Young *et al*. Supplemental Figure 1

Detailed methods for solution phase synthesis of DDM-Thre and DDM-Ser.

Experimental Section

All of the anhydrous solvents, reagent grade solvents for chromatography and starting materials were purchased from either Aldrich Chemical Co. (Milwaulkee, WI) or Fisher Scientific (Suwanee, GA). General methods of purification of compounds involved the use of silica cartridges purchased from Analogix, Inc. (Madison, WI) and/or recrystallization. The reactions were monitored by thin-layer chromatography (TLC) on precoated Merck 60 F_{254} silica gel plates and visualized using UV light (254 nm). All compounds were analyzed for purity and characterized by ^{1}H and ^{13}C NMR using a Varian 300MHz NMR and aVarian 500MHz NMR spectrometer. Chemical shifts are reported in ppm (δ) relative to the residual solvent peak and coupling constants (*J*) are reported in hertz (Hz) (s = singlet, bs = broad singlet, d = doublet, dd = double doublet, bd = broad doublet, ddd = double doublet of dublet, t = triplet, tt – triple triplet, q = quartet, m = multiplet) and analyzed using MestReC NMR data processing. Mass Spectra values are reported as *m/z*. All reactions were conducted under argon unless otherwise noted. Solvents were removed *in vacuo* on a rotary evaporator. The yields quoted are unoptimized. The HPLC analyses were carried out on a Waters 2695 instrument consisting of a photodiode array detector 996, using the Waters symmetry C_{18} 5 μm reverse phase column. Mobile phases: (a) 50% HPLC grade acetonitrile in Millipore purified water at a flow rate of 1.0 mL/min^{-1} and UV detection at 254 nm. ACN = acetonitrile; DCM = dichloromethane; DMF = dimethylformamide; EtOAc = ethyl acetate; HOAc = acetic acid; DAST = Bis(2-methoxyethyl)amino-sulfur trifluoride.

Details of solution phase synthesis

Z-(*L*)-Caprolactam 2: To Z-(*L*)-lysine (3.00 g, 0.0107 mol), HOAt (1.60 g, 0.0118 mol), in 600 mL of 5:1 CH_3CN/DMF at room temperature was added EDC (2.47 g, 0.0128 mol). The solution was stirred at room temperature for 60 h. The CH_3CN was evaporated under reduced pressure and the residual solution diluted with 1N HCl. The aqueous solution was extracted three times with EtOAc. The combined organic extracts were dried with NaS_2O_4, filtered and concentrated. The residue was purified by silica gel chromatography (1:1 to 2:1 EtOAc:hexanes) to give 2.66 g of the product as an amorphous white solid in 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.26 (m, 5H), 6.39 (br s, 1H), 5.10 (s, 2H), 4.36 (dd, 1H, J = 6.05, 11.18 Hz), 3.26-3.22 (m, 2H), 2.13-1.98 (m, 2H), 1.85-1.71 (m, 2H), 1.59-1.35 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6, 155.7, 136.8, 128.7, 128.2, 128.1, 66.8, 53.9, 42.3, 32.3, 29.1, 28.2; IR (Neat) 3251, 2933, 1720, 1667 cm^{-1}; HRFABMS for $C_{14}H_{19}N_2O_3$ (MH^+) calcd 263.1396, found 263.1411.

Pre-cobactin T 3a: A solution of **2** (0.264 g, 0.001 mol) in 10 mL of MeOH was purged with nitrogen. Pd-C (10% of 10% by weight) was added and a hydrogen atmosphere was established with a balloon. The solution was stirred for 45 min at room temperature and was filtered through Celite. In a separate flask, (R)-3-hydroxy butyric acid sodium salt (0.126 g, 0.001 mol) and MeOH/water washed Dowex 50X8-400 acidic ion exchange resin in 10 mL MeOH were stirred for 30 min. The resin was filtered and the filtrate concentrated. To the amine, acid, HOAt (0.136 g, 0.001 mol), DMAP (0.122 g, 0.001 mol) in 15 mL CH_2Cl_2 was added EDC (0.211 g, 0.0011 mol) and the mixture was stirred at room temperature for 24 h. Without workup, the reaction mixture was applied to a silica gel column and eluted (EtOAc to 4:1 EtOAc:MeOH) to give 0.204 g of the product as an amorphous white solid in 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02 (br s, 1H), 6.05 (br s, 1H), 4.21-4.13 (m, 1H), 3.98 (d, 1H, J = 3.21Hz), 2.42 (dd, 1H, 3.10, 15.29 Hz), 2.30 (dd, 1H, J = 8.77, 15.41 Hz), 2.11-2.00 (m, 2H), 1.91-1.74 (m, 2H), 1.56-1.37 (m, 2H), 1.22 (d, 3H, J = 6.42 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.7, 171.8, 65.0, 52.2, 43.9, 42.3, 31.6, 29.0, 28.1, 22.8; ^{1}H NMR (600 MHz, d_6-DMSO) δ 7.78 (d, 1H, J = 6.59 Hz), 7.72 (br t, 1H, J = 5.86 Hz), 4.63 (br s, 1H), 4.39 (ddd, 1H, J = 1.56, 6.87, 11.17 Hz), 3.97-3.91 (m, 1H), 3.19-3.13 (m, 1H), 3.08-3.03 (m, 1H), 2.24 (dd, 1H, J = 6.87, 14.19 Hz), 2.21 (dd, 1H, J = 5.59, 14.19 Hz), 1.87-1.59 (m, 4H), 1.40-1.17 (m, 2H), 1.08 (t, 3H, J = 6.23 Hz) ^{13}C NMR (150 MHz, d_6-DMSO) δ 174.2, 169.7, 64.0, 51.2, 45.0, 40.6, 31.0, 28.8, 27.5, 23.2; IR (Neat) 3296, 2929, 1640 cm^{-1}; HRFABMS for $C_{10}H_{19}N_2O_3$ (MH^+) calcd 215.1396, found 215.1410.

Pre-cobactin S 3b: Synthesized in 72% yield from (S)-3-hydroxy butyric acid using the same process as described above for pre-cobaction **3a**. ^{1}H NMR (600 MHz, d_6-DMSO) δ 7.80 (d, 1H, J = 7.07 Hz), 7.76 (br t, 1H, J = 5.86 Hz), 4.70 (br d, 1H, J = 3.84 Hz), 4.38 (ddd, 1H, J = 1.70, 6.77, 11.17 Hz), 3.95-3.91 (m, 1H), 3.19-3.14 (m, 1H), 3.08-3.04 (m, 1H), 2.24 (dd, 1H, J = 7.24, 13.83 Hz), 2.20 (dd, 1H, J = 5.41, 13.83 Hz), 1.88-1.60 (m, 4H), 1.40-1.18 (m, 2H), 1.07 (t, 3H, J = 6.23 Hz) ^{13}C NMR (150 MHz, d_6-DMSO) δ 174.2, 169.8, 63.8, 51.3, 45.1, 40.6, 30.9, 28.7, 27.5, 23.1; IR (Neat) 3306, 2930, 1635 cm^{-1}; HRFABMS for $C_{10}H_{19}N_2O_3$ (MH^+) calcd 215.1396, found 215.1392.

Z-(*L*)-Lysine-N^5-Boc methyl ester 4: MeOH (25 mL) was cooled to 0 °C. $SOCl_2$ (4.24 g, 0.0356 mol) was added with a syringe. Z-(*L*)-Lysine (2.50 g, 0.0089 mol) was added and the solution was stirred for 12 h while coming to room temperature. The solution was concentrated and the residue taken up in 30 mL of 1:1 THF water. $NaHCO_3$ (0.900 g, 0.0107 mol) was added followed by Boc_2O (2.34 g, 0.0107 mol) and the solution stirred at room temperature for 48 h. The THF was removed under reduced pressure and the residue extracted four times with CH_2Cl_2. The combined organic extracts were dried with NaS_2O_4, filtered and concentrated. The residue was purified by silica gel chromatography (CH_2Cl_2 to 4:1 CH_2Cl_2:EtOAc) to give 3.36 g of the product as a clear oil which solidified upon standing in 96% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.30 (m, 5H), 5.44-5.42 (m, 1H), 5.10 (s, 2H), 4.59 (br s, 1H), 4.38-4.32 (m, 1H), 3.73 (s, 3H), 3.10-3.08 (m, 2H), 1.90-1.31 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 156.1, 155.9, 136.2, 128.5, 128.1, 128.0, 79.1, 67.0, 53.7, 52.3, 40.0, 32.1, 29.5, 28.4, 22.3; IR (Neat) 3348, 2952, 1694 cm^{-1}; HRFABMS for $C_{20}H_{31}N_2O_6$ (MH^+) calcd 395.2182, found 395.2205.

Boc protected pre-mycobactic acid methyl ester 6 (R=H): A solution of the **4** (0.578 g, 1.46 mmol) in 15 mL of MeOH was purged with nitrogen. 10% weight of 10% Pd-C was added and a hydrogen atmosphere was established with a balloon. The solution was stirred for 45 min at room temperature and was filtered through Celite. To this amine, oxazoline acid **5** (R=H) (0.312 g, 1.46 mmol), HOAt (0.199 g, 1.46 mmol), and catalytic DMAP in 10 mL of DMF was added EDC (0.308 g, 1.61 mmol) and the solution was stirred at room temperature for 12 h. The solution was diluted with water and extracted three times with EtOAc. The organic solution was dried with Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (1:1 to 1.5:1 EtOAc:hexanes) to give 0.531 g of the methyl ester in 74% yield as amorphous white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.41 (s, 1H), 7.72 (dd, 1H, J = 1.70, 7.79 Hz), 7.45 (dt, 1H, J = 1.70, 7.80 Hz), 7.07 (br d, 1H, J = 8.71 Hz), 6.96-6.88 (m, 2H), 4.99 (br t, 1H, J = 9.75 Hz), 4.74-4.58 (m, 4H), 3.79 (s, 3H), 3.10-3.05 (m, 2H), 1.95-1.65 (m, 2H), 1.56-1.26 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 170.6, 168.1, 160.0, 156.2, 134.5, 128.8, 119.3, 117.2, 110.2, 79.3, 69.7, 68.2, 52.8, 52.1, 40.3, 32.2, 29.6, 28.6, 22.7; ^{1}H NMR (600 MHz, d_6-DMSO) δ 8.68 (d, 1H, J = 7.50 Hz), 7.64 (dd, 1H, J = 1.74, 7.78 Hz), 7.46 (dt, 1H, J = 1.65, 8.61 Hz), 7.00 (d, 1H, J = 8.25 Hz), 6.94 (dt, 1H, 1.10, 8.65 Hz), 6.69 (br s, 1H), 5.01 (dd, 1H, J = 7.51, 10.26 Hz), 4.64 (dd, 1H, J = 8.33, 13.28 Hz) 4.52 (t, 1H, J = 7.88 Hz), 4.27 (dd, 1H, J = 8.33, 13.28 Hz), 3.64 (s, 3H), 2.92-2.86 (m, 2H), 1.78-1.64 (m, 2H), 1.38-1.26 (m, 13H); ^{13}C NMR (150 MHz, d_6-DMSO) δ 172.2, 169.7, 165.8, 159.0, 155.5, 133.9, 127.9, 118.9, 116.5, 109.8, 77.2, 69.1, 67.1, 52.1, 51.8, 30.3, 28.9, 28.2, 22.6; IR (Neat) 3326, 2932, 1744, 1681, 1639 cm^{-1}; HRFABMS for $C_{22}H_{32}N_3O_7$ (MH^+) calcd 450.2240, found 450.2243.

Boc protected pre-mycobactic acid methyl ester 6 (R=Me): A solution of **4** (0.106 g, 0.267 mmol) in 5 mL of MeOH was purged with nitrogen. 10% weight of 10% Pd-C was added and a hydrogen atmosphere was established with a balloon. The solution was stirred for 45 min at room temperature and was filtered through Celite and concentrated. To this amine, oxazoline acid **5** (R=Me, 0.060 g, 0.270 mmol), HOAt (0.037 g, 0.270 mmol), and catalytic DMAP in 3 mL of DMF was added EDC (0.057 g, 0.297 mmol) and the solution was stirred at room temperature for 12 h. The solution was diluted with water and extracted three times with EtOAc. The organic solution was dried with Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (1:1 to 1.5:1 EtOAc:hexanes) to give 0.093 g of the methyl ester in 74% yield as amorphous white solid. ^{1}H NMR (600 MHz, d_6-DMSO) δ 11.91 (s, 1H); 8.48 (d, 1H, J = 7.58 Hz), 7.61 (dd, 1H, J = 1.78, 7.80 Hz), 7.46 (dt, 1H, J = 1.67, 8.59 Hz), 6.99 (d, 1H, J = 8.03 Hz), 6.94 (t, 1H, 1.10, 7.37 Hz), 6.71 (br s, 1H), 5.16 (m, 1H), 4.99 (d, 1H, J = 9.81 Hz) 4.30 (dd, 1H, J = 8.14, 13.49 Hz), 3.64 (s, 3H), 2.90 (dd, 2H, 6.81, 13.05 Hz), 1.76-1.63 (m, 2H), 1.38-1.26 (m, 16H); ^{13}C NMR (150 MHz, d_6-DMSO) δ 172.1, 167.8, 166.1, 159.2, 155.5, 133.8, 127.9, 118.8, 116.4, 110.0, 77.8, 77.2, 69.5, 51.8, 51.7, 30.3, 28.9, 28.2, 22.6, 15.4; IR (Neat) 3343, 2934, 1743, 1680, 1639 cm^{-1}.

DDM-ser [Pre-Mycobactin 1a (R=H)]: To **6** (R=H) (0.155 g, 0.337 mmol) in 5 mL of 1:1 THF/water was added LiOH (0.040 g, 1.69 mmol) at room temperature. The mixture was stirred for 12 h and the THF was removed under reduced pressure. The aqueous solution was acidified to pH = 4 with 1N citric acid and the solution was extracted three times with EtOAc. The combined organic extracts were washed with water and brine. The organic solution was dried with Na_2SO_4, filtered, and concentrated. The residue was used without purification. Crude ^{1}H NMR (600 MHz, d_6-DMSO) δ 8.43 (d, 1H, J = 7.69 Hz), 7.64 (dd, 1H, J = 1.74, 7.78 Hz), 7.46 (dt, 1H, J = 1.74, 7.25 Hz), 6.99 (dd, 1H, J = 1.10, 8.24 Hz), 6.94 (dt, 1H, 1.15, 7.28 Hz), 6.59 (br s, 1H), 5.01 (dd, 1H, J = 7.51, 10.26 Hz), 4.64 (dd, 1H, J = 8.41, 10.24 Hz) 4.52 (t, 1H, J = 7.87 Hz), 4.23-4.20 (m, 1H), 2.93-2.85 (m, 2H), 1.79-1.63 (m, 2H), 1.42-1.25 (m, 13H). To this acid, pre-cobactin T **3a** (0.072 g, 0.337 mmol), HOAt (0.046 g, 0.337 mmol), DMAP (0.041 g, 0.337 mmol) in 5 mL CH_2Cl_2 was added EDC (0.071 g, 0.371 mmol) at room temperature. After 24h, the solution was taken up in EtOAc and washed two times with 5% $NaHCO_3$, water, and brine. The organic solution was dried with Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (EtOAc to 4.5:1 EtOAc:MeOH) to give 0.051 g of the Boc protected pre-mycobactin T (R = H) in 24% yield as a white amorphous solid. ^{1}H NMR 4:1 ratio of rotational isomers present, major isomers reported when possible. (600 MHz, d_6-DMSO) δ 8.57 (d, 1H, J = 7.87 Hz), 7.81 (d, 1H, J = 6.96 Hz), 7.73-7.69 (m, 1H), 7.64 (dd, 1H, J = 1.83, 7.87 Hz), 7.46 (dt, 1H, J = 1.74, 8.70 Hz), 7.01 (dd, 1H, J = 1.10, 8.24 Hz), 6.95 (dt, 1H, J = 1.10, 8.07 Hz), 6.72-6.65 (m, 1H), 5.14-5.09 (m, 1H), 5.00 (dd, 1H, J = 7.51, 10.26 Hz), 4.63 (dd, 1H, J = 8.33, 10.16 Hz), 4.52 (t, 1H, J = 7.97 Hz), 4.40-4.36 (m, 1H), 4.23-4.19 (m, 1H), 3.18-3.13 (m, 1H), 3.07-3.03 (m, 1H), 2.93-2.84 (m, 2H), 2.52 (dd, 1H, J = 7.97, 14.93 Hz), 2.39 (dd, 1H, J = 5.68, 14.65 Hz), 1.87-1.58 (m, 6H), 1.41-1.24 (m, 15H), 1.18 (d, 3H, J = 6.23 Hz); ^{13}C NMR Rotational isomers present, major peaks reported when possible. (150 MHz, d_6-DMSO) δ 174.0, 170.9, 169.6, 165.8, 159.0, 155.5, 133.9, 127.9, 118.9, 116.5, 109.9, 77.2, 69.2, 68.7, 67.1, 52.1, 51.3, 41.3, 40.5, 31.0, 30.4, 29.0, 28.8, 28.2, 27.5, 22.7, 19.4; IR (Neat) 3317, 2932, 1744, 1640 cm^{-1}; HRFABMS for $C_{31}H_{46}N_5O_9$ (MH^+) calcd 632.3296, found 632.3300.

To Boc protected pre-mycobactin T (R = H) (0.095 g, 0.150 mmol) in 5 mL of EtOAc at 0 °C was added 5 mL of an HCl saturated EtOAc solution. The solution was stirred at 0 °C for 1h and the solvents were removed under reduced pressure. To this amine salt was added eicosanoic acid (0.047 g, 0.150 mmol), HOAt (0.020 g, 0.150 mmol), DMAP (0.018 g, 0.150 mmol), and DIPEA (0.021 g, 0.165 mmol). The reagents were dissolved in 5 mL of DMF and 4 mL of CH_2Cl_2 at room temperature EDC (0.032 g, 0.165 mmol) was added and the solution was stirred for 48h and diluted with EtOAc. The organic solution was washed with 1M HCl, water, 5% $NaHCO_3$, and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (EtOAc to 4.5:1 EtOAc:MeOH) to give 0.066 g of thePre-mycobactin **1a** in 53% yield as a white amorphous solid. ^{1}H NMR 4:1 ratio of rotational isomers present, major peaks reported when possible. (600 MHz, d_6-DMSO) δ 8.54 (d, 1H, J = 7.69 Hz), 7.80 (d, 1H, J = 6.95 Hz), 7.70 (br t, 1H, J = 6.13 Hz), 7.65-7.69 (m, 2H), 7.46 (dt, 1H, J = 1.65, 7.14 Hz), 7.00 (dd, 1H, J = 1.01, 8.33 Hz), 6.94 (dt, 1H, J = 1.10, 8.15 Hz), 5.13-5.08 (m, 1H), 5.00 (dd, 1H, J = 7.69, 10.25 Hz), 4.63 (dd, 1H, J = 8.43, 10.26 Hz), 4.51 (t, 1H, J = 7.97 Hz), 4.40-4.35 (m, 1H), 4.23-4.19 (m, 1H), 3.17-2.98 (m, 4H), 2.52 (dd, 1H, J = 7.69, 14.65 Hz), 2.39 (dd, 1H, J = 5.68, 14.65 Hz), 2.03 (t, 2H, J = 7.51 Hz), 1.86-1.59 (m, 6H), 1.49-1.22 (m, 40H), 1.19 (d, 3H, J = 6.40 Hz), 0.86 (t, 3H, J = 7.05 Hz); ^{13}C NMR Rotational isomers present, major peaks reported when possible. (150 MHz, d_6-DMSO) δ 174.0, 171.8, 170.9, 169.6, 167.7, 165.8, 159.0, 133.9, 127.9, 118.9, 116.5, 109.8, 69.2, 68.7, 67.1, 52.1, 51.3, 41.3, 40.5, 38.0, 35.4, 31.2, 30.9, 30.4, 28.8, 28.8, 28.7, 28.6, 28.5, 27.5, 25.2, 22.7, 21.9, 19.4, 13.8; IR (Neat) 3305, 2923, 2826, 1738, 1641 cm^{-1}; HRFABMS for $C_{46}H_{76}N_5O_8$ (MH^+) calcd 826.5694, found 826.5667.

DDM-Thr [Pre-Mycobactin 1b (R=Me)]: To **6** (R=Me, 0.087 g, 0.186mmol) in 4 mL of 1:1 THF/water was added LiOH (0.018 g, 0.745 mmol) at room temperature. The mixture was stirred for 12 h and the THF was removed under reduced pressure. The aqueous solution was acidified to pH = 4 with 1N citric acid and the solution was extracted three times with EtOAc. The combined organic extracts were washed with water and brine. The organic solution was dried with Na_2SO_4, filtered, and concentrated. The residue was used without purification. To this acid, pre-cobactin T **3a** (0.039 g, 0.186 mmol), HOAt (0.025 g, 0.186 mmol), DMAP (0.006 g, 0.047 mmol) in 3 mL of DMF was added EDC (0.039 g, 0.205 mmol) at room temperature. After 24 h, the solution was taken up in EtOAc and washed two times with 5% $NaHCO_3$, water, and brine. The organic solution was dried with Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (EtOAc to 4.5:1 EtOAc:MeOH) to give 0.024 g of the Boc protected pre-mycobactin T (R=Me, *=(*S*) in 20% yield as a white amorphous solid. ^{1}H NMR 1:1 ratio of rotational isomers present. (600 MHz, d_6-DMSO) δ 11.95-11.91 (m, 1H); 8.41-8.39 (m, 1H), 7.87-7.84 (m, 1H), 7.73-7.69 (m, 1H), 7.62-7.60 (m, 1H), 7.46 (t, 1H, J = 7.82 Hz), 7.00 (d, 1H, 8.27 Hz), 6.94 (t, 1H, J = 7.07 Hz), 6.71(br s, 1H), 5.15-5.09 (m, 1H), 4.99-4.97 (m, 1H), 4.40-

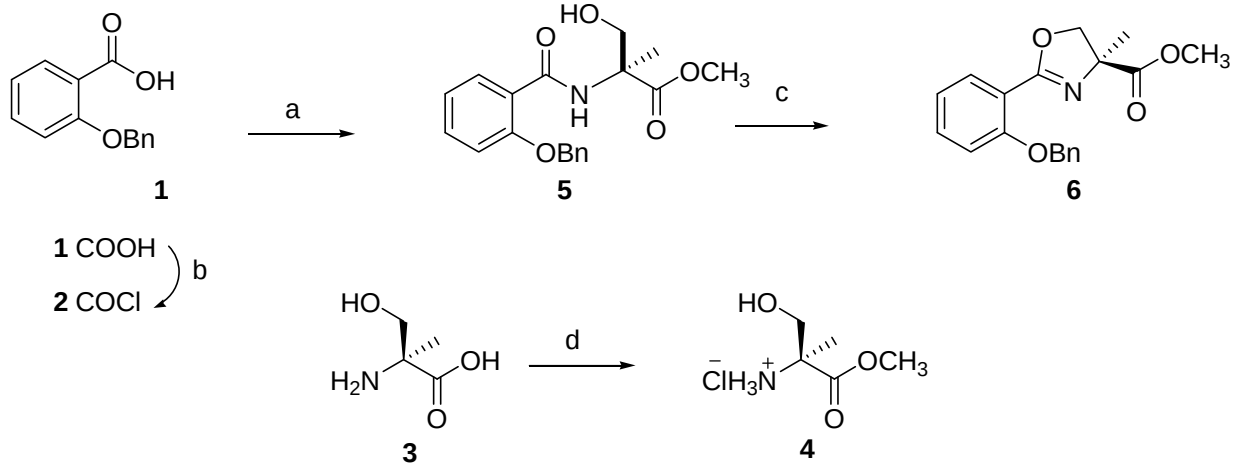
4.36 (m, 1H), 4.27-4.19 (m, 1H), 3.18-3.09 (m, 2H), 2.93-2.84 (m, 2H), 2.56-2.52 (m, 1H), 2.44-2.39 (m, 1H), 1.86-1.58 (m, 6H), 1.41-1.16 (m, 21H); ^{13}C NMR Rotational isomers present. (150 MHz, d_6-DMSO) δ 174.0, 171.0, 170.8, 167.8, 167.7,, 167.6, 159.2, 155.4, 133.8, 127.9, 118.8, 116.4, 110.1, 110.0, 77.8, 77.7,, 77.2, 69.8, 69.5, 68.8, 52.0, 51.9, 51.3, 41.3, 41.2, 40.5, 31.0, 30.9, 30.6, 30.3, 29.0, 28.7, 28.2, 27.5, 22.6, 22.5, 19.4, 19.3, 15.5, 15.4; IR (Neat) 3325, 2963, 1652 cm^{-1};

To Boc protected pre-mycobactin T (R=Me, *=(*S*) (0.020 g, 0.031 mmol) in 1 mL of EtOAc at 0 °C was added 2 mL of an HCl saturated EtOAc solution. The solution stirred at 0 °C for 1h and the solvents were removed under reduced pressure. To this amine salt was added eicosanoic acid (0.010 g, 0.031 mmol), HOAt (0.004 g, 0.031 mmol), DMAP (0.004 g, 0.031 mmol), and DIPEA (0.034 g, 0.034 mmol). The reagents were dissolved in 1 mL of DMF and 2.5 mL of CH_2Cl_2 at room temperature EDC (0.007 g, 0.034 mmol) was added and the solution was stirred for 60 h and diluted with EtOAc. The organic solution was washed with 1M HCl, water, 5% $NaHCO_3$, and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (EtOAc to 4.5:1 EtOAc:MeOH) to give 0.006 g of the pre-mycobactin **1b** (R=Me) in 23% yield as a white amorphous solid. ^{1}H NMR 1:1 ratio of rotational isomers present. (600 MHz, d_6-DMSO) δ 11.94-11.89 (m, 1H); 8.40-8.37 (m, 1H), 7.86-7.84 (m, 1H), 7.72-7.69 (m, 1H), 7.68-7.63 (m, 1H), 7.61 (t, 1H, J = 7.04 Hz), 7.46 (t, 1H, J = 7.71 Hz), 6.99 (d, 1H, J = 8.26 Hz), 6.93 (t, 1H, J = 7.15 Hz), 5.15-5.05 (m, 1H), 4.99-4.97 (m, 1H), 4.40-4.35 (m, 1H), 4.28-4.20 (m, 1H), 3.18-3.10 (m, 1H), 3.08-2.96 (m, 3H), 2.56-2.52 (m, 1H), 2.44-2.39 (m, 1H), 2.03-2.00 (m, 2H), 1.88-1.16 (m, 52H), 0.85 (t, 3H, J = 6.93 Hz); ^{13}C NMR Rotational isomers present. (150 MHz, d_6-DMSO) δ 174.0, 171.8, 171.0, 170.8, 167.8, 167.7,, 167.6, 159.3, 159.2, 155.4, 133.8, 127.9, 118.7, 116.4, 110.1, 110.0, 77.8, 77.7, 77.2, 69.8, 69.5, 68.8, 52.0, 51.9, 51.3, 41.3, 38.0,37.9, 35.3, 33.6, 31.2, 31.0, 30.9, 30.5, 30.3, 28.9, 28.7, 28.8, 28.7, 28.6, 28.5, 28.4, 27.5, 25.2, 24.4, 22.7, 22.5, 22.0, 19.4, 19.3, 15.5, 15.4, 13.8; IR (Neat) 3306, 2919, 2851, 1643 cm^{-1}

Synthesis steps leading to protected oxazoline used in solid phase synthesis

(*S*)-Methyl 4,5-dihydro-4-methyl-2-phenyloxazole-4-carboxylate, **6**, was prepared by first converting 2-(benzyloxy)benzoic acid, **1**, to the corresponding acid chloride derivative **2** and immediate coupling of the acid chloride with freshly prepared[1] (*S*)-methyl 2-amino-2-(hydroxymethyl)propanoate hydrochloride, **4**, in the presence of a hindered organic base like diisopropylethylamine (DIPEA) to give intermediate amide **5**. Then dehydrative cyclization of β-hydroxy amide **5** with bis(2-methoxyethyl)amino-sulfur trifluoride (DAST) as reported by Wipf[2] gave the desired oxazoline product **6** in good yield (Scheme 1).

Scheme 1[a]



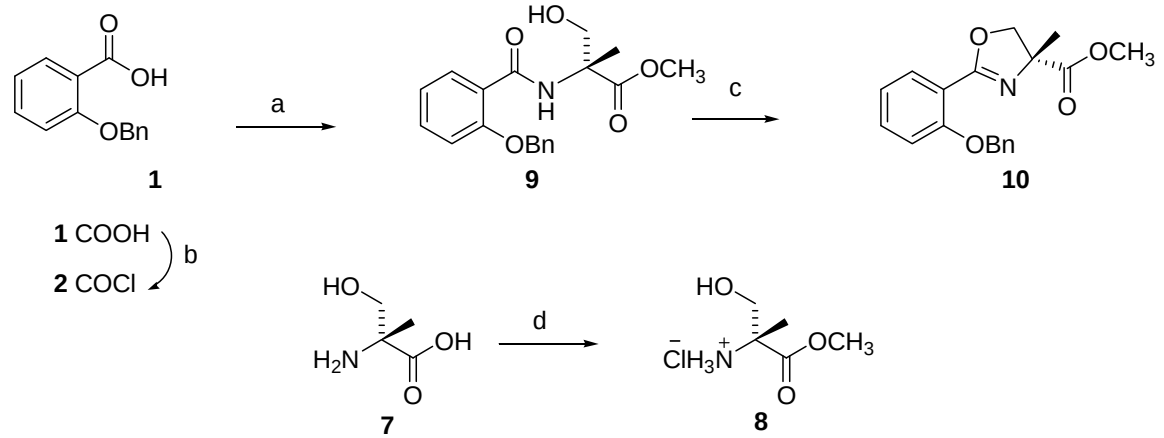
[1]Purchased amino acid was differentially protected as the methyl ester hydrochloride by the one step procedure found in Huang, Y.; David R. Dalton, D.R.; and Carroll, P.J. *J. Org. Chem.*, **1997**, *62*, 372-376 for D-serine methyl ester hydrochloride.

[2]Wipf, P. et. al., *Organic Letters*, **2000**, *2*, 1165-1168.

[a]Reagents: (a) (*S*)-methyl 2-amino-2-(hydroxymethyl)propanoate hydrochloride**, 2**, DIPEA, 50 °C, 14 h, 59%; (b) oxalyl chloride, CH_2Cl_2, DMF (one drop), 4 h, room temp., quantitative yield; (c) Bis(2-methoxyethyl)amino-sulfur trifluoride, K_2CO_3, CH_2Cl_2, -78 °C to RT, 1 h, 90%; (d) acetyl chloride, methanol, 0 °C, 10 min., **3**, reflux, 5 h, quantitative yield.

In an identical way, (*R*)-methyl 2-(2-(benzyloxy)phenyl)-4,5-dihydro-4-methyloxazole-4-carboxylate, **10**, was prepared by coupling of the same benzoic acid chloride derivative **2** with freshly prepared[1] (*R*)-methyl 2-amino-2-(hydroxymethyl)propanoate hydrochloride **8** in the presence of a diisopropylethyl amine to give β-hydroxy amide, **9**. Then- dehydrative cyclization of **9** with DAST gave the desired oxazoline **10** in similar good yields (Scheme 2).

Scheme 2[a]



[a]Reagents: (a) (*R*)-methyl 2-amino-2-(hydroxymethyl)propanoate hydrochloride, **2**, DIPEA, 50 °C, 14 h, 71%; (b) oxalyl chloride, CH_2Cl_2, DMF (drop), 4 h, room temp., quantitative yield; (c) Bis(2-methoxyethyl)amino-sulfur trifluoride, K_2CO_3, CH_2Cl_2, -78 °C to RT, 1 h, 84%; (d) acetyl chloride, methanol, 0 °C, **3**, reflux, 5 h, quantitative yield.

2-(benzyloxy)benzoyl chloride (2)
2-(Benzyloxy)benzoic acid (**1**, 1.0 g, 4.30 mmol) was dissolved in 20 mL of anhydrous dichloromethane (DCM) and oxalyl chloride (0.75 mL, 0.60 mmol) was added drop wise followed by addition of 25 μL of dimethylformamide (DMF) to initiate the reaction. The reaction mixture bubbled and was stirred at room temperature for four hours, where it became a homogeneous yellow solution. The reaction mixture was concentrated to a yellow paste which was subsequently concentrated *in vacuo* from toluene and then chloroform to give a crude yellow solid (1.1 g, 98.7% yield) which was used immediately. R_f = 0.5 (EtOAc, red streak) ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (1 H, dd, *J* = 7.9, 1.7 Hz), 7.60-7.28 (6 H, m), 7.06 (2 H, dd, *J* = 12.2, 5.2 Hz), 5.22 (2 H, s).

(*S*)-Methyl 2-amino-2-(hydroxymethyl)propanoate hydrochloride (4)
Dry methanol (2 mL) was chilled in an ice bath before slow addition of acetyl chloride (0.22 mL, 3.1 mmol) under argon. After 10 min of stirring, *S*-(+)-2-amino-2-methyl-3-hydroxypropanoic acid (**3**, 150 mg, 1.2 mmol) was added in one portion and the reaction mixture was warmed to room temperature and heated at reflux for 5 h. The reaction mixture was concentrated to a yellow paste which was subsequently concentrated *in vacuo* from toluene to give a crude white solid (0.21 g, 100% yield) which was used immediately. R_f product = 0.6 yellow-orange stained spot (5: 0.1: 0.1 MeOH : H_2O : HOAc, ninhydrin stain). ^{1}H NMR (300 MHz, CD_3OD) δ 3.89 (1 H, d, *J* = 11.5 Hz), 3.80 (3 H, s), 3.59 (1 H, d, *J* = 11.5), 1.43 (3 H, s).

(*S*)-Methyl 2-(2-(benzyloxy)benzamido)-3-hydroxy-2-methylproponate (**5**)
2-(Benzyloxy)benzoyl chloride **(2**, 358 mg, 1.38 mmol) was dissolved in 4 mL of DCM and then freshly prepared (*S*)-methyl 2-amino-2-(hydroxymethyl)propanoate hydrochloride (**4**, 213 mg, 1.26 mmol) and diisopropylethylamine (0.66 mL, 3.77 mmol) was added while reaction was chilled in an ice bath (0 ^{o}C bath temperature). The reaction mixture was warmed to room temperature and then heated to reflux for 14 h. The reaction mixture was concentrated, then dissolved in DCM and washed with 10% aq. $NaHCO_3$ solution (2x), 0.5 N citric acid (2x), and brine. The organic phase was collected

and dried over Na_2SO_4, filtered and concentrated *vacuo*. Crude material was purified using silica gel column chromatography with 5% to 50% ethyl acetate : DCM as the solvent system to give 255 mg (59%) of a clear oil which crystallized upon standing. R_f product = 0.45 (20% EtOAc:DCM). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (1 H, bs, OH), 8.17 (1 H, dd, *J* = 7.8, 1.7 Hz), 7.55-7.33 (6 H, m), 7.20-7.02 (2 H, m), 5.19 (2 H, s), 3.97 (1 H, d, *J* = 11.3 Hz), 3.73 (4 H, m, CH_2 and OCH_3), 1.25 (3 H, s).

(S)- M**ethyl 4,5-dihydro-4-methyl-2-phenyloxazole-4-carboxylate** (**6)**
(*S*)- Methyl 2-(2-(benzyloxy)benzamido)-3-hydroxy-2-methylproponate (**5**, 225 mg, 0.74 mmol) was dissolved in DCM (7 mL) and cooled to -78 ^{o}C (bath temp, dry ice-acetone). Bis(2-methoxyethyl)amino-sulfur trifluoride (DAST, 0.11 mL, 0.85 mmol) was added slowly to the cold mixture under argon where it stirred for 30 min. Then K_2CO_3 (277 mg, 2.0 mmol) was added and the reaction mixture was allowed to warm to room temperature. Once compete (by TLC) the reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with DCM. The organic layer was washed again with saturated $NaHCO_3$ solution then brine, dried over Na_2SO_4, filtered and then concentrated *in vacuo* to give a yellowish oil. Crude material was purified using silica gel column chromatography with a 5% to 20% ethyl acetate : DCM gradient solvent system to give a clear oil (218 mg, 90% yield). R_f product = 0.6 (20% EtOAc:DCM). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (1 H, dd, *J* = 7.7, 1.6 Hz), 7.51 (2 H, d, *J* = 7.3 Hz), 7.46-7.27 (4 H, m), 6.99 (2 H, t, *J* = 7.5, 7.5 Hz), 5.18 (2 H, s), 4.81 (1 H, d, *J* = 8.7 Hz), 4.18 (1 H, d, *J* = 8.7 Hz), 3.78 (3 H, s), 1.64 (3 H, s). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.9, 164.2, 157.6, 136.9, 132.59, 131.57, 128.8, 128.4, 128.4, 127.6, 126.8, 120.7, 117.5, 113.6, 75.7, 74.3, 70.5, 52.7, 25.3. HREMSI calcd. $C_{19}H_{19}NO_4$ 326.1392 found 326.1396. $[\alpha]_D$ at 25 ^{o}C = +42.1 $CHCl_3$, HPLC retention time 9.8 min (97% pure).

(*R*)-methyl 2-amino-2-(hydroxymethyl)propanoate hydrochloride (8)
Dry methanol (2 mL) was chilled in an ice bath before slow addition of acetyl chloride (0.22 mL, 3.1 mmol) under argon. After 10 min of stirring, the *R*-(-)-2-amino-2-methyl-3-hydroxypropanoic acid (**7**, 150 mg, 1.2 mmol) was added in one portion and the reaction mixture was first allowed to warm to room temperature and then heated at reflux for 5 h. The reaction mixture was concentrated to a yellow paste which was subsequently concentrated *in vacuo* from toluene to give a crude white solid (0.21 g, 100% yield) which was used immediately. ^{1}H NMR (300 MHz, CD3OD) δ 3.90 (1 H, d, J = 11.5), 3.81 (3 H, s), 3.60 (1 d, *J* = 11.5), 1.44 (3 H, s).

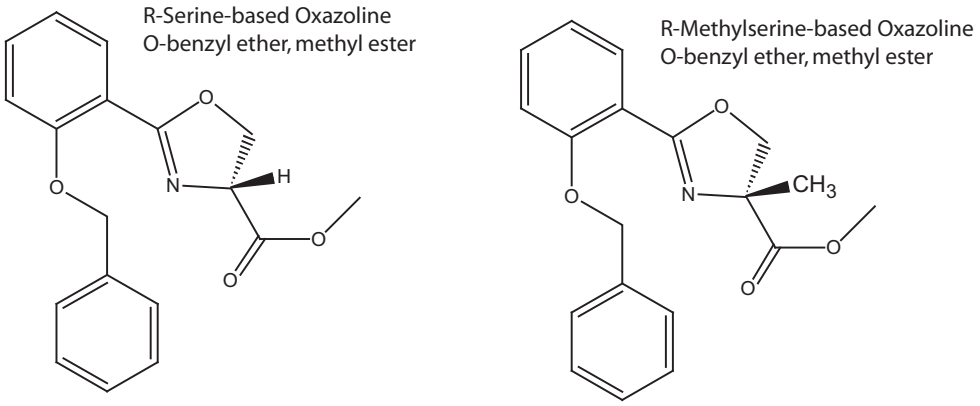
(*R*)-methyl 2-(2-(benzyloxy)benzamido)-3-hydroxy-2-methylproponate (**9)**
2-(Benzyloxy)benzoyl chloride (**2**, 358 mg, 1.38 mmol) was dissolved in 4 mL of DCM and then freshly prepared (*S*)-methyl 2-amino-2-(hydroxymethyl)propanoate hydrochloride (**4**, 213 mg, 1.26 mmol) and diisopropylethylamine (0.66 mL, 3.77 mmol) was added while the reaction was chilled in an ice bath (0 ^{o}C bath temperature). The reaction was allowed to warm to room temperature then heated to reflux, for 14 h. The reaction mixture was concentrated then dissolved in DCM and washed with 10% aq. $NaHCO_3$ solution (2x), 0.5 N citric acid (2x), and brine. The organic phase was collected and dried over Na_2SO_4, filtered and then concentrated *in vacuo*. The resulting crude material was purified using silica gel column chromatography with 5% to 50% ethyl acetate:DCM as the solvent system to give 306 mg (71% yield) of a clear oil. R_f product = 0.45 (20% EtOAc:DCM). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (1H, bs, OH), 8.18 (1 H, dd, *J* = 7.8, 1.8 Hz), 7.53-7.37 (6 H, m), 7.15-7.04 (2 H, m), 5.20 (2 H, s), 3.98 (1 H, d, *J* = 11.1 Hz), 3.79-3.66 (4 H, m, CH_2 and OCH_3), 1.26 (3 H, s).

(*R*)-methyl 2-(2-(benzyloxy)phenyl)-4,5-dihydro-4-methyloxazole-4-carboxylate (**10)**
(*R*)- Methyl 2-(2-(benzyloxy)benzamido)-3-hydroxy-2-methylproponate (**9**, 306 mg, 0.89 mmol) was dissolved in DCM (8 mL) and cooled to -78 ^{o}C (bath temp, dry ice-acetone). Bis (2-methoxyethyl)amino-sulfur trifluoride (DAST, 0.13 mL, 1.0 mmol) was added slowly to a cold mixture under argon where it stirred for 30 min. Then K_2CO_3 (332 mg, 2.4 mmol) was added and the reaction mixture was warmed to room temperature.
Once complete (by TLC analysis) the reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with DCM. The organic layer was washed again with saturated $NaHCO_3$ solution then brine, dried over Na_2SO_4, filtered and then concentrated *in vacuo* to a yellowish oil. Crude material was purified using silica gel column chromatography with 5% to 20% ethyl acetate : DCM gradient solvent system to give a clear oil (243 mg, 84% yield). R_f product = 0.6 (20% EtOAc:DCM). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (1 H, dd, *J* = 7.5, 1.8 Hz), 7.51 (2 H, d, *J* = 7.3 Hz), 7.48-7.27 (4 H, m), 6.99 (2 H, t, *J* = 7.5, 7.5 Hz), 5.17 (2 H, s), 4.81 (1 H, d, *J* = 8.7 Hz), 4.18 (1 H, d, *J* = 8.7 Hz), 3.77 (3 H, s), 1.63 (3

H, s). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.7, 164.0, 157.4, 136.81, 132.44, 131.40, 128.7, 128.3, 128.2, 127.4, 126.6, 120.5, 117.4, 113.4, 75.5, 74.1, 70.4, 52.6, 25.1. HREMSI calcd. $C_{19}H_{19}NO_4$ 326.1392 found 326.1402. $[\alpha]_D$ at 25 °C = +37.2 $CHCl_3$, HPLC retention time 9.8 min (92% pure).

Young *et al*. Supplemental Figure 2

A



B

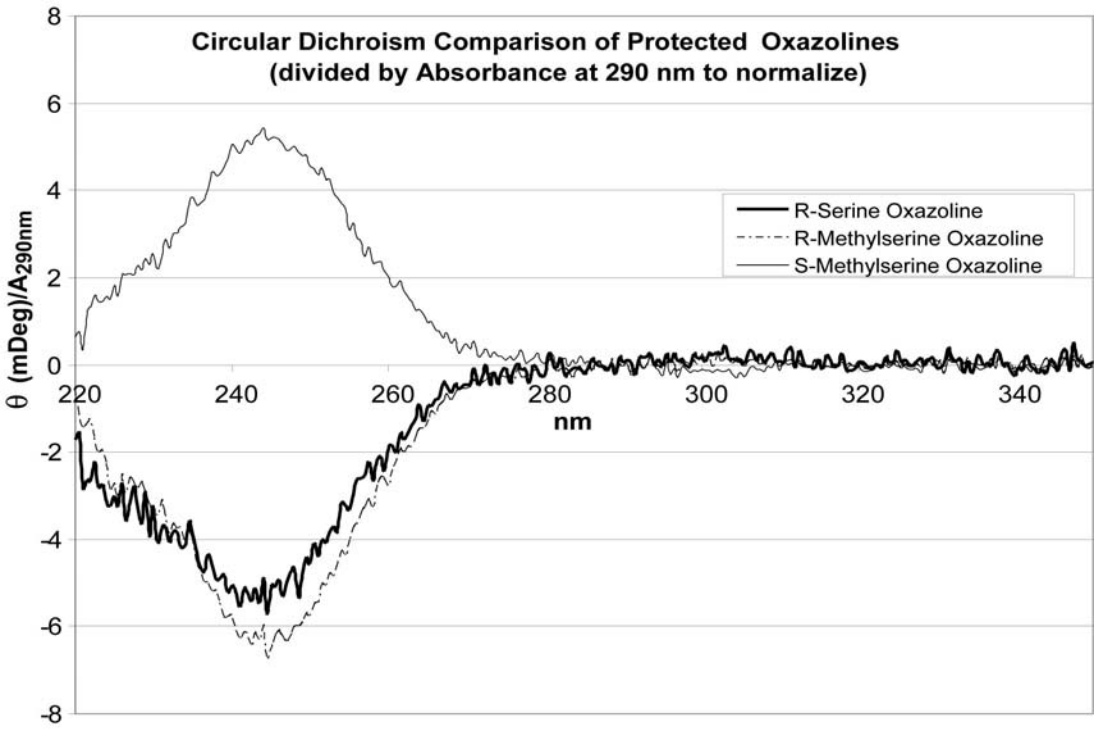


Figure S2. Circular dichroism analysis for oxazoline precursors made with serine or α-methylserine. The compounds of indicated (A) structure were protected by forming a methyl ester on the serine methylserine groups and a benzyl ether on the phenolic hydroxy group in the salicylic acid portion of the molecules. The S-methyl-serine used in synthesis was confirmed to show opposite rotation prior to its incorporation in DDM, and this was maintained after incorporation (Fig. 4).

Young *et al*. Supplemental Figure 3

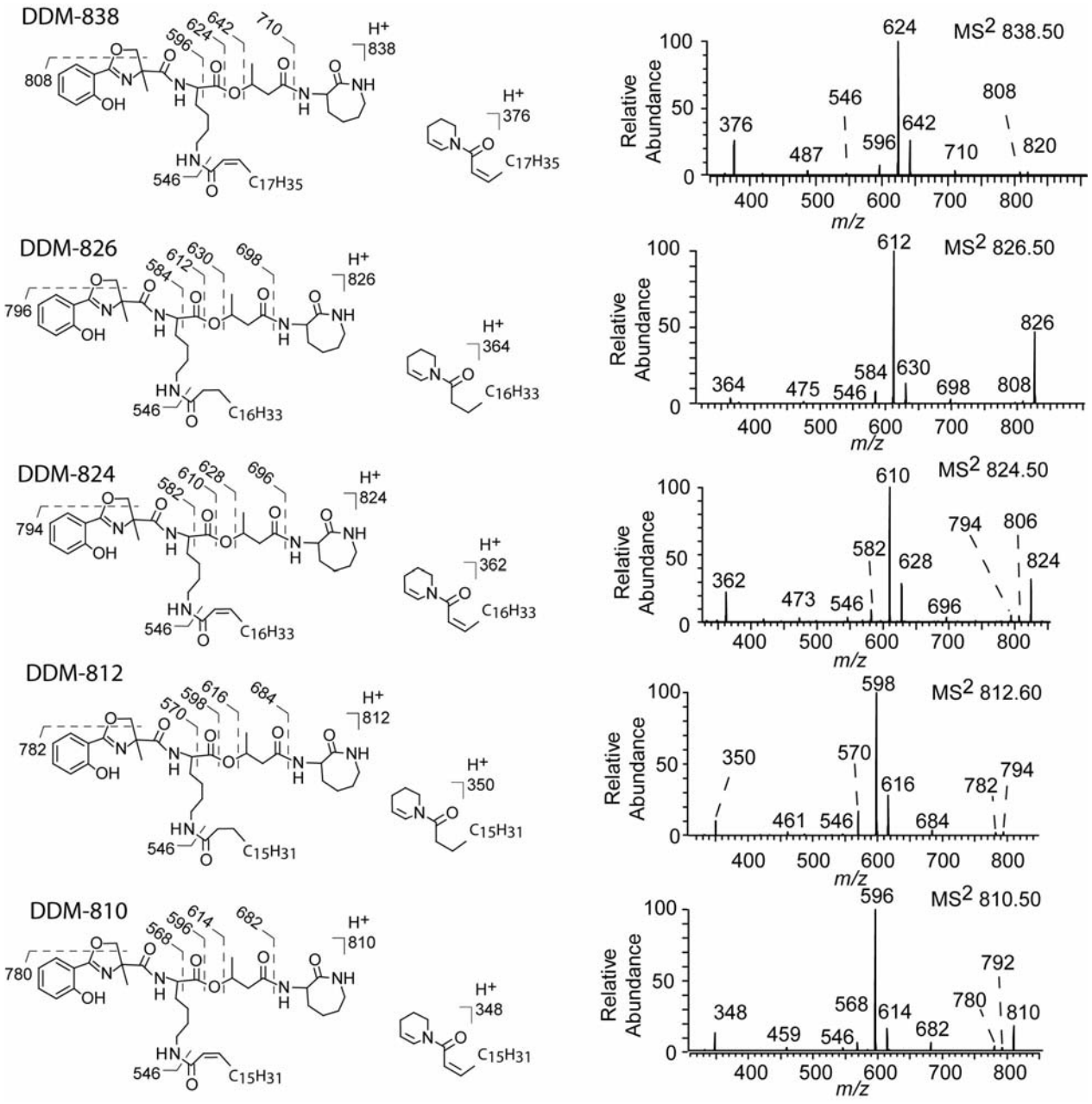


Figure S3. Collision-induced dissociation mass spectra of natural dideoxymycobactins shows that the lipid and not the peptide differs among individual members of the series. The differences in mass among individual members of the series could be explained if DDM-840 DDM-838, DDM-826, DDM-824, DDM-812, and DDM-810 carry the same peptide backbone but are acylated with C_{20}, $C_{20:1}$, C_{19}, $C_{19:1}$, C_{18}, $C_{18:1}$, respectively. Consistent with this interpretation, the fragment *m/z* 546, which corresponds to the loss of fatty acid chains, is present in all spectra. More generally, fragments corresponding to proposed cleavages in the peptide moiety show the same mass interval in all 6 spectra. In contrast, fragments interpreted as containing fatty acyl groups differ in mass, and those differences match the deduced mass of the differing acyl chains in each compound.

Young *et al*. Supplemental Figure 4

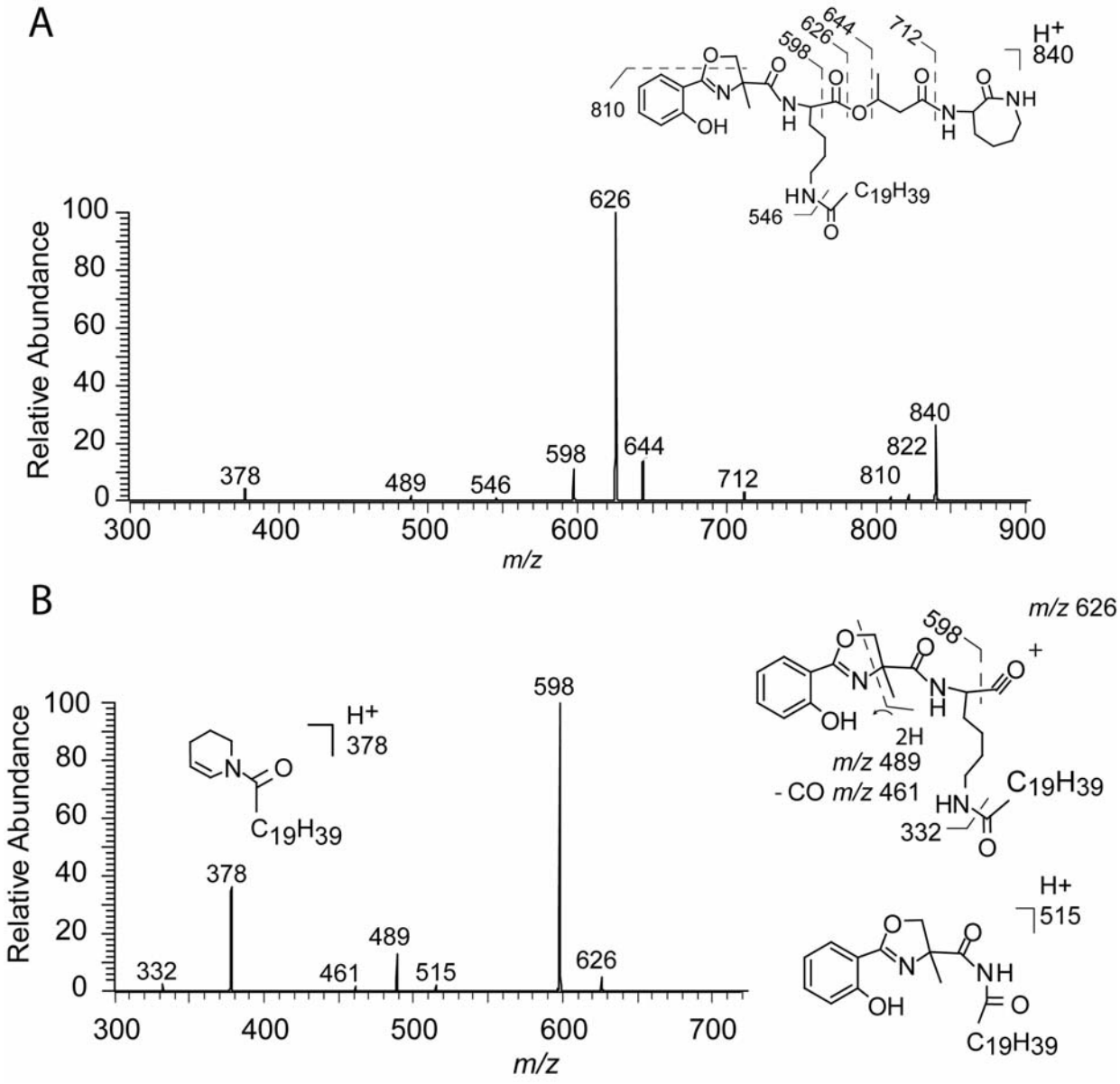


Figure S4. Multistage mass spectrometric analysis demonstrates the detailed structure of a natural *M. tuberculosis* dideoxymycobactin (DDM-840). A. MS/MS analysis of the $[M+H]^+$ of the intact compound (m/z 840) yields expected products corresponding to the mycobactic acid fragment (*m/z* 626, 644) generated after loss of the cobactin moiety. Fragments corresponding to the loss of dehydrated lysine (*m/z* 712), cleavage through the oxazoline ring (m/z 810) and loss of a $C_{20:1}$ fatty acid (m/z 546) are apparent. **B**. All major ions apparent in the MS^3 spectrum of the *m/z* 626 product are consistent with this depicted DDM structure. The cleavage leading to *m/z* 332 helps to confirm the presence of a C_{20} acyl group in this substructure of the originally postulated DDM structure. Ions at m/z 378 and 515 arise from more complex fragmentation likely involving the postulated rearrangements. Hydrogen transfer is implied in fragmentations adjacent to -NH- or -O- in amide or ester bonds. Fragment ion assignments are supported by FTMS accurate mass measurements (Moody et al, 2004)

Young *et al*. Supplemental Figure 5

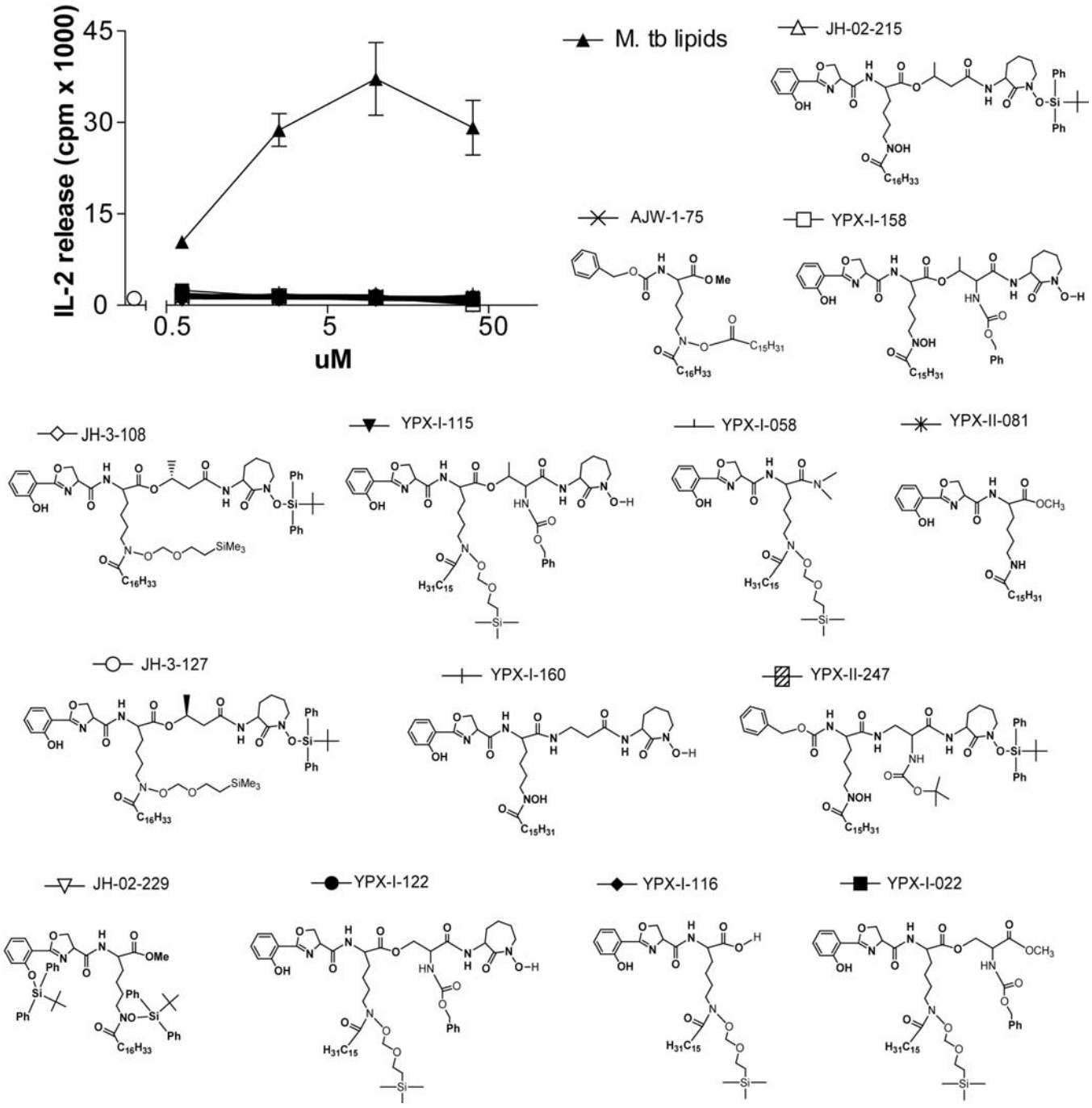


Figure S5. T cell response to synthetic compounds related to Mycobactin T as compared to response to lipid extract of *M. tuberculosis*. CD8-2 T cells were incubated with CD1a-expressing antigen presenting cells and the indicated mycobactin-like compound or DDM-containing lipid extracts from *M. tb* (M. tb lipids) and subjected to IL-2 release by the HT-2 bioassay.

Young *et al*. Supplemental Figure 6

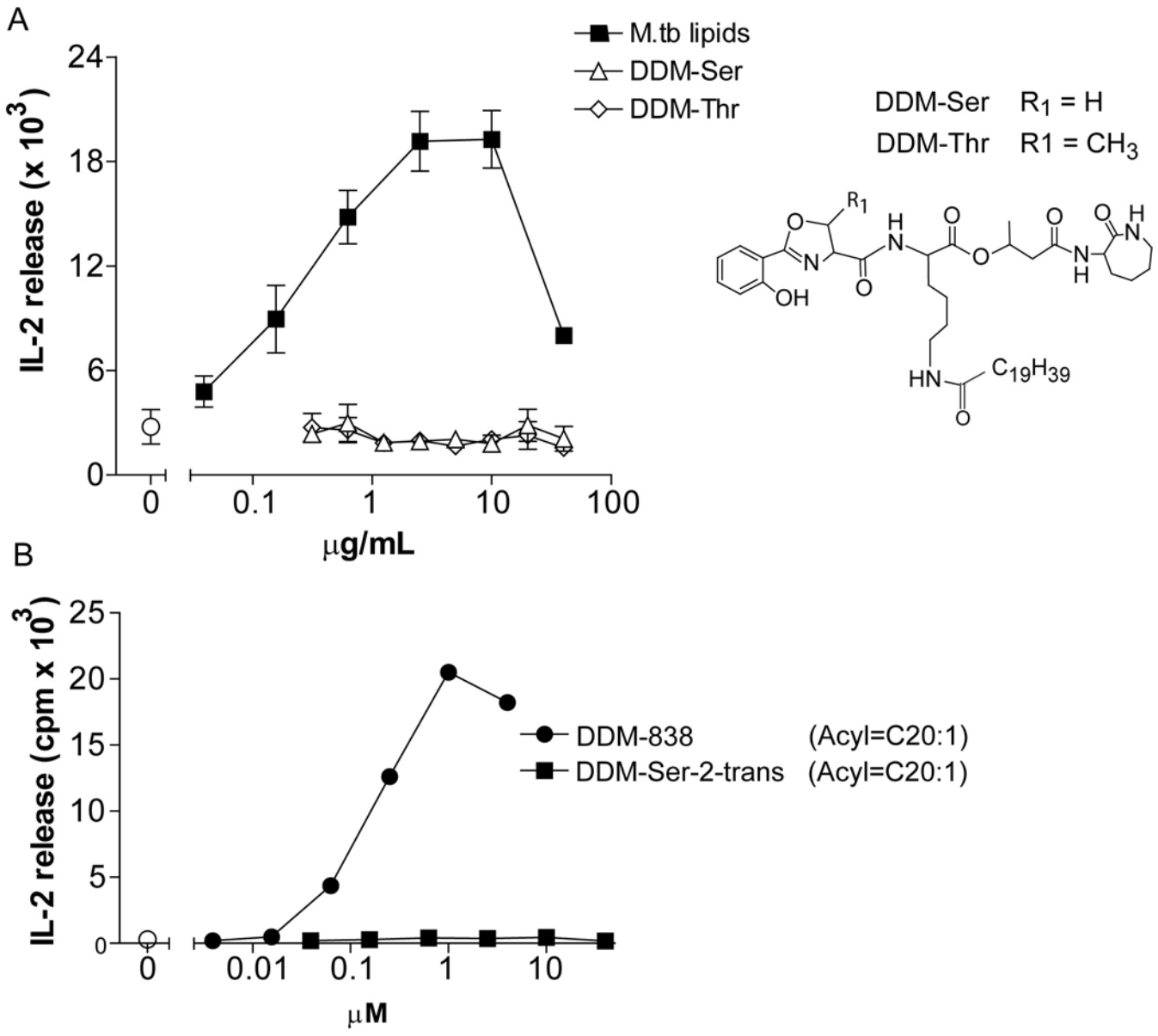


Figure S6. Synthetic DDMs containing serine and threonine do not stimulate CD1a-restricted T cells. A. T cells were treated with synthetic dideoxymycobactins synthesized using serine- and threonine-based oxazoline rings and containing unsaturated or saturated C_{20} fatty acids. DDM-containing lipid extracts from *M. tb* (M. tb lipids) or pure natural DDM-838 were used to determine the maximal IL-2 release by T cells.

Young *et al*. Supplemental Figure 7

H1 NMR of DDM-838SR

ppm	Description including integration	Coupling constant Hz	TOCSY connectivity
7.70	Doublet 1H	7.5	6.92
7.44	triplet 1H	7.5	6.92,7.02
7.02	doublet 1H	8.0	7.44
6.92	triplet 1H	8.0	7.44,7.70
5.94	complex multiplet 1H		5.70,2.59
5.70	doublet 1H	11	5.94
5.31	multiplet 1H		1.34,2.59
4.70	doublet	9.5	4.27
4.54	complex multiplet 1H		2.00,1.80,1.50
4.46	complex multiplet 1H		1.95, 1,80, 1.30
4.27	doublet 1H	9.0	4.70
3.26	unresolved		1.80, 1.34
3.18	multiplet	7.0	1.52, 1.34
2.59	complex multiplet		1.34, 1.40, 5.94
2.00	overlapping multiplet		1.8
1.80	overlapping multiplet		2.0
1.62	singlet		none
1.52	complex multiplet		1.4, 2.0, 3.2, 4.5
1.40	multiplet		
1.34	doublet	7.5	2.60, 5.31
1.27	broad (large)		0.90
0.89	triplet 3H	7.0	1.28

Figure S7. The NMR spectrum of DDM-838SR in deuterated chloroform and methanol. Data were acquired using an Inova 500 MHz NMR spectrometer. The resonances, coupling constants, integration values and connectivity determined using a tocsy 2D NMR experiment were all consistent with the expected structure of DDM. These results for synthetic DDMs were similar to a previously reported spectrum from natural DDM (Moody et al, 2004), and hydrogen atoms distant from the hydroxyamide functionality were similar to those seen in mycobactinfrom *M. smegmatis* . The spin coupled resonances at 4.70 ppm, and 4.27 ppm have chemical shifts similar to those observed with oxazolines based on serine and threonine, but with a simpler splitting pattern, as expected for an α-methyl serine containing DDM. The single hydrogen at 5.31 ppm coupling with both 1.34 ppm and 1.27 ppm is also consistent with the assignment of chiral center *3 as being identical to that observed in *M. smegmatis* (Greatbanks et al., 1969).

Young *et al*. Supplemental Figure 8

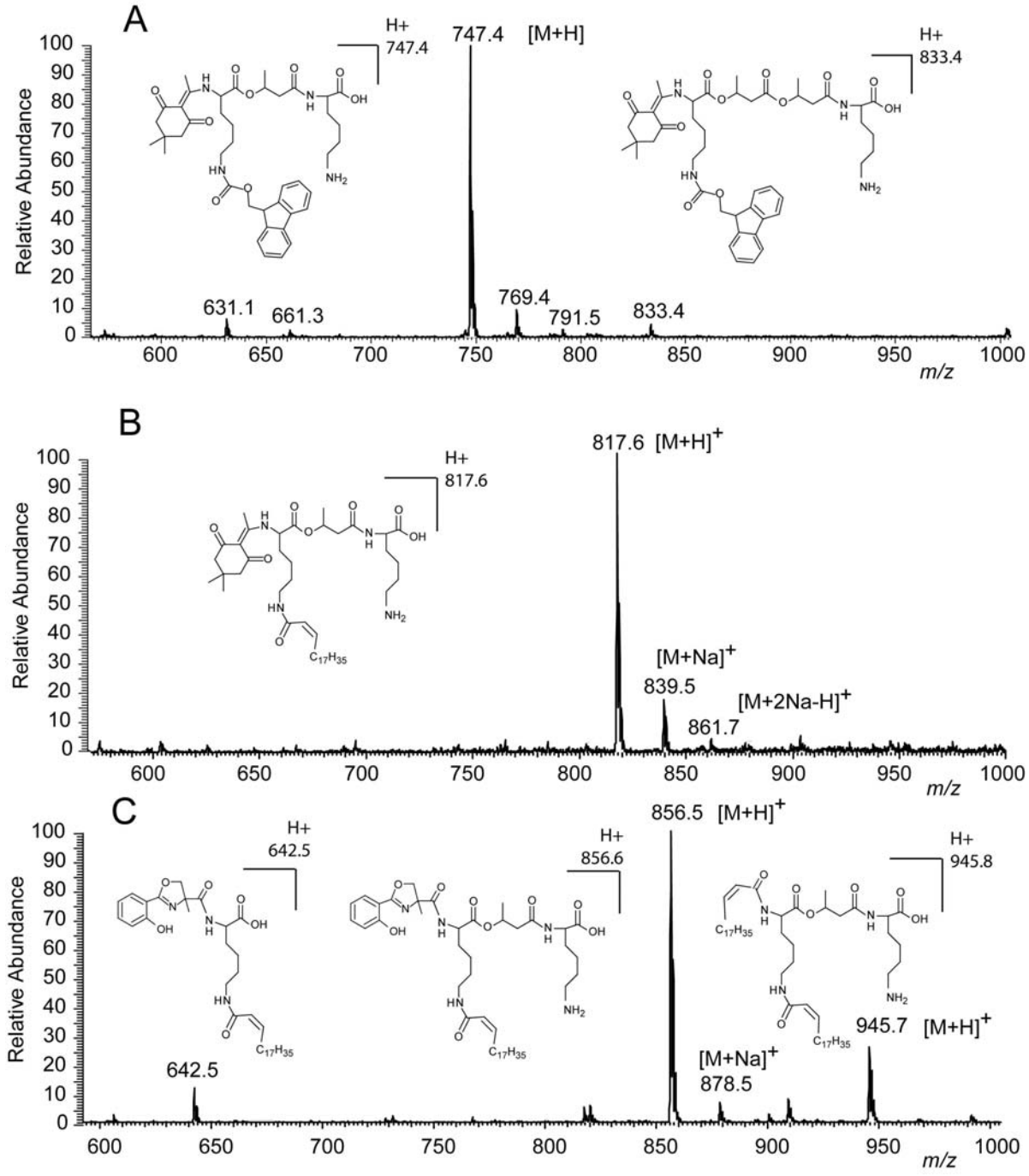


Figure S8. Nanoelectrospray MS analysis of intermediates generated in the solid phase peptide synthesis. For each of the last 3 steps in synthesis (Fig. 3A), a small portion beads was recovered and treated with 2 % trifluoroacetic acid in methylene chloride to release intermediates. The resulting solution was diluted in isopropyl alcohol and analyzed by nanoelectrospray mass spectrometry. In all cases the major ion corresponds to the proton adduct of the expected intermediates generated before (A) and after (B) acylation or after coupling of the hydroxybenzo-oxazoline moiety (C).

Young *et al*. Supplemental Figure 9

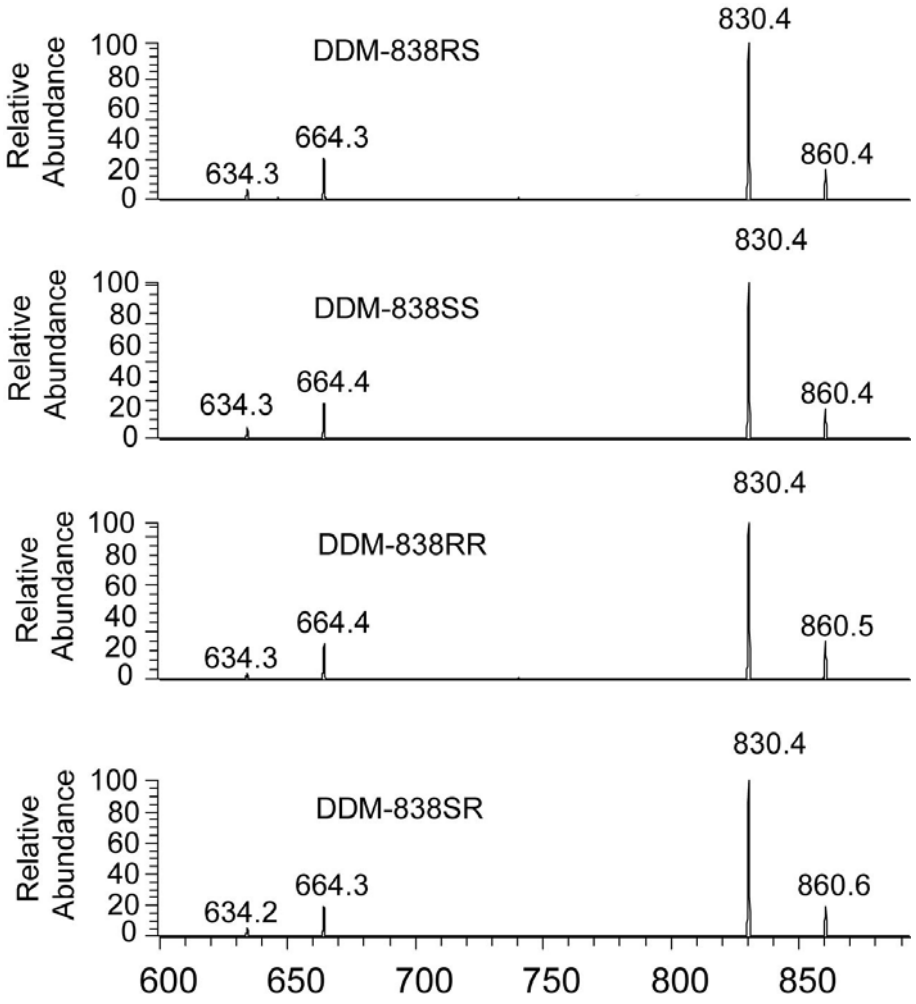


Figure S9. CID-MS analysis of synthetic DDM-838 stereisomers. MS/MS data of [M+Na]+ parent ions from four isomers with R or S stereoconfiguration at the *1 or *3 stereocenters described in Fig. 1 (DDM-838RS, DDM-838SS, DDM-838RR, and DDM-838SR). All compounds have the expected mass of sodium adducts and nearly identical fragmentation spectra. As expected, *m/z* 830.4, corresponding to the loss of CH_2O from the oxazoline ring, and *m/z* 664.4, which corresponds cleavage of ester bond, are seen for all compounds.

Young *et al*. Supplemental Figure 10

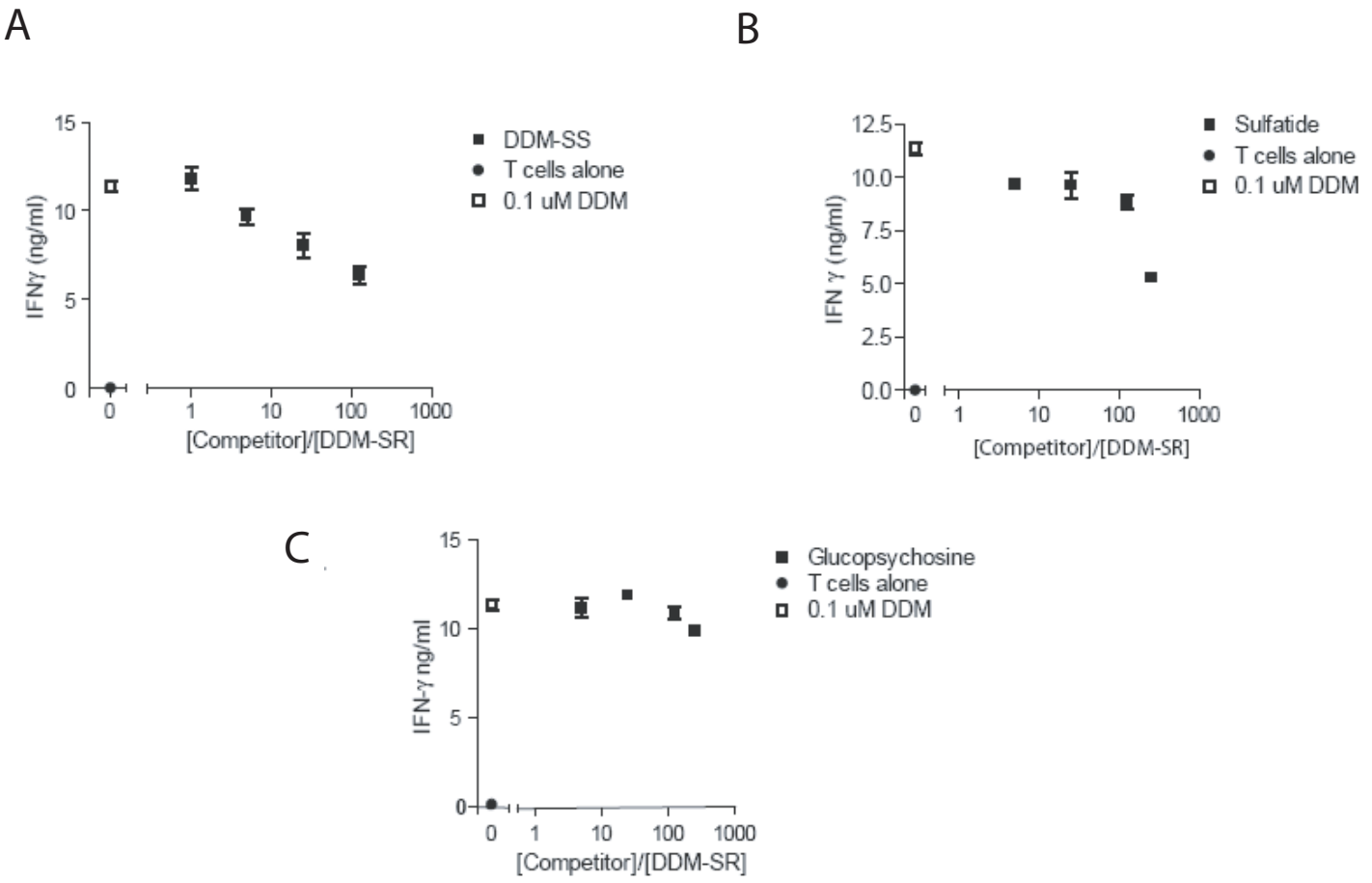


Figure S10. Both non-stimulatory DDM-SS (A) and sulfatide (B) bind to CD1a. CD1a-Fc coated plates were incubated with non-stimulatory ligands (DDM-SS or sulfatide) or an irrelevant ligand (glucopsychosine, C) for 8 hours prior to the addition of 0.1 uM DDM-SR. After an additional 12 hours of incubation, the plate was washed three times and 10^5 CD8-2 T cells were added per well. 24 hours later supernatants were harvested and tested for cytokine production by Interferon-γ ELISA.