Supporting Information

Synergy of Prediction Rule and Total Synthesis in Solving the Stereochemical Puzzle of Eucalactam B

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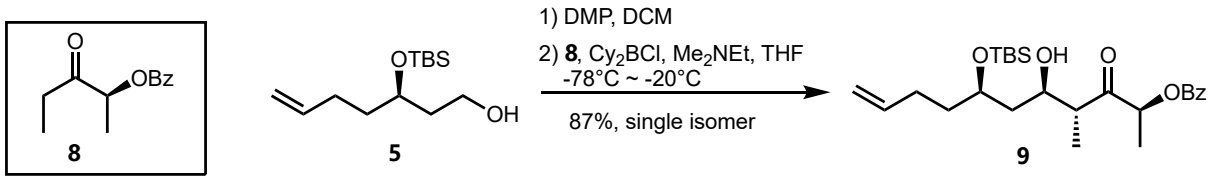
I. General Information

All reactions were conducted in flame-dried or oven-dried glassware under an atmosphere of dry nitrogen or argon unless otherwise stated. Oxygen and/or moisture-sensitive solids and liquids were transferred appropriately.

The concentration of solutions in vacuo was accomplished using a rotary evaporator fitted with a water aspirator. All reaction solvents were purified before use: Tetrahydrofuran was distilled from sodium. Toluene was distilled over molten sodium metal. Dichloromethane, dimethylformamide, diethylamine, triethylamine and diisopropylethylamine were distilled from CaH_2. Methanol was distilled from Mg/I_2. Flash column chromatography was performed using the indicated solvents on E. Qingdao silica gel 60 (230 – 400 mesh ASTM). TLC was carried out using pre-coated sheets (Qingdao silica gel 60-F250, 0.2 mm). Compounds were visualized with UV light, iodine, p-anisaldehyde stain, ceric ammonium molybdate stain, Ninhydrin stain or phosphomolybdic acid in EtOH. ^{1}H NMR spectra were recorded on Bruker Avance 400 MHz, Avance 500 MHz or Quantum-I Plus 400 spectrometers. Chemical shifts were reported in parts per million (ppm), relative to either a tetramethylsilane (TMS) internal standard or the signals due to the solvent. The following abbreviations are used to describe spin multiplicity: s = singlet, d =doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, br = broad, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartets, ddd = doublet of doublet of doublets; other combinations are derived from those listed above. Coupling constants (J) are reported in Hertz (Hz) for corresponding solutions, and chemical shifts are reported as parts per million (ppm) relative to residual $CHCl_3$ δH (7.26 ppm). ^{13}C Nuclear magnetic resonance spectra were recorded using a 101 MHz or a 126 MHz spectrometer for corresponding solutions, and chemical shifts are reported as parts per million (ppm) relative to residual $CDCl_3$ δC (77.16 ppm). High-resolution mass spectrometry was measured on Thermofisher Q-Exactive Focus. Accurate masses are reported for the molecular ion (M+H, M+Na, M-H), or a suitable fragment ion. Optical rotations were recorded on an Anton Parr MCP 4100 polarimeter at 589 nm, 2.5 mm cell. Data were reported as follows: optical rotation (c (g/100 mL), solvent).

II. Experimental Details and Spectral Data

(2S,4R,5R,7R)-7-((tert-butyldimethylsilyl)oxy)-5-hydroxy-4-methyl-3-oxoundec-10-en-2-yl benzoate (9)



To a stirred solution of **5**[1] (1.16 g, 4.74 mmol, 1.0 eq.) in anhydrous DCM (50 mL) was added $NaHCO_3$ (1.60 g, 18.96 mmol, 4.0 eq) followed by Dess-Martin periodinane (4.02 g, 9.48 mmol, 2.0 eq.) at 0°C under argon. The mixture was stirred at ambient temperature, and after 3 h at 0 ºC, the reaction was completed by TLC analysis. The solvent was removed in vacuo and the residue was purified by flash chromatography (2% EtOAc/hexanes) to afford the corresponding aldehyde **7** (*ca.* 1.13 g).

To a solution of **8** (638.0 mg, 3.1 mmol, 0.67 eq.) in anhydrous diethyl ether (50 mL, 0.06 M) was added Cy_2BCl (4.74 mL, 4.74 mmol, 1.0 M in diethyl ether, 1.0 eq.) at -78°C under argon, followed by Me_2NEt (408.0 mg, 5.58 mmol, 1.2 eq.). The mixture was kept at -78°C for 5 min and allowed to warm to 0°C and stirred for 1 h. Then the mixture was cooled back to -78°C, and a solution of the aldehyde **7** (*ca.* 1.13 g) in diethyl ether (10 mL) was added dropwise via cannula. The reaction mixture was stirred at -20 °C overnight before it was quenched by the addition of MeOH (5 mL) and pH = 7 Buffer (5 mL) and stirred for 5 min. After the slow addition of 30% H_2O_2 (10 mL) and stirring for another 30 min at ambient temperature, the reaction mixture was then diluted with MTBE, the organic layer was separated, and the aqueous phase was extracted with MTBE. The combined organic phases were washed with saline and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo and the crude product was purified with flash chromatography (2% ~ 5% EtOAc/hexanes) to give the hydroxyketone **9** as a colorless oil (1.49 g, 87%).

R_f = 0.35 (10% EtOAc/hexanes), PMA stain.

$[\alpha]_D^{20}$ = +6.2 (c 1, $CHCl_3$);

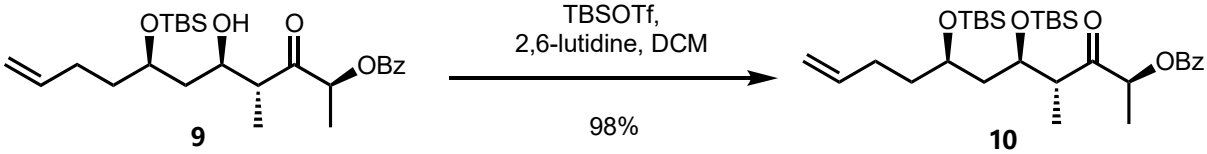
HRMS (ESI, m/z) for $C_{25}H_{40}O_5SiNa^+$ $[M + Na]^+$: Calcd. 471.2538; Found 471.2537.

IR (KBr) ν_{max} 3520, 2953, 2926, 2856, 1708, 1699, 1641, 1602, 1454, 1257, 1114, 1070, 1001, 908, 839, 773, 711, 509 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 – 8.02 (m, 2H), 7.62 – 7.54 (m, 1H), 7.51 – 7.40 (m, 2H)f 5.79 (ddt, *J* = 16.8, 10.0, 6.6 Hz, 1H), 5.43 (q, *J* = 7.0 Hz, 1H), 5.22 – 4.75 (m, 2H), 3.97 (dd, *J* = 7.5, 5.3 Hz, 1H), 3.91 (t, *J* = 8.6 Hz, 1H), 3.31 (brs, 1H), 2.91 (p, *J* = 7.0 Hz, 1H), 2.29 – 1.96 (m, 2H), 1.71 (ddd, *J* = 14.2, 5.3, 1.9 Hz, 1H),1.65 – 1.50(m, 3H) 1.56 (d, *J* = 7.0 Hz, 3H), 1.22 (d, *J* = 7.1 Hz, 3H), 0.87 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 211.2, 166.0, 138.5, 133.5, 129.9, 129.6, 128.6, 114.8, 75.0, 72.3, 72.1, 48.5, 40.1, 36.8, 29.1, 26.0, 18.1, 15.9, 13.6, -4.0, -4.5.

(2*S*,4*R*,5*R*,7*R*)-5,7-bis((*tert*-butyldimethylsilyl)oxy)-4-methyl-3-oxoundec-10-en-2-yl benzoate (10)



9 **10**

To a stirred solution of **9** (1.49 g, 3.32 mmol, 1.0 eq.) in anhydrous DCM (30 mL) was added 2,6-lutidine (8.30 mmol, 0.97 mL, 2.5 eq.) followed by TBSOTf (3.99 mmol, 0.92 mL, 1.2 eq.) at 0°C under argon. The reaction mixture was stirred at ambient temperature for 3 h before it was quenched with water and diluted with DCM. The phases were separated and the organic layer was washed with 0.5 M $CuSO_4$ solution and saline. The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo.* The crude product was purified with flash chromatography (2% ~ 3% EtOAc/hexanes) to give the ketone **10** as a colorless oil (1.83 g, 98%).

R_f = 0.6 (5% EtOAc/hexanes), PMA stain.

$[\alpha]_D^{21}$ = +0.6 (c 1, $CHCl_3$);

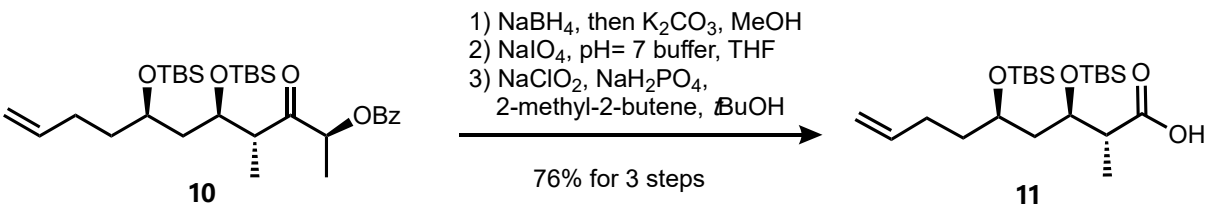
HRMS (ESI, m/z) for $C_{31}H_{54}O_5Si_2Na^+$ $[M + Na]^+$: Calcd. 585.3402; Found 585.3402.

IR (KBr) ν_{max} 3074, 2954, 2929, 2856, 1720, 1463, 1259, 1112, 1070, 837, 775, 771 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 – 7.97 (m, 2H), 7.63 – 7.53 (m, 1H), 7.50 – 7.41 (m, 2H), 5.89 – 5.74 (m, 1H), 5.42 (qd, *J* = 6.9, 1.4 Hz, 1H), 5.06 – 4.89 (m, 2H), 4.11 – 4.02 (m, 1H), 3.92 (p, *J* = 6.0 Hz, 1H), 3.15 – 3.04 (m, 1H), 2.20 – 1.99 (m, 2H), 1.72 (dt, *J* = 13.3, 6.3 Hz, 1H), 1.66 – 1.55 (m, 2H), 1.52 (dd, *J* = 6.9, 1.4 Hz, 3H), 1.39 – 1.22 (m, 1H), 1.19 (dd, *J* = 7.1, 1.3 Hz, 3H), 0.88 (d, *J* = 1.4 Hz, 9H), 0.85 (d, *J* = 1.3 Hz, 9H), 0.06 (d, *J* = 1.3 Hz, 3H), 0.04 (d, *J* = 1.3 Hz, 3H), 0.01 (d, *J* = 1.4 Hz, 3H), -0.04 (d, *J* = 1.3 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 209.0, 165.9, 139.1, 133.5, 130.0, 129.7, 128.6, 114.4, 74.7, 70.1, 68.6, 48.7, 40.6, 36.5, 29.2, 26.1, 26.0, 18.2, 18.1, 16.0, 12.2, -4.0, -4.2, -4.4, -4.5.

(2*R*,3*R*,5*R*)-3,5-bis((*tert*-butyldimethylsilyl)oxy)-2-methylnon-8-enoic acid (11)



10 **11**

To a stirred solution of **10** (1.62 g, 2.87 mmol, 1.0 eq.) in anhydrous methanol (30 mL) at 0°C was added

$NaBH_4$ (5.74 mmol, 217.0 mg, 2.0 eq.) under argon. The mixture was allowed to warm to ambient temperature and stirred for 30 min. After cooling to 0°C, anhydrous K_2CO_3 (1.59 g, 11.48 mmol, 4.0 eq.) was added, and the suspension was allowed to warm to ambient temperature and stirred overnight. The reaction was then quenched with pH = 7 buffer and diluted with MTBE. The layers were separated and the organic layer was washed with aqueous saturated NH_4Cl and saline. The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give rise to the crude product.

To a solution of the crude product in a mixed solvent of THF and pH = 7 buffer (35 mL total, 5.5:1, v/v) at 0°C was added Na_2IO_4 (2.45 g, 11.54 mmol, 4.0 eq.). The reaction mixture was allowed to warm to ambient temperature and stirred for 3 h. The resulting suspension was diluted with EtOAc (100 mL) and poured into 100 mL of water. The organic layer was separated and the aqueous layer was extracted with EtOAc (3×35 mL). The combined organic extracts were washed with saline, dried over Na_2SO_4 filtered, and concentrated *in vacuo* to offer the crude aldehyde.

To a solution of the crude aldehyde in a mixed solvent of *t*-BuOH and H_2O (45 mL total, 2:1, v/v) at 0°C under argon was added NaH_2PO_4 (4.40 g, 28.70 mmol, 10.0 eq.), 2-methyl-2-butene (11.3 mL) and $NaClO_2$ (4.00 g, 80 wt%, 22.96 mmol, 8.0 eq.). The resulting yellow solution was stirred at ambient temperature for 1 h before it was quenched by the addition of saturated aqueous NH_4Cl solution (10 mL). and extracted with EtOAc (3×15 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo.* The crude product was purified by flash chromatography (1% ~ 10% EtOAc/hexanes) to give **11** as a colorless oil (0.94 g, 76% over 3 steps).

R_f = 0.65 (20% EtOAc/hexanes w/ 1‰ HCOOH), PMA stain.

$[\alpha]_D^{21}$= -0.4 (c 1, $CHCl_3$);

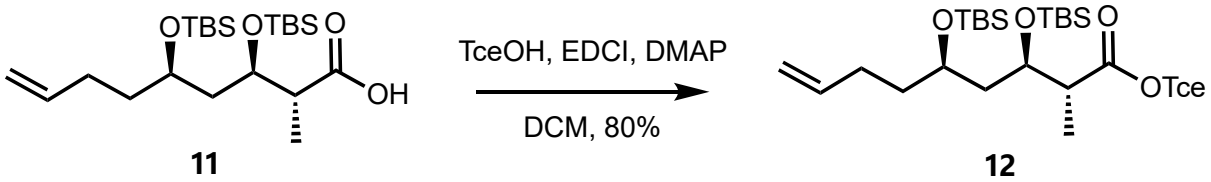
HRMS (ESI, m/z) for $C_{22}H_{45}O_4Si_2^-$ $[M - H]^-$: Calcd. 429.2862; Found 429.2861.

IR (KBr) ν_{max} 2956, 2929, 2859, 1712, 1641, 1471, 1257, 1095, 835, 773, 667 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.17 – 4.86 (m, 2H), 4.02 (ddd, *J* = 8.1, 5.3, 3.0 Hz, 1H), 3.88 – 3.68 (m, 1H), 2.71 (qd, *J* = 7.2, 3.0 Hz, 1H), 2.19 – 1.97 (m, 2H), 1.73 (ddd, *J* = 13.7, 8.3, 5.3 Hz, 1H), 1.67 – 1.60 (m, 1H), 1.60 – 1.49 (m, 2H), 1.24 (d, *J* = 7.2 Hz, 3H), 0.92 (s, 9H), 0.88 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H), 0.06 (s, 3H), 0.03 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 177.1, 138.5, 114.9, 71.7, 68.7, 44.1, 42.0, 36.8, 29.0, 26.0, 25.9, 18.1, 18.0, 14.1, -4.0, -4.2, -4.4, -4.7.

2,2,2-trichloroethyl (2*R*,3*R*,5*R*)-3,5-bis((*tert*-butyldimethylsilyl)oxy)-2-methylnon-8-enoate (12)



11 **12**

To a stirred solution of **11** (0.94 g, 2.18 mmol, 1.0 eq.) in anhydrous DCM (30 mL) at ambient temperature was sequentially added 2,2,2-Trichloroethanol (TceOH) (0.67 g, 4.36 mmol, 2.0 eq.), EDCI (0.64 g, 3.27 mmol, 1.5 eq.), DMAP (0.13 g, 1.09 mmol, 0.5 eq.) under argon. The reaction mixture was stirred overnight before it was diluted with EtOAc (20 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (3×10 mL). The combined organic extracts were washed with saturated aqueous NH_4Cl solution (15 mL), saturated aqueous $NaHCO_3$ solution, and brine; then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (1% ~ 2% EtOAc/hexanes) to give **12** as a colorless oil (0.98 g, 80%).

R_f = 0.6 (2% EtOAc/hexanes), PMA stain.

$[\alpha]_D^{20}$= -8.0 (c 1, $CHCl_3$)

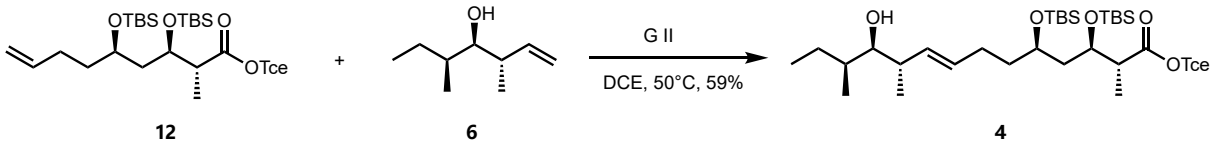
HRMS (ESI, m/z) for $C_{24}H_{47}Cl_3O_4Si_2Na^+$ $[M + Na]^+$: Calcd. 583.1971; Found 583.1975.

IR (KBr) ν_{max} 2954, 2927, 2856, 1755, 1641, 1463, 1382, 1257, 1097, 1051, 835, 773, 721, 663, 572 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.05 – 4.90 (m, 2H), 4.77 – 4.66 (m, 2H), 4.09 (dt, *J* = 7.4, 4.7 Hz, 1H), 3.92 – 3.79 (m, 1H), 2.85 (qd, *J* = 7.0, 4.1 Hz, 1H), 2.24 – 1.94 (m, 2H), 1.72 (ddd, *J* = 13.5, 7.5, 5.9 Hz, 1H), 1.64 – 1.58 (m, 2H), 1.45 (ddt, *J* = 13.6, 10.5, 6.0 Hz, 1H), 1.21 (d, *J* = 7.1 Hz, 3H), 0.89 (s, 9H), 0.88 (s, 9H), 0.07 (s, 3H), 0.07 (s, 3H), 0.06 (s, 3H), 0.06 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 139.0, 114.5, 95.1, 74.1, 70.9, 68.9, 45.2, 41.0, 36.5, 29.4, 26.0, 25.9, 18.2, 18.1, 11.6, -4.1, -4.2, -4.2, -4.6.

2,2,2-trichloroethyl (2*R*,3*R*,5*R*,10*S*,11*R*,12*S*,*E*)-3,5-bis((*tert*-butyldimethylsilyl)oxy)-11-hydroxy-2,10,12-trimethyltetradec-8-enoate (4)



12 **6** **4**

To a stirred solution of **12** (184.0 mg, 0.33 mmol, 1.0 eq.) and **6**[2] (142.0 mg, 0.99 mmol, 3.0 eq.) in degassed DCE (10 mL) under argon.was added Grubbs II catalyst (28.0 mg, 0.033 mmol, 0.1 eq.). The reaction mixture was stirred and heated in a 50 °C oil bath for 24 h. The reaction was then cooled to

ambient temperature and the solvent was removed *in vacuo.* The crude product was purified by flash chromatography (1% ~ 2% EtOAc/hexanes) to give **4** as a colorless oil (131.0 mg, 59%).

R_f = 0.3 (3.3% EtOAc/hexanes), PMA stain.

$[\alpha]_D^{20}$ = -3.5 (c = 1, $CHCl_3$)

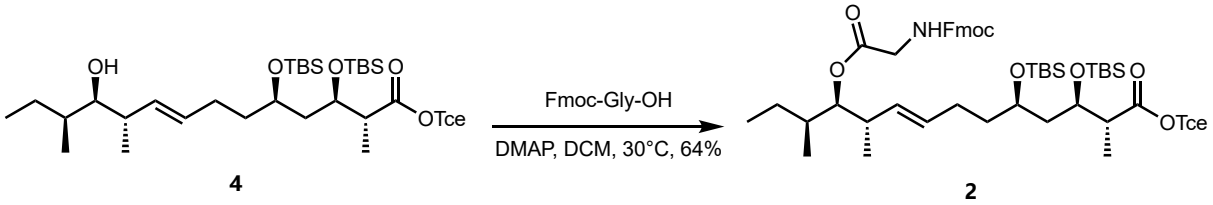
HRMS (ESI, m/z) for $C_{31}H_{61}Cl_3O_5Si_2Na^+$ $[M + Na]^+$: Calcd. 697.3016; Found 697.3020.

IR (KBr) ν_{max} 3581, 2956, 2929, 2887, 2856, 1755, 1728, 1652, 1555, 1471, 1377, 1257, 1097, 1062, 835, 773, 721, 663, 572 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.53 (dt, *J* = 15.3, 6.6 Hz, 1H), 5.31 (dd, *J* = 15.3, 8.7 Hz, 1H), 4.78 – 4.65 (m, 2H), 4.08 (dt, *J* = 7.6, 4.6 Hz, 1H), 3.85 (td, *J* = 6.2, 6.2 Hz, 1H), 3.17 (dd, *J* = 8.0, 3.2 Hz, 1H), 2.84 (qd, *J* = 7.0, 4.1 Hz, 1H), 2.28 – 2.18 (m, 1H), 2.17 – 1.97 (m, 3H), 1.71 (dt, *J* = 13.5, 6.7 Hz, 1H), 1.66 – 1.38 (m, 4H), 1.31 (tdd, *J* = 15.3, 9.6, 5.4 Hz, 1H), 1.21 (d, *J* = 7.0 Hz, 3H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.92 (d, *J* = 7.6 Hz, 3H), 0.90 – 0.84 (m, 21H), 0.07 (s, 3H), 0.06 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 133.0, 132.8, 95.1, 76.9, 74.1, 70.9, 68.9, 45.1, 41.1, 41.0, 37.1, 36.4, 28.3, 27.2, 26.0, 25.9, 18.2, 18.1, 17.3, 12.5, 12.0, 11.6, -4.1, -4.2, -4.6.

2,2,2-trichloroethyl (2*R*,3*R*,5*R*,10*S*,11*R*,12*S*,*E*)-11-(((((9*H*-fluoren-9-yl)methoxy)carbonyl)glycyl)oxy)-3,5-bis((*tert*-butyldimethylsilyl)oxy)-2,10,12-trimethyltetradec-8-enoate (2)



4 **2**

To a stirred solution of **4** (151.0 mg, 0.22 mmol, 1.0 eq.) in anhydrous DCM (10 mL) was sequentially added Fmoc-Gly-OH (200.0 mg, 0.66 mmol, 3.0 eq.), DCC (231.0 mg, 1.10 mmol, 5.0 eq.) and DMAP (13.6 mg, 0.11 mmol, 0.5 eq.) under argon. The resulting white suspension was stirred and heated in a 30°C oil bath for 15 h. The reaction was then cooled to ambient temperature and the solvent was removed *in vacuo.* The residue was purified by flash chromatography (1.6% ~ 5% EtOAc/hexanes) to give **2** as a colorless oil (136.0 mg, 64%).

R_f = 0.35 (10% EtOAc/hexanes), PMA stain.

$[\alpha]_D^{24}$ = +2.4 (c 0.25, $CHCl_3$)

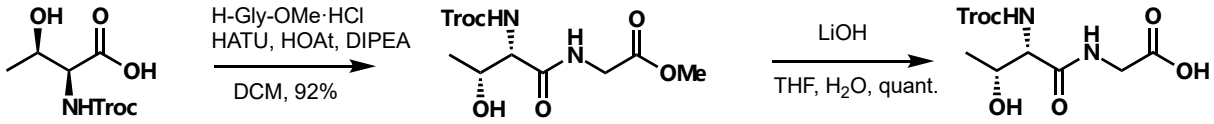
HRMS (ESI, m/z) for $C_{48}H_{74}Cl_3NO_8Si_2Na^+$ $[M + Na]^+$: Calcd. 976.3911; Found 976.3914.

IR (KBr) ν_{max} 3394, 2958, 2927, 2858, 1728, 1506, 1450, 1377, 1259, 1201, 1097, 1051, 802, 740 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 – 7.73 (m, 2H), 7.64 – 7.57 (m, 2H), 7.44 – 7.37 (m, 2H), 7.37 – 7.27 (m, 2H), 5.42 (dt, *J* = 15.2, 6.5 Hz, 1H), 5.36 – 5.31 (m, 1H), 5.26 (dd, *J* = 15.3, 8.7 Hz, 1H), 4.82 (dd, *J* = 6.9, 5.1 Hz, 1H), 4.71 (dd, *J* = 13.6, 11.7 Hz, 2H), 4.44 – 4.33 (m, 2H), 4.23 (t, *J* = 7.2 Hz, 1H), 4.08 (dt, *J* = 7.6, 4.9 Hz, 1H), 4.04 – 3.92 (m, 2H), 3.91 – 3.76 (m, 1H), 2.85 (qd, *J* = 7.1, 4.2 Hz, 1H), 2.42 (dt, *J* = 8.4, 6.6 Hz, 1H), 2.18 – 1.88 (m, 1H), 1.77 – 1.49 (m, 6H), 1.45 – 1.31 (m, 2H), 1.27 (d, *J* = 12.5 Hz, 2H), 1.21 (d, *J* = 7.1 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.93 – 0.82 (m, 24H), 0.07 (s, 3H), 0.06 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 170.0, 156.3, 144.0, 141.4, 131.6, 127.8, 127.2, 125.3, 120.1, 95.1, 81.2, 74.1, 70.9, 69.0, 67.3, 47.3, 45.1, 42.9, 40.9, 39.3, 37.1, 35.9, 28.1, 26.3, 26.0, 25.9, 18.2, 18.1, 17.9, 13.7, 11.6, 11.6, -4.1, -4.2, -4.6.

Methyl ((2,2,2-trichloroethoxy)carbonyl)-*L*-threonylglycinate (S1)



S1 **3**

To a stirred solution of Troc-Thr-OH (568.5 mg, 1.93 mmol, 1.0 eq.) in DCM (30 mL) at 0°C was sequentially added HATU (811.4 mg, 6.16 mmol, 1.5 eq.), HOAt (394.1 mg, 6.16 mmol, 1.5 eq.), and DIPEA (748.3 mg, 5.79 mmol, 3.0 eq.). After being stirred for 5 min., H-Gly-OMe·HCl (266.6 mg, 2.12 mmol, 1.1 eq.) was added, and the reaction mixture was then stirred at ambient temperature overnight. The reaction was then quenched by the addition of saturated aqueous NH_4Cl (20 mL) and extracted with EtOAc (3×20 mL). The combined organic phases were washed with saturated aqueous NH_4Cl, $NaHCO_3$, and brine, dried over Na_2SO_4, and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (30% ~ 70% EtOAc/hexanes) to give **S1** as an amorphous solid (649.1 mg, 92%).

To a stirred solution of **S1** (365.0 mg, 1.00 mmol, 1.0 eq.) in THF (10 mL) and H_2O (10 mL) at 0°C was added $LiOH·H_2O$ (210.0 mg, 5.00 mmol, 5.0 eq.). After being stirred at 0°C for 30 min, the reaction mixture was diluted with EtOAc (20 mL) and water (10 mL) and acidified with 1.0 M HCl aq. to pH = 3. The mixture was partitioned, and the aqueous phase was extracted with EtOAc (3×15 mL). The combined organic phases were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to produce Troc-Thr-Gly-OH (**3**, 350.0 mg, quant.), which was directly used in the next step without further purification.

R_f = 0.3 (66% EtOAc/hexanes), PMA stain.

$[\alpha]_D^{24}$ = -27.0 (c 1, $CHCl_3$)

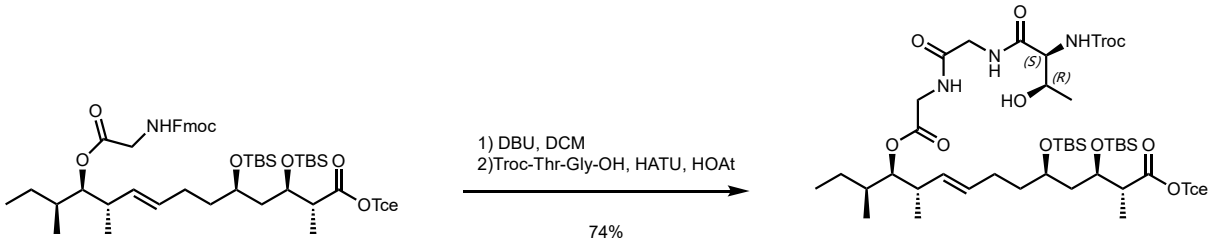
HRMS (ESI, m/z) for $C_9H_{12}Cl_3N_2O_6^-$ [M - H]$^-$: Calcd. 348.9766; Found 348.9766.

IR (KBr) ν_{max} 3645, 3350, 3057, 2954, 2916, 2849, 1828, 1737, 1660, 1586, 1435, 1373, 1215, 1114, 1045, 975, 819, 734, 570 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (t, *J* = 5.7 Hz, 1H), 6.29 (d, *J* = 8.0 Hz, 1H), 4.83 – 4.68 (m, 2H), 4.40 (qd, *J* = 6.5, 2.7 Hz, 1H), 4.25 (dd, *J* = 8.0, 2.6 Hz, 1H), 4.06 (dd, *J* = 5.7, 1.7 Hz, 2H), 3.75 (s, 3H), 1.22 (d, *J* = 6.4 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 170.5, 155.3, 95.4, 74.9, 59.3, 52.7, 41.4, 18.3.

(3*S*,4*R*,5*S*,10*R*,12*R*,13*R*,*E*)-10,12-bis((*tert*-butyldimethylsilyl)oxy)-3,5,13-trimethyl-14-oxo-14-(2,2,2-trichloroethoxy)tetradec-6-en-4-yl (*S*)-1,1,1-trichloro-6-((*R*)-1-hydroxyethyl)-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate (13)



To a stirred solution of **2** (88.9 mg, 0.093 mmol, 1.0 eq.) in anhydrous DCM (10 mL) at 0°C was added DBU (14.2 mg, 0.093 mmol, 1.0 eq.) via syringe under argon. After being stirred at 0°C for 30 min, the reaction mixture was allowed to warm to ambient temperature and stirred for an additional 30 min, at which point the starting material **13** was consumed as judged by TLC analysis. The reaction mixture was cooled to 0°C, and a solution of Troc-Thr-Gly-OH (**3**, 49.1 mg, 0.14 mmol, 1.5 eq.) in DCM was added, followed by HOAt (38.0 mg, 0.28 mmol, 3.0 eq.). The mixture was stirred at 0°C for 5 min before HATU (106.1 mg, 0.28 mmol, 3.0 eq.) was added. The reaction mixture was slowly warmed up to ambient temperature and stirred overnight, and then quenched by the addition of saturated aqueous NH_4Cl (20 mL) and extracted with EtOAc (3×20 mL). The combined organic phases were washed with saturated aqueous NH_4Cl (20 mL) and $NaHCO_3$ (20 mL) and brine, dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (30% ~ 40% EtOAc/hexanes) to give **13** as an amorphous solid (73.4 mg, 74%).

R_f = 0.2 (50% EtOAc/hexanes), PMA stain.

$[\alpha]_D^{23}$= +3.3 (c =0.3, $CHCl_3$)

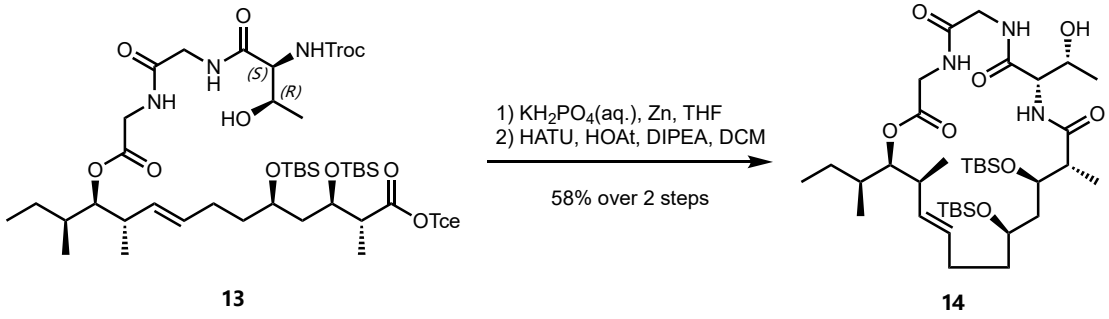
HRMS (ESI, m/z) for $C_{42}H_{75}Cl_6N_3O_{11}Si_2Na^+$ [M + Na]$^+$: Calcd. 1086.2964; Found 1086.2965.

IR (KBr) ν_{max} 3331, 2958, 2929, 2856, 1739, 1647, 1537, 1463, 1382, 1257, 1211, 1095, 1060, 837, 804, 721, 570 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (t, *J* = 5.9 Hz, 1H), 6.75 (t, *J* = 5.1 Hz, 1H), 6.12 (d, *J* = 7.9 Hz, 1H), 5.39 (dt, *J* = 15.3, 6.6 Hz, 1H), 5.23 (dd, *J* = 15.3, 8.7 Hz, 1H), 4.88 – 4.65 (m, 5H), 4.44 (qd, *J* = 6.5, 2.3 Hz, 1H), 4.24 (dd, *J* = 7.9, 2.4 Hz, 1H), 4.14 – 4.02 (m, 3H), 4.01 – 3.90 (m, 2H), 3.89 – 3.77 (m, 1H), 2.90 – 2.79 (m, 1H), 2.41 (dt, *J* = 8.6, 6.6 Hz, 1H), 2.10 – 1.87 (m, 1H), 1.78 – 1.66 (m, 1H), 1.66 – 1.61 (m, 1H), 1.61 – 1.56 (m, 1H), 1.56 – 1.44 (m, 1H), 1.41 – 1.31 (m, 1H), 1.29 – 1.19 (m, 8H), 1.18 – 1.07 (m, 1H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.91 – 0.86 (m, 21H), 0.86 – 0.84 (m, 1H), 0.84 (d, *J* = 6.8 Hz, 3H), 0.06 (dd, *J* = 6.0, 4.4 Hz, 12H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 171.3, 170.1, 168.9, 155.1, 131.7, 131.4, 95.4, 95.1, 81.9, 74.9, 74.2, 70.9, 69.0, 67.3, 59.8, 45.2, 43.1, 41.4, 40.9, 39.3, 37.1, 35.9, 28.3, 26.2, 26.1, 25.9, 18.8, 18.2, 18.1, 17.9, 13.8, 11.5, -4.1, -4.2, -4.6.

(9*S*,12*R*,13*R*,15*R*,20*S*,21*R*,*E*)-21-((*S*)-*sec*-butyl)-13,15-bis((*tert*-butyldimethylsilyl)oxy)-9-((*R*)-1-hydroxyethyl)-12,20-dimethyl-1-oxa-4,7,10-triazacyclohenicos-18-ene-2,5,8,11-tetraone (14)



13 **14**

To a solution of **13** (73.4 mg, 69.0 μmol, 1.0 eq.) in a mixed solvent of THF and 1N KH_2PO_4 aq. (20 mL total, 1:1, v/v,) at ambient temperature under argon, was added activated Zn powder (89.0 mg, 1.38 mmol, 20.0 eq.). The reaction mixture was stirred vigorously for 6 h, at which point TLC and HRMS analysis indicated that starting **13** was completely consumed. The reaction was worked up by the addition of water (10 mL) and EtOAc (20 mL). The aqueous phase was extracted with EtOAc (3×10 mL), then treated with aqueous $NaHCO_3$ solution to pH = 7 and extracted with EtOAc (3×10 mL). The combined organic phases were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure The resulting crude amino acid was directly subjected to the next step without further purification.

To the solution of the above crude product in anhydrous DCM (70 mL, 0.001 M) at 0°C under argon was sequentially added HOAt (47.0 mg, 0.35 mmol, 5.0 eq.) and DIPEA (89.2 mg, 0.69 mmol, 10.0 eq.). After being stirred for 5 min, HATU (131.2 mg, 0.35 mmol, 5.0 eq.) was added at 0°C to the solution. The reaction mixture was allowed to slowly warm to ambient temperature and stirred for an additional 24 h before it was quenched by the addition of saturated aqueous NH_4Cl (10 mL). The aqueous layer was separated and extracted with EtOAc (3×20 mL) and the combined organic phases were washed with aqueous 5% citric acid solution, saturated aqueous NH_4Cl solution, saturated aqueous $NaHCO_3$ solution,

and brine; then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (30% ~ 70% EtOAc/hexanes) to give **14** as an amorphous solid (29.7 mg, 58%).

R_f = 0.15 (67% EtOAc/hexanes), PMA stain.

$[\alpha]_D^{20}$= -28.0 (c 1, $CHCl_3$)

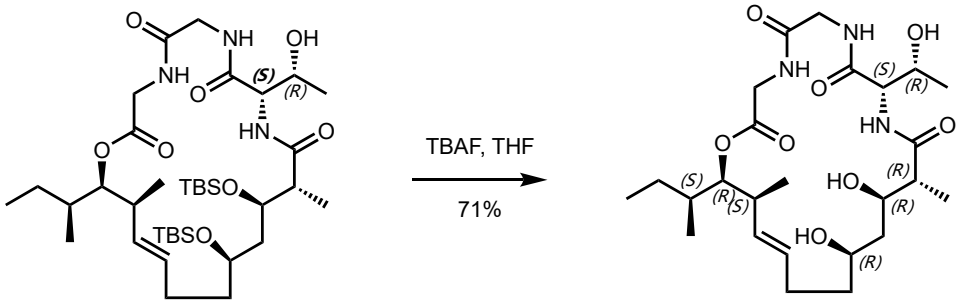
HRMS (ESI, m/z) for $C_{32}H_{71}N_3O_{18}Si_2Na^+$ $[M + Na]^+$: Calcd. 764.4672; Found 764.4673.

IR (KBr) ν_{max} 3408, 3339, 2956, 2926, 2854, 1739, 1647, 1525, 1464, 1377, 1258, 1207, 1080, 1053, 970, 837, 559cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.4 Hz, 1H), 7.51 (d, *J* = 6.1 Hz, 1H), 6.20 (s, 1H), 5.33 (ddd, *J* = 15.2, 8.8, 4.1 Hz, 1H), 5.14 (dd, *J* = 14.9, 9.4 Hz, 1H), 4.82 (dd, *J* = 10.3, 2.1 Hz, 1H), 4.53 – 4.42 (m, 1H), 4.41 – 4.33 (m, 1H), 4.31 (dd, *J* = 8.3, 1.6 Hz, 1H), 4.24 (dd, *J* = 17.0, 6.1 Hz, 1H), 3.98 – 3.86 (m, 2H), 3.76 (dd, *J* = 17.0, 3.2 Hz, 1H), 3.62 – 3.52 (m, 1H), 2.72 – 2.62 (m, 1H), 2.36 (td, *J* = 9.6, 6.6 Hz, 1H), 2.22 – 2.00 (m, 1H), 1.89 – 1.72 (m, 2H), 1.70 – 1.46 (m, 4H), 1.31 (d, *J* = 7.3 Hz, 3H), 1.29 – 1.16 (m, 2H), 1.14 (d, *J* = 6.5 Hz, 3H), 0.96 – 0.86 (m, 28H), 0.13 (s, 3H), 0.12 (s, 3H), 0.06 (s, 3H), 0.03 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 177.1, 172.5, 170.0, 167.9, 132.3, 132.2, 80.5, 72.0, 68.5, 65.9, 56.4, 44.6, 42.4, 41.9, 40.8, 40.3, 37.0, 35.2, 27.3, 26.1, 26.0, 25.5, 18.8, 18.2, 18.2, 17.5, 17.3, 12.5, 12.0, -3.9, -4.1, -4.6, -4.7.

(9*S*,12*R*,13*R*,15*R*,20*S*,21*R*,*E*)-21-((*S*)-*sec*-butyl)-13,15-dihydroxy-9-((*R*)-1-hydroxyethyl)-12,20-dimethyl-1-oxa-4,7,10-triazacyclohenicos-18-ene-2,5,8,11-tetraone (1)



14 **1**

To a stirred solution of **14** (12.2 mg, 16.4 μmol, 1.0 eq.) in anhydrous THF (10 mL) at 0°C was added TBAF (1.0 M solution in THF, 0.33 mL, 0.33 mmol, 20.0 eq.) via syringe. The reaction mixture was stirred at ambient temperature for 6 h before it was diluted with EtOAc (20 mL) and quenched with saturated aqueous NH_4Cl solution. The organic phase was separated and washed with water (3×10 mL) and brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product

was purified by flash chromatography (0% ~ 10% MeOH/DCM) to give **1** as an amorphous solid (6.0 mg, 72%).

R_f = 0.4 (10% MeOH/DCM), PMA stain.

$[\alpha]_D^{20}$= -17.7 (c 0.3, MeOH)

HRMS (ESI, m/z) for $C_{25}H_{42}N_3O_8^-$ $[M - H]^-$: Calcd. 512.2977; Found 512.2974.

IR (KBr) ν_{max} 3490, 3438, 3339, 2966, 2936, 2854, 1677, 1657, 1525, 1464, 1207, 1078, 970, 836, 560 cm^{-1}

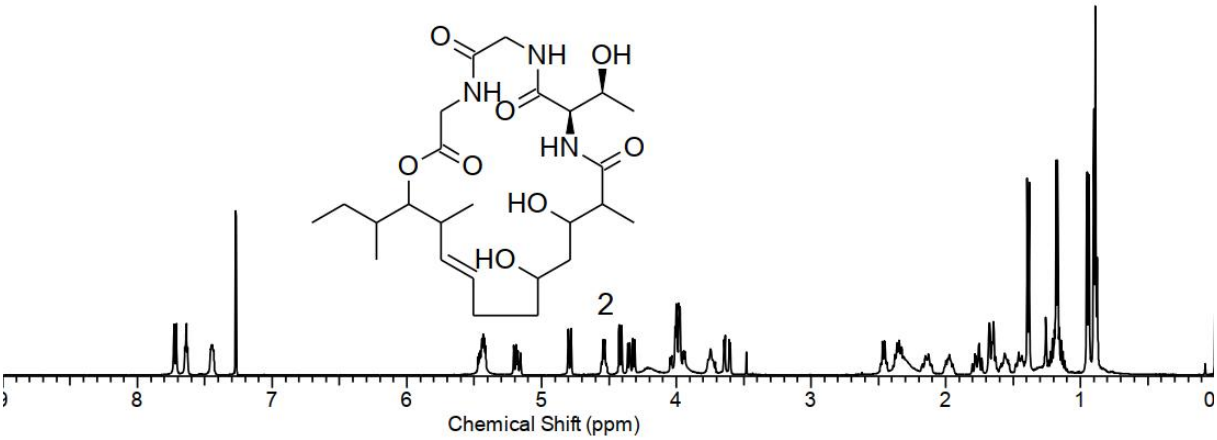
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.60 (m, 2H), 7.30 (s, 1H), 5.44 (ddd, *J* = 15.1, 8.6, 4.7 Hz, 1H), 5.17 (dd, *J* = 15.2, 9.0 Hz, 1H), 4.79 (dd, *J* = 10.3, 2.0 Hz, 1H), 4.54 (qd, *J* = 6.4, 1.7 Hz, 1H), 4.42 (dd, *J* = 9.0, 1.6 Hz, 1H), 4.34 (dd, *J* = 18.4, 7.6 Hz, 1H), 4.07 – 3.90 (m, 3H), 3.79 – 3.70 (m, 1H), 3.61 (dd, *J* = 18.4, 3.6 Hz, 1H), 2.46 (qd, *J* = 7.2, 2.7 Hz, 1H), 2.35 (td, *J* = 9.4, 6.7 Hz, 1H), 2.13 (td, *J* = 15.1, 6.7 Hz, 1H), 2.04 – 1.90 (m, 1H), 1.75 (dt, *J* = 14.7, 10.4 Hz, 1H), 1.71 – 1.59 (m, 2H), 1.56 (t, *J* = 12.8 Hz, 1H), 1.50 – 1.39 (m, 1H), 1.38 (d, *J* = 7.3 Hz, 3H), 1.20 – 1.14(m, 1H), 1.17 (d, *J* = 6.4 Hz, 3H), 1.12 (dd, *J* = 13.8, 7.1 Hz, 1H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H), 0.88 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 176.4, 172.1, 170.5, 168.8, 132.7, 130.8, 80.7, 74.3, 73.5, 66.1, 57.2, 48.1, 42.5, 41.5, 40.9, 39.6, 37.7, 35.3, 28.5, 27.1, 19.3, 16.9, 16.3, 12.2, 11.9.

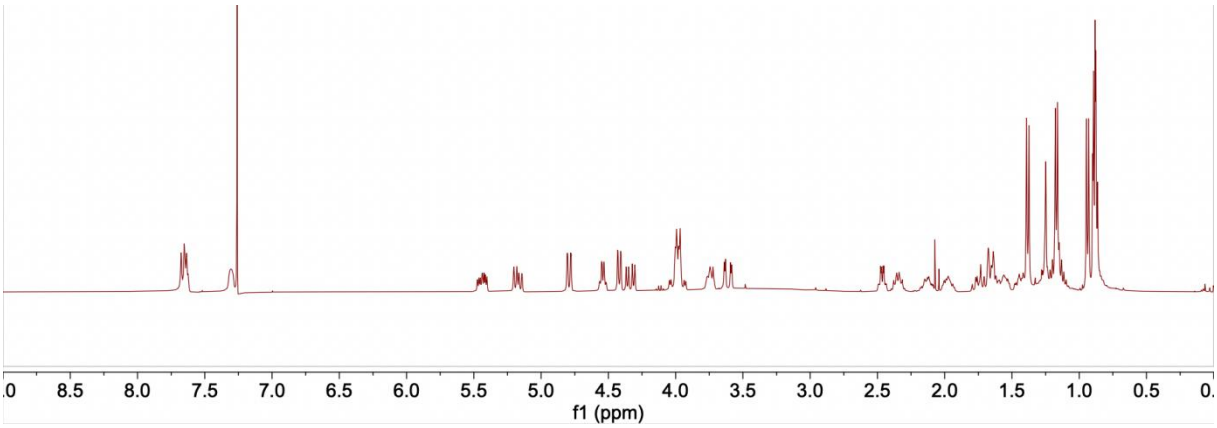
Natural Eucalactam B[3]**:** colorless, amorphous solids; $[\alpha]_D^{27}$ −17 (c 0.23, MeOH); IR (KBr) ν_{max} 3489, 3440, 3355, 3280, 2971, 2937, 1744, 1680, 1659, 1511, 1450, 1214 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Table S1; ESIMS m/z 512.02 $[M - H]^-$, 547.93 $[M + Cl]^-$; ESIMS m/z 536.19 $[M + Na]^+$; HRESIMS m/z 512.2982 ($[M - H]^-$, calcd for $C_{25}H_{42}N_3O_8^-$, 512.2972).

III Comparision of NMR Spectra of natural and synthetic Eucalactam B

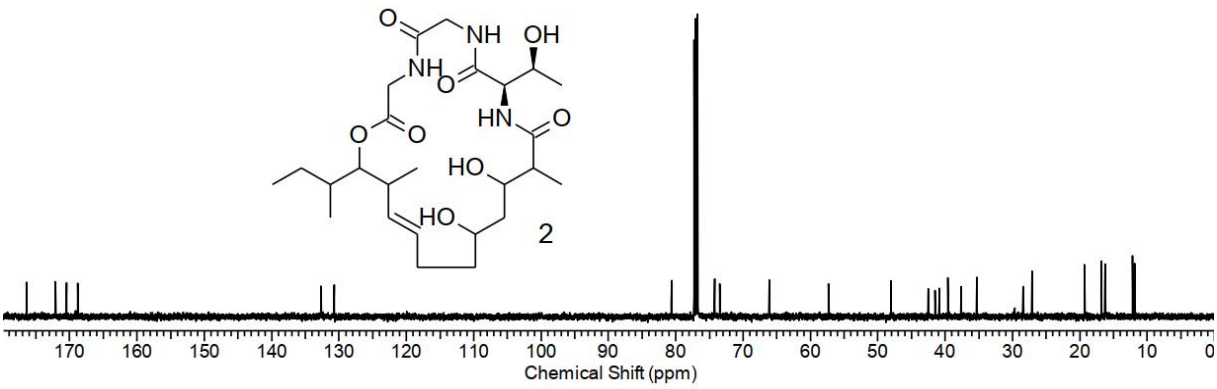
^{1}H NMR (natural product, 400 MHz, $CDCl_3$)



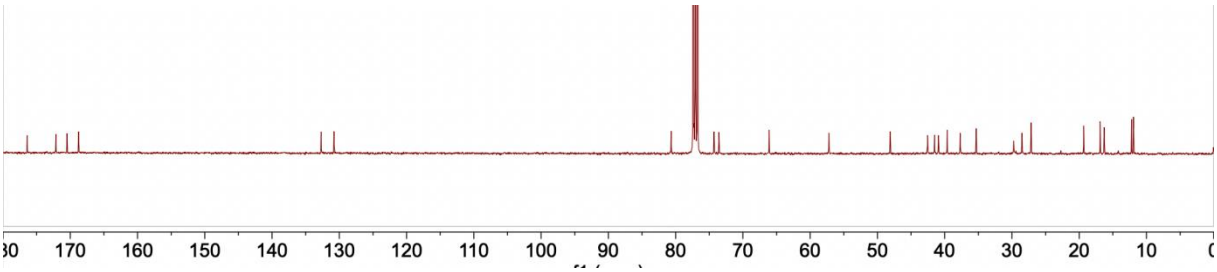
^{1}H NMR (synthetic product, 400 MHz, $CDCl_3$)



^{13}C NMR (natural product, 101 MHz, $CDCl_3$)



^{13}C NMR (synthetic product, 101 MHz, $CDCl_3$)

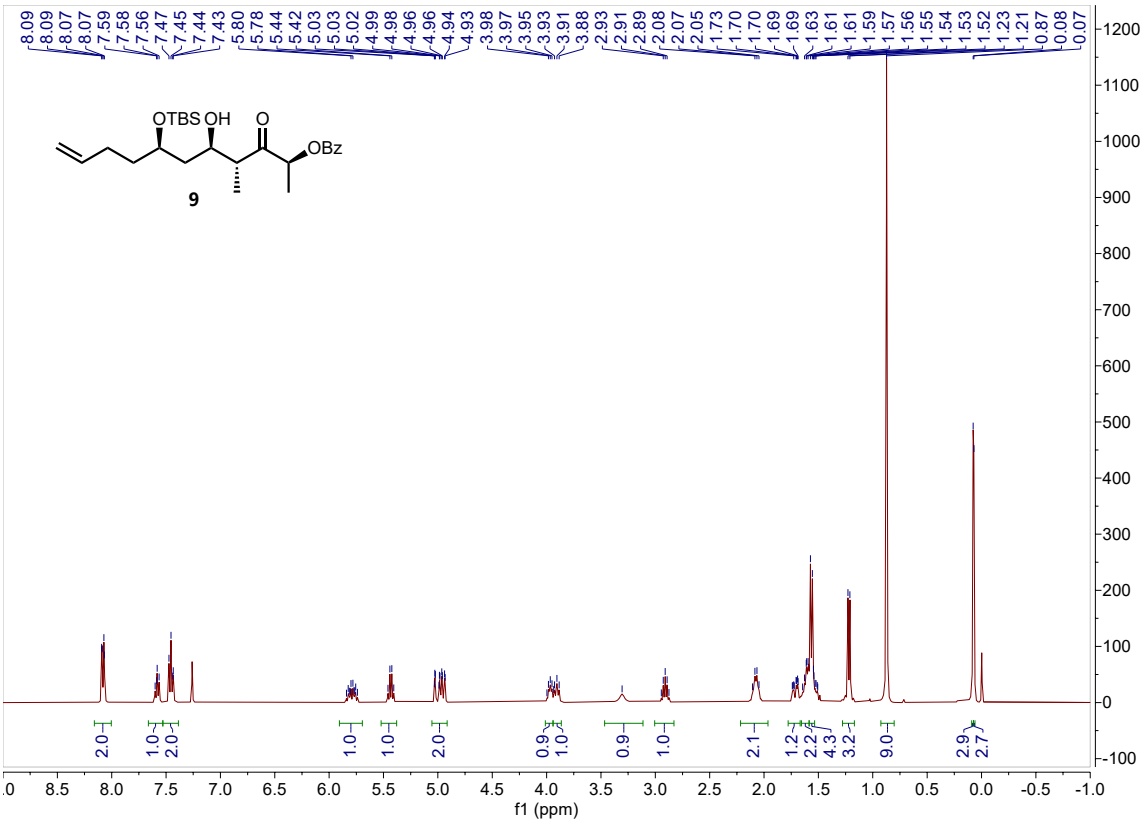


IV Table S1 Comparison of NMR data of Eucalactam B

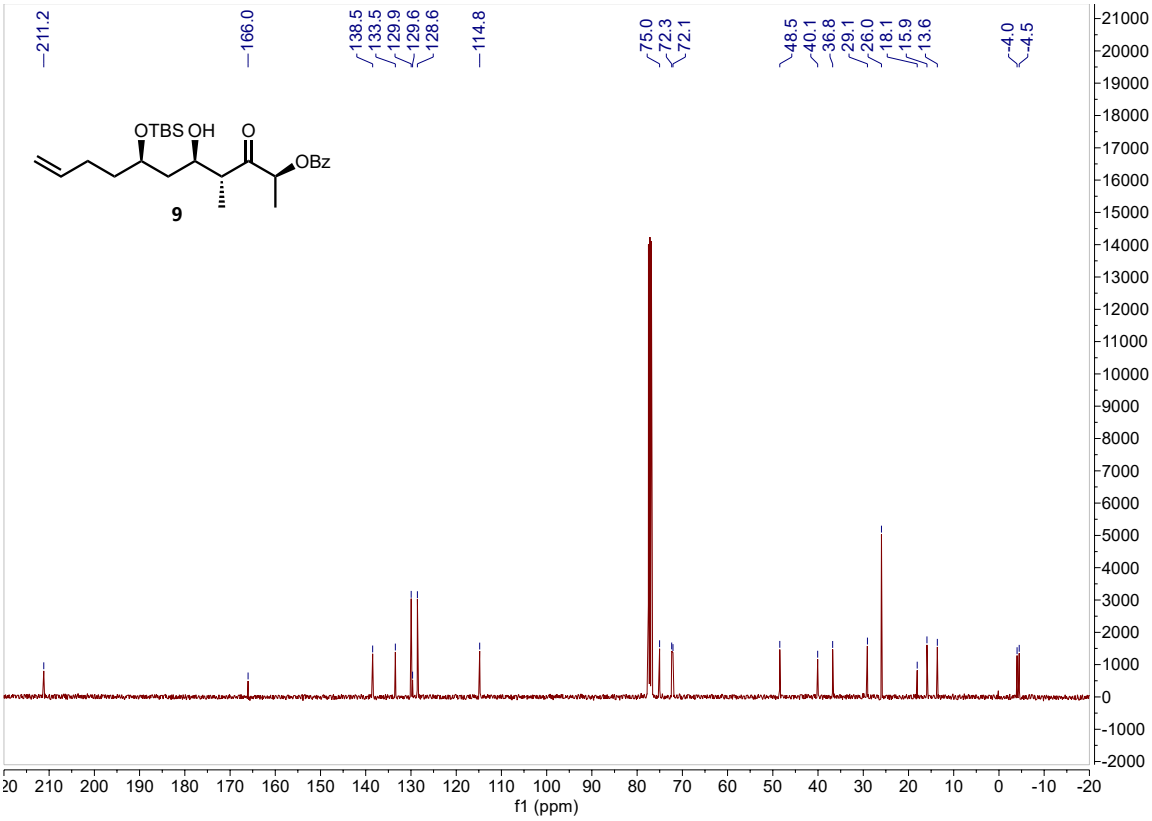
Position	δ_H(Nat)/ ppm (mult., J)	δ_H(Syn)/ ppm (mult., J)	δ_C(Nat)/ ppm	δ_C(Syn)/ ppm	$\Delta\delta_C$(Syn-Nat)/ppm
1			176.4	176.4	0.0
2	2.46(dq, 7.3, 2.7)	2.46(dq, 7.2, 2.7)	48.1	48.1	0.0
3	3.99(m, overlapped)	3.99(m, overlapped)	74.2	74.3	0.1
4	1.65(m, overlapped)	1.66(m, overlapped)	41.5	41.5	0.0
	1.77(dt, 14.7,10.6)	1.75(dt, 14.7, 10.4)			
5	3.73(m, overlapped)	3.74(m)	73.5	73.6	0.1
6	1.46(m)	1.45(m)	37.7	37.7	0.0
	1.56(m)	1.56(t, 12.8)			
7	1.98(m)	1.97(m)	28.5	28.5	0.0
	2.14(m)	2.13(dt, 15.1, 6.7)			
8	5.44(m)	5.44(ddd, 15.1, 8.6, 4.7)	130.7	130.8	0.1
9	5.18(dd, 15.3, 9.1)	5.17(dd, 15.2, 9.0)	132.7	132.7	0.0
10	2.35(m)	2.35(dt, 9.4, 6.7)	39.6	39.6	0.0
11	4.79(dd, 10.4, 1.6)	4.79(dd, 10.3, 2.0)	80.6	80.7	0.1
12	1.65(m, overlapped)	1.66(m, overlapped)	35.3	35.3	0.0
13	1.13(m)	1.12(dd, 13.8, 7.1)	27.1	27.2	0.0
14	0.87(t, 6.9)	0.88(t, 7.4)	12.2	12.2	0.0
15	1.39(d, 7.6)	1.38(d, 7.3)	16.3	16.3	0.0
16	0.94(d, 6.6)	0.94(d, 6.8)	16.8	16.9	0.1
17	0.89(d, 6.0)	0.89(d, 6.9)	11.9	11.9	0.0
18			170.5	170.5	0.0
NH	7.45(dd, 7.2, 3.6)	7.30(brs)			
19	3.62(dd, 18.3, 3.6)	3.61(dd, 18.4, 3.6)	40.9	40.9	0.0
	4.33(dd, 18.3, 7.2)	4.34(dd, 18.4, 7.6)			
20			168.8	168.8	0.0
NH	7.64(t, 4.7)	7.65(m, overlapped)			
21	3.99(m, overlapped)	3.99(m, overlapped)	42.5	42.5	0.0
22			172.1	172.1	0.0
NH	7.72(d, 6.3)	7.65(m, overlapped)			
23	4.42(br.d, 9.1)	4.42(dd, 9.2, 1.6)	57.3	57.2	-0.1
24	4.54(qd, 6.3, 0.9)	4.54(qd, 6.4, 1.7)	66.1	66.1	0.0
25	1.18(d, 6.3)	1.17(d, 6.4)	19.3	19.3	0.0

V. ^{1}H and ^{13}C NMR Spectra

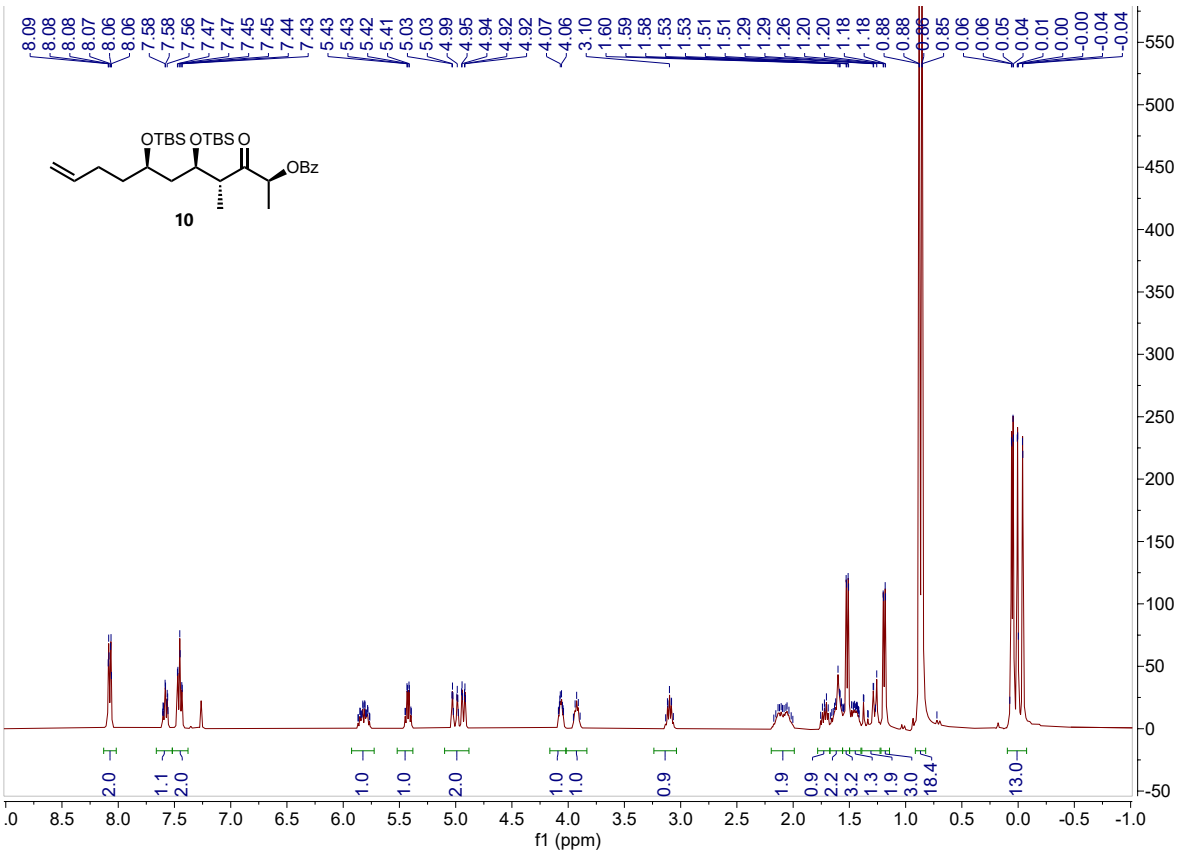
^{1}H NMR (400 MHz, $CDCl_3$)



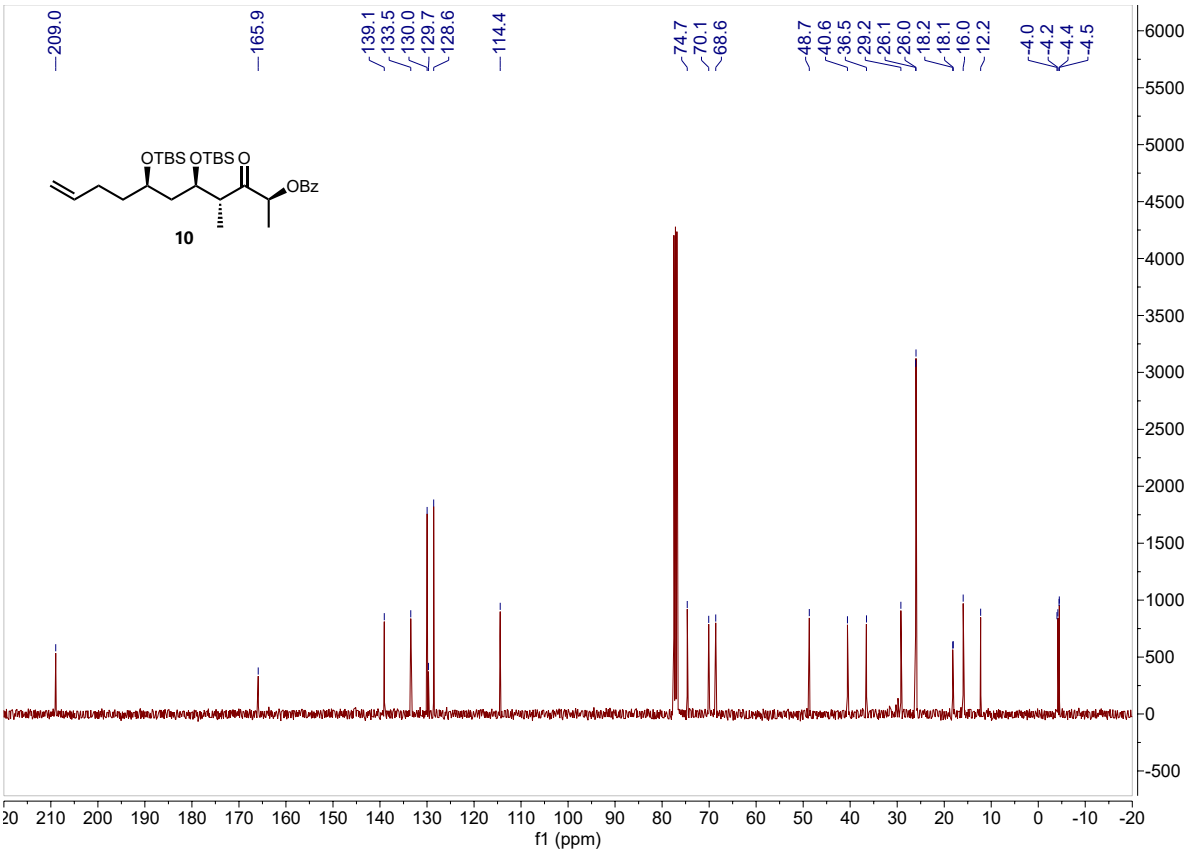
^{13}C NMR (400 MHz, $CDCl_3$)



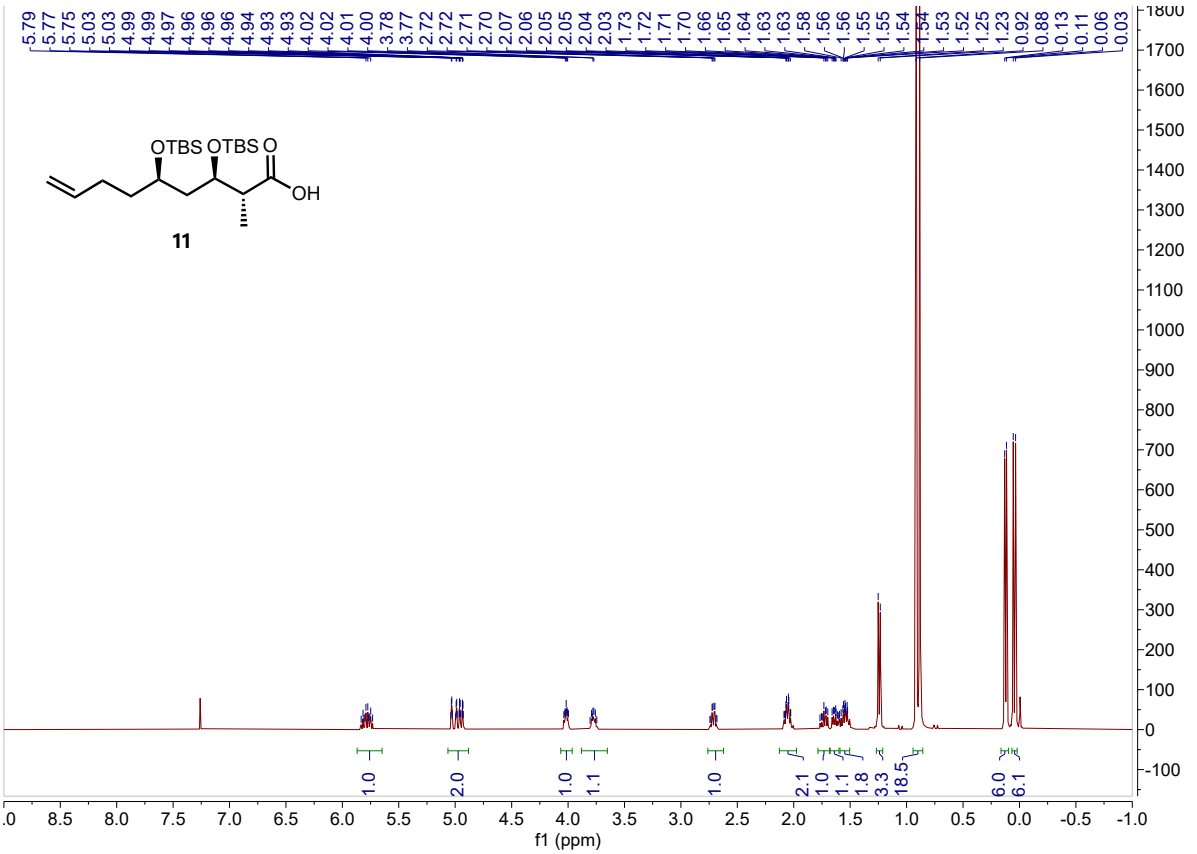
^{1}H NMR (400 MHz, $CDCl_3$)



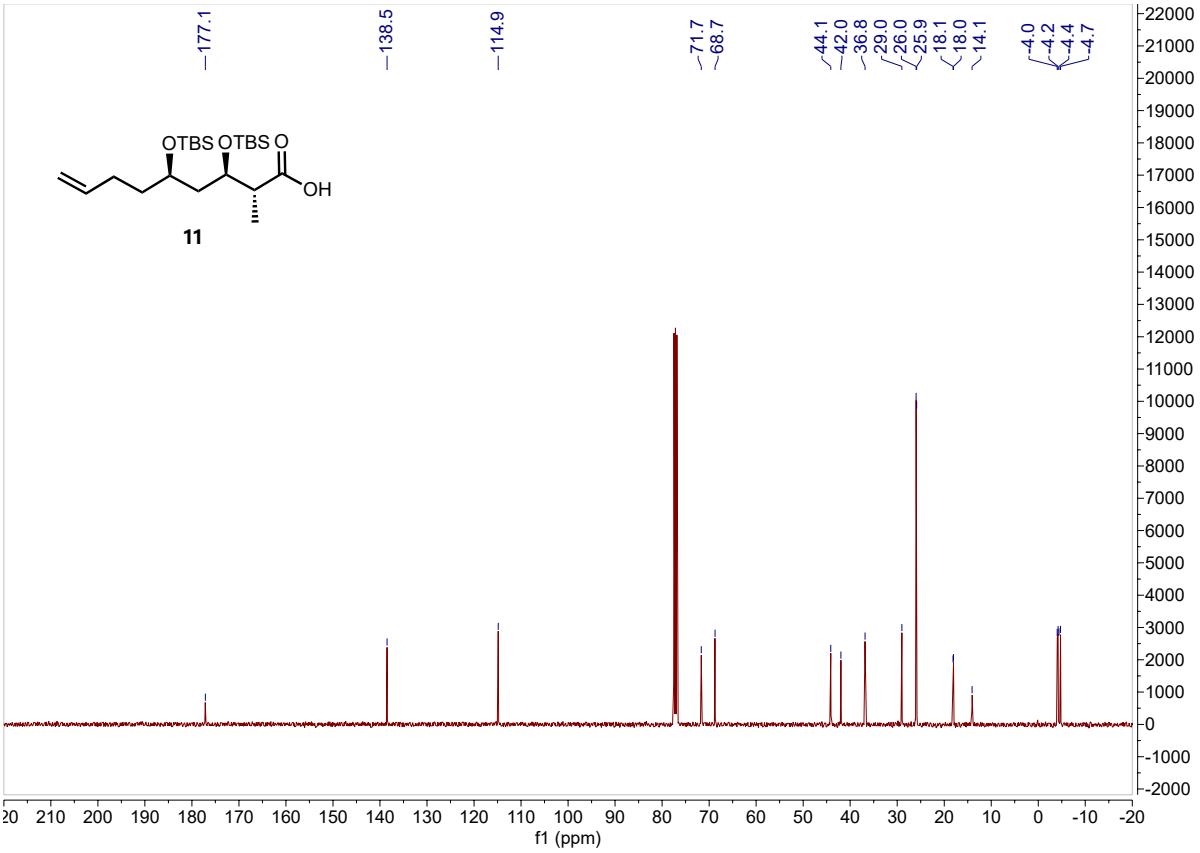
^{13}C NMR (101 MHz, $CDCl_3$)



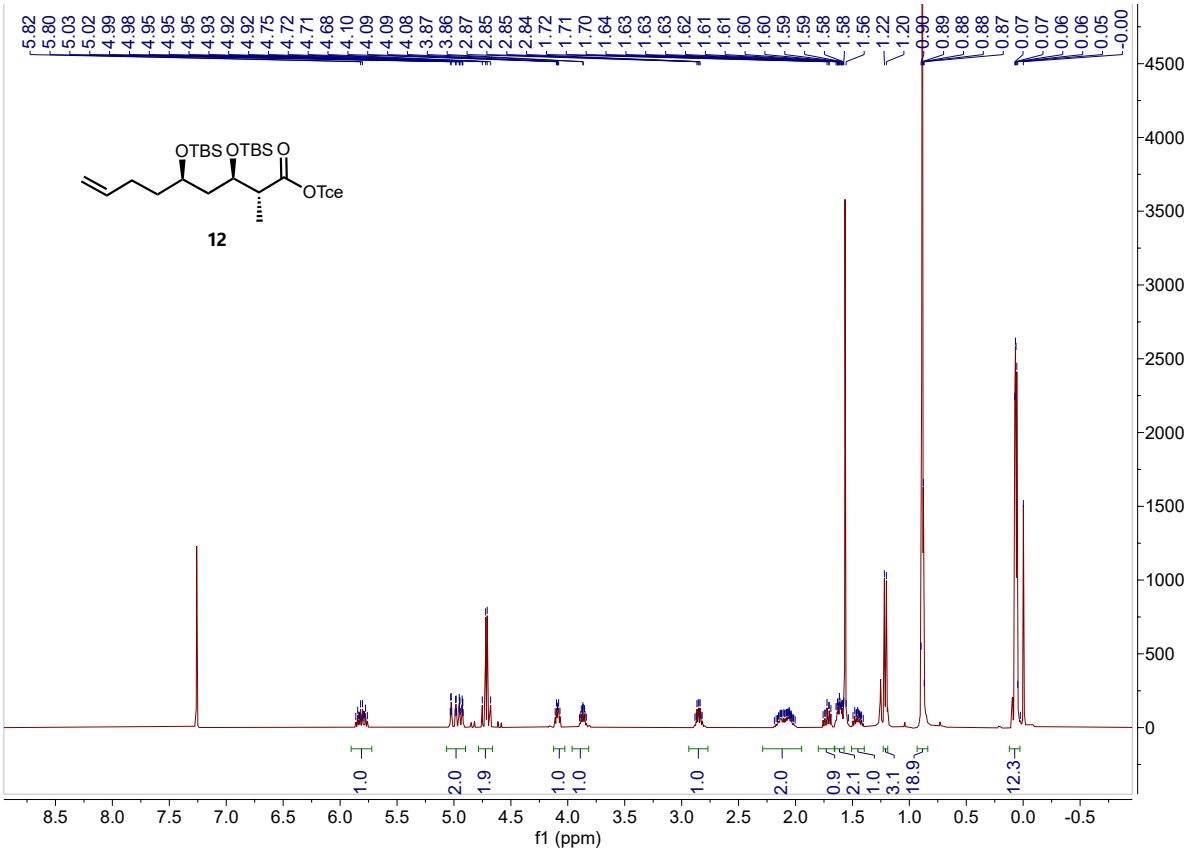
^{1}H NMR (400 MHz, $CDCl_3$)



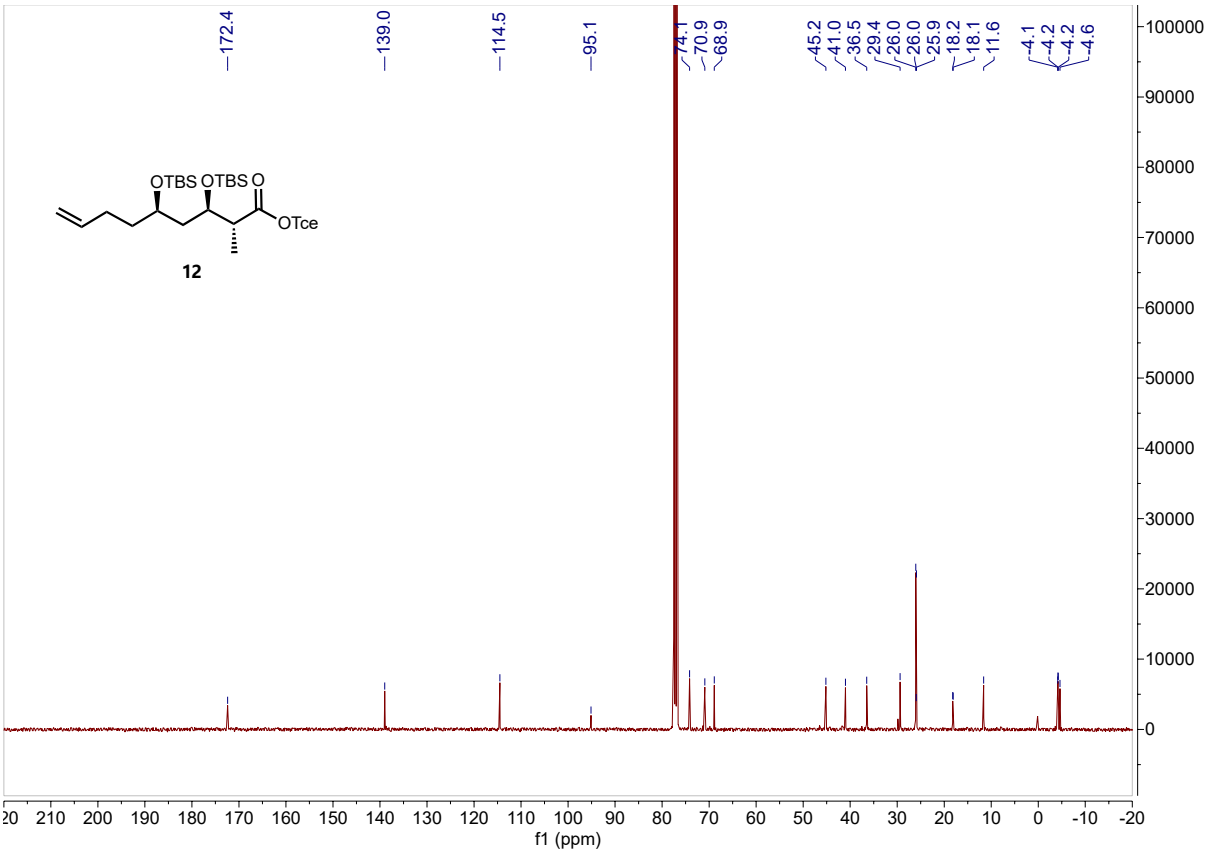
^{13}C NMR (101 MHz, $CDCl_3$)



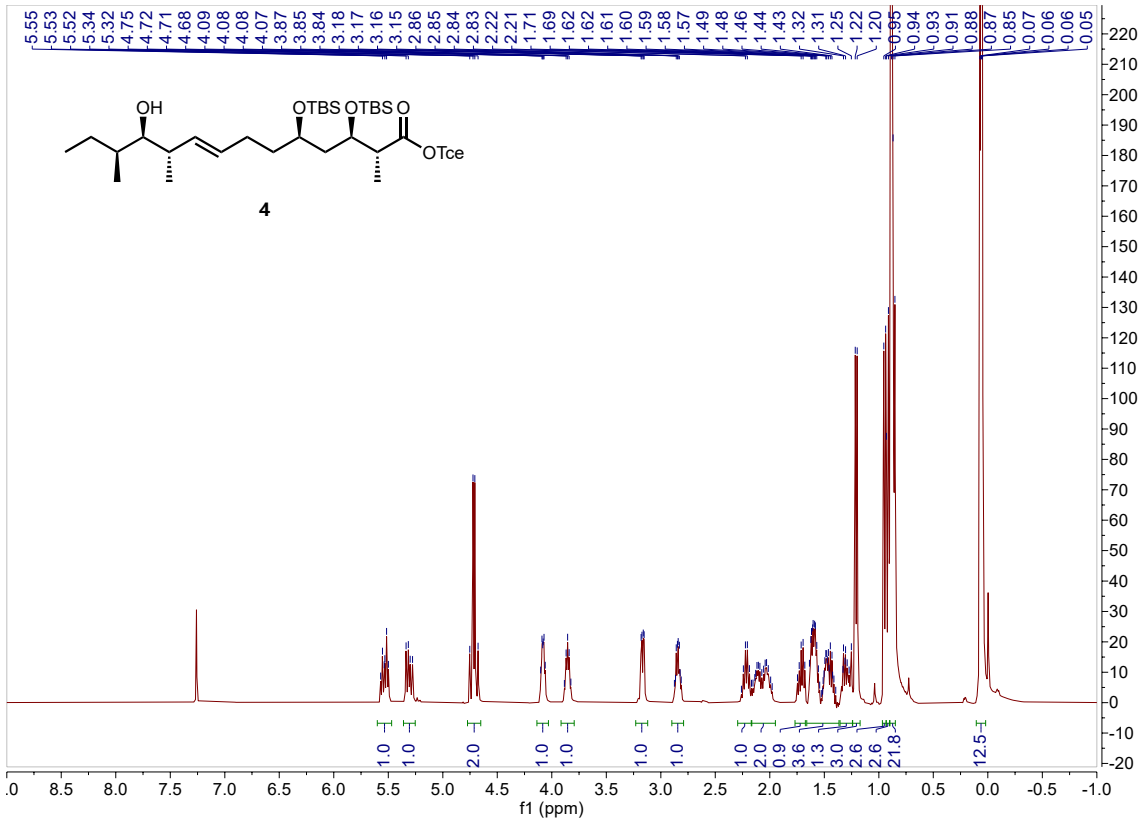
^{1}H NMR (400 MHz, $CDCl_3$)



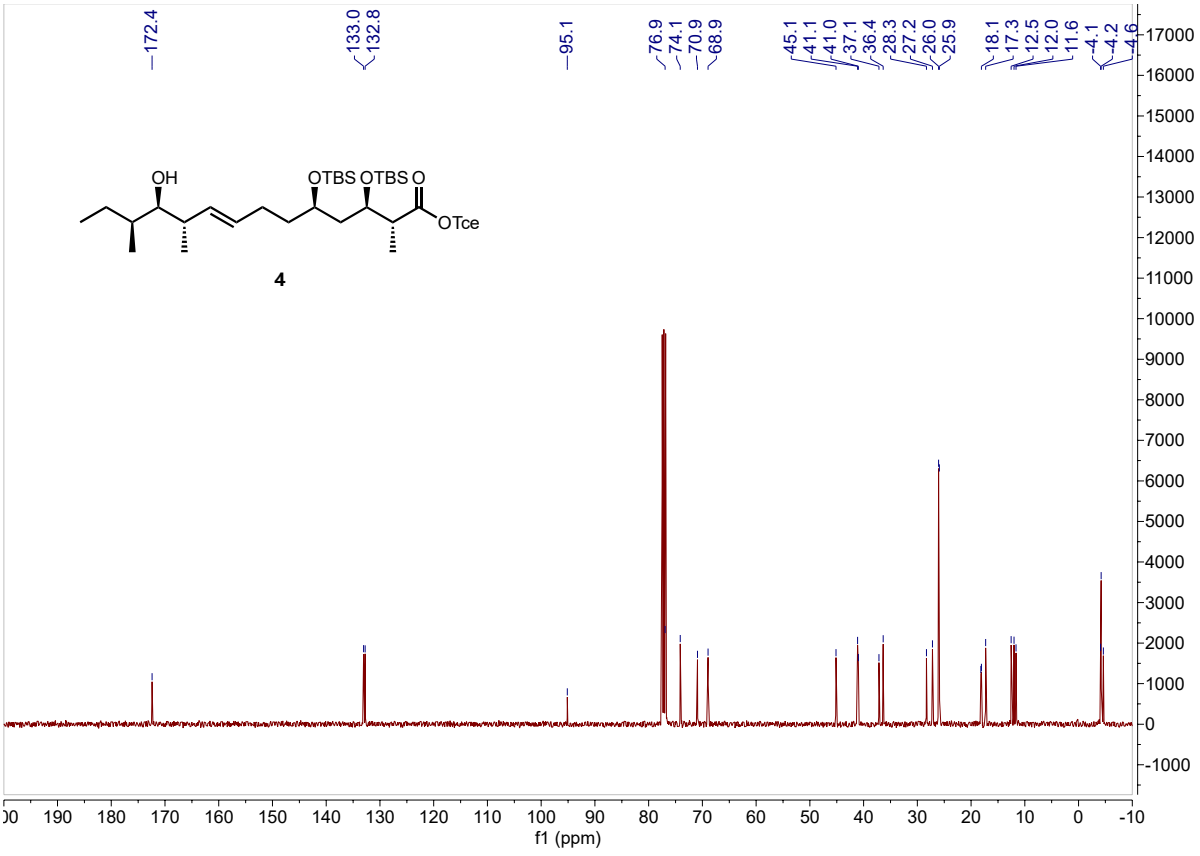
^{13}C NMR (101 MHz, $CDCl_3$)



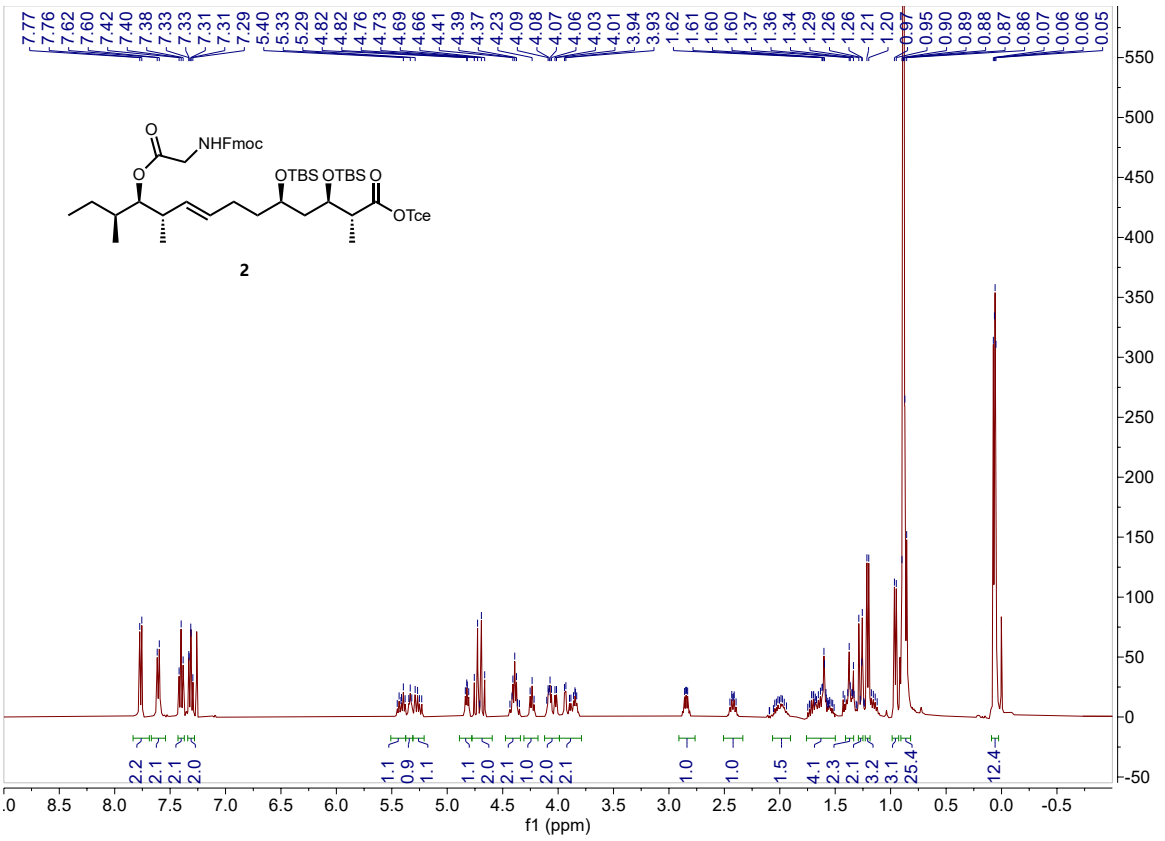
^{1}H NMR (400 MHz, $CDCl_3$)



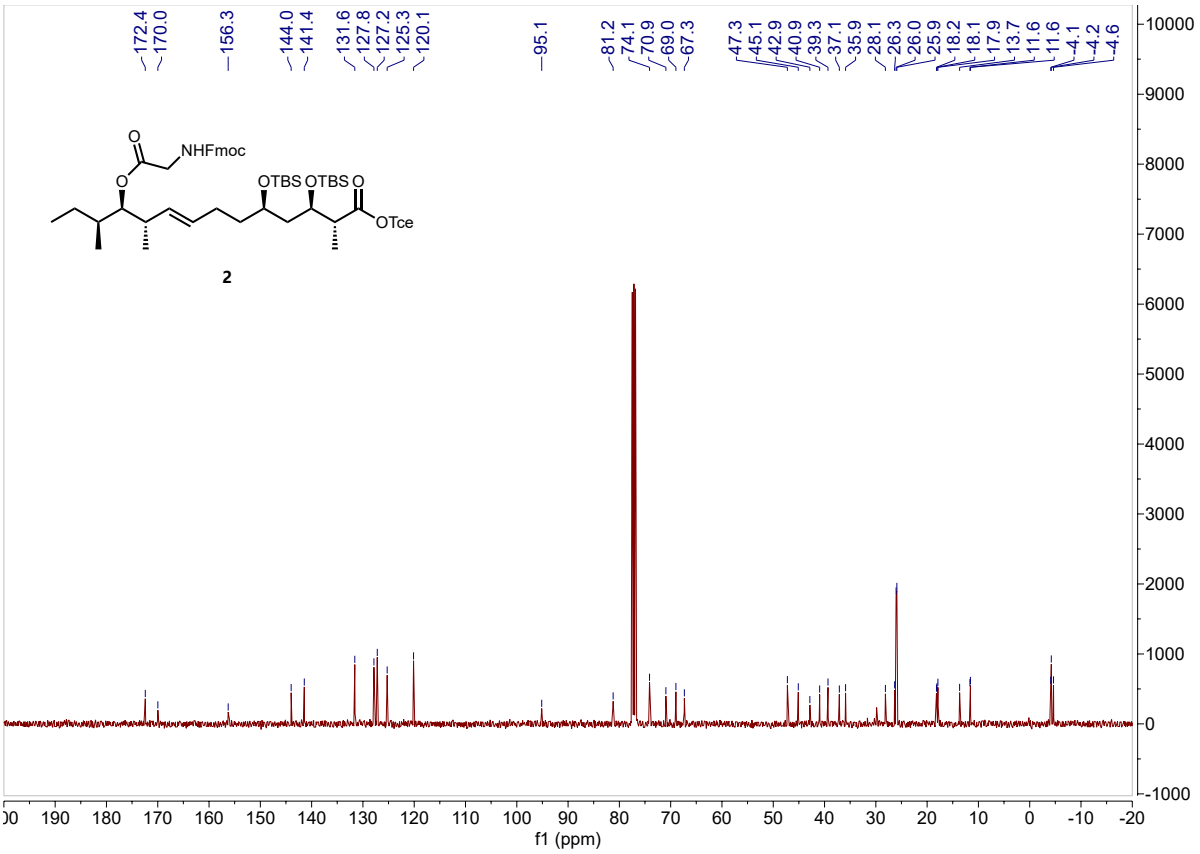
^{13}C NMR (101 MHz, $CDCl_3$)



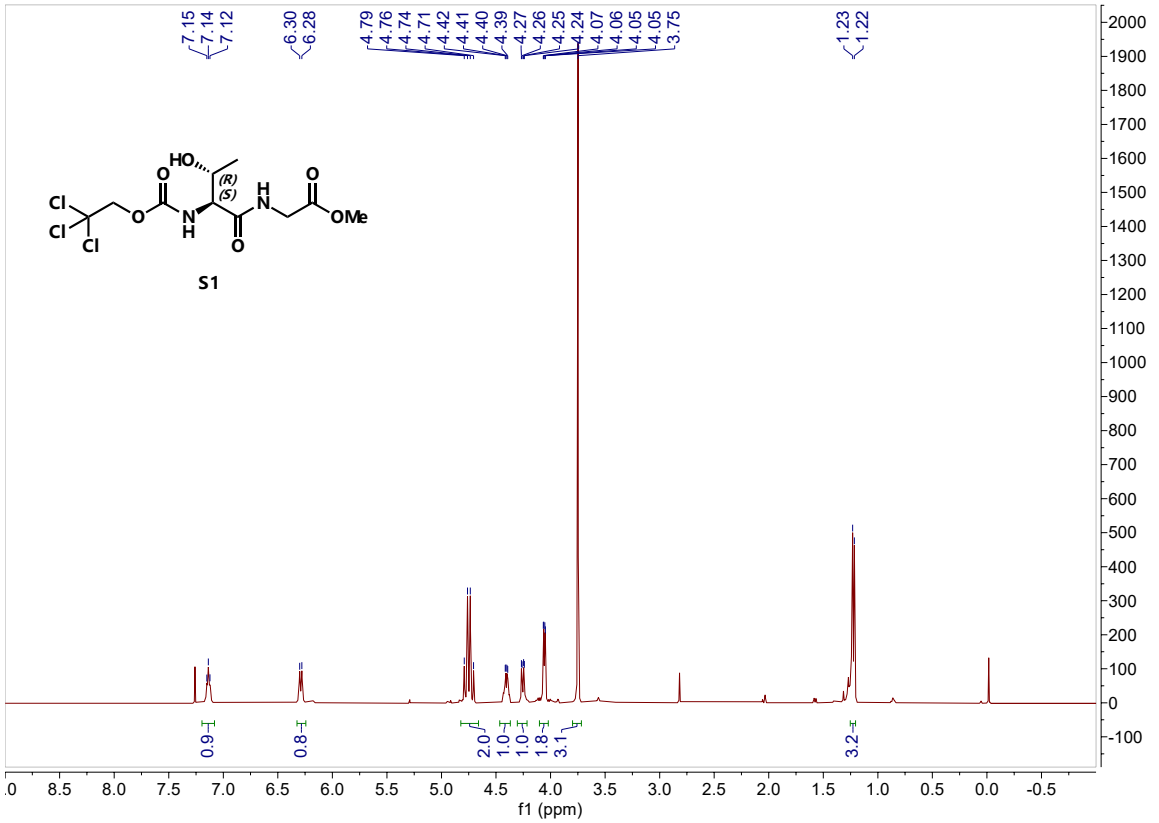
^{1}H NMR (400 MHz, $CDCl_3$)



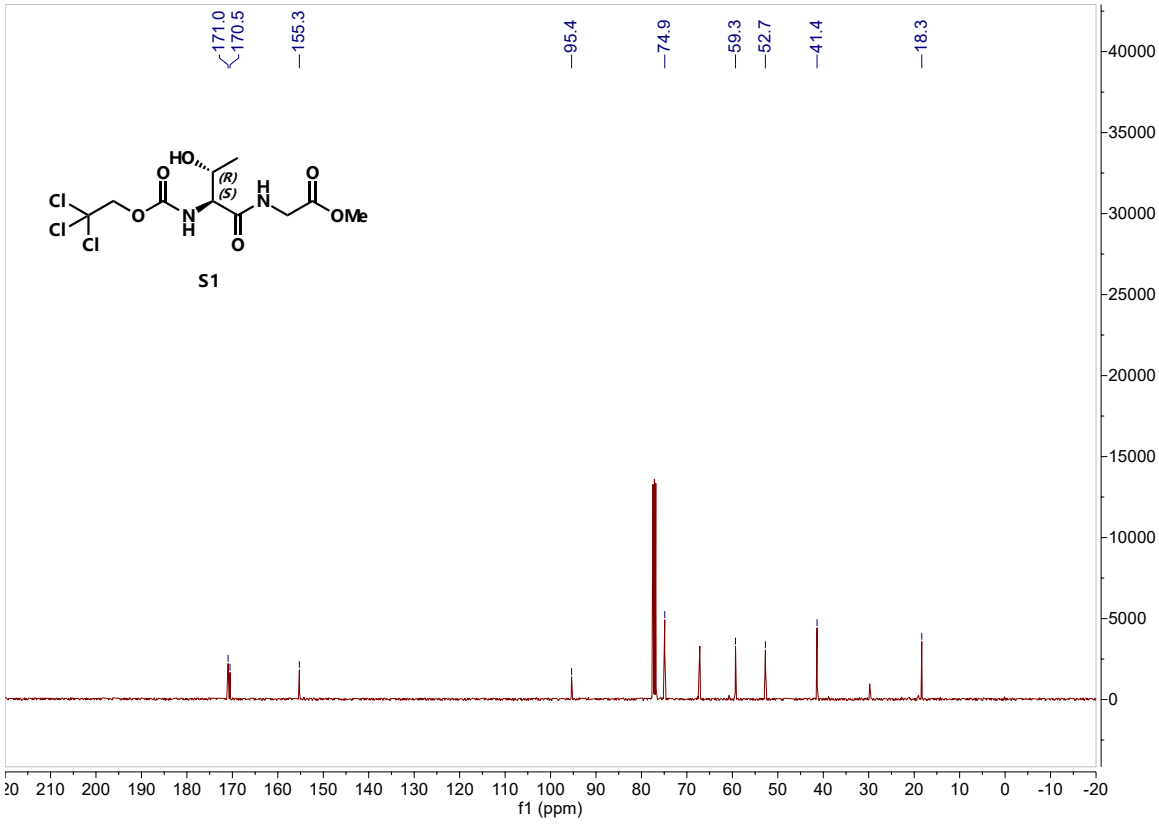
^{13}C NMR (101 MHz, $CDCl_3$)



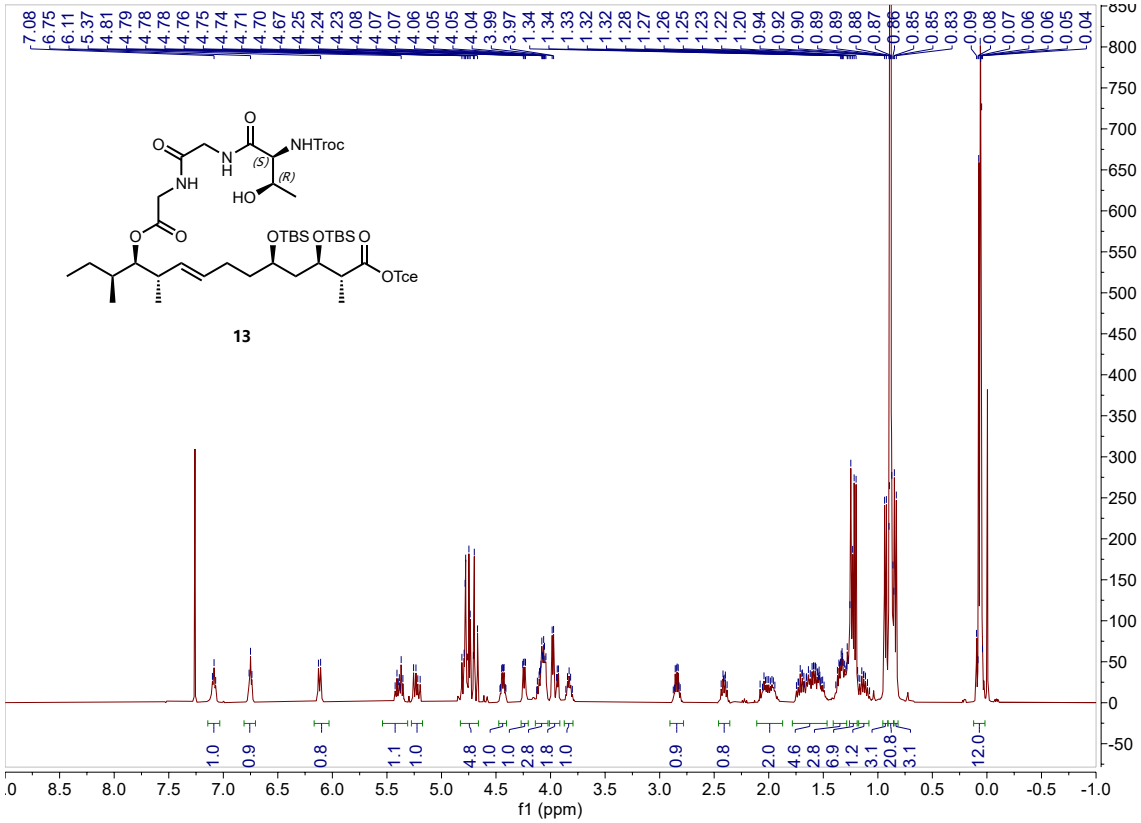
^{1}H NMR (400 MHz, $CDCl_3$)



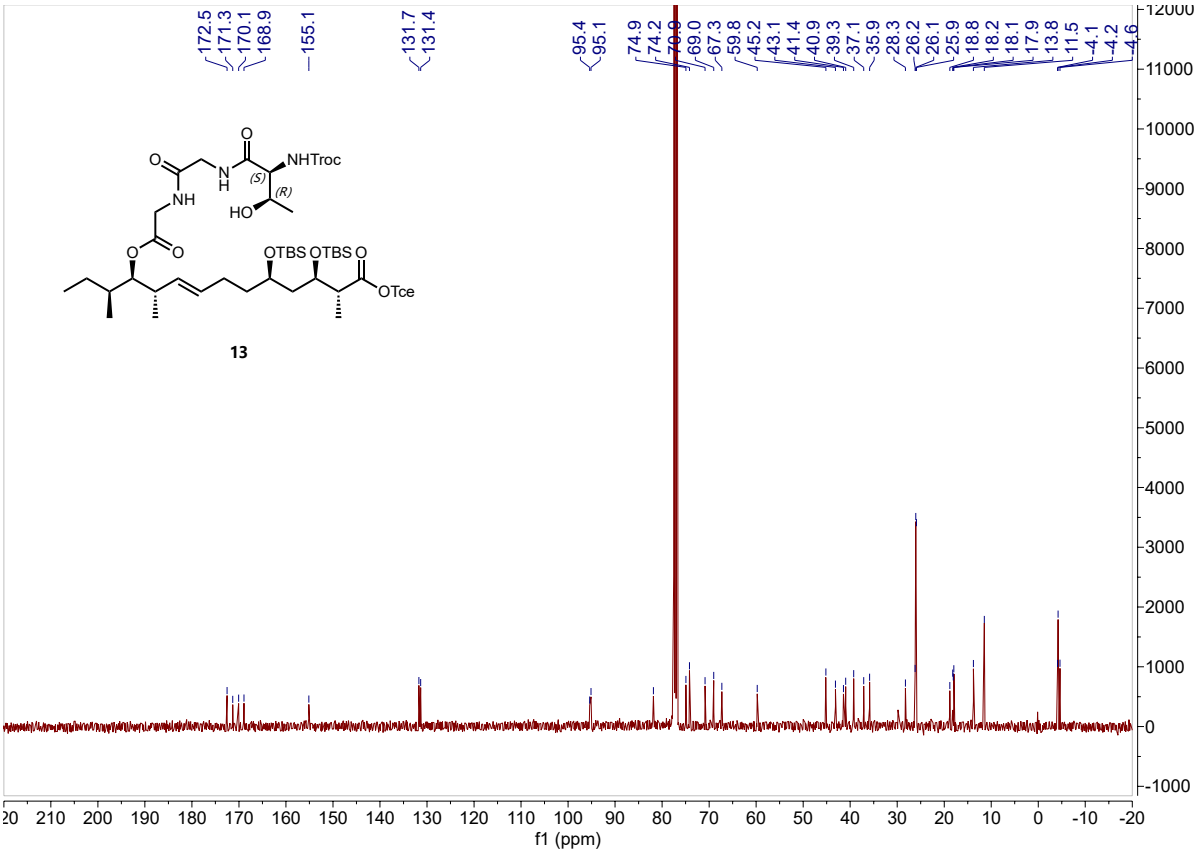
^{13}C NMR (101 MHz, $CDCl_3$)



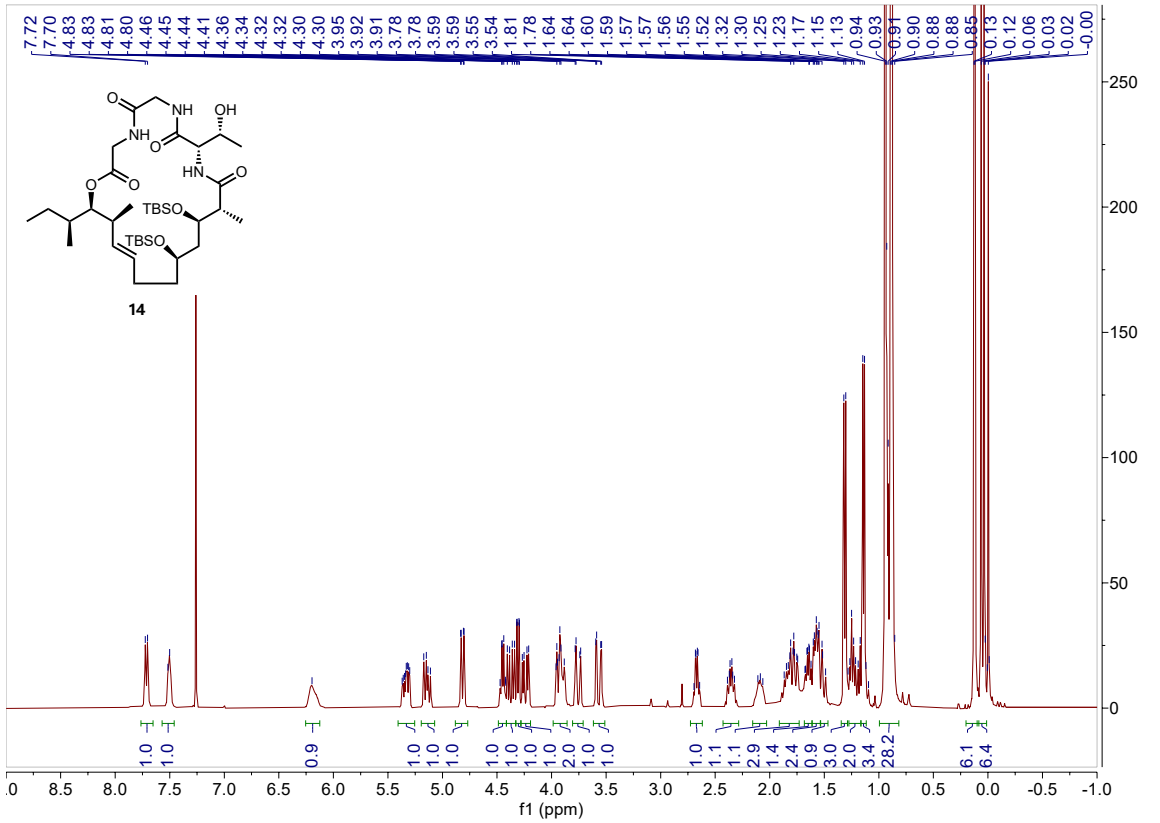
^{1}H NMR (400 MHz, $CDCl_3$)



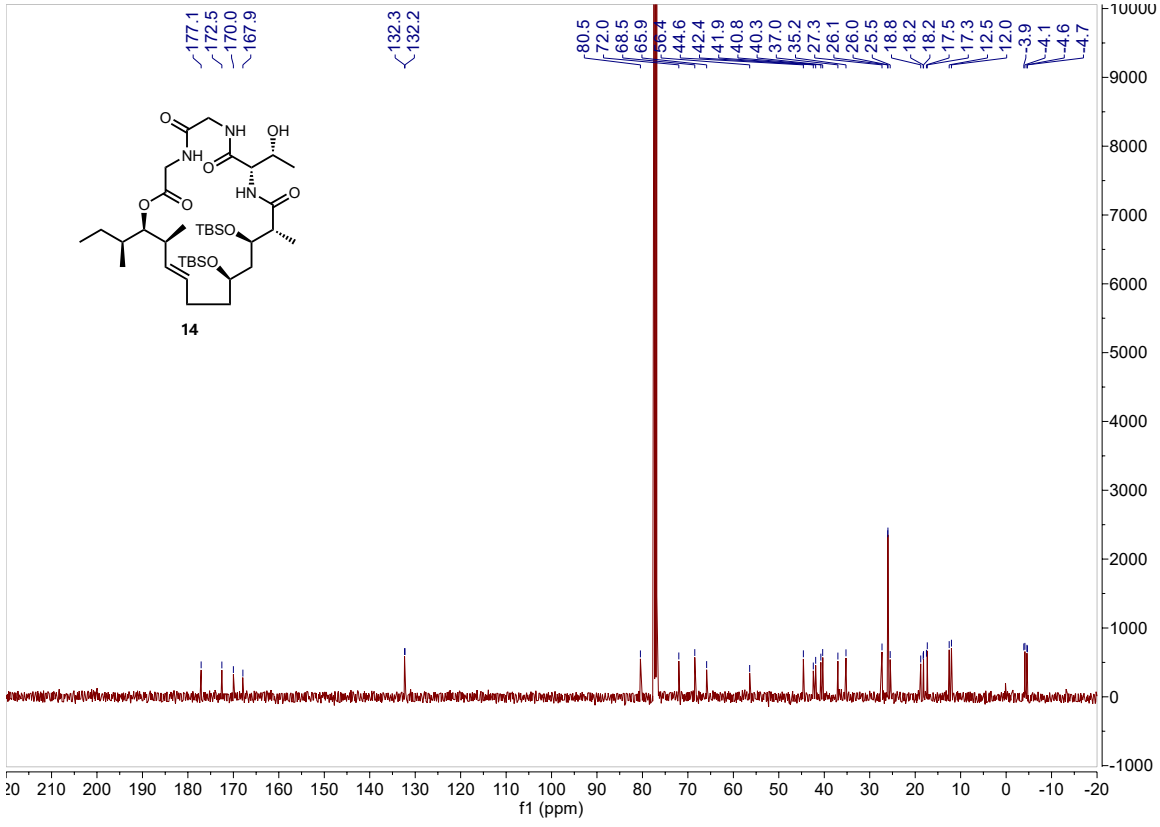
^{13}C NMR (101 MHz, $CDCl_3$)



^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (101 MHz, $CDCl_3$)



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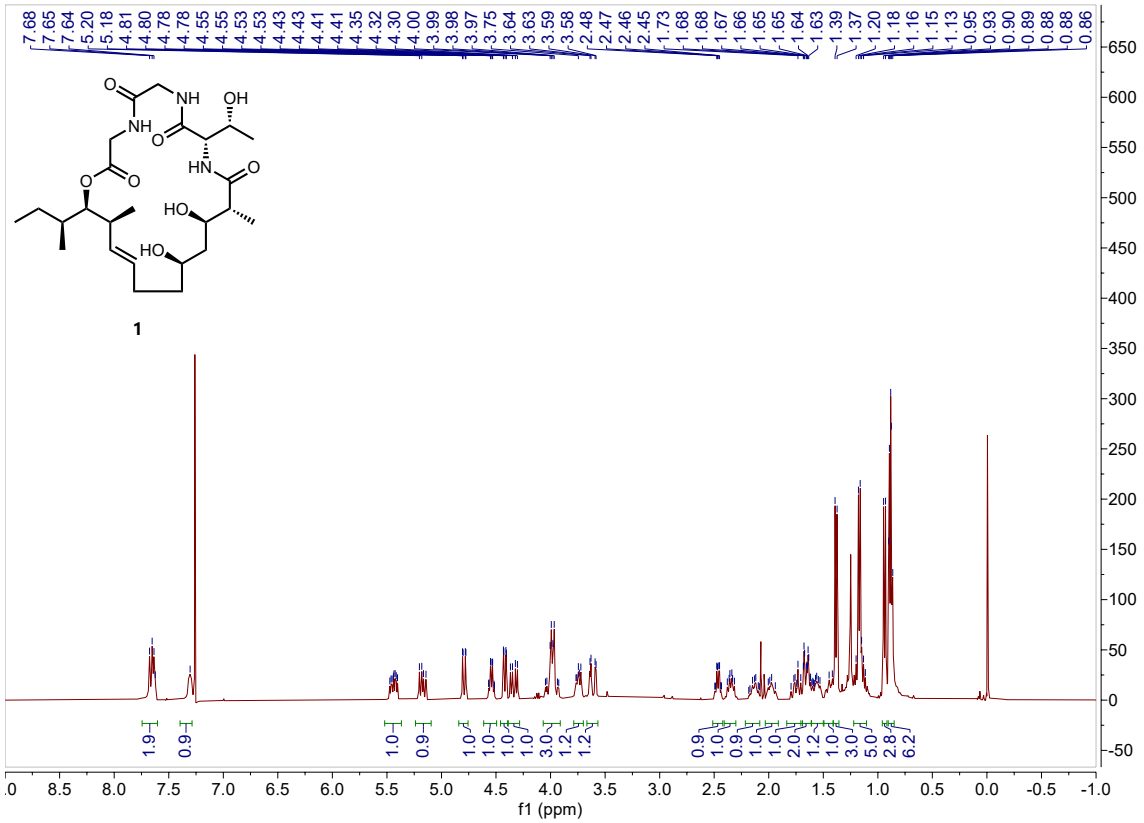

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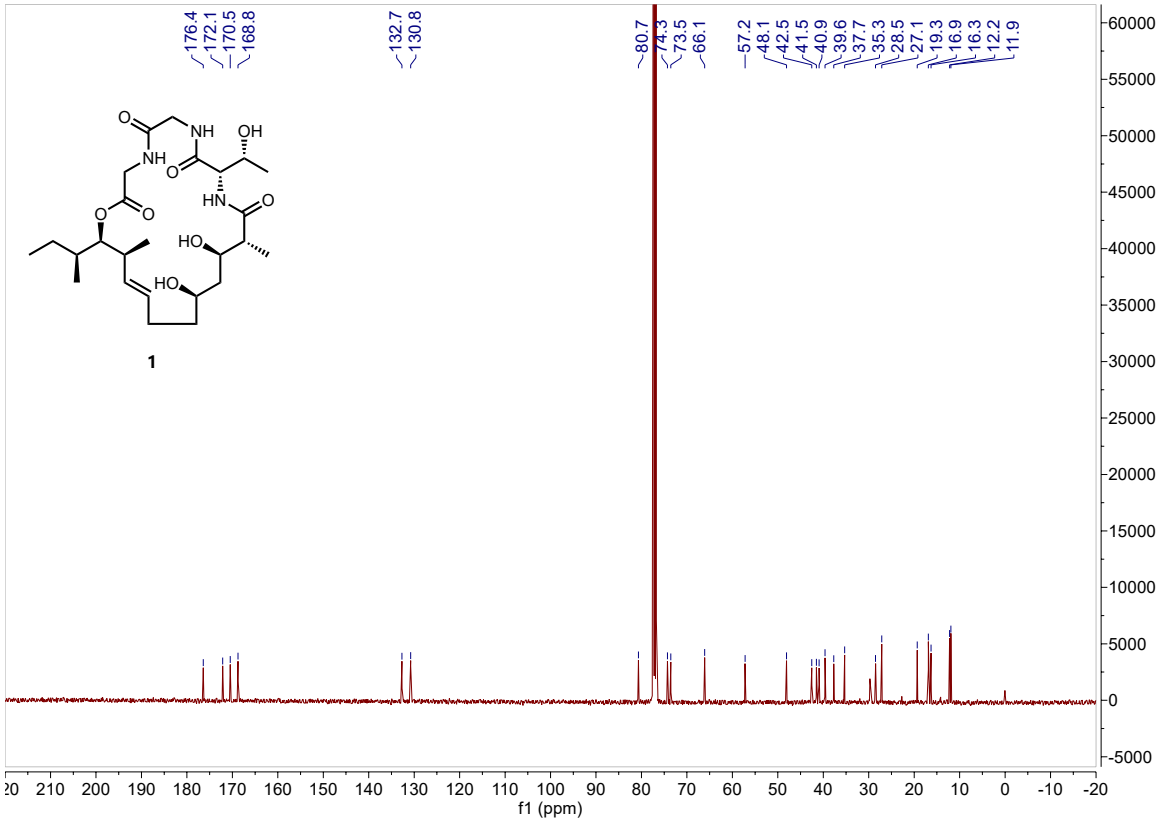

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