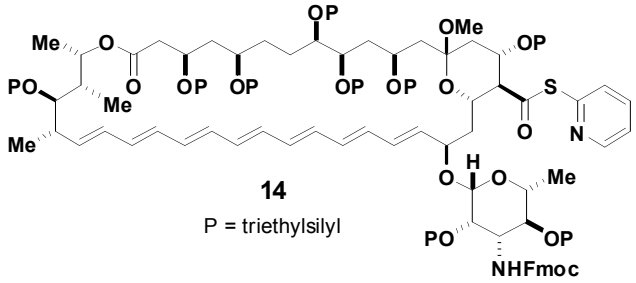
HRMS (ESI)

calculated for $C_{117}H_{211}NO_{19}Si_9$ $(M + Na)^+$:	2209.3397
found:	2209.3303



2-pyridylthioester 14

To a stirred solution of **7** (4.9 g, 2.24 mmol, 1 eq) in diethyl ether (90 mL) at 0 °C was added triethylamine (0.40 mL, 2.91 mmol, 1.3 eq). 2-thiopyridyl chloroformate (4 mL, 4 mmol, 1.8 eq, 1M in CH_2Cl_2) was added and the solution was stirred for 30 minutes at 0 °C. The formation of a precipitate was observed as the reaction progressed. The mixture was then diluted with diethyl ether (200 mL) and the solids were removed via Büchner filtration using Whatman 50 filter paper. The filtrate was concentrated *in vacuo* to give crude **14** as a yellow solid. Purification by flash chromatography (SiO_2; hexanes:diethyl ether 10:1 → 3:1) afforded **14** as an orange solid (4.65 g, 2.04 mmol, 91%).



TLC (hexanes:diethyl ether 2:1)

R_f = 0.76, visualized by UV

^{1}H NMR (500 MHz, acetone *d*-6)

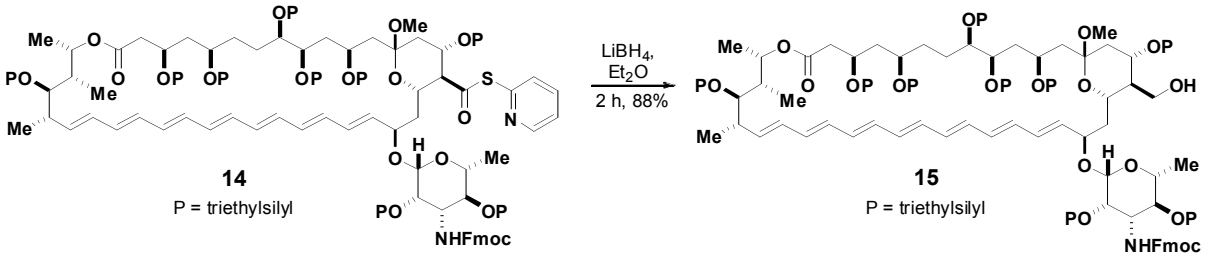
δ 8.71 (d, *J* = 3.5 Hz, 1H), 7.94 (dt, *J* = 1.5, 7.5 Hz, 1H), 7.86 (d, *J* = 7.5 Hz, 2H), 7.73-7.68 (m, 3H), 7.43-7.31 (m, 5H), 6.59-6.52 (m, 3H), 6.46-6.11 (m, 9H) 5.97 (dd, *J* = 5.5, 16 Hz, 1H), 5.49 (dd, *J* = 9.5, 15 Hz, 1H), 5.28 (d, *J* = 10 Hz, 1H), 4.78 (bs, 1H), 4.65 (bs, 1H), 4.62 (s, 1H), 4.52-4.43 (m, 3H), 4.33 (dd, *J* = 7, 10.5 Hz, 1H), 4.23 (app t, *J* =6.5 Hz, 2H), 4.14 (t, *J* = 10 Hz, 1H), 4.06 (t, *J* = 9.0 Hz, 1H), 4.00 (bs, 1H), 3.91 (d, *J* = 2.5 Hz, 1H), 3.85 (dd, *J* = 2, 8.5 Hz, 1H), 3.70-3.66 (m, 3H), 3.61 (dd, *J* = 4, 10.5 Hz, 1H), 3.44-3.37 (m, 2H), 3.13 (s, 3H), 2.71 (t, *J* = 10 Hz, 1H), 2.58 (d, *J* = 6.5 Hz, 1H), 2.43 (app dd, *J* = 7, 9 Hz, 1H), 2.32 (dd, *J* = 6.5, 15 Hz, 1H) 2.12-2.08 (m, 2H) , 1.93-1.83 (m, 3H), 1.79-1.62 (m, 7H), 1.53-1.50 (m, 1H), 1.22 (d, *J* = 5.5 Hz, 3H), 1.17 (d, *J* = 6 Hz, 3H), 1.10-0.90 (m, 87H), 0.81-0.56 (m, 54H).

^{13}C NMR (125 MHz, acetone *d*-6)

δ 197.5, 169.9, 163.5, 155.6, 151.5, 151.0, 144.5, 141.6, 138.3, 137.8, 135.1, 135.0, 134.5, 133.7, 133.3, 131.9, 131.8, 131.6, 131.3, 130.7, 130.0, 129.5, 127.8, 127.2, 127.1, 125.2, 125.1, 124.1, 120.1, 100.7, 97.2, 76.0, 74.0, 73.7, 73.6, 73.4, 72.8, 72.7, 70.5, 68.5, 67.2, 66.8, 66.7, 66.6, 64.8, 57.2, 47.6, 47.4, 43.5, 42.7, 42.0, 40.9, 40.1, 35.0, 34.7, 26.9, 19.3, 18.7, 18.3, 10.5, 7.01, 6.89, 6.88, 6.72, 6.68, 6.65, 6.59, 5.80, 5.56, 5.29, 5.28, 5.24, 5.21, 5.16, 5.02.

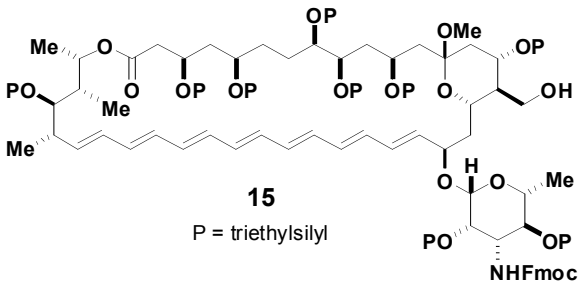
HRMS (ESI)

calculated for $C_{122}H_{214}N_2O_{18}SSi_9$ $(M + Na)^+$:	2302.3424
found:	2302.3328



Hydroxymethyl derivative 15

To a stirred solution of **14** (4.6 g, 2.02 mmol, 1 eq) in diethyl ether (100 mL) at 23 °C was added dropwise a solution of lithium borohydride in THF (2M, 10 mL, 20 mmol, 10 eq). The solution was stirred for 2 hours and then cooled to 0 °C and quenched by addition over 5 minutes of saturated aqueous ammonium chloride (100 mL). The two layers were separated and the organic phase was diluted with diethyl ether (100 mL). The organic phase was washed with saturated ammonium chloride (1 x 20 mL), water (3 x 20 mL) and brine (1 x 20 mL). The combined aqueous washings were back-extracted with diethyl ether (1 x 50 mL) and the combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. Purification of the resulting residue by flash chromatography (SiO_2; hexanes:diethyl ether 20:1 → 5:1) furnished **15** as a yellow solid (3.87 g, 1.78 mmol, 88%).



TLC (hexanes:diethyl ether 1:1)

R_f =0.67, visualized by UV

^{1}H NMR (500 MHz, acetone *d-6*)

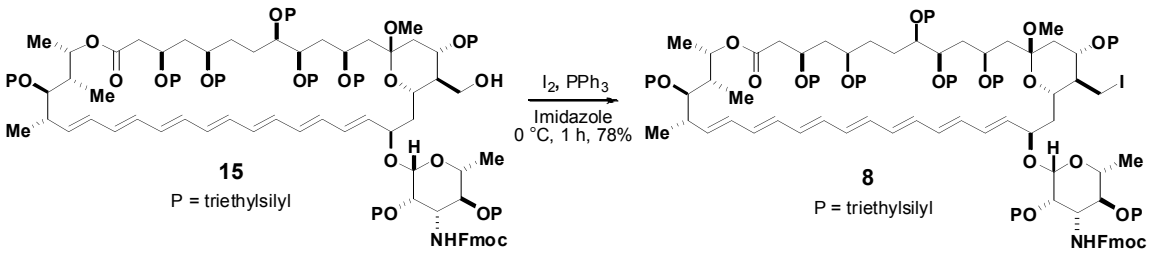
δ 7.86 (d, *J* = 8 Hz, 2H), 7.69 (d, *J* = 7.5 Hz, 2H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 6.53-6.10 (m, 12H), 6.05 (dd, *J* = 6.5, 15.5 Hz, 1H), 5.50 (dd, *J* = 9.5, 15 Hz, 1H), 5.32 (d, *J* = 10 Hz, 1H) 4.77 (s, 1H), 4.68 (app t, *J* = 6 Hz, 2H), 4.46 (dd, *J* = 6.5, 10.5 Hz, 1H), 4.32 (dd, *J* = 6.5, 10.5 Hz, 1H), 4.23 (app t, *J* = 6.5 Hz, 2H), 4.18 (dt, *J* = 5,10.5 Hz, 1H), 4.11 (t, *J* = 10.5 Hz, 1H), 3.99 (m, 1H), 3.91 (d, *J* = 3 Hz, 2H), 3.86-380 (m, 4H), 3.70-3.67 (m, 2H), 3.65-3.59 (m, 3H), 3.46 (t, *J* = 9 Hz, 1H), 3.34 (app dd, *J* = 6.5, 8.5 Hz, 1H), 3.11 (s, 3H), 2.56-2.54 (m, 2H), 2.45-2.38 (m, 2H), 2.10 (dd, *J* = 4.5, 12.5 Hz, 1H), 2.02-2.00 (m, 1H), 1.94-1.61 (m, 10H), 1.52-1.49 (m, 1H), 1.24 (d, *J* = 6.5 Hz, 3H), 1.17 (d, *J* = 6 Hz, 3H), 1.06-0.890 (m, 87H), 0.77-0.57 (m, 54H).

^{13}C NMR (125 MHz, acetone *d-6*)

δ 170.0, 155.8, 144.4, 141.6, 138.6, 135.2, 134.7, 134.1, 133.3, 132.5, 132.4, 132.2, 131.9, 130.8, 130.4, 130.1, 127.9, 127.3, 127.2, 125.2, 125.1, 120.2, 100.4, 97.3, 92.4, 76.1, 75.0, 73.8, 73.4, 72.8, 70.4, 67.4, 66.9, 66.7, 66.6, 66.1, 58.4, 57.5, 49.6, 47.3, 47.1, 44.0, 43.4, 42.7, 40.8, 35.1, 26.8, 19.3, 18.3, 7.01, 6.88, 6.72, 6.68, 5.72, 5.51, 5.23, 5.16, 5.12, 4.99.

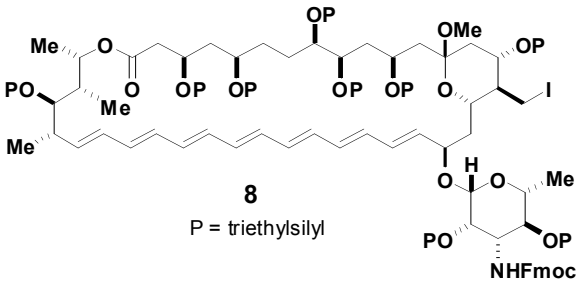
HRMS (ESI)

calculated for $C_{117}H_{213}NO_{18}Si_9$ $(M + Na)^+$:	2195.3604
found:	2195.3503



Iodomethyl derivative 8

Prior to the reaction, **15** was azeotropically dried via coevaporation with benzene (3 x 25 mL) and left under vacuum for a minimum of eight hours. The resulting yellow solid (3.8 g, 1.74 mmol, 1 eq) was dissolved in THF (60 mL) and cooled 0 °C. To this solution was added imidazole (0.355 g, 5.22 mmol, 3 eq), triphenyl phosphine (0.912 g, 3.48 mmol, 2 eq), and iodine (0.880 g, 3.48 mmol, 2 eq). The resulting brown solution was stirred for 1 hour at 0 °C and then quenched with the addition of saturated aqueous sodium bisulfite (50 mL). The two phases were separated and the organic layer was diluted with diethyl ether (50 mL). The organic layer was washed with saturated aqueous sodium bisulfite (1 x 20 mL), water (3 x 20 mL), and brine (1 x 20 mL). The combined aqueous washings were back-extracted with diethyl ether (1 x 20 mL). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. Purification of the resulting residue by flash chromatography (SiO_2; hexanes:diethyl ether 20:1 → 7:1) furnished **8** as an orange solid (3.10 g, 1.36 mmol, 78%) and recovered **15** as a yellow solid (0.545 g, 0.251 mmol, 14%).



8

P = triethylsilyl

TLC (hexanes:diethyl ether 2:1)

R_f = 0.79, visualized by UV

^{1}H NMR (500 MHz, acetone *d*-6)

δ 7.86 (d, *J* = 7.5 Hz, 2H), 7.69 (d, *J* = 6.5 Hz, 2H), 7.41 (t, *J* = 7 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 6.54-6.10 (m, 12H), 6.04 (dd, *J* = 6, 15.5 Hz, 1H), 5.50 (dd, *J* = 9.5, 14.5 Hz, 1H), 5.30 (d, *J* = 10 Hz, 1H), 4.87 (s, 1H) 4.72 (bs, 1H), 4.67 (bs, 1H), 4.46 (dd, *J* = 6.5, 10.5 Hz, 1H), 4.34 (dd, *J* = 6.5, 10.5 Hz, 1H), 4.22 (app t, *J* = 6 Hz, 2H), 4.14-4.04 (m, 3H), 4.00 (bs, 1H) 3.84 (app d, *J* = 7 Hz, 1H), 3.76 (t, *J* = 9 Hz, 1H), 3.72-3.69 (m, 3H) 3.63-3.61 (m, 2H), 3.50 (app d, *J* = 8 Hz, 1H), 3.46 (t, 9.5 Hz, 1H), 3.41-3.37 (m, 2H), 3.14 (s, 3H), 2.57 (bs, 2H), 2.43 (app dd, *J* = 9, 15 Hz, 1H), 2.27 (dd, *J* = 7.5, 14.5 Hz, 1H), 2.16 (dd, *J* = 5, 12.5 Hz, 1H), 1.93-1.86 (m, 3H), 1.81-1.74 (m, 6H) 1.65-1.61 (m, 2H), 1.49 (bs, 1H), 1.25 (d, *J* = 6 Hz, 3H), 1.16 (d, *J* = 6 Hz, 3H), 1.06-0.86 (m, 87H), 0.77-0.55 (m, 54H).

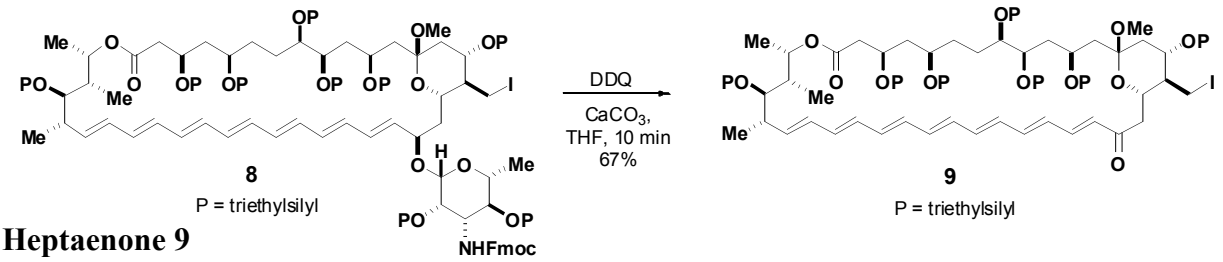
^{13}C NMR (125 MHz, acetone *d*-6)

δ 170.0, 155.7, 144.7, 144.4, 141.6, 138.5, 135.1, 134.6, 134.5, 134.0, 133.2, 132.4, 132.3, 132.0, 130.9, 130.2, 127.9, 127.2, 125.2, 125.1, 120.2, 100.4, 97.3, 76.1, 74.0, 73.4, 72.8, 70.5, 69.9, 68.3, 66.9, 66.7, 66.5, 57.5, 47.4, 47.2, 45.9, 43.8, 42.8, 42.0, 40.6, 40.3, 35.1, 33.5, 26.8, 19.3, 18.6, 18.3, 10.7, 9.23, 7.04, 7.03, 6.92, 6.91, 6.79, 6.77, 6.73, 6.69, 5.80, 5.55, 5.48, 5.31, 5.28, 5.24, 5.20, 5.17.

HRMS (ESI)

calculated for $C_{117}H_{212}INO_{17}Si_9$ $(M + Na)^+$: 2305.2621

found: 2305.2617

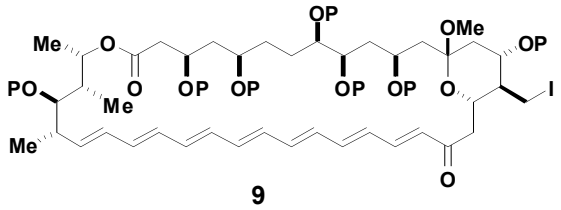


8
P = triethylsilyl

9
P = triethylsilyl

Heptaenone 9

Prior to the reaction, calcium carbonate was dried via storage under vacuum for a minimum of eight hours in the presence phosphorus pentoxide desiccant. Also, **8** was azeotropically dried via coevaporation with benzene (3 x 25 mL) and left under vacuum for a minimum of eight hours. The resulting orange solid (2.45 g, 1.07 mmol, 1 eq) was dissolved in THF (50 mL) at 23 °C and calcium carbonate (1.07 g, 10.7 mmol, 10 eq) was added. DDQ (0.364 g, 1.61 mmol, 1.5 eq) was added and the reaction mixture immediately transitioned to a dark red color. The mixture was stirred for 10 minutes and then quenched with saturated aqueous sodium bicarbonate (250 mL). The resulting red emulsion was extracted with dichloromethane (10 x 100 mL). The combined organic extracts were washed with brine (1 x 100 mL), dried over sodium sulfate, and concentrated *in vacuo*. Purification of the resulting residue by flash chromatography (SiO_2; hexanes:diethyl ether 20:1 → 9:1) afforded **9** as a deep red solid (1.21 g, 0.717 mmol, 67%).



9
P = triethylsilyl

TLC (hexanes:diethyl ether 2:1)

R_f = 0.48, visualized by eye

^{1}H NMR (500 MHz, acetone *d*-6)

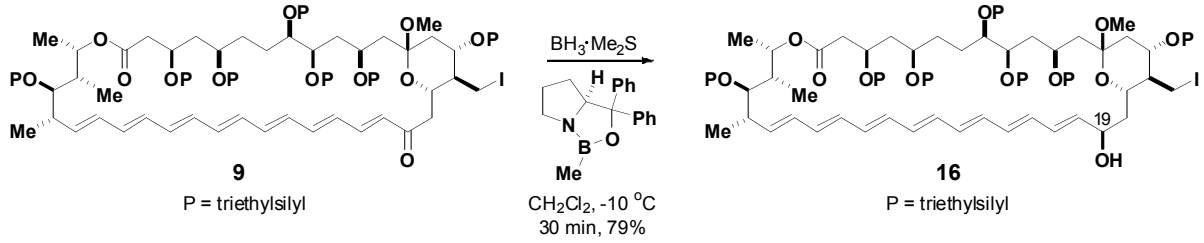
δ 7.84 (dd, *J* = 11.5, 16 Hz, 1H), 7.11 (dd, *J* = 11.5, 14.5 Hz, 1H), 6.80 (dd, *J* = 11, 15 Hz, 1H), 6.61-6.54 (m, 2H), 6.49-6.14 (m, 7H), 6.08 (d, *J* = 15.5 Hz, 1H), 5.52 (dd, *J* = 9.5, 15 Hz, 1H), 4.47-4.44 (m, 1H), 4.27 (app t, *J* = 10.5 Hz, 1H), 4.16-4.07 (m, 3H), 3.96 (dd, *J* = 3, 9 Hz, 1H), 3.75-3.63 (m, 4H), 3.46 (dd, *J* = 3, 10.5 Hz, 1H), 3.01 (dd, *J* = 10.5, 12 Hz, 1H), 2.90 (s, 3H), 2.66 (dd, *J* = 4, 18 Hz, 1H), 2.60 (dd, *J* = 9.5, 18 Hz, 1H) 2.47-2.42 (m, 2H), 2.26 (app t, *J* = 10.5 Hz, 1H), 2.10 (dd, *J* = 5, 12 Hz, 1H), 2.00 (app t, *J* = 10.5 Hz, 1H), 1.93-1.72 (m, 8H), 1.65 (app d, *J* =12.5 Hz, 1H), 1.47 (app t, *J* = 10.5 Hz, 1H), 1.15 (d, *J* = 6 Hz, 3H), 1.11 (t, *J* = 8 Hz, 10H), 1.05-0.96 (m, 51H), 0.89 (t, *J* = 7.5 Hz, 9H), 0.84 (q, *J* = 8 Hz, 6H), 0.76-0.51 (m, 36H).

^{13}C NMR (125 MHz, acetone *d*-6)

δ 199.4, 169.4, 148.1, 142.8, 140.2, 139.4, 138.6, 137.6, 136.5, 131.5, 131.1, 130.9, 130.4, 130.3, 130.2, 129.6, 100.4, 76.1, 73.5, 73.0, 72.9, 70.9, 68.2, 67.2, 66.4, 47.8, 46.5, 46.4, 43.2, 42.7, 42.6, 40.7, 40.3, 36.5, 34.6, 27.2, 24.6, 23.2, 19.5, 19.1, 7.11, 6.90, 6.84, 6.67, 6.62, 5.94, 5.50, 5.38, 5.23, 5.21, 5.08, 4.97.

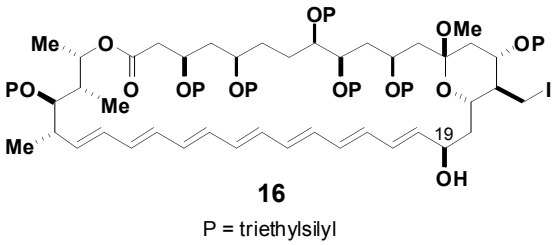
HRMS (ESI)

calculated for $C_{84}H_{161}O_{12}ISi_7$ $(M + Na)^+$: 1707.9316
found: 1707.9270



Allylic alcohol 16

Prior to the reaction, **9** was azeotropically dried via coevaporation with benzene (3 x 10 mL) and was left under vacuum for a minimum of eight hours. To a stirred solution of borane dimethyl sulfide complex (27 μL, 0.270 mmol, 1.2 eq), and (*S*)-2-methyl-CBS-oxazaborolidine (0.225 mL, 0.225 mmol, 1 eq, 1M in toluene) in dichloromethane (1 mL) at -10 °C was added **9** (380 mg, 0.225 mmol, 1 eq) dropwise as a solution in dichloromethane (6.5 mL). The resulting solution was stirred for 30 minutes at -10 °C and during this time a color change from deep red to pale orange was observed. The reaction was quenched at -10 °C with saturated aqueous ammonium chloride (10 mL) and diluted with dichloromethane (50 mL). The two layers were separated and the organic layer was washed with saturated aqueous ammonium chloride (1 x 20 mL), water (3 x 20 mL), and brine (1 x 20 mL). The combined aqueous washings were back-extracted with dichloromethane (1 x 50 mL). The combined organic extracts were dried over sodium sulfate and concentrated *in vacuo*. Purification of the resulting residue by flash chromatography (SiO_2; hexanes:diethyl ether 19:1 → 6:1) furnished **16** (300 mg, 0.177 mmol, 6:1 d.r., 79%). The major diastereomer was assigned as having the (*R*) configuration at C-19 based on extensive NMR characterization of final product **2** as described in detail on pages S19-S21.



TLC (hexanes:diethyl ether 2:1)

R_f = 0.35, stained by anisaldehyde

^{1}H NMR (500 MHz, acetone *d*-6)

δ 6.48-6.10 (m, 13H), 5.53 (dd, *J* = 9.5, 15 Hz, 1H), 4.71 (bs, 1H), 4.52 (bs, 1H), 4.25 (bs, 1H), 4.10 (t, *J* = 10 Hz, 1H), 4.06-3.98 (m, 2H), 3.88 (app d, *J* = 1.5, 1H) 3.80 (app t, *J* = 9 Hz, 2H), 3.68-3.61 (m, 4H), 3.37 (app d, *J* = 10 Hz, 1H), 3.13 (s, 3H), 2.55 (t, *J* = 6 Hz, 2H), 2.42 (app dd, *J* = 7, 14 Hz, 1H), 2.17 (dd, *J* = 5, 10.5 Hz, 1H), 1.99-1.84 (m,

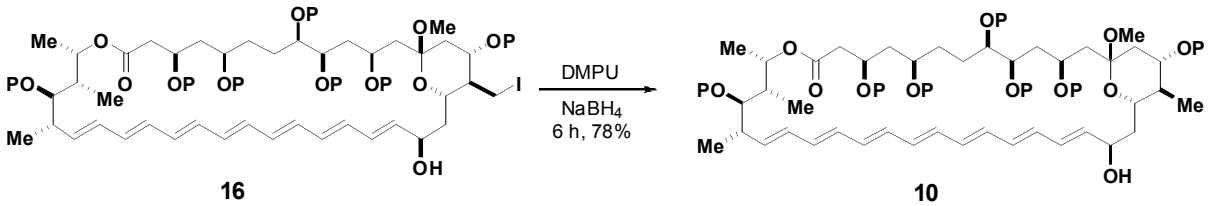
5H), 1.78-1.69 (m, 5H), 1.62-1.61 (m, 3H), 1.53-1.47 (m, 1H), 1.17 (d, *J* = 6 Hz, 3H), 1.06-0.95 (m, 69H), 0.75-0.62 (m, 42H).

^{13}C NMR (125 MHz, acetone *d*-6)

δ 173.6, 142.7, 141.7, 137.9, 137.7, 136.9, 136.3, 136.0, 135.9, 135.6, 135.5, 134.5, 134.4, 133.8, 130.7, 104.0, 79.6, 77.0, 76.1, 73.9, 73.4, 72.0, 71.9, 70.4, 70.3, 50.9, 50.8, 50.6, 49.1, 47.5, 46.4, 45.9, 44.2, 43.9, 42.4, 40.1, 38.6, 30.3, 28.2, 22.7, 22.0, 14.2, 12.5, 10.6, 10.4, 10.2, 9.32, 9.09, 9.03, 8.82, 8.75, 8.73.

HRMS (ESI)

calculated for $C_{84}H_{163}O_{12}ISi_7$ $(M + Na)^+$: 1709.9472
found: 1709.9456

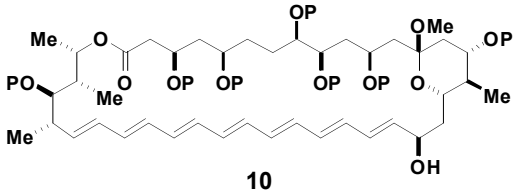


16
P = triethylsilyl

10
P = triethylsilyl

C(41)-methyl derivative 10

Prior to the reaction, **16** was azeotropically dried via coevaporation with benzene (3 x 25 mL) and left under vacuum for a minimum of eight hours. The resulting orange solid (775 mg, 0.459 mmol, 1 eq) was dissolved in DMPU (15 mL) at 23 °C and sodium borohydride (87 mg, 2.3 mmol, 5 eq) was added. The solution was stirred for 6 hours and was then quenched with saturated aqueous ammonium chloride (5 mL). The resulting yellow emulsion was diluted with hexanes (50 mL) and the mixture washed with water (5 x 10 mL) and brine (1 x 10 mL). The combined aqueous washings were back-extracted with hexanes (25 mL). The combined organic extracts were dried over sodium sulfate and concentrated *in vacuo*. Purification of the resulting residue by flash chromatography (SiO_2; hexanes:diethyl ether 20:1→10:1) yielded **10** as an orange solid (557 mg, 0.356 mmol, 78%).



10
P = triethylsilyl

TLC (hexanes:diethyl ether, 2:1)

R_f = 0.28, stained by anisaldehyde

^{1}H NMR (500 MHz, acetone *d*-6)

δ 6.49-6.10 (m, 13H), 5.52 (dd, *J* = 9.5, 15 Hz, 1H), 4.71 (app t, *J* = 6.5 Hz, 1H), 4.47 (bs, 1H), 4.28-4.24 (m, 1H), 4.10 (t, *J* = 10 Hz, 1H), 4.00-3.97 (m, 1H), 3.85 (d, *J* = 4 Hz, 1H), 3.82 (dd, *J* = 2.5, 9 Hz, 1H) 3.76-3.67 (m, 3H), 3.62 (dd, *J* = 4.5, 10.5 Hz, 1H), 3.56

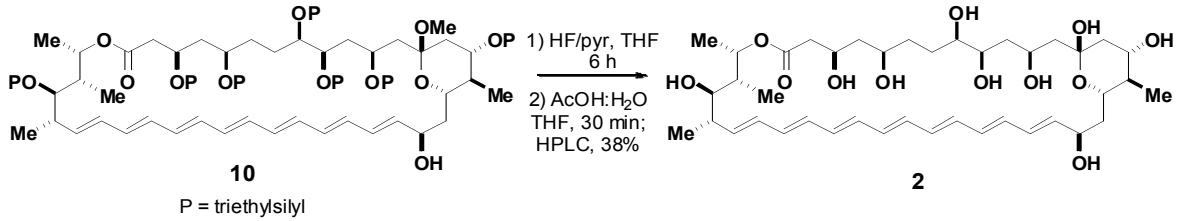
(app t, J = 8.5 Hz, 1H), 3.10 (s, 3H), 2.55 (t, J = 6.5 Hz, 2H), 2.42 (app dd, J = 9, 16 Hz, 1H), 2.01-1.97 (m, 2H), 1.93-1.84 (m, 3H), 1.80-1.74 (m, 3H), 1.73-1.70 (m, 2H) 1.68-1.62 (m, 3H), 1.53-1.50 (m, 1H), 1.36-1.27 (m, 2H) 1.17 (d, J = 6 Hz, 3H), 1.06-0.94 (m, 72H), 0.75-0.61 (m, 42H).

^{13}C NMR (125 MHz, acetone *d*-6)

δ 170.1, 139.1, 138.2, 134.3, 134.2, 133.4, 132.7, 132.5, 132.4, 132.1, 132.0, 131.0, 130.9, 130.2, 128.5, 127.2, 100.5, 76.1, 73.5, 72.6, 71.6, 70.7, 70.4, 68.7, 68.5, 67.0, 66.8, 47.2, 47.1, 44.1, 42.9, 42.8, 40.8, 40.4, 39.5, 35.1, 26.8, 19.2, 18.5, 13.3, 10.7, 10.6, 7.04, 7.01, 6.90, 6.71, 6.69, 5.78, 5.57, 5.31, 5.27, 5.24, 5.22.

HRMS (ESI)

calculated for $C_{84}H_{164}O_{12}Si_7$ $(M+Na)^+$:	1584.0506
found:	1584.0481



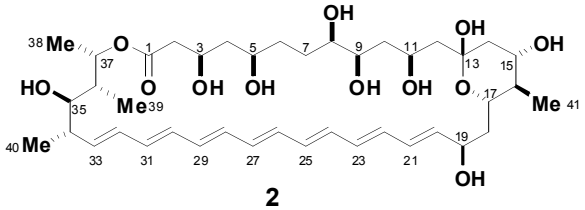
10

P = triethylsilyl

2

C41-Methyl amphoteronolide B (2)

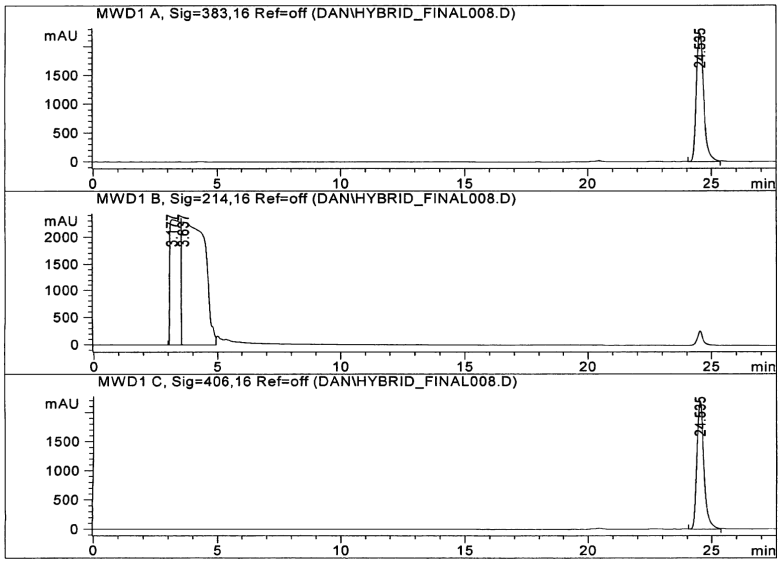
To a stirred solution of **10** (50 mg, 0.032 mmol) in THF:pyridine 3:2 (5 mL) at 0 °C was added dropwise 70% HF/pyridine complex (320 μL). The solution was stirred for 6 hours at 0 °C and was then quenched with the addition of trimethylsilyl ethoxide (2 mL). The solution was concentrated *in vacuo* and the resulting residue was dissolved in THF (1 mL) and AcOH:H_2O 1:1 (1 mL). The solution was stirred for 30 minutes and was then concentrated *in vacuo*. The resulting orange solid was immediately dissolved in DMSO (1.5 mL) and purified by preparative RP-HPLC (Waters SunFire Prep C_{18} OBD 5 micron 30 x 150 mm Lot # 168I161701 300 μL injection volume, 25 mL/min flow rate, MeCN:H_2O 1:19 → 19:1, over 25 minutes) to yield C(41)-methyl amphoteronolide B (**2**) as a yellow solid (9 mg, 0.012 mmol, 38% over two steps).



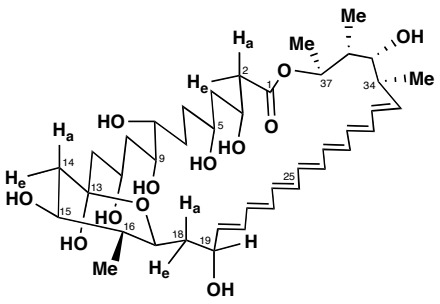
2

HPLC

tR = 24.9 min, flow rate = 25 mL/min, gradient of 5 → 95% MeCN in H_2O over 25 min.



As described in detail in Section III, proton and coupling constant assignments were made using a variety of two-dimensional NMR techniques including phase-sensitive COSY experiments combined with amplitude constrained multiplet evaluation (ACME).[3] Briefly, as demonstrated in section III, the polyene macrolide skeletons of **1**-**4** are quite rigid and hydrogen atoms can be assigned as having pseudoequatorial (e) and pseudoaxial (a) orientations.[4] The labeling scheme used herein is consistent with that utilized in Ref 19a of the text.



^{1}H NMR (600 MHz, pyridine *d*-5, CD_3OD 10:1)

δ 6.83 (dd, J = 10.8, 14.7 Hz, 1H, **H-22**), 6.77 (dd, J = 10.8, 15 Hz, 1H, **H-24**), 6.70 (dd, $J_{19,20}$ = 8.9 Hz, $J_{20,21}$ = 15 Hz, 1H, **H-20**), 6.60 (dd, J = 11.4, 14.4 Hz, 1H, **H-23**), 6.55-6.35 (m, 9H), 5.79 (app d, $J_{36,37}$ = 3.1 Hz, 1H, **H-37**), 5.55 (dd, $J_{32,33}$ = 15.6, $J_{33,34}$ = 9.9 Hz, 1H, **H-33**), 4.87 (app t, $J_{10a,11}$ = 1.3 Hz, $J_{10e,11}$ = 10.9 Hz, $J_{11,12a}$ = 1.2 Hz, $J_{11,12e}$ = 10.7 Hz, 1H, **H-11**), 4.77 (m, $J_{18a,19}$ = 3.9, $J_{18e,19}$ = 5.5 Hz, $J_{19,20}$ = 8.9 Hz, 1H, **H-19**), 4.65 (m, $J_{2e,3}$ = 9.2 Hz, $J_{3,4a}$ = 4.3, $J_{3,4e}$ = 10.9 Hz, $J_{16,17}$ = 10.8 Hz, $J_{17,18a}$ = 8.1 Hz, 2H, **H-3**, **H-17**), 4.20 (app dt, $J_{14e,15}$ = 2.1, $J_{14a,15}$ = 11.5 Hz, $J_{15,16}$ = 10.8 Hz, 1H, **H-15**), 4.12 (app t, $J_{4a,5}$ = 1.8, $J_{4e,5}$ = 10.3 Hz, $J_{5,6a}$ = 1.1, $J_{5,6e}$ = 11.5 Hz, 1H, **H-5**), 4.03 (app dd, $J_{8,9}$ = 3.2 Hz, $J_{9,10a}$ = 2.3, $J_{9,10e}$ = 11.7 Hz, 1H, **H-9**), 3.60 (app dd, $J_{7e,8}$ = 2.4, $J_{7a,8}$ = 11.3 Hz, $J_{8,9}$ = 3.2 Hz, 1H, **H-8**), 3.44 (app d, $J_{34,35}$ = 10.0 Hz, $J_{35,36}$ = 2.6 Hz, 1H, **H-35**), 2.68 (m, $J_{33,34}$ = 9.9 Hz, $J_{34,35}$ = 10.0 Hz, 1H, **H-34**), 2.62 (dd, $J_{2a,2e}$ = 16.2 Hz, $J_{2e,3}$ = 9.2 Hz, 1H, **H-2e**), 2.49-2.44 (m, $J_{14e,15}$ = 2.1 Hz, $J_{18e,19}$ = 5.5 Hz, 3H, **H-2a**, **H-14e**, **H-18e**), 2.37-2.34 (m, $J_{6a,7a}$ = 10.6 Hz, $J_{6e,7a}$ = 5.7 Hz, $J_{7a,8}$ = 11.3, 1H, **H-7a**), 2.19-2.15 (m, $J_{9,10e}$ = 11.7 Hz, $J_{10e,11}$ = 10.9 Hz, 1H, **H-10e**) 2.13-2.10 (m, $J_{35,36}$ = 2.6 Hz, $J_{36,37}$ = 3.1 Hz, 1H, **H-36**), 2.02 (ddd, $J_{17,18a}$ = 8.1 Hz, $J_{18a,18e}$ = 14.4 Hz, $J_{18a,19}$ = 3.9 Hz, 1H, **H-18a**), 1.99-1.95 (m, $J_{5,6e}$ = 11.5 Hz, $J_{6e,7a}$ = 5.7 Hz, $J_{6e,7e}$ = 13.5 Hz, $J_{11,12e}$ = 10.7 Hz, 2H, **H-6e**, **H-12e**), 1.85-1.74 (m, $J_{3,4e}$ = 10.9 Hz, $J_{4e,5}$ = 10.3 Hz, $J_{6e,7e}$ = 13.5 Hz, $J_{7e,8}$ = 2.4 Hz, $J_{11,12a}$ = 1.2 Hz, $J_{14a,15}$ = 11.5 Hz, 4H, **H-4e**, **H-7e**, **H-12a**, **H-14a**), 1.72-1.66 (m, $J_{5,6a}$ = 1.1 Hz, $J_{6a,7a}$ = 10.6 Hz, $J_{15,16}$ = 10.8 Hz, 2H, **H-6a**, **H-16**), 1.63 (app dt, $J_{3,4a}$ = 4.3 Hz, $J_{4a,4e}$ = 14.1 Hz, $J_{4a,5}$ = 1.8 Hz, 1H, **H-4a**), 1.56 (app dd, $J_{9,10a}$ = 2.3Hz, $J_{10a,10e}$ = 14.4 Hz, $J_{10a,11}$ = 1.3 Hz, 1H, **H-10a**), 1.45 (d, $J_{37,38}$ = 6.6 Hz, 3H, **H-38**), 1.34 (d, $J_{16,41}$ = 6 Hz, 3H, **H-41**), 1.31 (d, $J_{34,40}$ = 6 Hz, 3H, **H-40**), 1.24 (d, $J_{36,39}$ = 7.2 Hz, 3H, **H-39**).

^{13}C NMR (150 MHz, pyridine *d*-5 : CD_3OD, 10:1)

δ 172.1, 141.4, 137.5, 135.1, 134.8, 134.2, 133.6, 133.4, 133.1, 132.8, 132.7, 129.1, 98.3, 78.7, 76.3, 75.3, 75.2, 72.2, 71.4, 70.4, 69.7, 68.5, 48.2, 46.7, 45.1, 44.4, 43.8, 43.0, 41.5, 41.2, 41.0, 36.6, 31.9, 30.2, 29.8, 19.1, 17.4, 14.1, 12.8.

HRMS (ESI)

calculated for $C_{41}H_{64}O_{12}$ $(M + Na)^+$: 771.4295
found: 771.4268

[4] Ganis, P.; Avitabile, G.; Mechlinski, W.; Schaffner, C.P. *J. Am. Chem. Soc.* **1971**, *93*, 4560-4564.

The following data are consistent with the assignment of the C19 stereocenter of MeAmdeB (2) as 19-(*R*):

1. The 19-(*R*) assignment is consistent with preference for peripheral hydride attack precedented by Nicolaou and coworkers and the matched/mismatched relationships observed with the CBS catalysts.

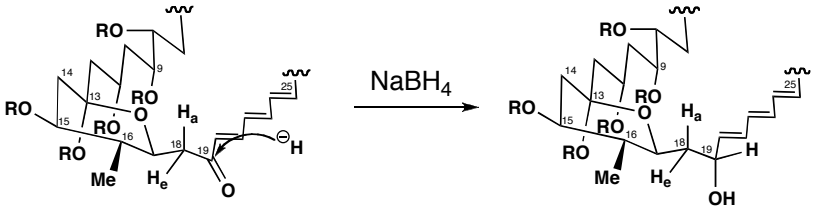
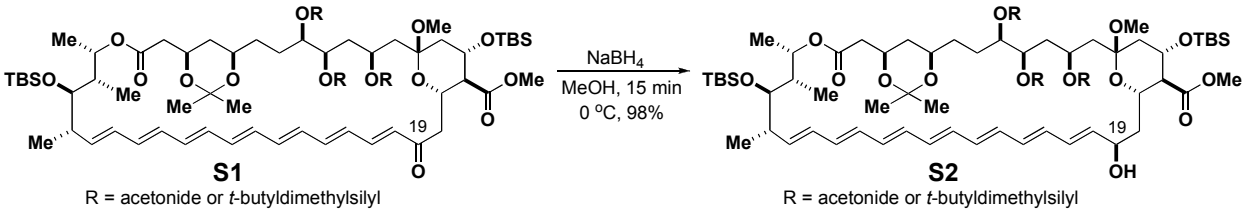


Figure S1. The peripheral mode of hydride attack.

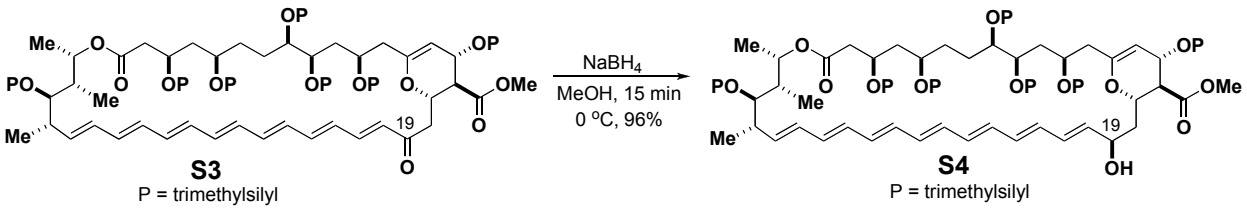
Specifically, in the context of degradative synthetic studies with amphotericin B,[5] Nicolaou and coworkers performed the following transformation of **S1** into **S2**:



S1
R = acetonide or *t*-butyldimethylsilyl

S2
R = acetonide or *t*-butyldimethylsilyl

A single stereoisomer at C(19) was observed, consistent with a predicted strong preference for peripheral hydride attack as shown in Fig. S1. The assignment of this stereocenter as the 19-(*R*) isomer was ultimately confirmed unambiguously via total synthesis of amphotericin B.[6] Nicolaou and coworkers also demonstrated that the protective groups can be changed without altering the observed diastereomeric outcome.[5] For example, octatrimethylsilyl ether derivative **S3** was reduced to allylic alcohol **S4** as the only reduction product.[5]



S3
P = trimethylsilyl

S4
P = trimethylsilyl

We observed the same result (a single diastereomer) for the reduction of enone **19** to allylic alcohol **11** using $NaBH_4$ in the context of our alternative synthesis of amphoteronolide B **3** (see S23-S24). However, the $NaBH_4$-mediated reduction of enone **9** to allylic alcohol **16** en route to

[5] Nicolaou, K.C.; Chakraborty, T.K.; Ogawa, Y.; Daines, R.A.; Simpskins, N.S.; Furst, G.T. *J. Am. Chem. Soc.* **1988**, *110*, 4660-4672.
[6] a) Nicolaou, K.C.; Daines, R.A.; Chakraborty, T.K.; Ogawa, Y. *J. Am. Chem. Soc.* **1987**, *109*, 2821-2822. B) Nicolaou, K.C.; Daines, R.A.; Chakraborty, T.K. *J. Am. Chem. Soc.* **1987**, *109*, 2208-2210.

MeAmdeB (**2**) was less selective (~2:1 mixture of diastereomers). Based on the precedent described above, we tentatively assigned the major isomer as 19-(*R*). Consistent with this, when we performed this same reduction of **9** to **16** with (*S*)-2-methyl-CBS-oxazaborolidine, the catalyst enantiomer predicted to deliver the desired 19 (*R*) isomer (the matched case), we obtained a significant enhancement in diastereoselectivity with the same major diastereomer being formed with a d.r. of 6:1. Alternative use of the (*R*)-2-methyl-CBS-oxazaborolidine (the predicted mismatched case) led to a 1:1 mixture of diastereomeric products.

2. The observed $J_{18a,19}$ and $J_{18e,19}$ coupling constants for MeAmdeB (2) are consistent with those calculated for the 19-(*R*) configuration and with those observed for AmB derivatives known to have the 19-(*R*) configuration.

As described in detail in Section III, we have determined the skeletons of **1**-**4** to be quite rigid, making it possible to calculate predicted coupling constants using the Altona Karplus equation.[7] Moreover, we have utilized phase-sensitive COSY (COSYPS) techniques and amplitude constrained multiplet evaluation[3] to unambiguously assign the $J_{18a,19}$ and $J_{18e,19}$ coupling constants. As shown in Fig. S2A, for the 19-(*R*) isomer, it is predicted that both $J_{18a,19}$ and $J_{18e,19}$ will be < 8 Hz. This is not the case for the 19-(*S*) isomer ($J_{18a,19}$ is predicted to be > 8 Hz). As shown in Fig. S2B, the observed $J_{18a,19}$ and $J_{18e,19}$ couplings for MeAmdeB (**2**) are consistent only with the calculated values for the 19-(*R*) isomer. Moreover, these values are consistent with those observed for AmB derivatives **17**[8] and **23** for which the C(19) stereocenter was not altered.

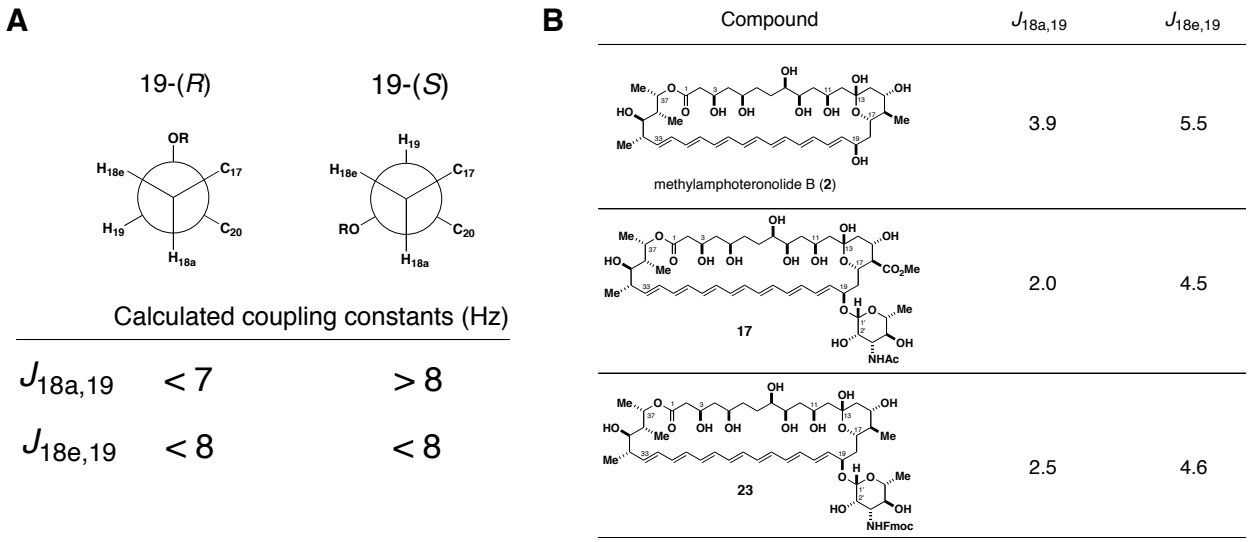


Figure S2. Coupling constants are consistent with the 19-(*R*) assignment for MeAmdeB (**2**).

[7] Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron*, **1980**, *36*, 2783-2792.
[8] a) Schaffner, C.P.; *J. Antibiot.* **1972**, *25*, 256. b) Pandey, R.C.; Rinehart, K.L., Jr. *J. Antibiot.* **1977**, *30*, 158.

3. The 19-(*R*) assignment for MeAmdeB (2) is consistent with NOEs observed in all four compounds 17, **2**, **21**, and **23** (See Section III for details).

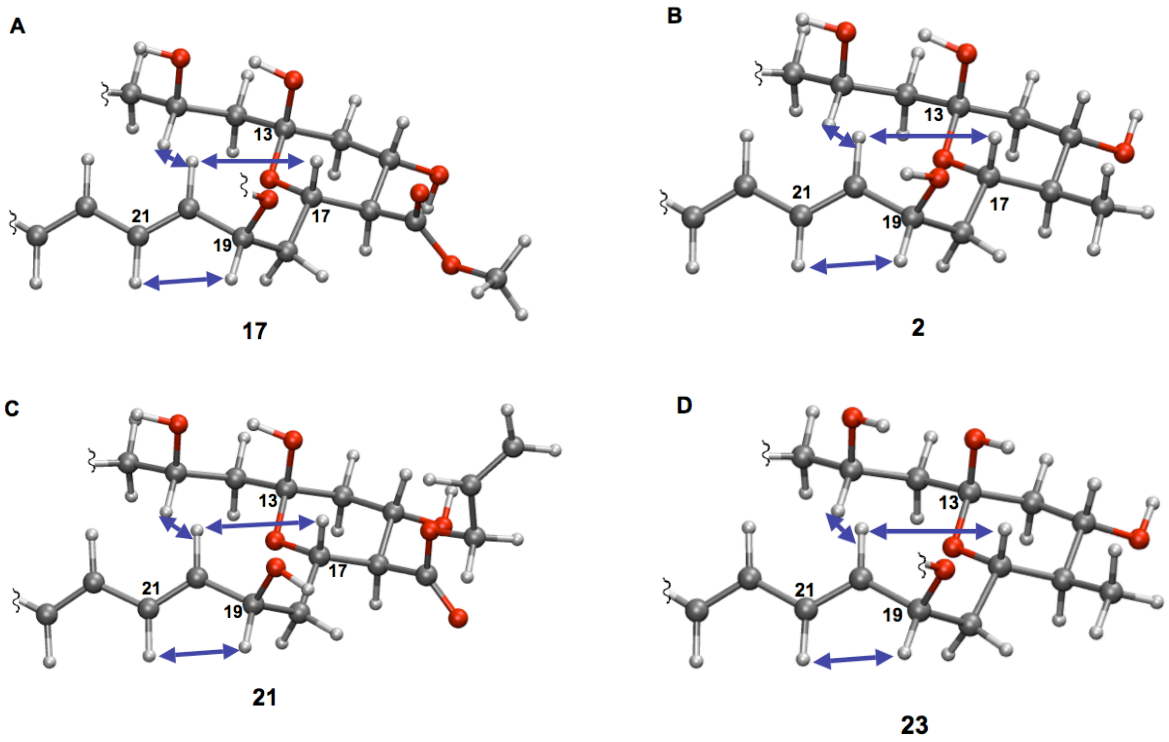
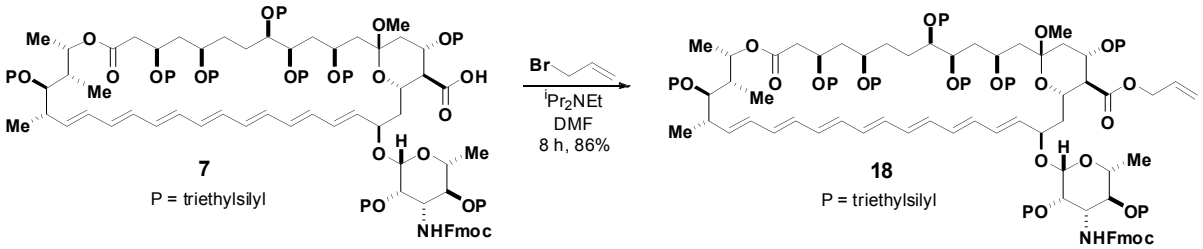


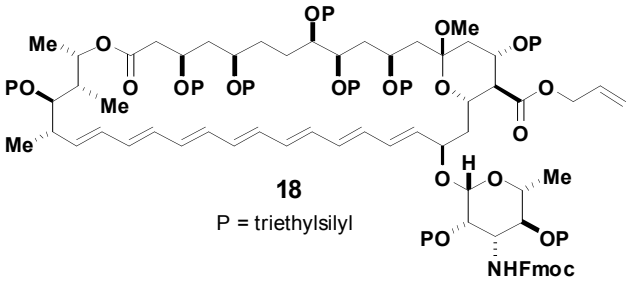
Figure S3. Observed NOEs (double-headed arrows) are consistent with the 19-(*R*) assignment for MeAmdeB (**2**).

In all four **17**, **2**, **21**, and **23** compounds an NOE correlation between H-19 and H-21 was observed, which is consistent with an $A_{1,3}$ type interaction between H-19 and H-21 (Figure S3A-D). This NOE is expected to be present only with the 19-*R* configuration.



Allyl ester 18

To a stirred solution of **7** (4.95 g, 2.26 mmol, 1 eq) in DMF:MeOH 10:1 (82.5 mL) at 23 °C was added allyl bromide (7.5 mL, 85.9 mmol, 38 eq) and diisopropyl ethyl amine (1.75 mL, 9.9 mmol, 4.4 eq). The solution was stirred for 8 hours and then diluted with water:saturated aqueous sodium bicarbonate 1:1 (250 mL). The aqueous phase was extracted with diethyl ether (4 x 100 mL) and the combined organic extracts were washed with brine (1 x 50 mL), dried over sodium sulfate and concentrated *in vacuo*. Purification of the resulting residue by flash chromatography (SiO_2; hexanes:diethyl ether 20:1 → 10:1) yielded **18** as a yellow solid (4.33 grams, 1.94 mmol, 86%).



TLC (hexanes:diethyl ether 2:1)

R_f = 0.85, visualized by UV

^{1}H NMR (500 MHz, acetone *d-6*)

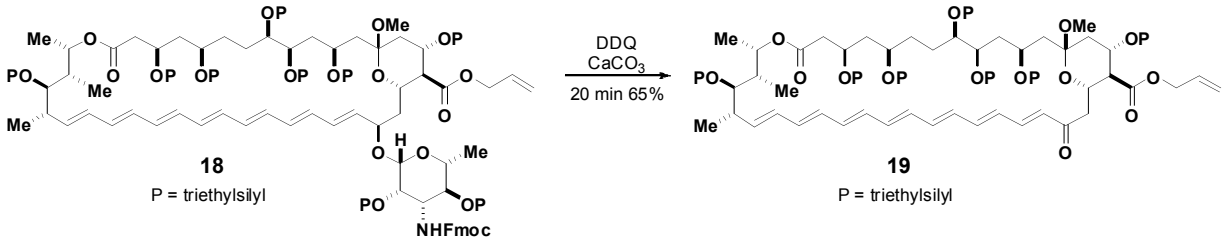
δ 7.87 (d, *J* = 7.5 Hz, 2H), 7.69 (d, *J* = 7.5 Hz, 2H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.34 (t, *J* = 7.5 Hz, 2H), 6.57-6.50 (m, 2H), 6.48-6.13 (m, 10H), 6.06-5.99 (m, 1H), 5.98 (dd, *J* = 5.5, 16 Hz, 1H), 5.49 (dd, *J* = 9.5, 14.5 Hz, 1H), 5.43 (dd, *J* = 1.5, 17 Hz, 1H), 5.34 (d, *J* = 10 Hz, 1H), 5.29 (d, *J* = 10.5 Hz, 1H), 4.74 (dd, *J* = 6, 13.5 Hz, 1H), 4.63-4.51 (m, 3H), 4.49-4.41 (m, 3H), 4.33 (dd, *J* = 6.5, 10.5 Hz, 1H), 4.26-4.23 (m, 2H), 4.12 (t, *J* = 10 Hz, 1H), 4.02-3.97 (m, 2H), 3.90 (d, *J* = 3 Hz, 1H), 3.85 (dd, *J* = 2.5, 6.5 Hz, 1H), 3.71-3.67 (m, 2H), 3.66-3.62 (m, 3H), 3.45 (t, *J* = 8.5 Hz, 1H), 3.43-3.30 (m, 1H), 3.13 (s, 3H), 2.58-2.56 (m, 2H) 2.43 (app dd, *J* = 9, 16 Hz, 1H), 2.36 (t, *J* = 10.5 Hz, 1H), 2.00-1.96 (m, 2H), 1.93-1.90 (m, 2H), 1.84-1.58 (m, 8H), 1.50 (bs, 1H), 1.23 (d, *J* = 6 Hz, 3H), 1.17 (d, *J* = 6 Hz, 3H), 1.07-0.89 (m, 87H), 0.78-0.56 (m, 54H).

^{13}C NMR (125 MHz, acetone *d*-6)

δ 172.9, 170.5, 156.3, 145.0, 144.9, 142.2, 142.1, 139.3, 135.6, 135.5, 135.0, 134.5, 134.2, 133.6, 133.4, 132.4, 132.3, 132.1, 131.6, 131.2, 130.6, 128.4, 127.8, 127.7, 125.8, 125.7, 120.7, 119.0, 101.4, 98.6, 76.6, 75.7, 74.5, 74.0, 73.9, 73.4, 73.2, 71.0, 68.9, 67.6, 67.4, 67.3, 67.2, 65.9, 58.1, 57.5, 48.1, 48.0, 47.8, 44.2, 43.3, 42.2, 41.4, 40.7, 36.2, 35.6, 27.4, 22.9, 19.9, 19.2, 18.8, 15.5, 11.1, 7.58, 7.53, 7.43, 7.42, 7.29, 7.23, 7.20, 7.19, 7.07, 6.30, 6.11, 5.84, 5.82, 5.81, 5.76, 5.72, 5.70, 5.58.

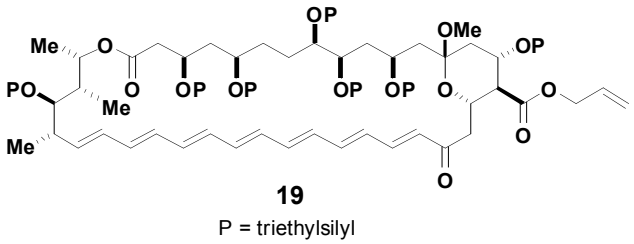
HRMS (ESI)

calculated for $C_{120}H_{215}NO_{19}Si_9$ $(M + Na)^+$:	2249.3710
found:	2249.3630



Heptaenone 19

Prior to the reaction, calcium carbonate was dried via storage under vacuum for a minimum of eight hours in the presence of phosphorus pentoxide desiccant. Also, **18** was azeotropically dried via coevaporation with benzene (3 x 25 mL) and left under vacuum for a minimum of eight hours. The resulting yellow solid (4.33 g, 1.94 mmol, 1 eq) was dissolved in THF (100 mL) at 23 °C and calcium carbonate (1.94 g, 19.4 mmol, 10 eq) was added. DDQ (0.660 g, 2.91 mmol, 1.5 eq) was added and an immediate color change to dark red was observed. This mixture was stirred for 20 minutes and then quenched with the addition of 100 mL saturated aqueous sodium bicarbonate. The resulting emulsion was diluted with water (300 mL) and extracted with dichloromethane (10 x 250 mL). The combined organic extracts were washed with brine (1 x 150 mL) and concentrated *in vacuo*. Purification of the resulting residue by flash chromatography (SiO_2; hexanes:Et_2O 20:1 → 6:1) yielded **19** as a dark orange solid (2.06 g, 1.26 mmol, 65%).



TLC (hexanes:Et_2O 2:1)

R_f = 0.44, visualized by eye

^{1}H NMR (500 MHz, acetone *d*-6)

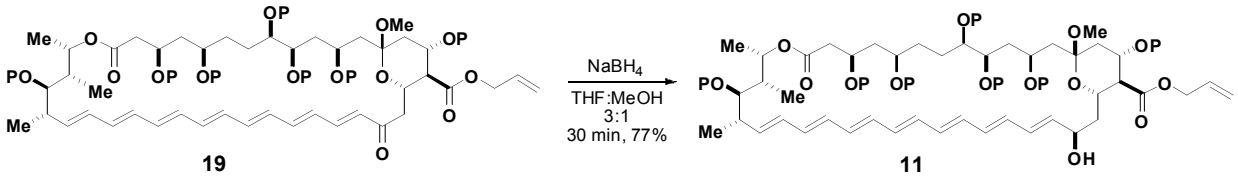
δ 7.84 (dd, *J* = 11, 15.5 Hz, 1H), 7.13 (dd, *J* = 11.5, 14.5 Hz, 1H), 6.81 (dd, *J* = 11, 14.5 Hz, 1H), 6.62-6.55 (m, 2H), 6.49-6.14 (m, 7H), 6.05 (d, *J* = 15.5 Hz, 1H), 6.01 (app ddd, *J* = 6, 10, 16 Hz, 1H), 5.51 (dd, *J* = 10, 15 Hz, 1H), 5.42 (dd, *J* = 1.5, 17.5 Hz, 1H), 5.27 (dd, *J* = 1.5, 10.5 Hz, 1H), 4.68 (app d, *J* = 5.5 Hz, 2H), 4.50 (dd, *J* = 5, 10.5 Hz, 1H), 4.45 (dd, *J* = 5.5, 10 Hz, 1H), 4.28 (app t, *J* = 10.5 Hz, 1H), 4.13-4.08 (m, 2H), 3.96 (dd, *J* = 3, 9.5 Hz, 1H), 3.77 (t, *J* = 10.5 Hz, 1H), 3.72-3.64 (m, 4H), 3.20 (dd, *J* = 11, 12.5 Hz, 1H), 2.92 (s, 3H), 2.70 (dd, *J* = 4, 18 Hz, 1H), 2.62 (dd, *J* = 10, 18 Hz, 1H), 2.46 (app dd, *J* = 9, 16 Hz, 1H), 2.40 (t, *J* = 10.5 Hz, 1H), 2.30 (app t, *J* = 11 Hz, 1H), 2.13 (d, *J* = 12 Hz, 1H), 1.96-1.86 (m, 4H), 1.83-1.76 (m, 4H), 1.67 (app d, *J* = 12.5 Hz, 1H), 1.50 (app t, *J* = 10.5 Hz, 1H), 1.17 (d, *J* = 6 Hz, 3H), 1.15 (t, *J* = 8 Hz, 9H), 1.09-0.96 (m, 60H), 0.89 (q, *J* = 8.5 Hz, 6H), 0.78-0.54 (m, 36H)

^{13}C NMR (125 MHz, acetone *d*-6)

δ 199.3, 172.1, 169.9, 149.2, 143.7, 140.8, 140.2, 139.2, 138.3, 137.1, 133.2, 132.0, 131.6, 131.3, 130.7, 130.6, 130.5, 130.1, 118.6, 101.2, 76.7, 74.1, 73.6, 71.5, 70.4, 68.9, 67.8, 67.0, 65.9, 57.9, 48.4, 47.1, 44.6, 43.5, 43.1, 40.9, 40.7, 39.9, 35.1, 27.7, 20.0, 19.7, 10.9, 7.66, 7.45, 7.44, 7.39, 7.21, 7.16, 6.98, 6.50, 6.06, 5.80, 5.77, 5.63, 5.54, 5.50.

HRMS (ESI)

calculated for $C_{87}H_{164}O_{14}Si_7$ $(M + Na)^+$:	1652.0404
found:	1652.0461

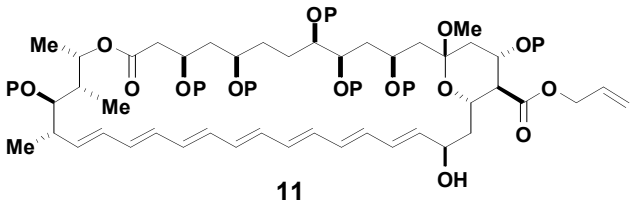


19
P = triethylsilyl

11
P = triethylsilyl

Allylic alcohol 11

Prior to the reaction **19** was azeotropically dried via coevaporation with benzene (3 x 25 mL) and left under vacuum for a minimum of eight hours. The resulting red solid (2.05 g, 1.26 mmol, 1 eq) was dissolved in THF:MeOH 3:1 (12 mL) the resulting solution was cooled to 0 °C and sodium borohydride (0.480 g, 12.6 mmol, 10 eq) was added. The solution was stirred for 30 minutes and a color change from dark red to light orange was observed during the course of the reaction. The reaction was then quenched at 0 °C by the addition of saturated aqueous ammonium chloride (100 mL). The resulting emulsion was diluted with diethyl ether (250 mL). The two layers were separated and the organic phase was washed with water (3 x 50 mL) and brine (1 x 50 mL). The combined aqueous washings were back-extracted with diethyl ether (1 x 100 mL) and the combined organic extracts were dried over sodium sulfate and concentrated *in vacuo*. Purification of the resulting residue by flash chromatography (SiO_2; hexanes:diethyl ether 20:1 → 4:1) furnished **11** as a pale orange solid (1.59 g, 0.974 mmol, > 20:1 dr, 77%).



11
P = triethylsilyl

TLC (hexanes:diethyl ether 2:1)

R_f = 0.25, stained by anisaldehyde

^{1}H NMR (500 MHz, acetone *d*-6)

δ 6.52-6.13 (m, 13H), 5.98 (app ddd, *J* = 6, 10.5, 16.5 Hz, 1H), 5.55 (dd, *J* = 9.5, 15 Hz, 1H), 5.40 (dd, *J* = 1.5, 16 Hz, 1H), 5.24 (dd, *J* = 1, 6.5 Hz, 1H), 4.70 (app t, *J* = 6.5 Hz, 1H), 4.63 (app t, *J* = 5.5 Hz, 2H), 4.52 (bs, 1H), 4.44 (dt, *J* = 4.5, 10.5 Hz, 1H), 4.24 (m, 1H), 4.14 (t, *J* = 9.5 Hz, 1H), 4.06 (dq, *J* = 3, 7.5 Hz, 1H), 4.04-3.99 (m, 1H), 3.95 (d, *J* = 3.5 Hz, 1H), 3.86 (dd, *J* = 2.5, 9 Hz, 1H), 3.73-3.70 (m, 2H), 3.65 (dd, *J* = 4.5, 10.5 Hz 1H), 3.17 (s, 3H), 2.58 (app t, *J* = 2 Hz, 1H), 2.44 (app dd, *J* = 9, 15.5 Hz, 1H), 2.34 (t, *J* = 10 Hz, 1H), 2.12 (dd, *J* = 4.5, 12.5 Hz, 1H) 2.07-2.05 (m, 2H), 2.05-1.99 (m, 1H), 1.97-1.59 (m, 10H), 1.52 (bs, 1H) 1.18 (d, *J* = 6 Hz, 3H), 1.08-0.95 (m, 69H), 0.78-0.59 (m, 42H).

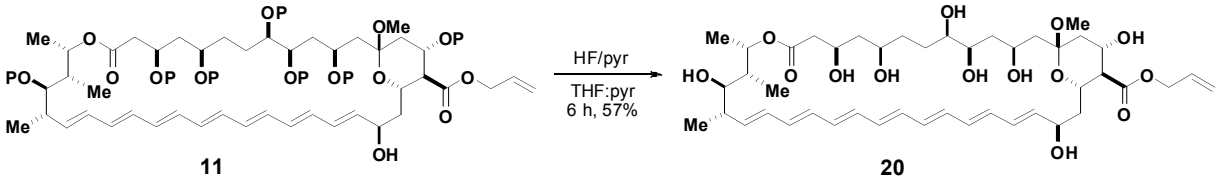
^{13}C NMR (125 MHz, acetone *d*-6)

δ 172.8, 170.6, 139.0, 135.0, 134.9, 134.1, 133.5, 133.1, 133.0, 132.6, 131.8, 131.5, 130.8, 128.1, 126.0, 118.4, 101.4, 76.7, 74.0, 71.0, 69.0, 67.5, 67.4, 65.6, 57.7, 48.1, 47.8, 44.4, 43.5, 42.4, 41.3, 40.7, 35.6, 30.7, 29.2, 27.4, 19.8, 19.1, 7.62, 7.59, 7.48, 7.30, 7.27, 7.12, 6.38, 6.17, 5.89, 5.83, 5.79, 5.64.

HRMS (ESI)

calculated for $C_{87}H_{166}O_{14}Si_7$ $(M + Na)^+$: 1654.0506
found: 1654.0493

For a detailed discussion of the 19-(*R*) stereochemical assignment, see S16-S18.



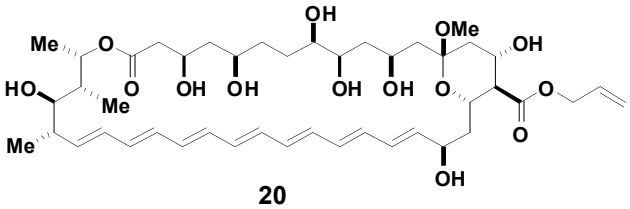
11

P = triethylsilyl

20

Polyol 20

To a stirred solution of **11** (1.55 g, 0.949 mmol, 1 eq) in THF (7 mL) in a polypropylene vial at 0 °C was added a chilled (0 °C) solution of 70% HF/pyridine complex (10 mL) in THF:pyridine 5:3 (160 mL). The resulting solution was allowed to warm to 23 °C and stirred for 6 hours. The reaction was then cooled to 0 °C and quenched by the addition of saturated aqueous sodium bicarbonate (500 mL). The resulting yellow emulsion was extracted with CH_2Cl_2:MeOH 5:1 (5 x 200 mL). The combined organic extracts were washed with brine (1 x 100 mL), dried over sodium sulfate and concentrated *in vacuo*. Purification of the resulting residue by flash chromatography (C_{18} bonded SiO_2; MeCN:H_2O 1:1) furnished **20** as an orange solid (0.442 g, 0.531 mmol, 56%).



20

TLC (CH_2Cl_2:MeOH 10:1)

R_f = 0.25, stained by anisaldehyde

HPLC

tR = 18.90 min; flow rate = 4 mL/min, gradient of 5 → 95% MeCN in 5 mM ammonium acetate over 25 min.

^{1}H NMR (500 MHz, CD_3OD)

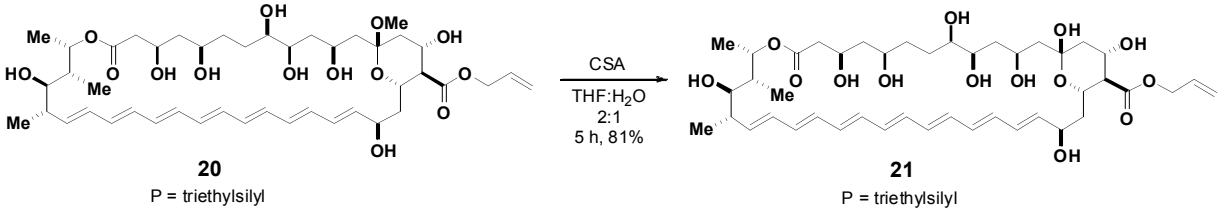
δ 6.43-6.13 (m, 12H), 5.98 (app ddd, *J* = 6, 10.5, 16 Hz, 1H), 5.80 (dd, *J* = 7.5, 15 Hz, 1H), 5.47 (dd, *J* = 9.5, 14 Hz, 1H), 5.38 (dd, *J* = 1.5, 16 Hz, 1H), 5.24 (dd, *J* = 1, 10.5 Hz, 1H), 4.66 (d, *J* = 5 Hz, 2H), 4.48 (app dd, *J* = 7, 11.5 Hz, 1H), 4.20-4.12 (m, 3H), 3.99 (dq, *J* = 3.5, 10.5 Hz, 1H), 3.91 (dd, *J* = 8.5, 12.5 Hz, 1H), 3.71 (app t, *J* =6 Hz, 1H), 3.53 (app d, *J* = 10 Hz, 1H), 3.26 (dd, *J* = 7.5, 9.5 Hz, 1H), 3.22 (app d, *J* = 12.5 Hz, 1H), 3.19 (s, 3H), 2.39 (app dd, *J* = 7, 9.5 Hz, 1H), 2.31 (dd, *J* = 10.5, 15.5 Hz, 1H), 2.22 (dd, *J* = 3.5, 16.5 Hz, 1H), 2.11 (dd, *J* = 5, 13 Hz, 1H), 1.90-1.81 (m, 4H), 1.72-1.61 (m, 4H), 1.51-1.26 (m, 8H), 1.19 (d, *J* = 6.5 Hz, 3H), 1.11 (d, *J* = 6.5 Hz, 3H), 1.00 (d, *J* = 7.5 Hz, 3H).

^{13}C NMR (125 MHz, pyridine *d*-5:CD_3OD 10:1)

δ 173.0, 171.4, 140.1, 135.4, 134.4, 134.2, 134.1, 133.6, 133.2, 133.1, 132.9, 132.8, 132.7, 132.4, 131.8, 128.5, 101.7, 77.7, 75.2, 74.5, 71.5, 70.5, 67.9, 67.7, 67.6, 67.4, 66.7, 66.5, 65.1, 57.0, 44.6, 43.5, 43.1, 42.9, 42.3, 41.3, 41.2, 38.9, 36.0, 33.9, 32.1, 30.5, 29.0, 24.7, 23.9, 23.1, 18.6, 18.5, 17.5, 13.9, 12.2, 10.9.

HRMS (ESI)

calculated for $C_{45}H_{68}O_{12}$ $(M + Na)^+$: 855.4507
found: 855.4482

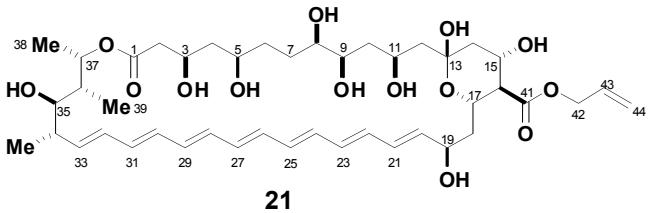


20
P = triethylsilyl

21
P = triethylsilyl

Amphoteronolide B allyl ester 21

To a stirred solution of **20** (10 mg, 0.012 mmol, 1 eq) in THF:H_2O 2:1 (1.2 mL) at 23 °C was added camphorsulfonic acid (0.6 mg, 0.003 mmol, 0.25 eq). The solution was stirred for 5 hours and was then diluted with THF (2 mL) and quenched by addition of solid sodium bicarbonate. The mixture was stirred vigorously for five minutes and the solids were removed by filtration through a pad of Celite. The filtrate was concentrated *in vacuo* and the resulting yellow solid was dissolved in THF (1.5 mL) and purified by prep RP-HPLC (Waters SunFire Prep C_{18} OBD 5 micron 30 x 150 mm; 300 µL injection volume, 25 mL/min flow rate, MeCN:H_2O 1:19 → 19:1, over 25 minutes) to yield amphoteronolide B allyl ester (**21**) as a yellow powder (8 mg, 0.0098 mmol, 81%).



21

HPLC

tR = 28.6 min; flow rate = 25 mL/min, gradient of 5 → 95% MeCN in H_2O over 25 min.

^{1}H NMR (500 MHz, pyridine *d*-5:CD_3OD 10:1)

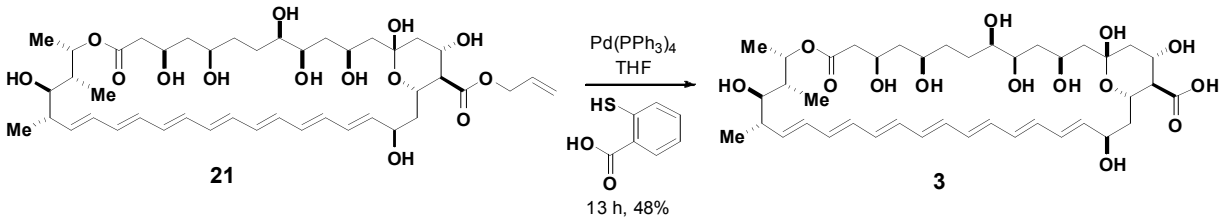
δ 6.83 (dd, J = 11, 15 Hz, 1H, **H-22**), 6.77 (dd, J = 11, 15 Hz, 1H, **H-24**), 6.69 (dd, $J_{19,20}$ = 9.5 Hz, $J_{20,21}$ = 15.5 Hz, 1H, **H-20**), 6.61 (dd, J = 14.5, 15 Hz, 1H, **H-26**), 6.54-6.35 (m, 9H), 6.04-5.96 (app ddd, $J_{42,43}$ = 6.5, $J_{43,44cis}$ = 11 Hz, $J_{43,44trans}$ = 17 Hz, 1H, **H-43**), 5.80 (app d, $J_{36,37}$ = 1.9 Hz, 1H, **H-37**), 5.55 (dd, $J_{32,33}$ = 15 Hz, $J_{33,34}$ = 10.1 Hz, 1H, **H-33**), 5.42 (dd, $J_{44cis,44trans}$ = 1.5 Hz, $J_{43,44trans}$ = 17.5 Hz, 1H, **H-44*trans***), 5.30 (app t, $J_{16,17}$ = 10.6 Hz, $J_{17,18e}$ = 0.9 Hz, $J_{17,18a}$ = 8.7 Hz, 1H, **H-17**), 5.14 (dd, $J_{44cis,44trans}$ = 1.5 Hz, $J_{43,44cis}$ = 10.5 Hz, 1H, **H-44*cis***), 5.06 (app dt, $J_{14e,15}$ = 3.5 Hz, $J_{14a,15}$ = 11.0 Hz, $J_{15,16}$ = 10.7 Hz, 1H, **H-15**), 4.87 (t, $J_{10a,11}$ = 3.1 Hz, $J_{10e,11}$ = 10.3 Hz, $J_{11,12a}$ = 3.4 Hz, $J_{11,12e}$ = 11.3 Hz, 1H, **H-11**), 4.80-4.73 (m, $J_{18e,19}$ = 6.0 Hz, $J_{19,20}$ = 8.8 Hz, 3H, **H-19**, **H-42**(2)), 4.64 (app t, $J_{2a,3}$ = 4.7 Hz, $J_{2e,3}$ = 9.2 Hz, $J_{3,4a}$ = 3.9 Hz, $J_{3,4e}$ = 10.1 Hz, 1H, **H-3**), 4.13 (app t, $J_{4a,5}$ = 4.4 Hz, $J_{4e,5}$ = 9.5 Hz, $J_{5,6a}$ = 5.4 Hz, $J_{5,6e}$ = 10.3 Hz, 1H, **H-5**), 4.04 (app d, $J_{8,9}$ = 3.0 Hz, $J_{9,10a}$ = 3.3 Hz, $J_{9,10e}$ = 10.7 Hz, 1H, **H-9**), 3.61 (app d, $J_{7e,8}$ = 2.8 Hz, $J_{7a,8}$ = 11.2 Hz, $J_{8,9}$ = 3.0 Hz, 1H, **H-8**), 3.43 (app d, $J_{34,35}$ = 9.8 Hz, $J_{35,36}$ = 2.6 Hz, 1H, **H-35**), 2.85 (t, $J_{15,16}$ = 10.7 Hz, $J_{16,17}$ = 10.6 Hz, 1H, **H-16**), 2.66 (m, $J_{33,34}$ = 10.1 Hz, $J_{34,35}$ = 9.8 Hz, 1H, **H-34**), 2.61 (dd, $J_{2a,2e}$ = 17 Hz $J_{2e,3}$ = 9.2 Hz, 1H, **H-2e**), 2.51 (dd, $J_{14a,14e}$ = 12 Hz $J_{14e,15}$ = 3.5 Hz, 1H, **H-14e**), 2.46 (dd, $J_{2a,2e}$ = 16.5, $J_{2a,3}$ = 4.7, 1H, **H-2a**), 2.41 (dd, $J_{17,18e}$ = 0.9 Hz, $J_{18a,18e}$ = 14 Hz, $J_{18e,19}$ = 6.0 Hz, 1H, **H-18e**), 2.37 (m, $J_{7e,8}$ = 2.8 Hz, 1H, **H-7e**), 2.19-2.15 (m, $J_{9,10e}$ = 10.7 Hz, $J_{10e,11}$ = 10.3 Hz, $J_{17,18a}$ = 8.7 Hz. 2H, **H-10e**, **H18a**), 2.10 (m, $J_{35,36}$ = 2.6 Hz, $J_{36,37}$ = 1.9 Hz, 1H, **H-36**), 2.00-1.95 (m, $J_{5,6e}$ = 10.3 Hz, $J_{11,12e}$ = 11.3 Hz, 2H, **H-6e**, **H-12e**), 1.85-1.80 (m, $J_{3,4e}$ = 10.1 Hz, $J_{4e,5}$ = 9.5 Hz, $J_{7a,8}$ = 11.2 Hz, $J_{14e,15}$ = 3.5 Hz, 3H, **H-4e**, **H-7a**, **H-14e**), 1.78-1.70 (m, $J_{5,6a}$ = 5.4 Hz, $J_{11,12a}$ = 3.4 Hz, 2H, **H-6a**, **H-12a**), 1.61 (app t, $J_{3,4a}$ = 3.9 Hz, $J_{4a,5}$ = 4.4 Hz, 1H, **H-4a**), 1.56 (app d, $J_{9,10a}$ = 3.3 Hz, $J_{10a,10e}$ = 14.5 Hz, $J_{10a,11}$ = 3.1 Hz, 1H, **H-10a**), 1.46 (d, $J_{37,38}$ = 6.5 Hz, 3H, **H-38**), 1.31 (d, $J_{34,40}$ = 6 Hz, 3H, **H-40**), 1.25 (d, $J_{36,39}$ = 7 Hz, 3H, **H-39**).

^{13}C NMR (150 MHz, pyridine *d*-5:CD_3OD, 10:1)

δ 173.5, 172.2, 140.9, 137.5, 135.0, 134.8, 134.7, 134.1, 133.7, 133.5, 133.4, 133.3, 133.2, 133.1, 132.9, 132.8, 129.1, 118.0, 98.6, 78.8, 76.3, 75.2, 72.2, 71.1, 70.4, 69.7, 68.5, 68.1, 67.0, 66.7, 65.4, 59.0, 47.6, 45.9, 45.1, 43.8, 43.0, 42.8, 41.1, 41.0, 39.4, 36.6, 31.8, 30.2, 29.5, 26.0, 24.4, 23.5, 19.1, 17.4, 12.9.

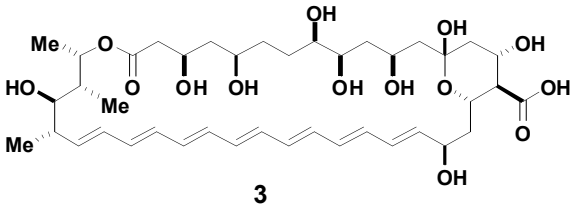
HRMS (ESI)

calculated for $C_{44}H_{66}O_{14}$ $(M+Na)^+$:	841.4350
found:	841.4369



Amphoteronolide B 3

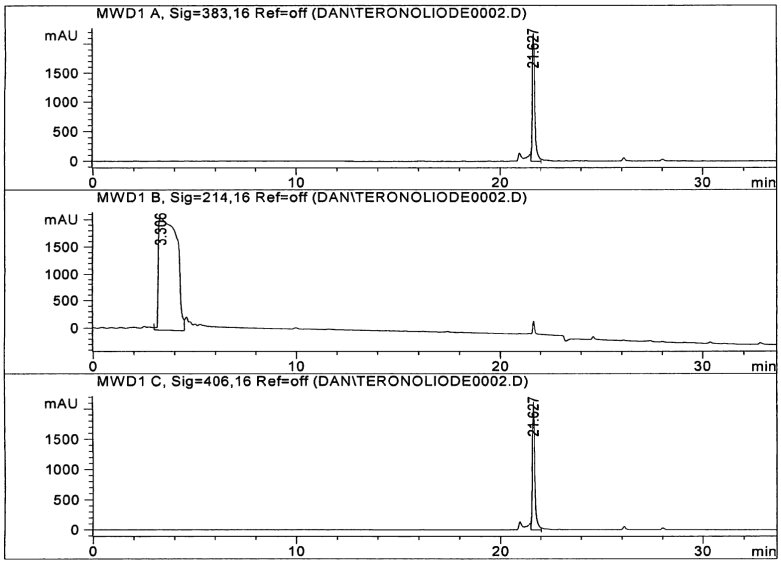
Prior to the reaction, **21** was azeotropically dried via coevaporation with acetonitrile (3 x 5 mL) and left under vacuum for a minimum of eight hours. The resulting yellow solid (20 mg, 0.024 mmol, 1 eq) was dissolved in THF (1.5 mL) at 23 °C and thiosalicylic acid (20 mg, 0.12 mmol, 5 eq). Palladium tetrakis(triphenylphosphine) (27 mg, 0.024 mmol, 1 eq) was added and the solution was stirred for 13 hours, during which time the formation of a precipitate was observed. The reaction was then concentrated *in vacuo* and the residue was triturated with cold (0 °C) diethyl ether (5 x 5 mL). The yellow solid was then dissolved in DMSO (1.5 mL) and purified by preparative RP-HPLC (Waters SunFire Prep C_{18} OBD 5 micron 30 x 150 mm; 250 µL injection volume, 25 mL/min flow rate, 5 → 75% MeCN in 10 mM NH_4OAc over 25 minutes) to afford amphoteronolide B (**3**) (9 mg, 0.012 mmol, 50%) as a yellow powder.



3

HPLC

tR: 21.6 min; flow rate = 25 mL/min, gradient of 5 → 75% MeCN in 10 mM ammonium acetate over 25 min.

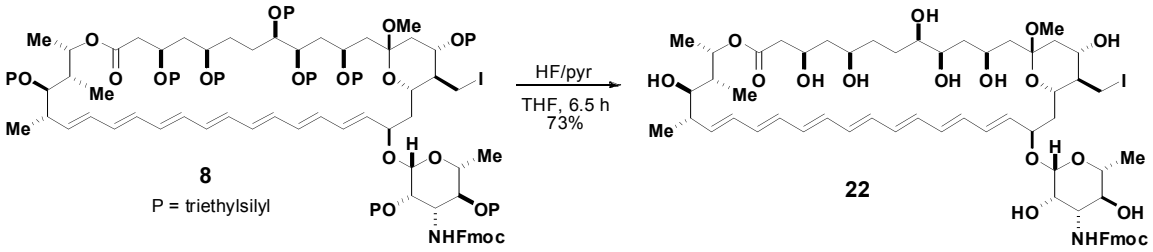


^{1}H NMR (pyridine *d*-5:CD_3OD 10:1)

δ 6.76-6.67 (m, 2H), 6.59-6.55 (m, 2H), 6.45-6.35 (m, 10H), 5.72 (app d, *J* = 5 Hz, 1H), 5.16 (app t, *J* = 10 Hz, 1H), 4.92 (bs, 1H), 4.78 (bs, 2H), 4.57 (app t, *J* = 10 Hz, 1H), 4.05 (app t, 9.5 Hz, 1H), 3.95 (app d, *J* = 10.5 Hz, 1H), 3.65 (app d, *J* = 4 Hz, 1H), 3.52 (app d, *J* = 10.5 Hz, 1H), 3.41 (app d, *J* = 9 Hz, 1H), 2.64 (bs, 2H), 2.56 (dd, *J* = 10, 16.5 Hz, 1H), 2.43-2.40 (m, 2H), 2.24 (bs, 2H), 2.10-2.05 (m, 2H), 1.95-1.90 (m, 2H), 1.79-1.65 (m, 5H), 1.58 (app d, *J* = 13.5 Hz, 1H), 1.51 (app d, *J* = 11 Hz, 1H), 1.41 (d, *J* = 6.5 Hz, 3H), 1.28 (d, *J* = 6.5 Hz, 3H), 1.21 (d, *J* = 7 Hz, 3H).

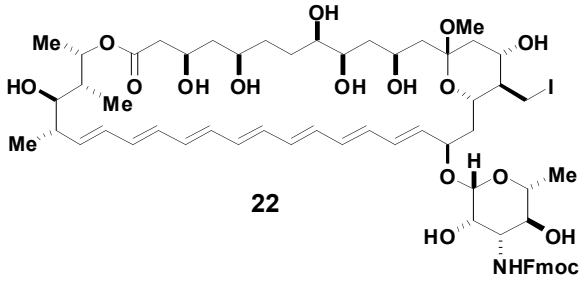
HRMS

Calculated for $C_{41}H_{62}O_{14}$ $(M+Na)^+$:	801.4037
found:	801.4039



Polyol 22

To a stirred solution of **8** (500 mg, 0.219 mmol) in THF (7 mL) in a polypropylene vial at 0 °C was added chilled (0 °C) 70% HF/pyridine complex (2.2 mL, 77 mmol, 350 eq) diluted with THF:pyridine 5:3 (40 mL). The solution was allowed to warm to 25 °C and stirred for 6.5 hours. The solution was subsequently cooled to 0 °C and quenched with the addition of saturated aqueous sodium bicarbonate (100 mL). The resulting yellow emulsion was extracted with CH_2Cl_2:MeOH 5:1 (5 x 100 mL). The combined organic extracts were washed with brine (1 x 25 mL), dried over sodium sulfate and concentrated *in vacuo*. Purification of the resulting residue by flash chromatography (SiO_2; DCM:MeOH 100:1 → 15:1) afforded **22** as a yellow solid (202 mg, 0.160 mmol, 73%).



TLC (DCM:MeOH 10:1)

R_f = 0.5, stained by anisaldehyde

HPLC

tR = 23.40 min; flow rate = 4 mL/min, gradient of 5 → 95% MeCN in 5 mM ammonium acetate over 25 min.

^{1}H NMR (500 MHz, CD_3OD)

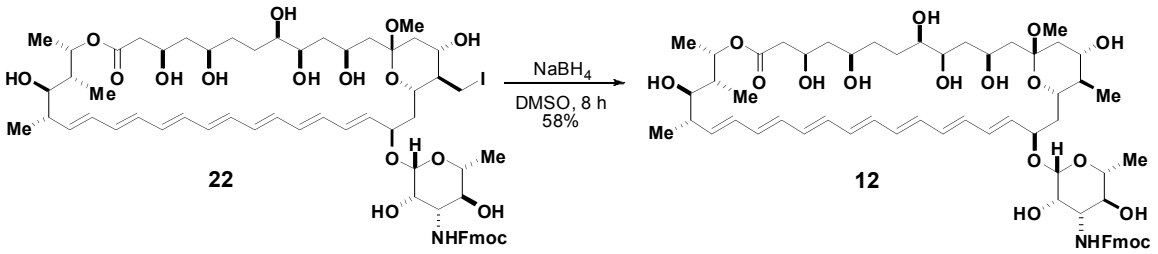
δ 7.79 (d, *J* = 7.5 Hz, 2H), 7.68 (dd, *J* = 4.5, 7.5 Hz, 2H), 7.39 (t, *J* = 7 Hz, 2H), 7.31 (t, *J* = 8 Hz, 2H), 6.41-6.14 (m, 12H), 5.86 (dd, *J* = 6.5, 14 Hz, 1H), 5.55 (dd, *J* = 9, 14 Hz, 1H), 5.14 (bs, 1H), 4.82 (s, 1H), 4.63 (t, *J* = 7 Hz, 1H), 4.36 (app d, *J* = 7 Hz, 2H), 4.23 (t, *J* = 7 Hz, 1H), 4.15-4.11 (m, 1H), 3.97-3.93 (m, 2H), 3.86 (dt, *J* = 5.5, 10 Hz, 1H), 3.80 (t, *J* = 9.5 Hz, 1H), 3.75 (d, *J* = 9 Hz, 1H), 3.72-3.68 (m, 1H), 3.64 (dd, *J* = 2.5, 9 Hz, 1H), 3.52 (app d, *J* = 10.5 Hz, 1H), 3.36-3.31 (m, 1H), 3.23 (app d, *J* = 9.5 Hz, 1H), 3.18 (s, 3H), 2.38 (app dd, *J* = 7.5, 15.5 Hz, 1H), 2.29 (dd, *J* = 9, 16.5 Hz, 1H), 2.23 (dd, *J* = 3, 16.5 Hz, 1H), 2.15 (dd, *J* = 5, 13 Hz, 1H), 1.86-1.83 (m, 1H), 1.80-1.75 (m, 1H), 1.69-1.54 (m, 8H), 1.50-1.39 (m, 9H), 1.30 (d, *J* = 5.5 Hz, 3H), 1.20 (d, *J* = 6.5 Hz, 3H), 1.11 (d, *J* = 6.5 Hz, 3H), 1.01 (d, *J* = 7.5, 3H).

^{13}C NMR (125 MHz, CD_3OD)

δ 172.6, 158.7, 145.4, 145.3, 142.6, 137.4, 135.0, 134.5, 134.1, 134.0, 133.9, 133.4, 133.2, 132.7, 128.8, 128.2, 126.3, 120.9, 102.3, 98.6, 79.0, 75.8, 75.4, 75.1, 74.6, 72.3, 71.9, 71.5, 71.2, 68.8, 68.3, 67.9, 66.8, 58.3, 46.2, 44.7, 43.3, 43.1, 41.5, 36.0, 35.3, 30.4, 18.9, 18.3, 17.9, 12.2.

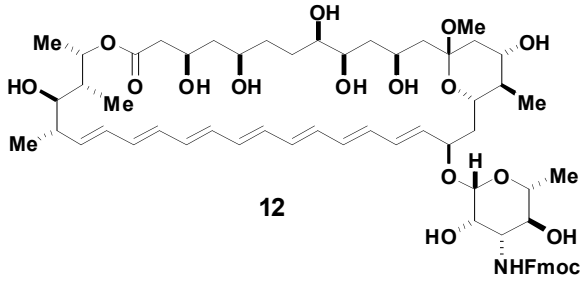
HRMS (ESI)

calculated for $C_{63}H_{86}INO_{17}$ $(M + Na)^+$:	1278.4838
found:	1278.4817



41-Methyl derivative 12

Prior to the reaction, **22** was azeotropically dried via coevaporation with acetonitrile (3 x 10 mL) and left under vacuum for a minimum of eight hours. The resulting yellow solid (50 mg, 0.0398 mmol, 1 eq) was dissolved in DMSO (1.3 mL) at 23 °C and sodium borohydride (7.5 mg, 0.199 mmol, 5 eq) was added. The solution was then stirred for 8.5 hours and subsequently quenched with the addition of saturated aqueous sodium bicarbonate (1 mL). The resulting emulsion was diluted with water (25 mL) and extracted with CH_2Cl_2:MeOH 5:1 (5 x 10 mL). The combined organic phases were washed with brine (1 x 20 mL), dried over sodium sulfate and concentrated *in vacuo*. Purification of the residue by flash chromatography (SiO_2; DCM:MeOH 20:1 → 10:1) yielded **12** as a yellow solid (26 mg 0.023 mmol, 58%).



TLC (CH_2Cl_2:MeOH 10:1)

R_f = 0.5, stained by anisaldehyde

HPLC

tR = 22.59 min; flow rate = 4 mL/min, gradient of 5 → 95% MeCN in 5 mM ammonium acetate over 25 min.

^{1}H NMR (500 MHz, CD_3OD)

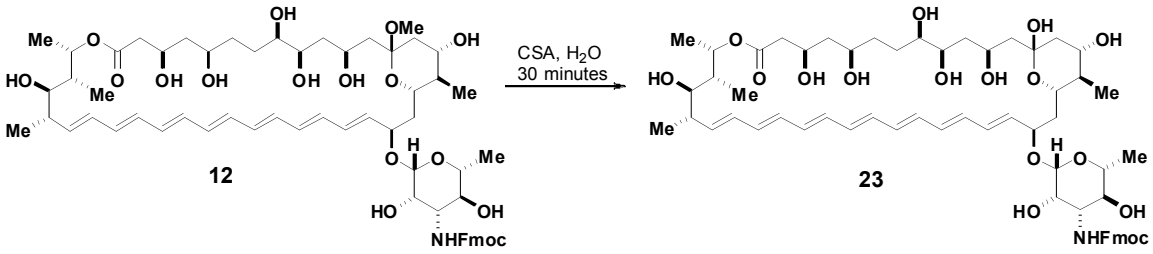
δ 7.79 (d, *J* = 7.5 Hz, 2H), 7.68 (t, *J* = 6.5 Hz, 2H), 7.39 (t, *J* = 7 Hz, 2H), 7.31 (t, 7.5 Hz, 2H), 6.44-6.15 (m, 12H), 5.83 (dd, *J* = 8, 15 Hz, 1H), 5.44 (dd, *J* = 10, 14 Hz, 1H), 5.28 (bs, 1H), 4.61 (s, 1H), 4.49 (dt, *J* = 2.5, 8.5 Hz, 1H), 4.40-4.33 (m, 2H), 4.23 (t, *J* = 7 Hz, 1H), 4.18-4.13 (m, 1H), 3.94-3.89 (m, 3H), 3.81 (d, *J* = 3 Hz, 1H), 3.72-3.67 (m, 2H), 3.65-3.62 (m, 1H), 3.59-3.52 (m, 4H), 3.27 (dd, *J* = 6, 8 Hz, 1H), 3.22 (dd, *J* = 1.5, 12.5 Hz, 1H), 3.16 (s, 3H), 2.38 (app dd, *J* = 9.5, 16 Hz, 1H), 2.29 (dd, *J* = 9, 16.5 Hz, 1H), 2.22 (dd, *J* = 3, 16.5 Hz, 1H), 2.09 (dd, *J* = 9, 13.5 Hz, 1H), 2.04-1.97 (m, 2H), 1.90-1.81 (m, 2H), 1.72-1.65 (m, 2H), 1.55-1.39 (m, 8H), 1.28 (d, *J* =5.5 Hz, 3H), 1.19 (d, *J* = 6 Hz, 3H), 1.11 (d, *J* = 6 Hz, 3H), 1.02 (d, *J* = 6.5 Hz, 3H), 1.01 (d, *J* = 7 Hz, 3H).

^{13}C NMR (125 MHz, CD_3OD)

δ 171.5, 157.6, 144.2, 144.1, 141.4, 136.2, 135.8, 133.9, 133.5, 133.4, 133.3, 133.0, 132.6, 132.4, 132.0, 130.9, 127.6, 127.0, 125.1, 125.0, 119.8, 107.7, 101.3, 98.4, 76.4, 74.7, 73.7, 71.4, 71.2, 70.6, 70.3, 70.0, 69.7, 69.1, 67.7, 67.5, 67.1, 66.7, 57.1, 43.6, 42.7, 42.0, 41.8, 41.4, 40.2, 35.0, 29.6, 28.9, 17.8, 17.0, 16.2, 12.1, 11.1.

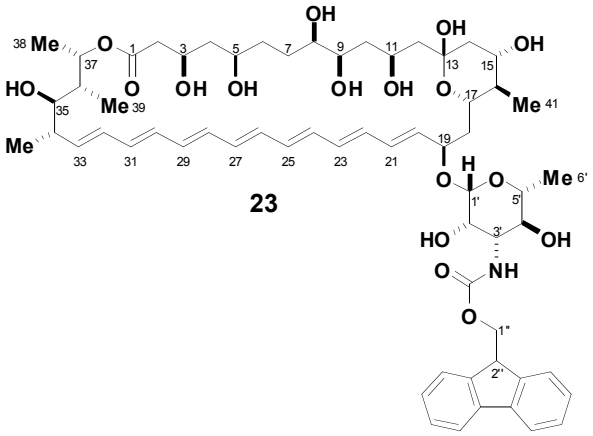
HRMS (ESI)

calculated for $C_{63}H_{87}NO_{17}$ $(M + Na)^+$:	1152.5872
found:	1152.5852



N-Fmoc-41-methyl amphotericin B 23

To a stirred solution of **12** (9 mg, 0.008 mmol) in THF:H_2O 2:1 (1 mL) at 23 °C was added camphorsulfonic acid (0.6 mg, 0.002 mmol, 0.25 eq). The solution was stirred for 30 minutes and was then diluted with THF (1 mL) and quenched by the addition of solid sodium bicarbonate. The solids were removed by filtration through a pad of Celite and the filtrate was concentrated *in vacuo* to give **23** as a yellow solid (9 mg, 0.008 mmol, ~100%). This material was used in the next step without further purification. Alternatively, **23** was purified by preparative RP-HPLC (Waters SunFire Prep C_{18} OBD 5 micron 30 x 150 mm; 300 µL injection volume, 25 mL/min flow rate 1:19 → 19:1 MeCN:10mM NH_4OAc, over 25 minutes) for use in NMR studies.



HPLC

tR = 25.1 minutes; flow rate = 25 mL/min, gradient of 5 → 95% MeCN in 5 mM ammonium acetate over 25 min.

^{1}H NMR (600 MHz, pyridine *d*-5:CD_3OD 10:1)

δ 7.85 (d, J = 7.2 Hz, 2H), 7.71 (dd, J = 6, 8.5 Hz, 2H), 7.41 (t, J = 7.2 Hz, 2H), 6.74 (m, 2H), 6.60-6.34 (m, 11H), 5.77 (app d, $J_{36,37}$ = 1.8 Hz, 1H, **H-37**), 5.54 (dd, $J_{32,33}$ = 15 Hz $J_{33,34}$ = 10.0, 1H, **H-33**), 4.97 (app s, $J_{1',2'}$ = 1.6 Hz, 1H, **H-1'**), 4.82-4.78 (m, $J_{10e,11}$ = 10.4 Hz, $J_{11,12e}$ = 11.0 Hz, $J_{18a,19}$ = 2.5 Hz, $J_{18e,19}$ = 4.6 Hz, $J_{19,20}$ = 8.0 Hz, 2H, **H-11**, **H-19**), 4.62 (app t, $J_{2a,3}$ = 1.8 Hz, $J_{2e,3}$ = 9.1 Hz, $J_{3,4a}$ = 2.0 Hz, $J_{3,4e}$ = 10.1 Hz, 1H, **H-3**), 4.51-4.43 (m, $J_{16,17}$ = 10.4 Hz, $J_{17,18a}$ = 7.5 Hz, 3H, **H-17**, **H-1''**(2)), 4.39 (app d, $J_{1',2'}$ = 1.6 Hz, 1H, **H-2'**), 4.34 (dd, $J_{2',3'}$ = 3 Hz, $J_{3',4'}$ = 9.7 Hz, 1H, **H-3'**), 4.29 (t, J = 7.2 Hz, 1H, **H-2''**), 4.12-4.07 (m, $J_{4a,5}$ = 1.0 Hz, $J_{4e,5}$ = 9.6 Hz, $J_{5,6a}$ = 1.8 Hz, $J_{5,6e}$ = 10.7 Hz, $J_{14e,15}$ = 2.6 Hz, $J_{14a,15}$ = 11.8 Hz, $J_{15,16}$ = 11.2 Hz, 2H, **H-5**, **H-15**), 4.02-3.99 (m, $J_{8,9}$ = 3.4 Hz, $J_{9,10a}$ = 2.6 Hz, $J_{9,10e}$ = 10.7 Hz, $J_{3',4'}$ = 9.7 Hz, $J_{4',5'}$ = 9.1 Hz, 2H, **H-9**, **H-4'**), 3.73 (app dd, $J_{4',5'}$ = 9.1 Hz, $J_{5',6'}$ = 6.6 Hz, 1H, **H-5'**), 3.58 (app d, $J_{7e,8}$ = 2.5 Hz, $J_{7a,8}$ = 10.6 Hz, $J_{8,9}$ = 3.4 Hz, 1H, **H-8**), 3.43 (app d, $J_{34,35}$ = 9.6 Hz, $J_{35,36}$ = 2.5 Hz, 1H, **H-35**), 2.67 (app dd, $J_{33,34}$ = 10.0 Hz, $J_{34,35}$ = 9.6 Hz, 1H, **H-34**), 2.60 (dd, $J_{2a,2e}$ = 16.8 Hz, $J_{2e,3}$ = 9.1 Hz, 1H, **H-2e**), 2.46 (app dd, $J_{18a,18e}$ = 16.8 Hz, $J_{18e,19}$ = 4.6 Hz, 1H, **H-18e**), 2.44-2.40 (m, $J_{2a,3}$ = 1.8 Hz, $J_{14e,15}$ = 2.6 Hz, 2H, **H-2a**, **H-14e**), 2.36-2.33 (m, $J_{6e,7e}$ = 13.4 Hz, $J_{7e,8}$ = 2.5 Hz, 1H, **H-7e**), 2.17-2.07 (m, $J_{9,10e}$ = 10.7 Hz, $J_{10e,11}$ = 10.4 Hz, $J_{35,36}$ = 2.5 Hz, $J_{36,37}$ = 1.8 Hz, 2H, **H-10e**, **H-36**), 2.02-1.90 (m, $J_{5,6e}$ = 10.7 Hz, $J_{6e,7a}$ = 4.4 Hz, $J_{6e,7e}$ = 13.4 Hz, $J_{11,12e}$ = 11.0 Hz, $J_{17,18a}$ = 7.5 Hz, $J_{18a,19}$ = 2.5 Hz, 3H, **H-6e**, **H-12e**, **H-18a**), 1.81-1.76 (m, $J_{3,4e}$ = 10.1 Hz, $J_{4e,5}$ = 9.6 Hz, $J_{6e,7a}$ = 4.4 Hz, $J_{6a,7a}$ = 12.7 Hz, $J_{7a,8}$ = 10.6 Hz, 3H, **H-4e**, **H-7a**, **H12a**), 1.73-1.69 (m, $J_{5,6a}$ = 1.8 Hz, $J_{6a,7a}$ = 12.7 Hz, $J_{14a,15}$ = 11.8 Hz, 2H, **H-6a**, **H-14a**), 1.62-1.60 (m, $J_{3,4a}$ = 2.0 Hz, $J_{4a,5}$ = 1.0 Hz, $J_{15,16}$ = 11.2 Hz, $J_{16,17}$ = 10.4 Hz, 2H, **H-4a**, **H-16**), 1.56 (d, $J_{5',6'}$ = 6.6 Hz, 3H, **H-6'**), 1.55-1.52 (m, $J_{9,10a}$ = 2.6 Hz, 1H, **H-10a**), 1.44 (d, $J_{37,38}$ = 6.6 Hz, 3H, **H-38**), 1.30 (d, $J_{34,40}$ = 6.6 Hz, 3H, **H-40**), 1.24 (d, $J_{36,39}$ = 7 Hz, 3H, **H-39**), 1.23 (d, $J_{16,41}$ = 5.2 Hz, 3H, **H-41**).[9]

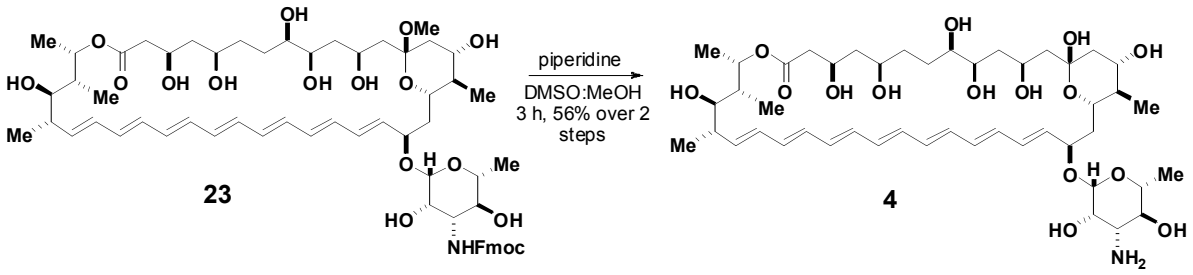
^{13}C NMR (150 MHz, pyridine *d*-5:CD_3OD 10:1)

δ 172.2, 158.0, 145.1, 144.9, 142.0, 141.9, 138.2, 137.6, 134.9, 134.8, 134.5, 134.1, 133.7, 133.5, 133.4, 133.2, 132.9, 130.3, 128.3, 127.8, 126.1, 123.6, 126.0, 120.7, 108.6, 99.6, 98.3, 78.8, 78.1, 76.3, 75.3, 75.0, 72.2, 71.9, 71.5, 70.4, 69.7, 69.5, 68.5, 67.6, 67.1, 58.7, 48.1, 45.1, 44.2, 43.1, 41.1, 36.6, 30.3, 19.1, 18.8, 17.5, 14.1, 12.8.

HRMS (ESI)

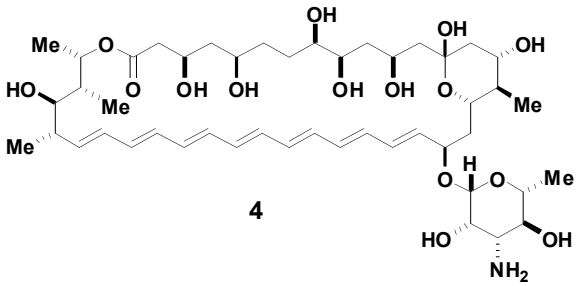
calculated for $C_{62}H_{85}NO_{17}$ $(M + Na)^+$:	1138.5715
found:	1138.5734

[9] Two of the Fmoc protons were obscured by the pyridine solvent peak.



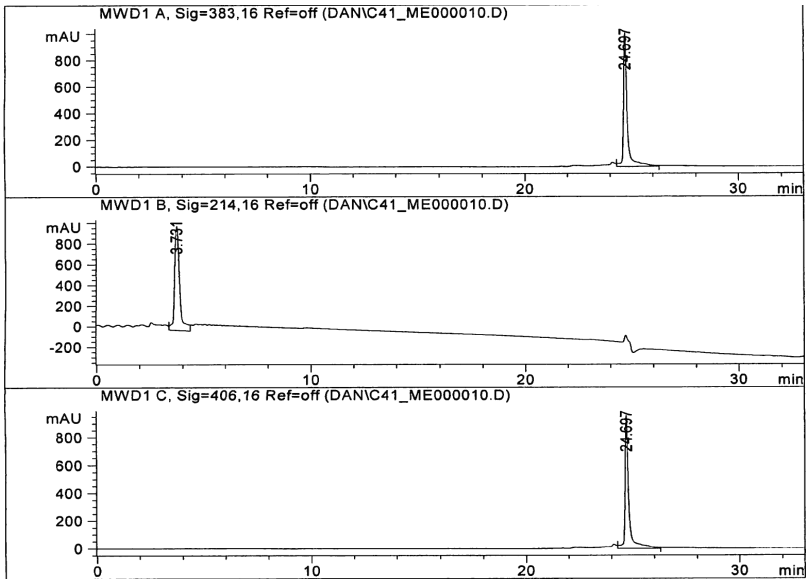
C(41)-Methyl amphotericin B 4

To a stirred solution of **23** (9 mg, 0.008 mmol) in DMSO:MeOH 4:1 (380 µL) was added piperidine (0.02 mmol, 2 µL, 2 eq). The solution was stirred for 3 hours and was then diluted with THF (1 mL) and purified by prep RP-HPLC (Waters SunFire Prep C_{18} OBD 5 micron 30 x 150 mm; 300 µL injection volume, 25 mL/min flow rate 1:19 → 19:1 MeCN:10mM NH_4OAc, over 25 minutes) to afford C(41)-methyl amphotericin B (**4**) as a yellow powder (4 mg, 0.0045 mmol, 56% over 2 steps).



HPLC

tR = 21.7 minutes; flow rate = 25 mL/min, gradient of 5 → 95% MeCN in 5 mM ammonium acetate over 25 min.



^{1}H NMR (500 MHz, pyridine *d*-5:CD_2OD 10:1)

δ 6.78-6.71 (m, 2H), 6.58-6.37 (m, 9H), 6.43-6.35 (m, 2H), 5.77 (app d, *J* = 5.5 Hz, 1H), 5.54 (dd, *J* = 10, 15 Hz, 1H), 4.95 (s, 1H), 4.81-4.77 (m, 2H), 4.61 (dt, *J* = 3, 12.5 Hz, 1H), 4.45-4.40 (m, 2H), 4.35 (d, *J* = 2.5 Hz, 1H), 4.11-4.03 (m, 2H), 4.00 (app d, *J* = 11 Hz, 1H), 3.77 (t, *J* = 9 Hz, 1H), 3.66-3.58 (m, 2H), 3.57 (app d, *J* = 11 Hz, 1H) 3.42 (app d, *J* = 9.5 Hz, 1H), 2.66 (app dd, *J* = 7, 9.5 Hz, 1H), 2.60 (dd, *J* = 9.5, 16.5 Hz, 1H), 2.49 (dd, *J* = 5.5, 14.5 Hz, 1H), 2.41 (app dd, *J* = 7.5, 12 Hz, 2H), 2.39-2.35 (m, 1H), 2.10 (app dd, *J* = 7.5, 15.5, 2H), 1.95-1.88 (m, 3H), 1.81-1.72 (m, 3H), 1.70-1.67 (m, 2H), 1.66-1.59 (m, 2H), 1.52 (d, *J* = 6 Hz, 3H), 1.44 (d, *J* = 6.5 Hz, 3H), 1.30 (d, *J* = 6.5 Hz, 3H), 1.24 (d, *J* = 7 Hz, 3H), 1.22 (d, *J* = 6.5 Hz, 3H).

HRMS (ESI)

calculated for $C_{47}H_{75}NO_{15}$ $(M + Na)^+$:	894.5191
found:	894.5182

III. NMR studies

Selection of compounds for NMR analysis. MeAmdeB **2** was used directly for NMR studies. Compounds **1**, **3**, and **4** were not amenable to high resolution NMR analysis due to their poor solubilities in appropriate NMR solvents. However, it has been demonstrated that AmB derivatives having covalent modifications of the carboxylic acid or amine functional groups have the same ground state conformation as judged by NMR[10] or X-ray crystallographic analysis,[11] respectively. Therefore, we chose suitably protected analogs AmB N-acyl methyl ester **17**, AmdeB allyl ester **21**, and N-Fmoc MeAmB **23** for the conformational analysis of **1**, **3**, and **4**, respectively.

gCOSY NMR spectra. 500 MHz and 600 MHz gCOSY NMR spectra were acquired at 30 °C with 2048 points, 256 or 512 increments and 1, 4, or 8 transients. Spectra were processed on a SUN Microsystems SPARCstation Ultra 5 computer using Varian VNMR software, version 6.1, revision C, with zero-filling to 4096 x 4096 and sine bell apodization such that sb = at/2 and sb1 = ni/(2*sw1).

H-H NOESY NMR spectra. Samples for NOESY NMR experiments were prepared in an Innovative Technologies, Inc. glove box using a NMR tube sealed with a PTFE screw cap. Sealed ampules of pyridine *d*-5 and CD_3OD with 0.03% tetramethylsilane were used as solvents for these experiments. 600 MHz NOESY spectra were acquired at 30 °C with 2048 points, 256 increments, 8 transients per increment, τ_{mix} = 0.7 s, and an interscan delay (d1) of 3*T1 (standard T1 relaxation experiments were performed for each compound). Spectra were processed using nmrPipe[12] as follows: 1) 4 points back prediction, 2) 90° shifted sinebell apodization, 3) zero-filling to 8192 points, 4) Fourier transformation and phasing, 5) linear prediction to 512 points, 6) 90° shifted sinebell apodization, 7) zero-filling to 2048 points, and 8) Fourier transformation and phasing. The Sparky program,[13] version 3.113 was used for peak-picking and integration of crosspeaks.

Phase-sensitive COSY (COSYPS) NMR spectra. 500 MHz COSYPS spectra were acquired at 30 °C with 2048 points, 256 increments, and 4 transients per increment. 600 MHz COSYPS spectra were acquired at 30 °C with 2458 points, 308 increments and 8 transients per increment. All COSYPS spectra were acquired with sufficient interscan delay to allow for full spin-relaxation (d1 = 23.2 seconds, as determined by T1 relaxation experiments, was sufficient for all compounds).

Gradient HMBC NMR spectrum. A gradient HMBC spectrum of **17** was acquired at 23 °C with 2048 points, 280 increments, and 128 transients. Parameters for C-H coupling were set such that j1xh = 140 Hz and jnxh = 8 Hz. The spectrum was processed on a SUN Microsystems SPARCstation Ultra 5 computer using Varian VNMR software, version 6.1 revision C, with zero-filling to 1024 points in the indirect dimension and sinebell apodization such that sb = at/2 and sb1 = ni/(2*sw1).

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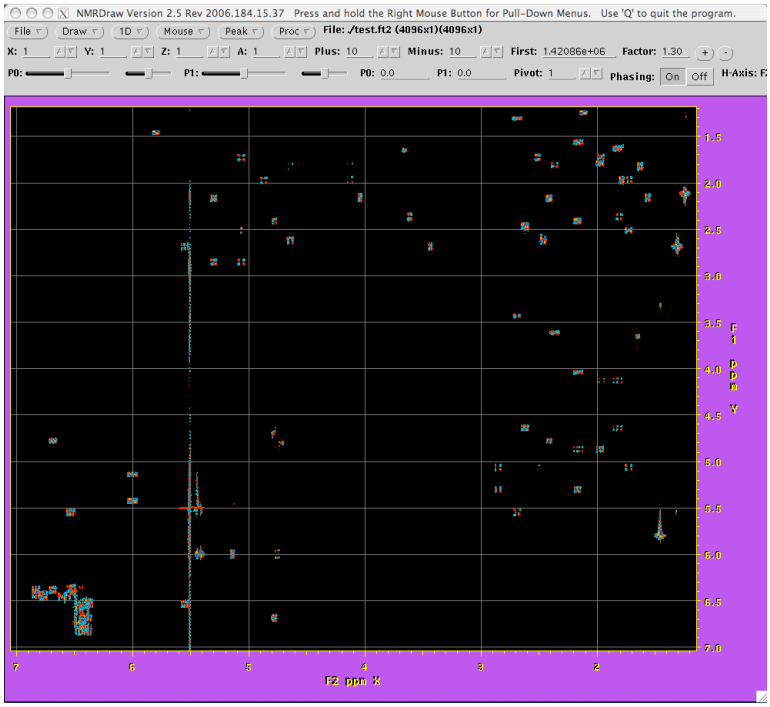
[10] Sowinski, P.; Pawlak, J.; Borowski, E.; Gariboldi, P. *Magn. Res. Chem.* **1992**, *30*, 275-279.
[11] Ganis, P.; Avitabile, G.; Mechliński, W.; Schaffner, C.P. *J. Am. Chem. Soc.* **1971**, *93*, 4560-4564.
[12] Delaglio, F.; Grzesiek, S.; Vuister, G. W.; Zhu, G.; Pfeifer, J.; Bax, A. *J. Biomol. NMR*, **1995**, *6*, 277-293.
[13] Goddard, T. D.; Kneller, D. G. SPARKY 3, University of California, San Francisco, http://www.cgl.ucsf.edu/home/sparky/

COSYPS processing and ^3J determination. Raw COSYPS data were processed as described by Bax and coworkers[14] to produce a diagonal-suppressed spectrum and a diagonal-only spectrum (Figures S4A and S4B, respectively show representative spectra for the AmdeB allyl ester **21**). Amplitude-constrained multiplet evaluation (ACME),[14] was used to determine ^3J H-H coupling constants (see Table S1 for all coupling constants calculated by ACME).

[14] Delaglio, F.; Wu, Z.; Bax, A. *J. Magn. Reson*. **2001**, *149*, 276-281

A



B

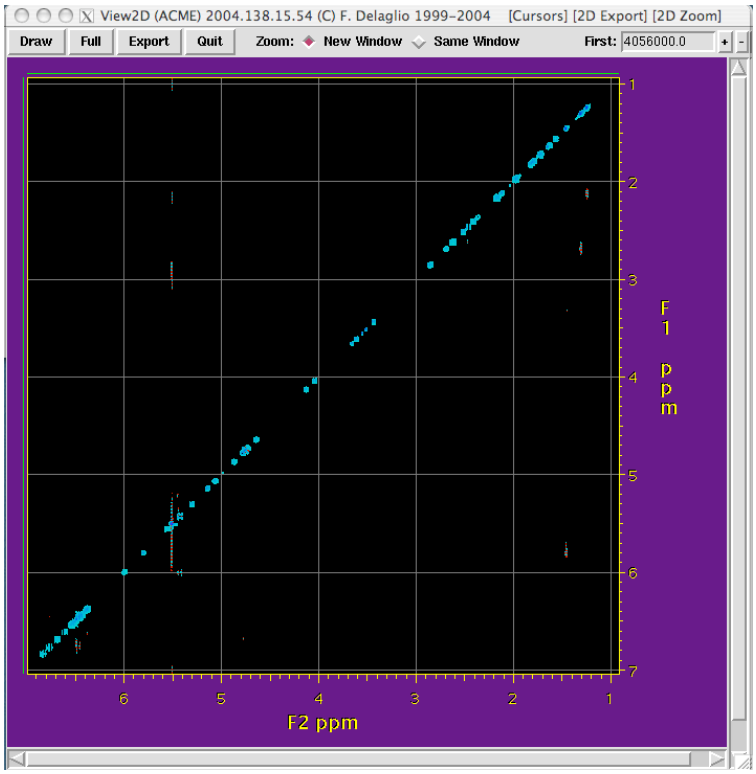


Figure S4. A. Diagonal-suppressed COSYPS spectrum of AmdeB allyl ester **21**. **B**. Diagonal-only COSYPS spectrum of Amde B allyl ester **21**.

Crosspeak Fitting. The ACME method for determining *J* values from COSYPS spectra is described in detail by Delaglio et al.[14] Briefly, the ACME program can accurately integrate the crosspeaks of a COSYPS spectrum provided the experiment is run with interscan delay time sufficient for the spins to fully relax. ACME integrates selected peaks from the diagonal-only spectrum and the resulting integration values are used to integrate crosspeaks in the diagonal-suppressed spectrum. Figure S5 shows the results of fitting two peaks from the COSYPS diagonal of AmdeB allyl ester **21**. Panels A, B, and C contain; A) the selected region of the spectrum (with peaks for fitting labeled 1 and 2), B) simulated peaks calculated in the fitting process, C) the residual between the experimental and calculated peaks. Therefore, in an accurate simulation, no residual is present for the selected peaks. Through this process, the average integration of selected peaks may be used to integrate crosspeaks from the diagonal-suppressed spectrum.

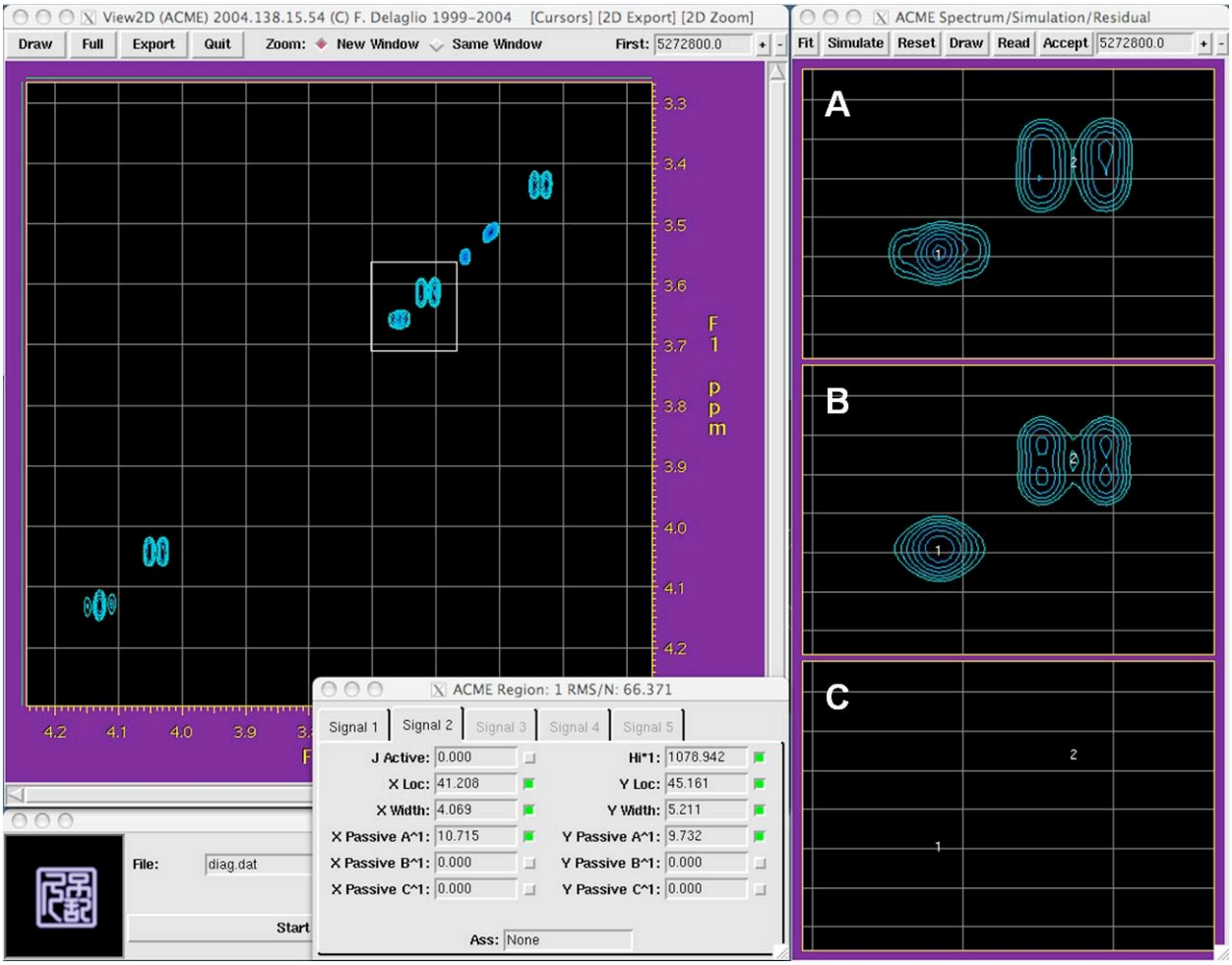


Figure S5. ACME results for fitting the AmdeB allyl ester **21** diagonal. A) The selected spectral region with peaks for fitting labeled 1 and 2. B) Simulated peaks calculated in fitting process. C) Residual between panels A) and B).

The fine structure of crosspeaks in a COSYPS spectrum can be used to obtain coupling constants in a manner analogous to obtaining coupling constants from a one-dimensional multiplet. In a two-dimensional crosspeak, the spacing between antiphase portions of the crosspeak correspond

to the *J* value for the associated spins. The ACME method employs the reference integration from the diagonal in a peak-fitting algorithm that integrates selected crosspeaks and calculates coupling constants. Figure S6 shows the results from the fitting algorithm applied to the cross-peak corresponding to protons H-18a and H-17 in AmdeB allyl ester **21**. Panel A again contains the selected peak from the spectrum. Panel B depicts the simulated crosspeak, and Panel C displays the residual. The lack of any significant residual is consistent with accurate reproduction of the multiplet fine structure in the fitting process. Similar analysis for each crosspeak in the spectra of compounds **17**, **2**, **21** and **23** was used to derive the coupling constants from which dihedral constraints were calculated.

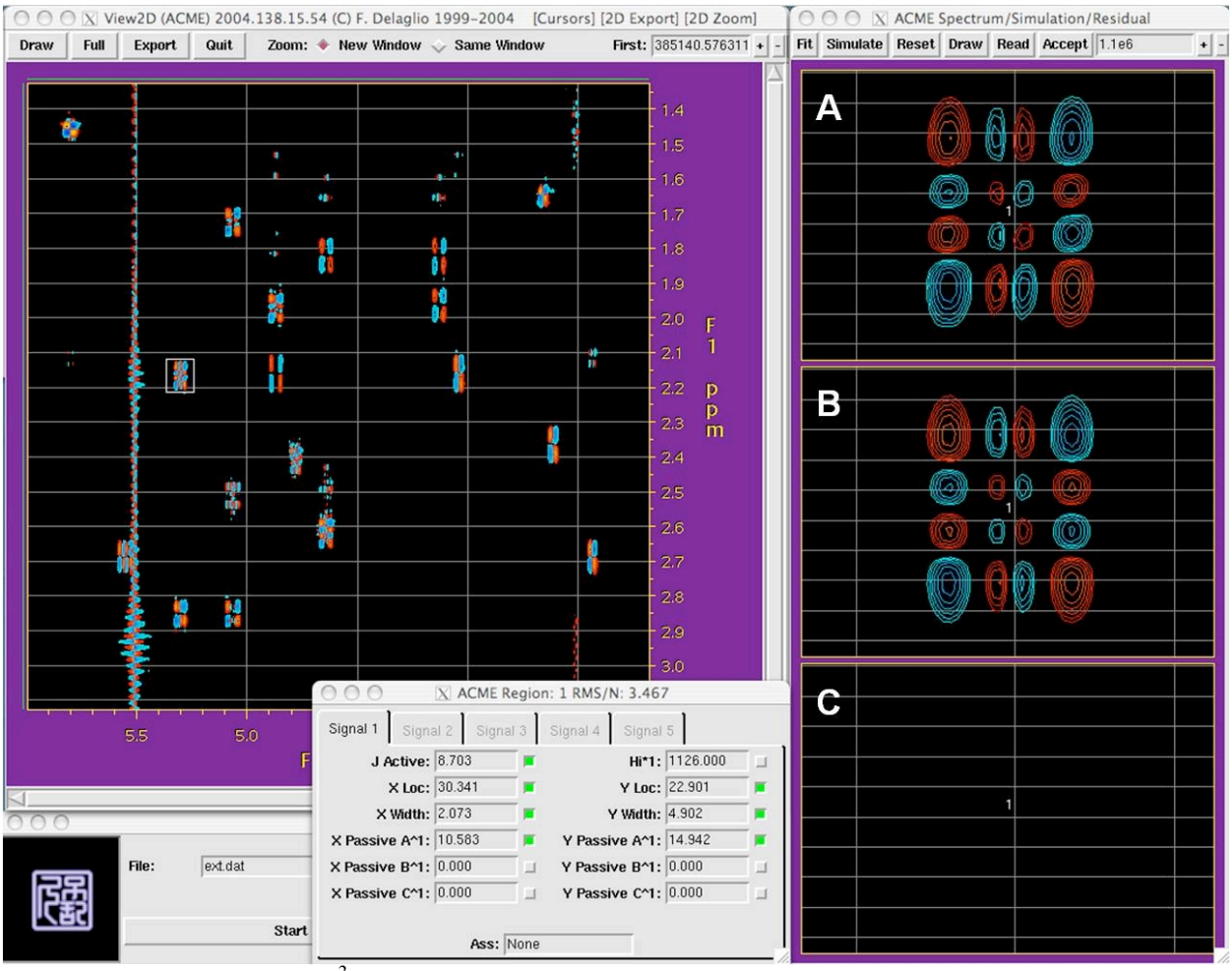


Figure S6. Calculation of ^{3}J for H-18a (pseudoaxial) and H-17 for AmdeB allyl ester **21**. ACME accurately reproduces the fine structure of the multiplet with no residual between the experimental and calculated peaks.

Dihedral angles were calculated from H-H ^{3}J values according to Altona's extended Karplus equation[15] using the "HLA (4 substituents)" setting in the MestReJ software.[16] However, each ^{3}J gives rise to 4 possible solutions to the Karplus equation. Methods for choosing the appropriate

[15] Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron*, **1980**, *36*, 2783-2792.
[16] http://www.mestrec.com/producto.php?id=8

value are well-precedented. For example, in the context of conformational analysis of the erythronolide B lactone, Aurichio[17] and Egan[18] chose dihedral values consistent with NOE data and the erythronolide B crystal structure, respectively. For our analyses, we chose angles consistent with both NOESY data and the AmB crystal structure.[19] In some cases, two solutions to the Karplus equation were consistent with both NOESY and crystal structure data, and both solutions were included. The selected dihedral angles (± 30°) were used as constraints in Monte Carlo conformational searches (see Section IV).

IV. Energy Minimization Calculations

NMR-Restrained Model Structures. Monte Carlo conformational searches were performed using the Molecular Operating Environment program (MOE), Version 2006.08,[20] with the empirical MMFF94x force field and a Born solvation model with no distance cutoffs for non-bonded interactions. Initial atomic coordinates and structure files were generated from the AmB crystal structure using MOE. NMR-derived distance and dihedral constraints were set with a weighting factor of 200. 3500 random conformations were generated and minimized with Gaussian distribution of dihedrals biased towards multiples of 30°, dihedral minimization (RMS = 100), 0.001 Cartesian minimization RMS gradient, 0.0001 Cartesian perturbation, 0.1 RMS tolerance, a maximum of 2000 energy minimization steps for each minimization, a failure limit of 5000, no chiral inversion, no rotation about π bonds or amide bonds, and an energy cutoff of 5 kcal/mol. Force field partial charges were calculated before each minimization. Default values were used for all other parameters.

Consistent with protein structural analysis techniques,[21] each H-H dihedral was constrained to the selected value ± 30º. When two solutions to the Karplus equation were selected, both values (± 30º) were allowed. Table S1 lists the dihedral constraints used in the conformational searching. Consistent with the standard convention for dihedral angles, values for the dihedral, θ, were defined over the range $-180° < \theta \leq 180°$. Consistent with the known *trans*-configuration of the seven double bonds of the polyene moiety, the π-bonds were constrained to 180 ± 10°.

Interproton distances were constrained for proton pairs exhibiting NOE correlations, with the lower limit set at 1.8 Å (twice the hydrogen van der Waals radius), and the upper limit set at 5.0 Å. Table S2 lists the NOE correlations used for conformational searching, and Figures S7 – S10 depict these correlations (red lines indicate NOE correlations, see Section VII for NOESY spectra). *Notably, all four compounds contain a diagnostic series transannular NOEs between protons of the polyol and those of the polyene*.

The Monte Carlo conformational search explores conformational space by randomly perturbing all dihedral angles in the molecule and then minimizing the resulting structures (taking into

[17] Auricchio, S.; Fronza, G.; Mele, A. *J. Org. Chem*. **1992**, *57* (2), 452-455.
[18] Egan, R. S.; Martin, J. R.; Perun, T. J.; Mitscher, L. A. *J. Am. Chem. Soc.* **1975**, *97* (16), 4578- 4583.
[19] Ganis, P.; Avitabile, G.; Mechliński, W.; Schaffner, C.P. *J. Am. Chem. Soc.* **1971**, *93*, 4560-4564.
[20] *Molecular Operating Environment*, version 2006.08; Chemical Computing Group: Montreal, Quebec, Canada.
[21] Cavanagh, J.; Fairbrother, W. J.; Palmer, A. G. III; Rance, M.; Skelton, N. J. *Protein NMR Spectroscopy: Principles and Practices, 2nd Ed.*; Academic Press: San Diego, 2006; p. 802.

account the restraints as described above). MOE repeated this process 3500 times, and the lowest energy conformation of each compound was used in rigid root mean square (RMS) atom alignment (see below).

	N-Acyl AmiB Methyl Ester			*N*-Fmoc C41-Me AmiB			AmdeB Allyl Ester			C41-Me AmdeB		
		θ constraint (degrees)			θ constraint (degrees)			θ constraint (degrees)			θ constraint (degrees)	
Protons	*J* (Hz)	Lower	Upper	*J* (Hz)	Lower	Upper	*J* (Hz)	Lower	Upper	*J* (Hz)	Lower	Upper
2ax - 3	3.1	-81	-21	1.8	-89	-29	4.7	-72	-12	ND[a]		
2eq -3	10.0	129	-120	9.1	123	-115	9.2	126	-174	9.2	124	-115
3 - 4ax	2.9	21	81	2.0	28	88	3.9	15	75	4.3	12	72
3 - 4eq	9.9	123	-133	10.1	124	-134	10.1	124	-134	10.9	133	-142
4ax - 5	2.9	-81	-21	1.0	-127	-37	4.4	-72	-12	1.8	-89	-29
4eq - 5	9.6	131	-120	9.6	132	-120	9.5	130	-120	10.3	136	-126
5 - 6ax	1.3	35	95	1.8	30	90	5.4	5	65	1.1	37	97
5 - 6eq	10.6	133	-145	10.7	137	-148	10.3	129	-141	11.5	150	-150
6eq - 7eq	13.9	150	-150	13.4	139	-139	ND[a]			13.5	141	-140
6eq - 7ax	8.1	5	65	4.4	22	82	ND[a]			5.7	16	76
6ax - 7ax	12.1	129	-129	12.7	133	-133	ND[a]			10.6	120	-120
6ax - 7eq	ND[a]			ND[a]			ND[a]			ND[a]		
7ax - 8	10.7	129	-140	10.6	128	-139	11.2	135	-147	11.3	138	-150
7eq - 8	2.7	23	83	2.5	24	84	2.8	22	82	2.4	25	85
8-9	3.1	-65	-5	3.4	-63	-3	3.0	-66	-6	3.2	-64	-4
9 - 10ax	1.8	29	89	2.6	24	84	3.3	19	79	2.3	33	93
9 - 10eq	11	128	-139	10.7	126	-136	10.7	126	-136	11.7	129	-128
10ax - 11	2.3	-85	-25	NA[a]	NA	NA	3.1	-80	-20	1.3	-94	-34
10eq - 11	10.0	134	-123	10.4	137	-131	10.3	136	-126	10.9	142	-132
11 - 12ax	2.7	23	83	ND[a]			3.4	19	79	1.2	35	95
11 - 12eq	11.3	132	-142	11.0	128	-138	11.3	132	-142	10.7	126	-136
14ax - 15	11.6	144	-134	11.8	147	-137	11.0	138	-127	11.5	143	-133
14eq - 15	4.5	24	84	2.6	35	95	3.5	29	89	2.1	39	99
15 - 16	10.4	132	-141	11.2	150	-150	10.7	148	-152	10.8	150	-150
16 - 17	10.8	130	-130	10.4	135	-144	10.6	165	-148	10.8	150	-150
17 - 18ax	8.8	124	-115	7.5	120	-120	8.7	123	-177	8.1	120	180
17 - 18eq	1.0	-98	-67	ND[a]			0.9	-99	-39	ND[a]		
18ax - 19	2.0	28	88	2.5	24	84	ND[a]			3.9	15	75
18eq - 19	4.5	-72	-12	4.6	-82	-22	6.0	-74	-14	5.5	-64	-4
19 - 20	8.8	120	180	8.0	120[b]	-120[b]	8.8	120[b]	-120[b]	8.9	120[b]	-120[b]
21 - 22	ND			ND			11.0	150[b]	-150[b]	10.8	150[b]	-150[b]
23 - 24	ND			ND			11.0	150[b]	-150[b]	10.8	150[b]	-150[b]
25 - 26	ND			ND			11.5	150[b]	-150[b]	ND		
33 - 34	10.2	133	-167	10.0	120[b]	-120[b]	10.1	120[b]	-120[b]	9.9	120[b]	-120[b]
34 - 35	9.6	125	-138	9.6	125	-138	9.8	128	-140	10.0	130	-143
35 - 36	2.3	-85	-25	2.5	-84	-24	2.6	-83	-23	2.6	-83	-23
36 - 37	4.2	-82	-22	1.8	-93	-33	1.9	-100	-40	3.1	-90	-30
1'-2'	1.0	34	118	1.6	24	84	NA			NA		
2'-3'	3.0	-82	-22	ND[a]			NA			NA		
3'-4'	10.5	152	-135	9.7	142	-124	NA			NA		
4'-5'	9.2	150	-150	9.1	150	-150	NA			NA		

[a]Associated cross peak was either too close to the diagonal or too weak to achieve an acceptable peak-fit.

[b]The generalized Karplus equation was utilized to determine dihedral constraint (Karplus, M.; *J. Phys. Chem.* **1959**, *30*, 11-15.).

Table S1. Dihedral constraints used in conformational searches using MOE. Also shown are the coupling constants (calculated by ACME) from which the dihedral constraints were derived.

Proton	N-Acyl AmB Methyl Ester	N-Fmoc C41-Me AmE	AmdeB Allyl Ester	C41-Me AmdeE
	NOE with proton(s)	NOE with proton(s)	NOE with proton(s)	NOE with proton(s)
3	**5, 28**	**5, 28**	**5, 28**	**5, 28**
5	3, 28	3, 28	3, 26, 28	3, 28
7eq	**9, 24, 26**	–	–	–
8	10ax	–	–	–
9	**7eq, 11, 22, 24**	**11, 22**	**11, 20, 22, 24, 26**	**11**
10ax	8	–	–	12ax
11	**9, 20, 22**	**9, 20, 22**	**9, 20, 22, 24**	**9, 20**
12ax	14ax	–	–	10ax
14ax	**12ax**	–	**16**	–
15	17	–	–	–
16	**14ax**	–	**14ax, 18ax**	–
17	15, 20	20	20	20
18ax	–	–	**16**	–
19	21, 1'	21	21	21
20	**11, 17**	**11, 17**	**9, 11, 17, 22**	**11, 17, 22**
21	19	19	19	19
22	**9, 11**	–	**9, 11, 20, 24**	**20**
24	7eq, 9	–	9, 11, 22, 26	–
26	**7eq**	–	**5, 9, 24**	–
28	3, 5	3, 5	–	3, 5
31	**33**	**33**	–	**33**
32	34, 37	34, 37	34, 37	34, 37
33	**31, 35, 36**	**31, 36**	**31, 35**	**31, 35**
34	32, 37	32	32, 37	32, 37
35	**33**	–	**33**	**33**
36	33	33	–	–
37	**34, 32**	**32**	**34, 32**	**34, 32**
1'	3', 5'	3', 5'	NA	NA

Table S2. Observed NOE correlations used to derive distance constraints in conformational searches using MOE.

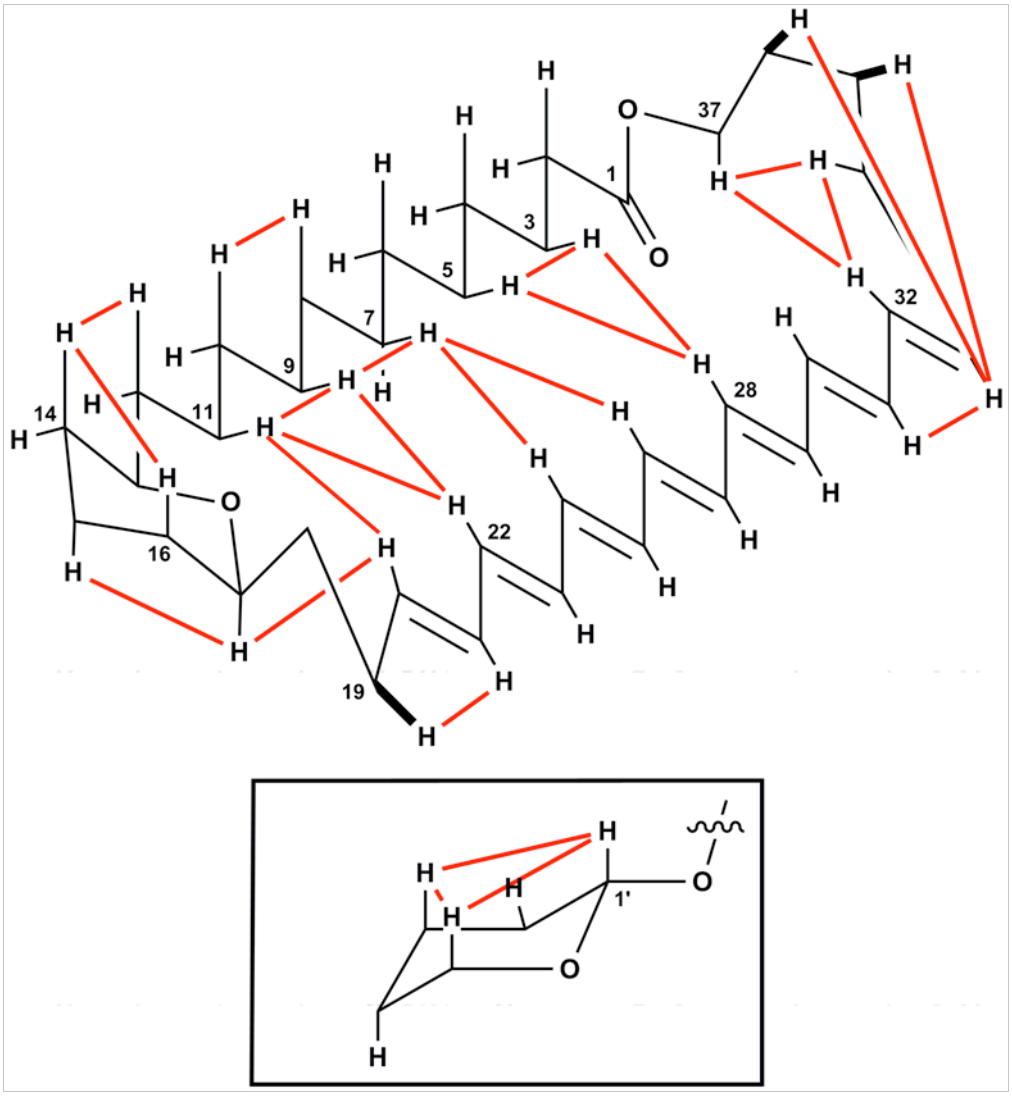


Figure S7. NOE correlations for N-acyl AmB Methyl ester **17**. For clarity, appendages other than protons have been removed from the macrolide skeleton. Selected carbon atoms are numbered.

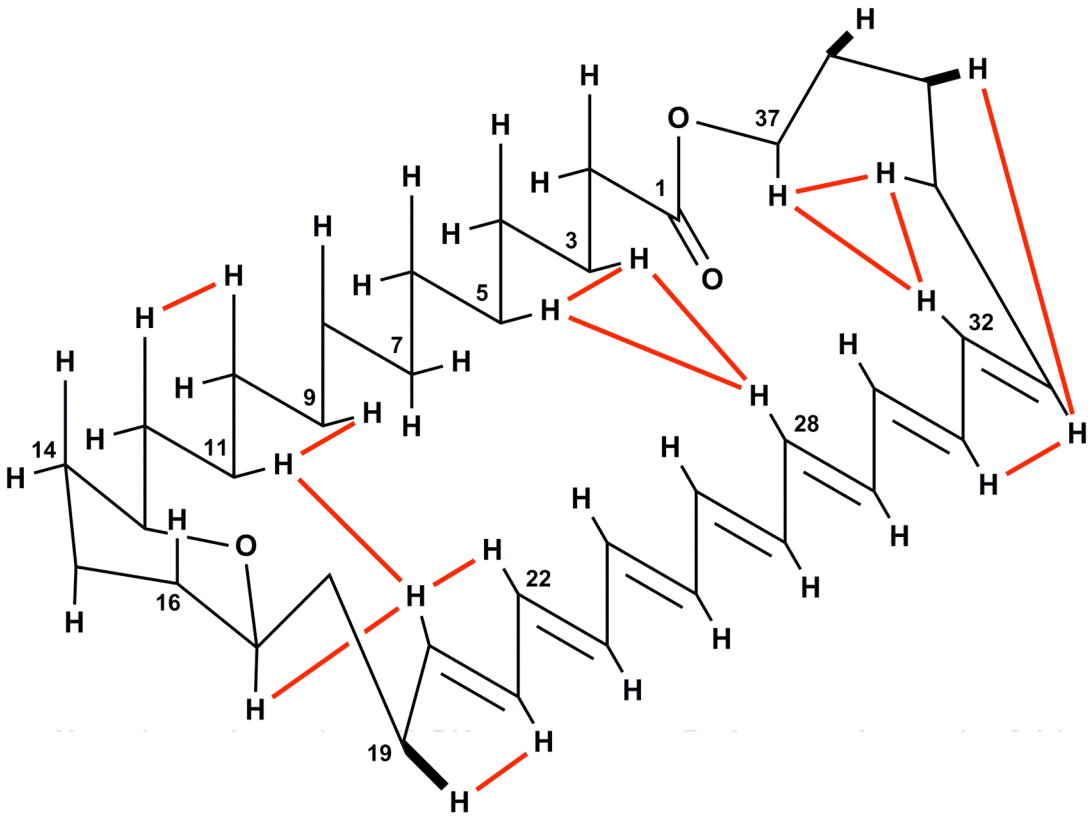


Figure S8. NOE correlations for MeAmdeB **2**. For clarity, appendages other than protons have been removed from the macrolide skeleton. Selected carbon atoms are numbered.

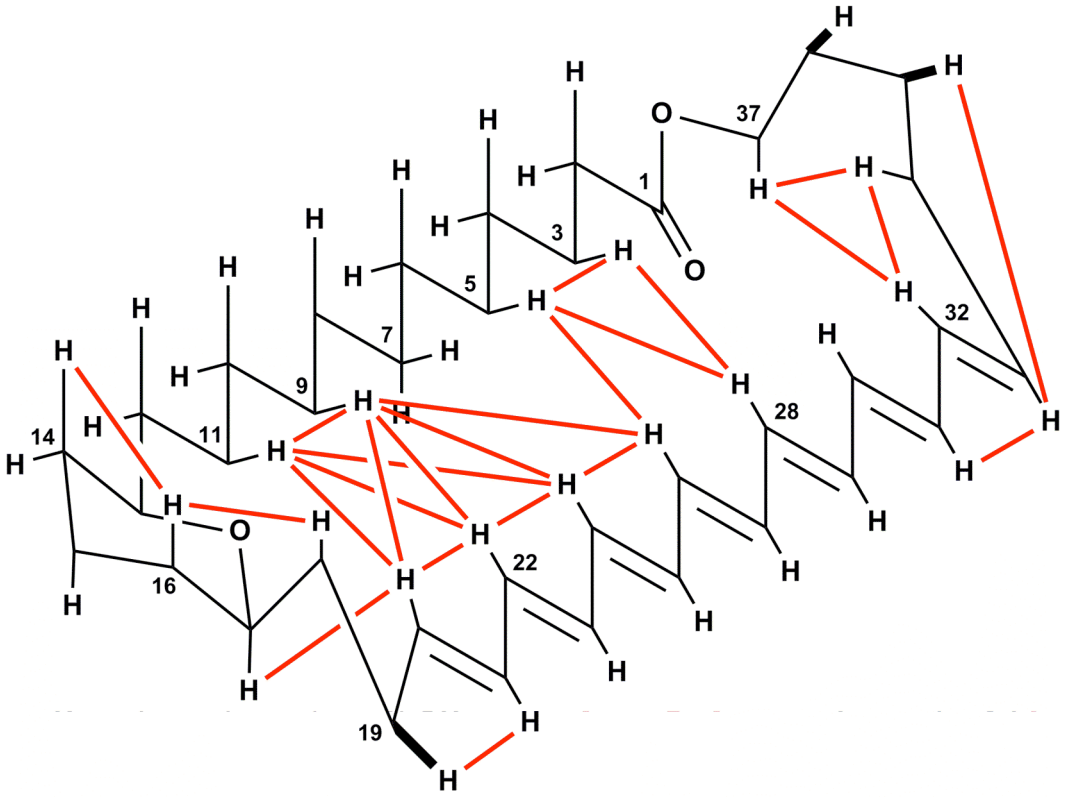


Figure S9. NOE correlations for AmdeB allyl ester **21**. For clarity, appendages other than protons have been removed from the macrolide skeleton. Selected carbon atoms are numbered.

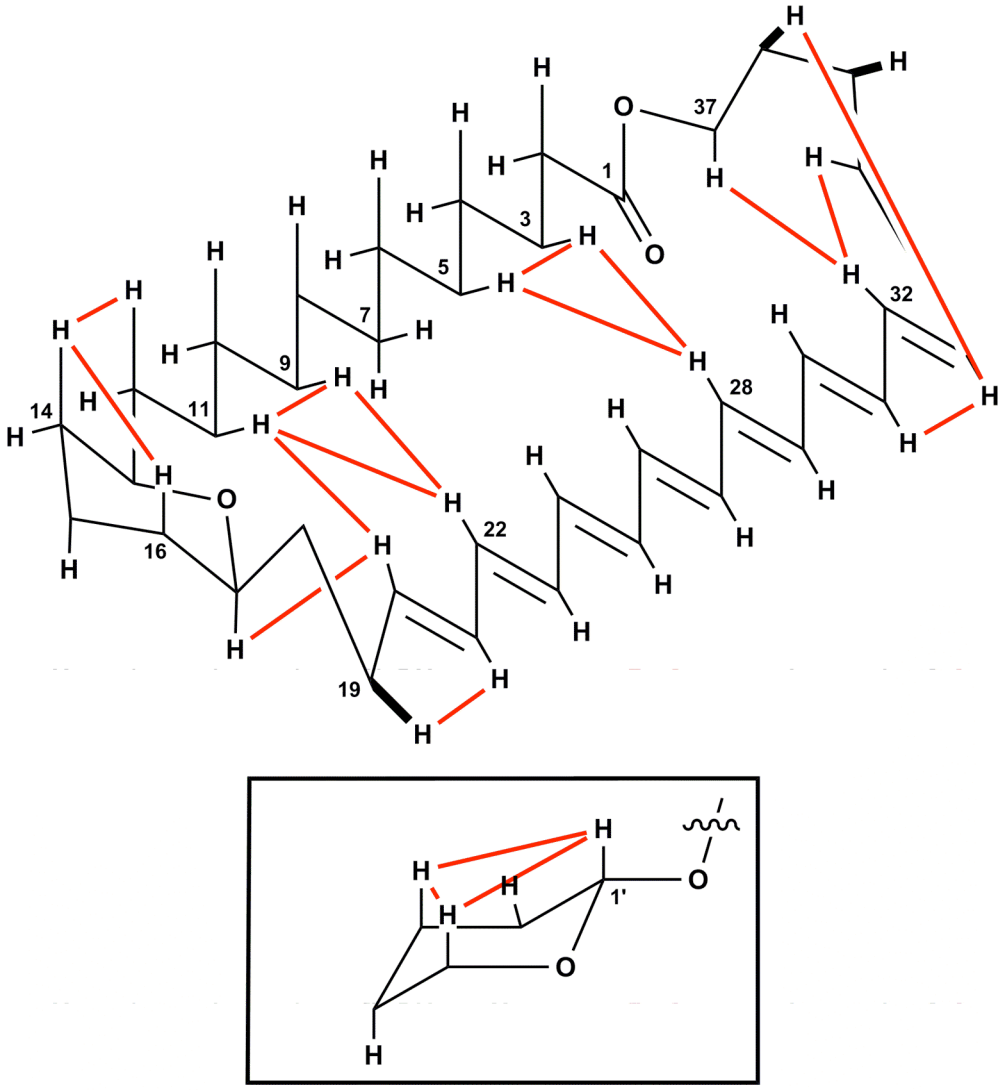


Figure S10. NOE correlations for *N*-Fmoc MeAmB **23**. For clarity, appendages other than protons have been removed from the macrolide skeleton. Selected carbon atoms are numbered.

Rigid RMS atom alignment for NMR-restrained model structures. Only the atoms of the macrolactone ring and the cyclic hemiketal were used for RMS alignment. All other atoms were deleted from the lowest-energy conformers of **17**, **2**, **21**, and **23**, and the resulting skeletons representing the ground-state conformations of AmB **1**, MeAmB **2**, AmdeB **3**, and MeAmdeB **4**, respectively were saved as MDL MOL files (*.mol) and imported into the Cerius[2] program, Version 4.11,[22] with no energy minimization or calculation of charges. Rigid RMS atom alignment revealed RMSD = 0.081 Å for the four structures. The aligned structures were saved as PDB files (*.pdb), and the overlay image (Figure 2 in the text and Figure S11) was generated using Visual Molecular Dynamics (VMD).[23]

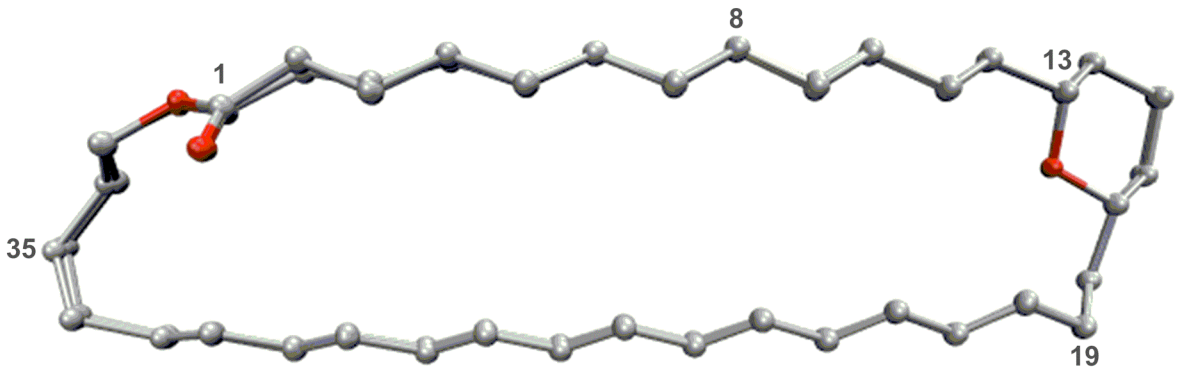


Figure S11. Superposition of the ground state conformation of the macrolactone skeletons of compounds **1**-**4** (or their more soluble analogs). Rigid RMS atom alignment revealed RMSD = 0.081 Å for the four structures.

[22] AccelRys Software, Inc. http://www.accelrys.com/products/cerius2/
[23] Humphrey, W.; Dalke, A; and Schulten, K., *J. Molec. Graphics* **1996**, *14*, 33-38.

V. Antifungal Assays

General procedure for extinction coefficient determination. 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.[24]

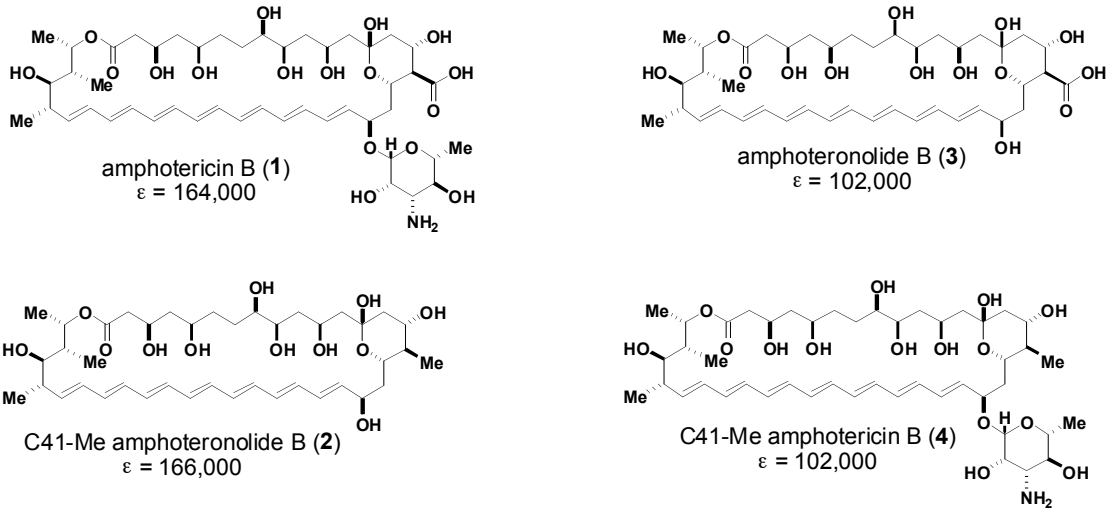


Figure S12. Extinction coefficients for compounds **1**-**4**.

Growth conditions for *Saccharomyces cerevisiae.* *S. cerevisiae* (ATCC 9763) cultures were incubated at 30 ºC on yeast peptone dextrose (YPD) agar plates or in YPD liquid cultures with rotary shaking. For liquid YPD medium, yeast extract (5 g), Bacto peptone (10 g), and MilliQ H_2O (475 mL) were combined and autoclaved at 250 ºC for 15 minutes. Sterile 40% w/v aqueous glucose (25 mL) was subsequently added (sterile glucose solutions were prepared by dissolving glucose in MilliQ water and autoclaving at 250 ºC for 12 min). For agar plates, the same procedure was used except using only 225 mL of water and combining with 250 mL sterile 4% w/v aqueous agar solution (sterile 4% w/v agar was prepared by adding agar to MilliQ water and autoclaving at 250 ºC for 15 min). Agar plates were prepared by pouring the hot YPD/agar mixture into sterile 15 mm x 100 mm culture dishes. The plates were allowed to cool at room temperature until the agar had solidified.

Growth conditions for *Candida albicans.* *C.albicans* (ATCC 90028) were cultured in the same manner as *S. cerevisiae* except that the cells were incubated at 37 °C rather than 30 °C.

[24] The value obtained for AmB in this manner agrees with the previously reported value; see McNamara, C.M.; Box, S.; Crawforth, J.M.; Hickman, B.S.; Norwood, T.J.; Rawlings, B.J. *J. Chem. Soc. Perkin Trans. I* **1998**, 83-87.

Disk Diffusion Assay. Protocols for disk diffusion assays were adapted from the National Committee of Clinical Laboratory Standards document M2-A8.[25] Yeast were streaked on YPD agar plates with a sterile toothpick and incubated at 30 ºC (*S. cerevisiae*) or 37 °C (*C. albicans*) until individual colonies could be identified by eye (~ 24 h). A single colony was suspended in 150-200 uL YPD liquid medium, and this suspension was added to ~50 mL YPD liquid medium. The liquid culture was incubated overnight at 30 °C (*S. cerevisiae*) or 37 °C (*C. albicans*) in a shaker incubator (200 rpm). The saturated cell culture was diluted with YPD medium to an OD_{600} of 0.1 (~ 3 x 10^7 cells/mL) as measured on a Shimadzu PharmaSpec UV-1700 UV/Visible spectrophotometer. This culture was used to inoculate a YPD plate by streaking the entire plate with a sterile cotton tip applicator three times, turning the plate approximately 60 ° after each application and finishing by swabbing the rim of the agar. The plate was allowed to dry for approximately 2 to 3 minutes before application of paper disks impregnated with compounds **1-4**. The disks were prepared in the following manner: 10 microliters of a 4 mg/ml (*S. cerevisiae*) or a 2 mg/mL (*C. albicans*) solution of each compound in DMSO was added to an 8 mm disk of Whatman 4 filter paper. Controls were prepared in a similar manner using only DMSO. The disks were then placed on the agar and gently pressed with forceps. All disks, including DMSO controls, were added within 15 minutes of inoculation. After disks were added to the plate the plate was inverted and incubated at 30 °C (*S. cerevisiae*) or 37 °C (*C. albicans*) for 36 to 48 hours prior to assessment. Those compounds which showed a visible zone of growth inhibition were judged to be active (Figure S13). This experiment was repeated for each yeast strain and yielded the same results.

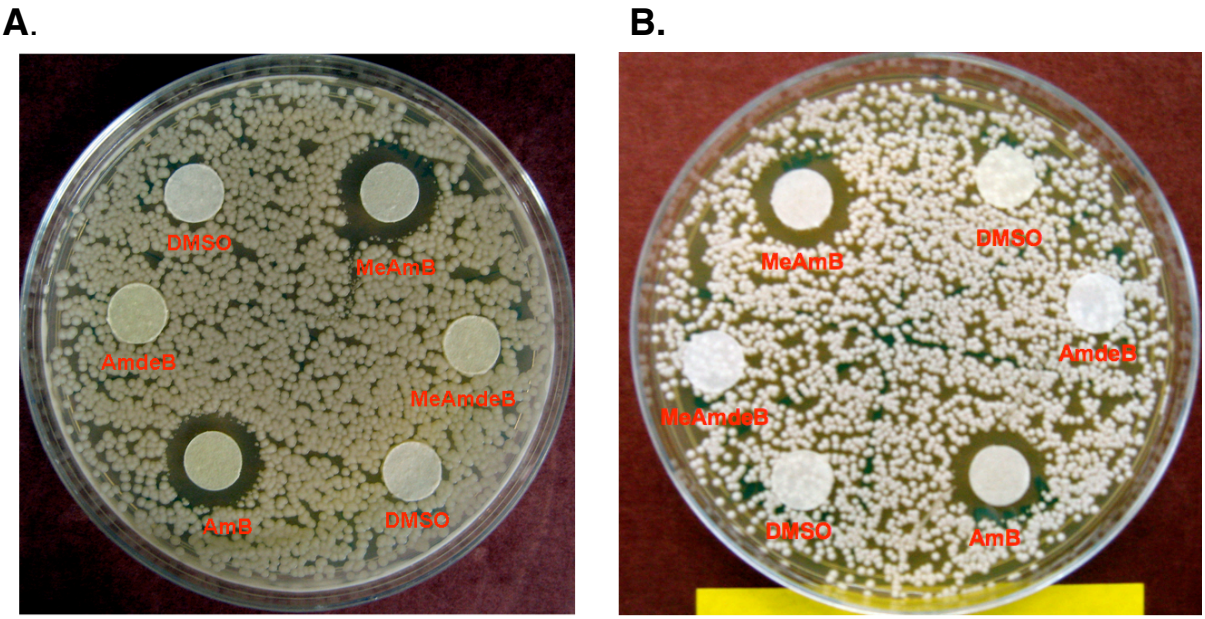


Figure S13. Representative results of disk diffusion assay for **A**. *S. cerevisae* and **B.** *C. albicans.*

[25] National Committee of Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk Susceptibility Tests, M2-A8 Approved Standard-8th Ed. Vol. 23, Number 1, 2003.

Broth microdilution minimum inhibitory concentration (MIC) assays. Protocols for broth microdilution assays were adapted from the National Committee of Clinical Laboratory Standards document M27-A2[26] and by the protocol reported by Paquet and Carreira.[27] Yeast were streaked on YPD agar plates with a sterile toothpick and incubated at 30 °C (*S. cerevisiae*) or 37 °C (*C. albicans*) until individual colonies could be identified by eye (~ 24 h). A single colony was suspended in 150-200 μL YPD liquid medium, and this suspension was added to ~50 mL YPD liquid medium. The liquid culture was incubated overnight at 30 °C (*S. cerevisiae*) or 37 °C (*C. albicans)* in a shaker incubator (200 rpm). The saturated cell culture was diluted with YPD medium to an OD_{600} of 0.1 (~ 3 x 10^7 cells/mL) as measured on a Shimadzu PharmaSpec UV-1700 UV/Visible spectrophotometer. Aliquots (195 μL) of the resulting cell suspension were added to a 96-well plate. Compounds for testing were prepared as 400 μM solution in DMSO and this stock solution was serially diluted to concentrations of 320, 240, 200, 160, 120, 80, 40, 20, 10, and 5 μM. Aliquots (5 μL) of each DMSO solution were added to the 96-well plate, with each row of the plate containing a different concentration. This 40-fold dilution resulted in final compound concentrations of 10, 8, 6, 5, 4, 3, 2, 1, 0.5, 0.25, and 0.125 μM. DMSO (5 μL) was added as a control to each well of the final row. Each concentration was tested in triplicate. Plates were covered with Corning Thermowell aluminum sealing tape and incubated at 30 °C (*S. cerevisiae*) or 37 °C (*C. albicans*) for 18 hours. MIC values were determined as concentration corresponding to the row which showed no visible growth. The assay was repeated three times and the reported values represent the average of these three experiments.

VI. Liposome Studies

Preparation of pyranine-containing liposomes

Liposomes were prepared using a modification of the protocol reported by Fujii and coworkers.[28] Hydrogenated soy phosphatidylcholine (HSPC) ergosterol (erg) and distearoyl phosphatidylcholine (DSPG) were dissolved in chloroform (0.1M, 0.01M and 0.01M respectively). To a 13 x 100 mm glass test tube was added 303 microliters of the HSPC solution, 750 microliters of the erg solution and 1.21 millilters of the DSPG solution. Samples were incubated at 65 °C for approximately five minutes and the solvent was evaporated with a stream of N_2 at 65 °C. The samples were left under vacuum for at least eight hours prior to hydration. The sample was hydrated with 2 mL pyranine SSB buffer [0.1 mM pyranine dye, 9% (w/w) sucrose 10 mM sodium succinate pH 5.5] and incubated at 65 °C for approximately five minutes. The samples were then vortexed vigorously to create a homogenous suspension. The homogenous suspension was probe sonicated 5 minutes (5 output watts, 2 mm probe) with a Sonics and Materials (Danbury, CT) Vibracell Sonicator model VC 130 keeping the internal temperature of the sample at 65 °C with a vigorously stirred water bath. The samples were then cooled to room temperature and passed through a 0.22 μm syringe tip filter (Millipore, Billerica MA). Unencapsulated pyranine was removed by gel exclusion chromatography with a Sephadex G50-150 column (1.5 x 25 cm) and SSB as the eluent. The liposome containing fractions were visualized with UV light (λ_{366}).

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[26] National Committee of Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing, M27-A2, Approved standard-2nd Ed. Vol. 22, Number 15, 2002.

[27] Paquet, V.; Carreira, E. M. *Org. Lett.* **2006**, *8*, 1807.

[28] a) Fujii, G.; Chang, J.-E.; Coley, T.; Steere, B. *Biochemistry* **1997**, 36, 4959-4968. b) Weakliem, C.L.; Fujii, G.; Chang, J.; Ben-Shaul, A.; Gelbart, W.A. *J. Phys. Chem*. **1995**, *99*, 7694-7697.

Liposome permeability assay

To prepare compounds **1** and **4** for the assay, a stock solution of 8 x 10^{-3} M was prepared. The stock solution was then serially dilute to the following concentrations: 6 x 10^{-3}, 4 x 10^{-3}, 2 x 10^{-3}, 1 x 10^{-3}, 8 x 10^{-4}, 6 x 10^{-4}, 4 x 10^{-4}, 2 x 10^{-4}, 1 x 10^{-4}, 8 x 10^{-5}, 6 x 10^{-5}, 4 x 10^{-5}, 2 x 10^{-5}, and 1 x 10^{-5}. The liposome suspension was prepared as described above and diluted by a factor of 10with SSB buffer prior to the experiment. A sample was then prepared for analysis by diluting 100 µL of the dilute liposome solution to 1.90 mL with phosphate buffer [9% sucrose, 10 mM (sodium) phosphate pH 7.4]. To this sample was added 2 µL of one of the drug containing solutions and time dependent emission spectra were observed over 300 seconds in 2 second increments. The excitation slitwidth was set at 4 nm and the emission slitwidth at 6.0 nm. The excitation wavelength was 454 nm and the emission wavelength was 513 nm.

This liposome permeability assay uses the pH dependent fluorescent dye pyranine to measure ion flux across the lipid bilayer.[28] As described above, this dye was trapped within small unilamellar vesicles (SUV's) having an internal pH of 5.5. The external buffer was then adjusted to pH 7.4 and increasing amounts of AmB or MeAmB were added to the exterior solution. Equilibration of the pH across the lipid bilayer, indicated by an increase in the fluorescence of the pyranine-impregnated liposome sample, signifies an increase in membrane permeability caused by the added compound. The negative log of the concentration of the compound was plotted against F_0/F_5 (the fluorescence intensity of the liposome suspension at time = 0 divided by the fluorescence intensity at time = 5 min.) to generate the plot shown in Figure S14.

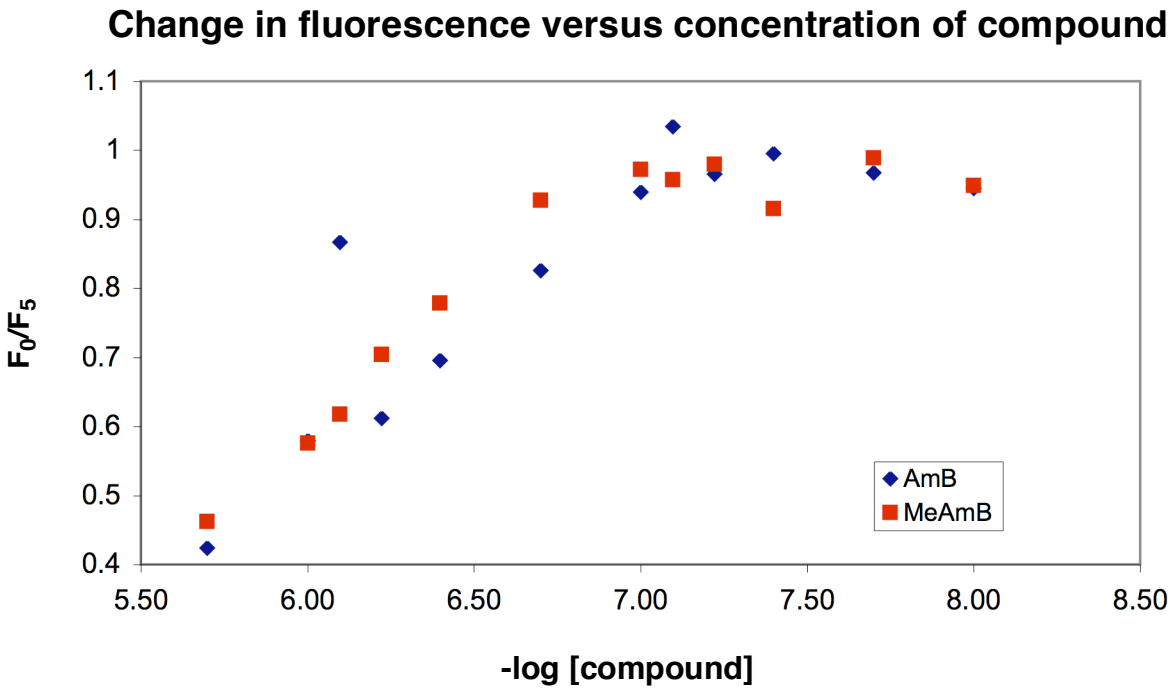

Figure S14. Change in fluorescence versus concentration of compound. F_0/F_5 represents the fluorescence intensity of the pyranine-impregnated liposome suspension at time = 0 divided by the fluorescence intensity at time = 5 min. A reduction in this ratio indicates the permeabilization of liposomes.[28] Data represent the average of two experiments.