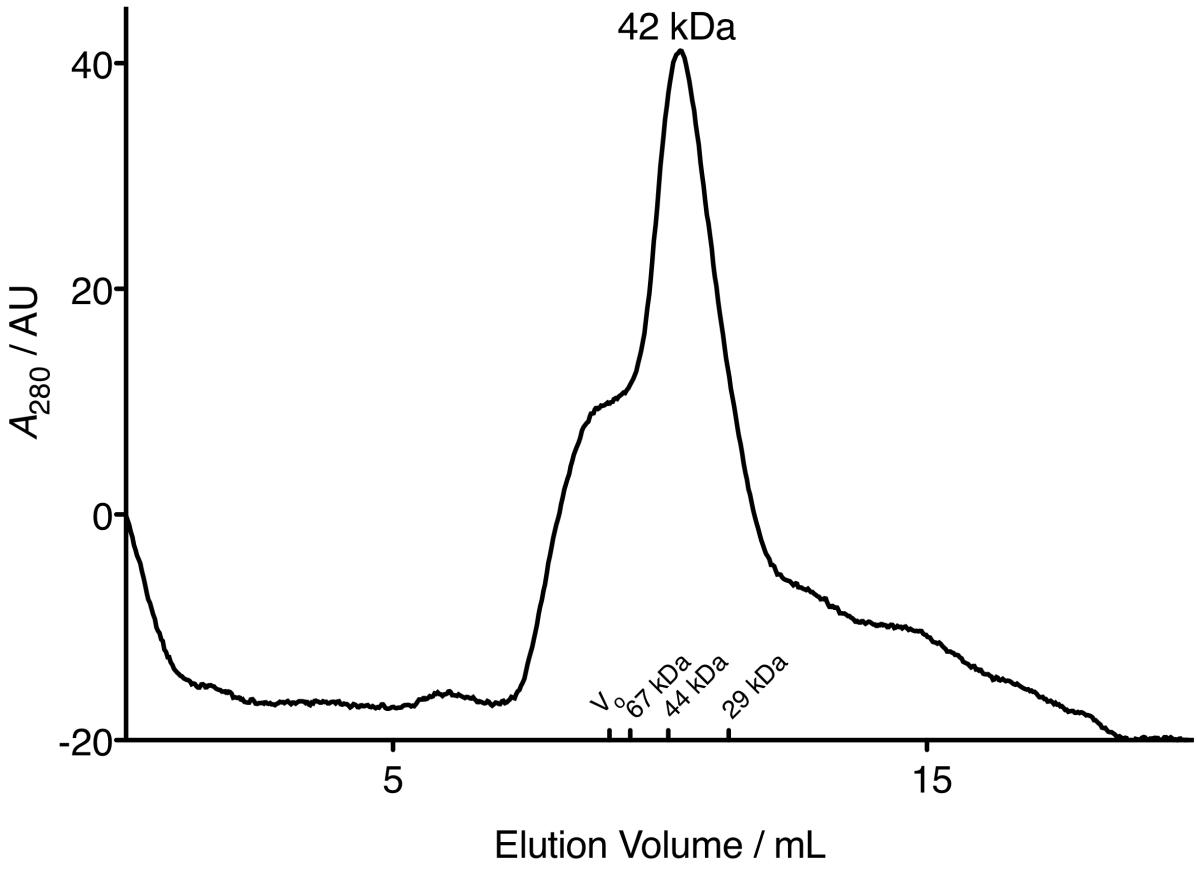
CHEMBIOCHEM

Supporting Information

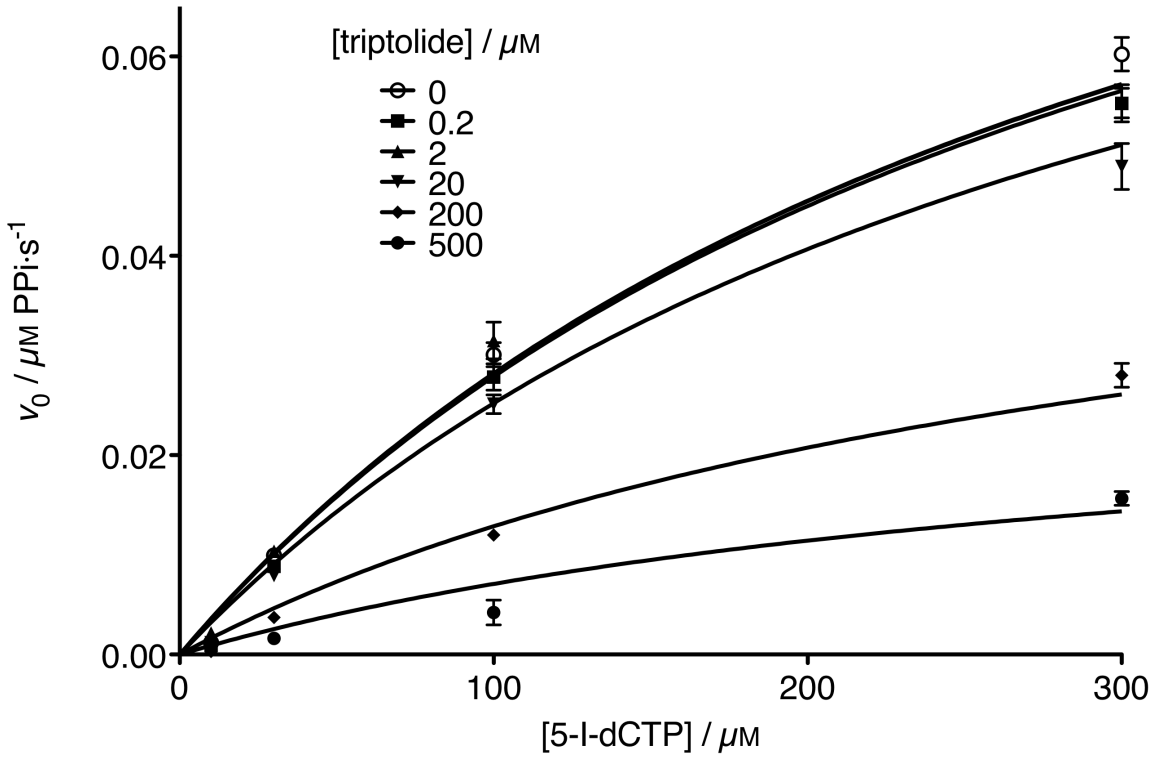
Triptolide Directly Inhibits dCTP Pyrophosphatase

Timothy W. Corson,*[a, b] Hüseyin Cavga,[a, c] Nicholas Aberle,[a] and Craig M. Crews[a, d]

cbic_201100007_sm_miscellaneous_information.pdf

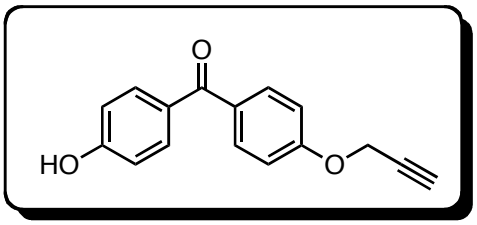


Supplementary Figure S1. Multimerization of recombinant DCTPP1. Size exclusion chromatography shows a single major peak for recombinant DCTPP1, corresponding to an interpolated size of 42 kDa (calculated dimer size = 38 kDa). A_{280}, absorbance at 280 nm; AU, arbitrary units; V_o, void volume determined with blue dextran.



Supplementary Figure S2. Michaelis-Menten kinetic analysis for inhibition of DCTPP1-mediated 5-iodo-dCTP (5-I-dCTP) hydrolysis by triptolide at the indicated concentrations. Initial velocity (v_0) was calculated as described in the Experimental Section.

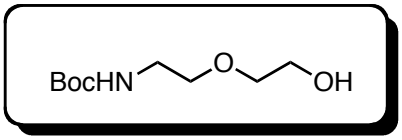
Synthesis of Triptolide Photoaffinity Reagent



S1

Propargyl bromide (80 wt % in toluene, 1.78 g, 12.0 mmol) was added to an orange suspension 4,4'-dihydroxybenzophenone (5.0 g, 23.4 mmol) and K_2CO_3 (1.65 g, 11.9 mmol) in anhydrous DMF (25 mL). The mixture was stirred at 70 °C for 18 h, then cooled to room temperature, diluted with EtOAc (40 mL) and washed with 2 M HCl (aq) (2 x 30 mL) then dried over $MgSO_4$, filtered and concentrated. The crude material was purified by flash chromatography (2:1 hexane:EtOAc) to give the product **S1** (1.92 g, 65%) as a white powder, along with recovered benzophenone starting material and a small amount of di-alkylated material.

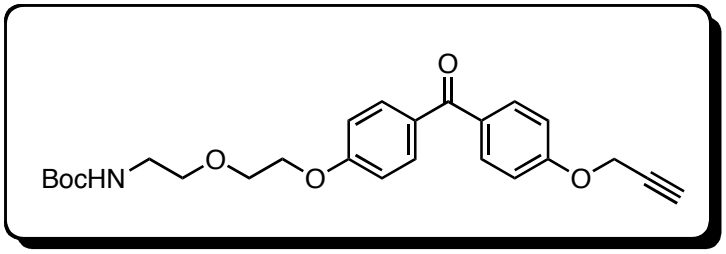
^{1}H NMR ($CDCl_3$ with a few drops of MeOD, 500 MHz) δ 7.65 (d, J = 8.40 Hz, 2 H), 7.59 (d, J = 8.35 Hz, 2 H), 6.93 (d, J = 8.40 Hz, 2 H), 6.77 (d, J = 8.30 Hz, 2 H), 4.66 (s, 2 H), 3.93 (bs, 1 H), 2.54 (s, 1 H).
^{13}C NMR ($CDCl_3$ with a few drops of MeOD, 125 MHz) δ 195.4, 161.3, 160.5, 132.5, 131.9, 131.2, 128.9, 114.9, 114.1, 76.0, 55.6, 21.6.
HRMS (ESI) Calcd for $(M + H)^+$ $C_{16}H_{12}O_3$ 253.0859; found 253.0851



S2

A solution of 2-(2-aminoethoxy)-ethanol (1.42 mL, 14.25 mmol) and KOH pellets (85%, 1.07 g, 16.2 mmol) in H_2O (10 mL) was cooled to 0 °C and treated dropwise with a solution of Boc_2O (3.53 g, 16.2 mmol) in 1,4-dioxane (5 mL). The mixture was allowed to warm to room temperature and stirred for 5 h, then extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were washed with brine, then dried ($MgSO_4$), filtered and concentrated. The crude residue was purified by flash chromatography (100% EtOAc) to give the desired product **S2** (2.78 g, 95%) as a clear oil.

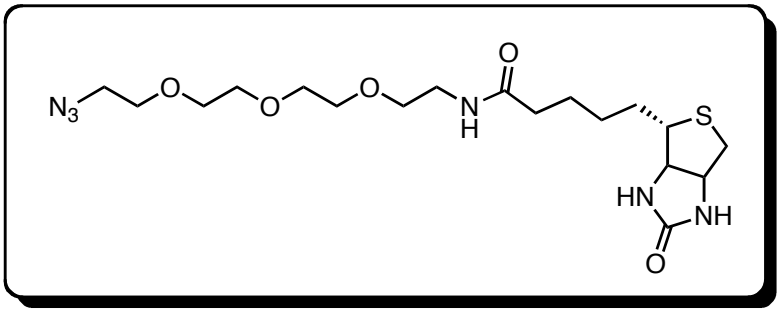
^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.92 (bs, 1 H), 3.71 (m, 2 H), 3.55 (m, 4 H), 3.32 (m, 2 H), 2.17 (t, J = 5.88 Hz, 1 H), 1.42 (s, 9 H).
LRMS (ESI) 228.36 (M + Na^+).



S3

Diisopropyl azodicarboxylate (0.72 mL, 3.66 mmol) was added dropwise to a suspension of phenol **S1** (1.16 g, 4.60 mmol), alcohol **S2** (0.51 g, 2.48 mmol) and PPh_3 (0.95 g, 3.62 mmol) in anhydrous THF (5.0 mL). A slight exotherm during the addition was tempered with an ice-bath, and the clear yellow solution was then stirred at room temperature for 48 h. The reaction was diluted with EtOAc (20 mL) then washed with H_2O (20 mL). The aqueous layer was back-extracted with more EtOAc and the combined organics were washed with brine, dried ($MgSO_4$), filtered and concentrated. Purification by flash chromatography afforded the desired product **S3** (0.53 g, 49%) as a white semi-solid.

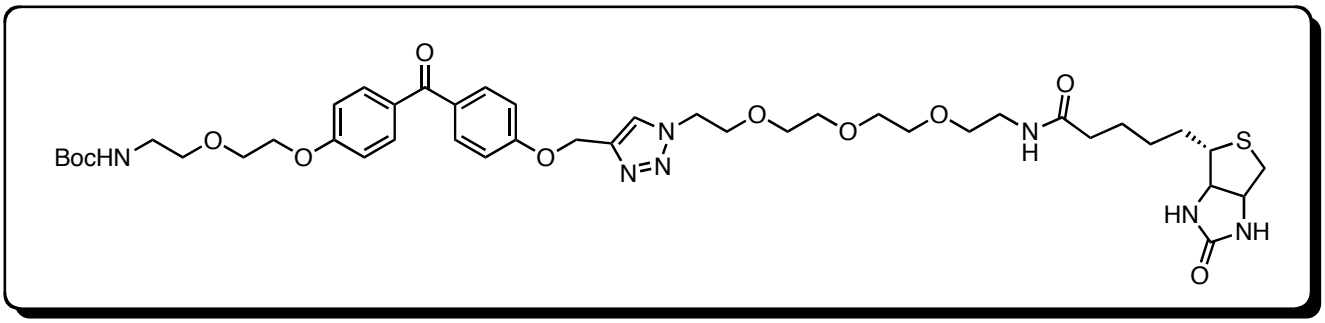
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (d, J = 8.76 Hz, 2 H), 7.78 (d, J = 8.76 Hz, 2 H), 7.04 (d, J = 8.80 Hz, 2 H), 6.98 (d, J = 8.80 Hz, 2 H), 4.96 (bs, 1 H), 4.78 (d, J = 2.36 Hz, 2 H), 4.20 (m, 2 H), 3.85 (m, 2 H), 3.62 (m, 2 H), 3.35 (m, 2 H), 2.57 (t, J = 2.36 Hz, 1 H), 1.44 (s, 9 H).
^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.3, 162.0, 160.6, 155.9, 132.2, 132.1, 131.4, 130.8, 114.3, 114.0, 79.3, 77.8, 76.1, 70.5, 69.2, 67.5, 55.8, 40.3, 28.4.



S4

Biotin (586 mg, 2.40 mmol), 11-azido-3,6,9-trioxaundecan-1-amine (588 mg, 2.88 mmol), HBTU (728 mg, 2.88 mmol) HOBt (389 mg, 2.88 mmol) and DIEA (1.25 mL, 7.20 mmol) were combined in DMF (20 mL) and stirred for 24 h. The reaction mixture was quenched with water (25 mL) and EtOAC (50 mL). The organic layer was separated, washed with water (x 3) and brine, then dried ($MgSO_4$), filtered and concentrated. The crude material was purified by flash chromatography (10:1 chloroform:methanol) to afford **S4** (off-white solid, 480 mg, 45%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.87 (t, *J* = 5.3 Hz, 1 H), 6.75 (s, 1 H), 5.90 (s, 1 H), 4.47 (m, 1 H), 4.28 (m, 1 H), 3.62 (m, 10 H), 3.54 (t, *J* = 5.0 Hz, 2 H), 3.39 (m, 4 H), 3.11 (m, 1 H), 2.87 (dd, *J* = 12.8, 5.0 Hz, 1 H), 2.71 (d, *J* = 12.8 Hz, 1 H), 2.20 (t, *J* = 7.5 Hz, 2 H), 1.61-1.72 (m, 4 H), 1.40 (m, 2 H).
^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4, 164.2, 70.5, 70.4, 70.0, 69.9, 69.9, 61.7, 60.1, 55.7, 50.6, 40.4, 39.0, 35.9, 28.2, 28.0, 25.6.
HRMS (ESI) Calcd for (M + H^+) $C_{18}H_{32}N_6O_5S$ 445.2227; found 445.2224.

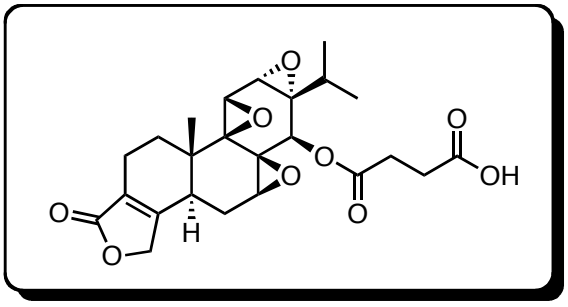


S5

Azide **S4** (116 mg, 0.261 mmol) and alkyne **S3** (115 mg, 0.262 mmol) were combined in methanol (2.5 mL) then treated with 0.78 mL of aqueous sodium ascorbate (13.6 mM solution) and 0.23 mL of aqueous $CuSO_4.5H_2O$ (10.4 mM solution). This slightly cloudy solution was stirred overnight, concentrated to dryness and partitioned between ethyl acetate and water. The aqueous layer was extracted with further EtOAc (3 x 5 mL) and the combined organic layers were washed with brine, then dried ($MgSO_4$), filtered and concentrated. Purification by flash chromatography (9:1 dichloromethane:methanol) afforded the product **S5** (166 mg, 72%) as a white foam.

^{1}H NMR (MeOD, 500 MHz) δ 8.17 (s, 1 H), 7.74 (m, 4 H), 7.14 (d, *J* = 8.8 Hz, 2 H), 7.05 (d, *J* = 8.8 Hz, 2 H), 5.27 (s, 2 H), 4.59 (t, *J* = 5.0 Hz, 2 H), 4.45 (dd, *J* = 7.7, 5.0, 1 H), 4.26 (dd, *J* = 7.8, 4.5 Hz, 1 H), 4.21 (m, 2 H), 3.88 (t, *J* = 5.0 Hz, 2 H), 3.83 (t, *J* = 4.5 Hz, 2 H), 3.56 (m, 10 H), 3.48 (m, 2 H), 3.30 (m, 2 H), 3.24 (m, 2 H), 3.14 (m, 1 H), 2.88 (dd, *J* = 12.7, 5.0 Hz, 1 H), 2.67 (d, *J* = 12.7 Hz, 1 H), 2.16 (t, *J* = 7.3 Hz, 2 H), 1.53-1.72 (m, 4 H), 1.42 (m, 11 H).
^{13}C NMR (MeOD, 125 MHz) δ 196.4, 176.0, 166.0, 164.0, 163.2, 158.4, 144.2, 133.4, 133.4, 132.1, 131.7, 126.4, 115.6, 115.3, 80.1, 71.5, 71.4, 71.4, 71.2, 70.5, 70.4, 70.3, 68.9, 63.3, 62.6, 61.6, 57.0, 54.3, 51.5, 41.2, 41.1, 40.3, 36.7, 29.7, 29.5, 28.8, 26.8.
HRMS (ESI) Calcd for (M + H^+) $C_{43}H_{61}N_7O_{11}S$ 884.4223; found 884.4177

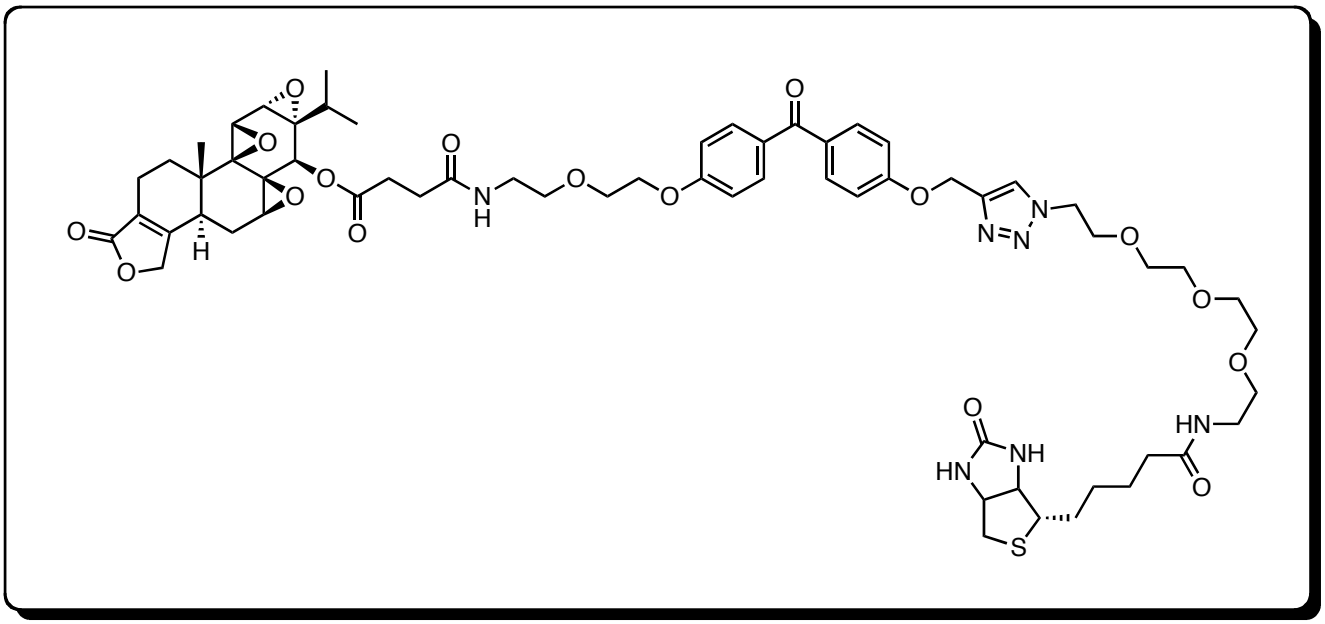
Photoaffinity compound **S5** (50 mg, 0.057 mmol) was dissolved in CH_2Cl_2 (5.0 mL) and treated with TFA (5.0 mL). After 2 h, the reaction was concentrated to dryness, then twice dissolved in CH_2Cl_2 and re-concentrated. The crude material (**3**) was on-reacted with **S6**.



S6

To a solution of triptolide **1** (11 mg, 0.031 mmol) in CH_2Cl_2 (1.0 mL) was added succinic anhydride (29 mg, 0.29 mmol), triethylamine (40 μL, 0.29 mmol) and DMAP (35 mg, 0.29 mmol). The mixture was stirred at room temperature for 48 h, then diluted with CH_2Cl_2 and washed with water then brine. The organic phase was dried over $MgSO_4$, filtered and concentrated. The crude material was purified by flash chromatography (9:1 dichloromethane:methanol), affording **S6** (13.2 mg, 94%) as a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.07 (s, 1 H), 4.67 (s, 2 H), 3.83 (d, J = 3.12 Hz, 1 H), 3.53 (d, J = 2.84 Hz, 1 H), 3.45 (d, J = 5.60 Hz, 2 H), 2.72 (m, 5H), 2.31 (m, 1 H), 2.14 (m, 2 H), 1.89 (m, 2 H), 1.56 (m, 1 H), 1.22 (m, 1 H), 1.04 (s, 3 H), 0.94 (d, J = 6.96 Hz, 3 H), 0.83 (d, J = 6.84 Hz, 3 H). Matches literature data.



2

HATU (11.3 mg, 0.0297 mmol) and DIEA (21 μL, 0.121 mmol) were added to a suspension of the triptolide acid **S6** (13.2 mg, 0.0287 mmol) and biotin-benzophenone-amine **3** (25 mg, 0.0279 mmol) in DMF (0.5 mL). The solution was stirred for 24 h, concentrated to dryness, then purified by flash chromatography (14:1 to 7:1 $CHCl_3$:MeOH). The product **2** was obtained as a clear oil (15.5 mg, 46%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.89 (s, 1 H), 7.78 (d, *J* = 8.8 Hz, 2 H), 7.77 (d, *J* = 8.8 Hz, 2 H), 7.07 (d, *J* = 8.8 Hz, 2 H), 6.99 (d, *J* = 8.8 Hz, 2 H), 6.63 (bs, 1 H), 6.22 (m, 1 H), 5.29 (m, 4 H), 5.05 (s, 1 H), 4.66 (m, 2 H), 4.57 (t, *J* = 4.92 Hz, 2 H), 4.48 (m, 1 H), 4.30 (m, 1 H), 4.20 (m, 2 H), 3.85 (m, 5 H), 3.39-3.67 (m, 15 H), 3.12 (m, 1 H), 2.88 (m, 1H), 2.65-2.82 (m, 5 H), 2.51 (m, 2 H), 2.11-2.26 (m, 6 H), 1.87 (m, 2 H), 1.65 (m, 5 H), 1.52 (m, 1 H), 1.41 (m, 3 H), 1.02 (s, 3 H), 0.92 (d, *J* = 7.0 Hz, 3 H), 0.82 (d, *J* = 6.8 Hz, 3 H).
^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.4, 173.5, 173.3, 172.2, 171.3, 163.8, 162.0, 161.5, 160.1, 143.2, 132.3, 132.3, 131.1, 130.9, 125.5, 124.4, 114.4, 114.1, 71.1, 70.5, 70.4, 70.0, 69.9, 69.4, 69.3, 63.6, 63.3, 62.0, 61.9, 61.3, 60.3, 59.7, 55.5, 54.9, 53.5, 50.7, 50.4, 40.5, 40.3, 39.3, 39.2, 35.8, 35.7, 31.1, 29.8, 28.1, 28.0, 28.0, 25.5, 23.4, 17.5, 17.1, 16.7, 13.8.
HRMS (ESI) Calcd for (M + H^+) $C_{62}H_{79}N_7O_{17}S$ 1226.5326; found 1226.5296

Synthesis of (5*R*)-5-hydroxytriptonide (6)

To a solution of triptonide (**4**) (3.7 mg, 10.4 μmol) in DMSO was added selenium dioxide (4.6 mg, 41.6 μmol). The reaction mixture was stirred at 120°C under N_2 for 12 h. After cooling to room temperature, the reaction mixture was neutralized with Na_2CO_3 solution, filtered through Celite and concentrated under vacuum. The crude product was purified via preparative TLC (ethyl acetate/hexane, 1:1) to yield desired product **6** (3.2 mg, 8.5 μmol, 82%)

^{1}H NMR (DMSO-d_6) δ 5.68 (1H, s, 5-OH), 4.90 (2H, s, H19), 4.12 (1H, d, *J* = 3, H11), 4.10 (1H, d, *J* = 2.5, H12), 3.43 (1H, d, *J* = 5, H7), 2.25 (1H, sept, *J* = 7, H15), 2.23 (1H, m, H6α), 2.16 (1H, t, *J* = 14, H6β), 2.13 (1H, brd, H2α), 2.02 (1H, m, H2β), 1.85 (1H, ddd, *J* = 6, 12, 12, H1α), 1.10 (1H, dd, *J* = 5.5, 12, H1β), 0.93 (3H, s, H20), 0.89 (3H, d, *J* =6.5, H17), 0.80 (3H, d, *J* = 7, H16).
^{13}C NMR (DMSO-d_6) δ 198.4 (C14), 173.2 (C18), 162.0 (C4), 124.5 (C3), 69.9 (C5), 68.7 (C19), 65.1 (C13), 64.3 (C9), 61.1 (C8), 59.0 (C7), 58.7 (C11), 56.0 (C12), 40.0 (C10), 29.7 (C6), 25.6 (C15), 24.2 (C1), 18.0 (C17), 16.8 (C2), 16.4 (C16), 16.0 (C20).
UV (EtOH) λ max 218 nm; IR (KBr) vmax 3509, 2961, 1764, 1710, 1679, 1037 cm^{-1}.
HRMS (ESI) Calcd for (M + H^+) $C_{20}H_{22}O_7$ 375.1433, found 375.1438

Synthesis of 14-α-triptolide (7)

14-α-triptolide (**7**) was synthesized by borohydride reduction of triptolide **1** as described,[22] and spectral data corresponded to published values.