Supplementary Information

Structure-Based Design of Potent and Selective *Leishmania N*-Myristoyltransferase Inhibitors

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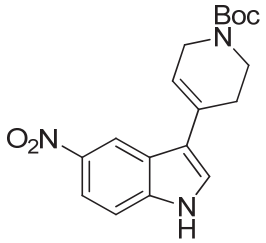
General Experimental. All chemicals were purchased from Sigma-Aldrich Ltd (Gillingham, UK), Acros Organics (Geel, Belgium), Alfa Aesar (Heysham, UK) or Apollo Scientific (Stockport, UK) and used without further purification. Silica gel normal phase column chromatography was performed either using Merck silica gel 60 (0.015-0.040 mm) or on an Isolera (Biotage, UK) automated

apparatus with SNAP silica cartridges (Biotage, UK). Final compounds were purified by reverse phase LC-MS on a Waters 2767 system equipped with a photodiode array and an ESI mass spectrometer using a XBridge Prep C18 (5 µm, 19 mm × 100 mm) column, equipped with an XBridge Prep C18 guard column (5 µm, 19 mm × 10 mm). The mobile phase consisted of H2O + 0.1 % formic acid (solvent A) and MeOH + 0.1% formic Acid (solvent B). The gradients used were: **Method A**: 0-10 min 5-98% B, 10-12 min 98% B, 12-13 min 98 to 5% B, 13-18 min 5% B. Flow rate: 20 mL/min. **Method B**: 0-10 min 50-98% B, 10-13 min 98% B, 13-14 min 98 to 50% B, 14-18 min 50% B. Flow rate: 20 mL/min. The purity of final compounds was verified by reverse phase LC-MS on a Waters 2767 system equipped with a photodiode array and an ESI mass spectrometer using a XBridge C18 (5 µm, 4.6 mm× 100 mm) column, equipped with an XBridge C18 guard column (5 µm, 4.6 mm × 20 mm). The following elution method was used: Gradient of solvent A and solvent B (as above): 0-10 min 5-98% B, 10-12 min 98% B, 12-13 min 98 to 5% B, 13-18 min 5% B. Flow rate: 1.2 mL/min. Purity of tested compounds was ≥ 95%, unless specified and the retention time is reported in minutes. ^{1}H and ^{13}C NMR spectra were recorded on 500 MHz and 126 MHz respectively Bruker AV instruments at room temperature and were referenced to residual solvent signals. Mass spectra were obtained from the Mass Spectrometry Service of Department of Chemistry, Imperial College London.

Synthetic procedures and characterization

***tert*-butyl 4-(5-nitro-1*H*-indol-3-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate 5**

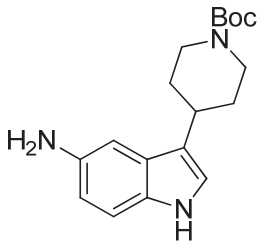


5

A solution of 5-nitroindole (2.00 g, 12.3 mmol) in dry ethanol (20 mL) was treated with pyrrolidine (3.08 mL, 37.0 mmol) followed by *N*-Boc-4-piperidone (4.91 g, 24.7 mmol) at room temperature. The solution was stirred under reflux for 3 days. The resulting solution was cooled to room temperature and a yellow precipitate formed. The precipitate was isolated by filtration and washed with cold

ethanol (3 x 10 mL) to give **5** as a bright yellow powder (3.40 g, 80%); R_f 0.45 (2:3 EtOAc - DCM); ^{1}H NMR (DMSO-d_6) 11.88 (s, 1H), 8.73 (d, 1H, J = 2.1 Hz), 8.04 (dd, 1H, J = 9.0, 2.1 Hz), 7.72 (s, 1H), 7.58 (d, 1H, J = 9.0 Hz), 6.22 (s, 1H), 4.09 (s, 2H), 3.59 (t, 2H, J = 5.7 Hz), δ 1.36 – 1.47 (m, 11H).

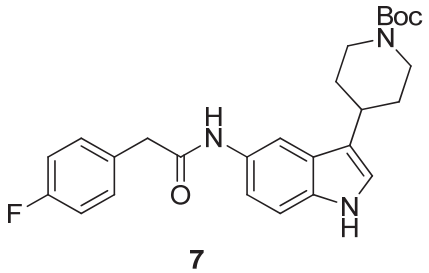
***tert*-butyl 4-(5-amino-1*H*-indol-3-yl)piperidine-1-carboxylate 6**



6

Ammonium formate (0.92 g, 14.6 mmol) and Pd/C (50 mg) were added to a solution of compound **5** (1.00 g, 2.91 mmol) in dry ethanol (15 ml). The reaction was stirred under reflux under a nitrogen atmosphere for 2 hours. The solution was cooled to room temperature and filtered through a bed of Celite. Ethanol was removed under reduced pressure to give a dark red oil which was purified by flash column chromatography (3:7 EtOAc - DCM, R_f 0.32) to give **6** as a colourless solid (0.87 g, 96%); ^{1}H NMR (DMSO-d_6) 10.30 (s, 1H), 7.03 (d, 1H, J = 8.5 Hz), 6.91 (d, 1H, J = 2.2 Hz), 6.71 (d, 1H, J = 1.9 Hz), 6.47 (dd, 1H, J = 8.5, 2.2 Hz), 4.44 (s, 2H), 4.09 – 4.05 (m, 2H), 2.82-2.77 (m, 3H), 1.92 – 1.89 (m, 2H), 1.54 – 1.41 (m, 11H).

***tert*-butyl 4-(5-(2-(4-fluorophenyl)acetamido)-1*H*-indol-3-yl)piperidine-1-carboxylate 7**

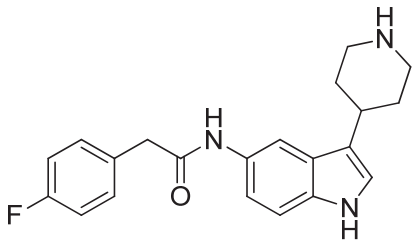


7

Triethylamine (0.67 mL, 4.76 mmol) was added to a solution of **6 (**0.5 g, 1.58 mmol) in anhydrous THF (10 mL) followed by *para*-fluorophenylacetyl chloride (0.26 mL, 1.90 mmol). Upon addition of

para-fluorophenylacetyl chloride the brown solution turned pale yellow and a precipitate formed. The reaction was stirred at room temperature for 2 hours. EtOAc (20 mL) was added and the mixture was washed with 1M NaOH (3 x 10 mL). The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure to give a brown solid which was purified by flash column chromatography (1:9 EtOAc - DCM, R_f 0.31) to give the product **7** as a yellow solid (0.65 g, 92%); ^{1}H NMR ($CDCl_3$) 7.97 (s, 1H), 7.89 – 7.87 (m, 1H), 7.43 – 7.29 (m, 3H), 7.11 – 7.06 (m, 2H), 7.02 – 6.98 (m, 1H), 4.25 – 4.23 (m, 2H), 3.73 (s, 2H), 3.01 – 2.87 (m, 3H), 2.06 – 2.01 (m, 2H), 1.65 – 1.61 (m, 11H).

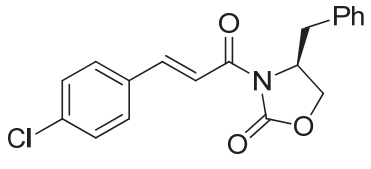
2-(4-fluorophenyl)-*N*-(3-(piperidin-4-yl)-1*H*-indol-5-yl)acetamide 1



1

5-6 N HCl in propan-2-ol (10 mL) was added to a solution of **7** in propan-2-ol and the reaction was stirred for 2 hours at room temperature. The solvent was removed under reduced pressure and the resulting crude red solid was recrystallized from methanol/EtOAc to give the HCl salt of **1** as a red solid (0.21 g, 43%); ^{1}H NMR (CD_3OD) δ 8.01 (d, 1H, J = 2.0 Hz), 7.45 – 7.40 (m, 2H), 7.33 (d, 1H, J = 8.0 Hz), 7.12 – 7.05 (m, 4H), 3.71 (s, 2H), 3.52 – 3.49 (m, 2H), 3.21 – 3.17 (m, 3H), 2.31 – 2.26 (m, 2H), 2.00 – 1.95 (m, 2H); ^{13}C NMR (100MHz, CD_3OD) δ 172.7, 166.2 (d, J = 224 Hz), 135.3, 132.4, 131.4, 130.7, 126.7, 124.5, 118.2, 115.9, 114.3, 112.6, 110.1, 46.9, 44.0, 36.2, 32.4; m/z: 352 ($[M+H]^+$); HRMS found 352.1823, $C_{21}H_{23}N_3OF$ requires 352.1825; LCMS Rt = 10.28 min.

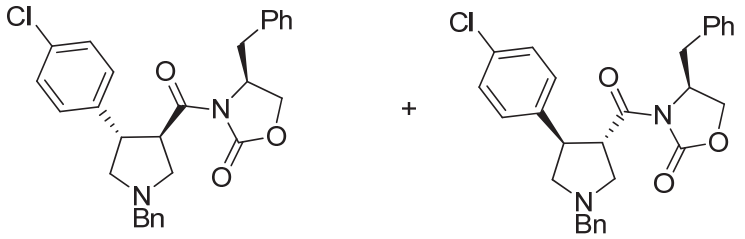
(*S*,*E*)-4-benzyl-3-(3-(4-chlorophenyl)acryloyl)oxazolidin-2-one 17



17

A solution of oxalyl chloride (3.89 mL, 46 mmol) in dichloromethane (20 mL) was added dropwise over 30 min to a suspension of (2*E*)-3-(4-chlorophenyl)acrylic acid **15** (4.20 g, 23 mmol) in dichloromethane (40 mL) and N,N-dimethylformamide (0.2 μL) at 0 °C. The reaction mixture was stirred for 24 hours at room temperature and a beige solution was obtained. The solution was added dropwise to a cooled solution of (4*S*)-4-benzyl-1,3-oxazolidin-2-one **16** (3.54 g, 20 mmol), triethylamine (12.82 mL, 92 mmol) and lithium chloride (3.90 g, 92 mmol) in dichloromethane (40 mL) at 5 °C. The reaction mixture was stirred for 15 min at 5 °C. Then, the ice bath was removed and the mixture was stirred for 2 hours at room temperature. Water (50 mL) and 5% citric acid solution (50 mL) were added. The mixture was extracted 3 times with dichloromethane (3 x 20 mL). The phases were separated and the organic phase was dried over magnesium sulfate, filtered and concentrated in vacuo. Purification by column chromatography on silica gel (1:3 EtOAc – hexane, R_f 0.40) afforded the desired product **17** as a white solid (1.98 g, 29%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 – 7.82 (m, 2H), 7.57 (d, J = 8.2 Hz, 2H), 7.45 – 7.20 (m, 7H), 4.81 (m, 1H), 4.24 (m, 2H), 3.38 (dd, J = 13.5, 3.4 Hz, 1H), 2.86 (dd, J =13.4, 9.4 Hz, 1H).

(*S*)-4-benzyl-3-((3*R*,4*S*)-1-benzyl-4-(4-chlorophenyl)pyrrolidine-3-carbonyl)oxazolidin-2-one 18a and (*S*)-4-benzyl-3-((3*S*,4*R*)-1-benzyl-4-(4-chlorophenyl)pyrrolidine-3-carbonyl)oxazolidin-2-one 18b

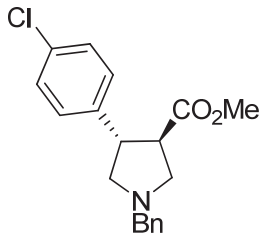


18a **18b**

To a cooled solution of **(***S*,*E*)-4-benzyl-3-(3-(4-chlorophenyl)acryloyl)oxazolidin-2-one **17** (1.92 g, 5.63 mmol) and *N*-benzyl-1-methoxy-*N*-[(trimethylsilyl)methyl]methanamine **9** (1.87 mL, 7.32 mmol) in dichloromethane (20 mL) was added trifluoroacetic acid (21 uL, 0.28 mmol,) at 0 °C. The reaction mixture was stirred at 0 °C for 20 minutes and then warmed to room temperature and stirred for 24 hours. Saturated aqueous $NaHCO_3$ (30 mL) was added and the reaction mixture was stirred for

20 minutes (pH=7). The phases were separated and the organic phase was dried over magnesium sulfate and the solvent was removed in vacuo to give a yellow oil. Purification by flash column chromatography on silica gel (100g SNAP) using a gradient of hexane/ethyl acetate (90/10-60/40) afforded the desired products; **18a** (first-eluding diastereoisomer); white powder, 960 mg (36%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 – 7.25 (m, 12H), 7.18 (m, 2H), 4.66 (m, 1H), 4.20 – 4.15 (m, 2H), 4.15 – 4.05 (m, 2H), 3.78 (d, J = 13 Hz, 1H) 3.67 (d, J = 13 Hz, 1H), 3.35 – 3.15 (m, 3H), 2.90 – 2.75 (m, 2H), 2.67 (m, 1H); **18b** (second-eluting diastereoisomer; white powder, 664 mg (25%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 – 7.13 (m, 12H), 6.99 (m, 2H), 4.62 (m, 1H), 4.25 – 3.95 (m, 4H), 3.70 (d, J = 13 Hz, 1H) 3.61 (d, J = 13 Hz, 1H), 3.25 – 3.05 (m, 3H), 2.60 – 2.80 (m, 3H).

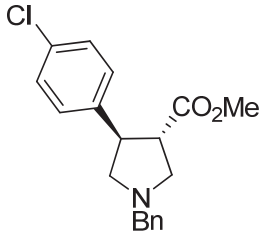
(3*R*,4*S*)-methyl 1-benzyl-4-(4-chlorophenyl)pyrrolidine-3-carboxylate 10a



10a

To a stirred solution of (*S*)-4-benzyl-3-((3*R*,4*S*)-1-benzyl-4-(4-chlorophenyl)pyrrolidine-3-carbonyl)oxazolidin-2-one **18a** (874.3 mg, 1.84 mmol) and dimethyl carbonate (775 uL, 9.20 mmol,) in dichloromethane (20 mL) was added sodium methoxide (497 mg, 9.20 mmol) at room temperature. The suspension was stirred for 72 hours (a mixture 2 :1 of starting material and expected product was observed by TLC); 5 equivalents of a 7N MeONa in MeOH solution were added. The reaction was stirred 1 h. The mixture was diluted with dichloromethane (20 mL) and saturated ammonium chloride (30 mL) and water (50 mL) were added. The phases were separated and the organic phase was washed with water dried over magnesium sulfate and concentrated in vacuo. The crude residue was purified by column chromatography on silica gel (50 g SNAP) using a gradient of hexane/EtOAc (100% to 75/25) to afford the desired product **10a** as a colourless oil, 292 mg (48%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.31 (m, 4H), 7.31 – 7.25 (m, 5H), 3.75 – 3.63 (m, 6H), 3.13 – 3.04 (m, 2H), 2.99 (m, 1H), 2.85 (dd, J = 8.4, 6.4 Hz, 1H), 2.77 (dd, J = 9.4, 4.9 Hz, 1H).

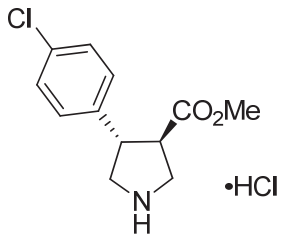
(3*S*,4*R*)-methyl 1-benzyl-4-(4-chlorophenyl)pyrrolidine-3-carboxylate 10b



10b

To a stirred solution of (*S*)-4-benzyl-3-((3*S*,4*R*)-1-benzyl-4-(4-chlorophenyl)pyrrolidine-3-carbonyl)oxazolidin-2-one **18b** (608 mg, 1.28 mmol) and dimethyl carbonate (539 uL, 6.40 mmol) in dichloromethane (20 mL) was added sodium methoxide (345.7 mg, 6.40 mmol) at room temperature. The suspension was stirred for 72 hours (a 2 :1 mixture of starting material and expected product was observed by TLC); 5 equivalent of a 7N MeONa in MeOH solution were added. The reaction was stirred 1h. The mixture was diluted with dichloromethane (20 mL) and saturated ammonium chloride (30 mL) and water (50 mL) were added. The phases were separated and the organic phase was washed with water, dried over magnesium sulfate and concentrated in vacuo. The crude residue was purified by column chromatography on silica gel (30 g SNAP) using n-hexane/EtOAc (100% - 75/25) as eluent to afford the desired product **10b** as a colourless oil, 167 mg (40%); ^{1}H NMR (300 MHz, $CDCl_3$) as for **10a**

(3*R*,4*S*)-methyl 4-(4-chlorophenyl)pyrrolidine-3-carboxylate hydrochloride 11a

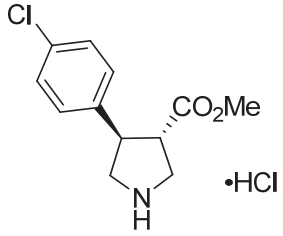


11a

To a solution of methyl (3*R*,4*S*)-methyl 1-benzyl-4-(4-chlorophenyl)pyrrolidine-3-carboxylate **10a** (265 mg, 0.804 mmol) in toluene (15 mL), was added 1-chloroethyl chloroformate (174 uL, 1.61 mmol). The solution was stirred for 10 min at room temperature (formation of a precipitate) and then stirred under reflux for 3.5 hours. Volatiles were removed under reduced pressure and methanol (15

mL) was added to the residue. The mixture was stirred under reflux for 45 min, cooled to room temperature and the methanol was evaporated to give **11a** as a beige powder (220 mg, quantitative); ^{1}H NMR (300 MHz, MeOD) δ 7.50 – 7.30 (m, 4H), 3.85 – 3.60 (m, 7H), 3.47 (q, J = 9.0 Hz, 1H), 3.37 (m, 1H).

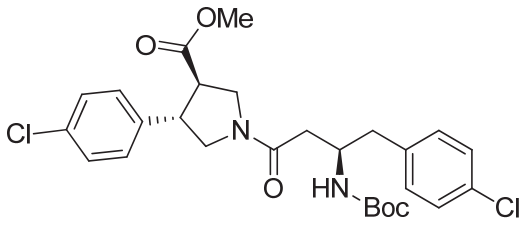
(3*S*,4*R*)-methyl 4-(4-chlorophenyl)pyrrolidine-3-carboxylate hydrochloride 11b



11b

To a solution of methyl (3*S*,4*R*)-methyl 1-benzyl-4-(4-chlorophenyl)pyrrolidine-3-carboxylate **10b** (143 mg, 0.432 mmol, 1 equiv) in toluene (10 mL), was added 1-chloroethyl chloroformate (94 uL, 0.865 mmol, 2 equiv). The solution was stirred for 10 min at room temperature (formation of a precipitate) and then under reflux for 3.5 hours. Volatiles were removed under reduced pressure and methanol (10 mL) was added to the brownish residue. The mixture was stirred at reflux for 45 min, cooled to room temperature and the methanol was evaporated to give **11b** as a beige powder (118 mg, quantitative); ^{1}H NMR as for **11b**.

(3*R*,4*S*)-methyl 1-((*R*)-3-((*tert*-butoxycarbonyl)amino)-4-(4-chlorophenyl)butanoyl)-4-(4-chlorophenyl)pyrrolidine-3-carboxylate 13a

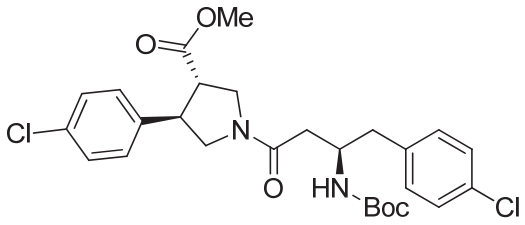


13a

To a solution of (3*R*,4*S*)-methyl 4-(4-chlorophenyl)pyrrolidine-3-carboxylate hydrochloride **11a** (104 mg, 0.375 mmol) in DMF (7 mL) was added beta-amino acid **12** (177 mg, 0.563 mmol), HOBt (76 mg, 0.563 mmol), EDCI (108 mg, 0.563 mmol) and DIPEA (163 uL, 0.938 mmol). The mixture was

stirred at room temperature for 3.5 h, and then diluted with EtOAc and brine. The phases were separated and the organic phase was washed with water dried over magnesium sulfate and concentrated in vacuo. The residue was purified by flash chromatography (10 gSNAP) using n-hexane/EtOAc as eluent (gradient 90/10 – 40/60). The product **13a** was isolated as a beige oil (174 mg, 86%); Amide rotamers were observed by ^{1}H NMR. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 – 7.23 (m, 4H), 7.21 – 7.11 (m, 4H), 5.89 (br s, 1H), 4.15 – 4.03 (m, 2H), 3.81 – 3.42 (m, 6H), 3.26 – 2.98 (m, 2H), 2.93 – 2.84 (m, 1H), 2.55 – 2.36 (m, 2H), 1.41 (s, 4.5H), 1.40 (s, 4.5H)

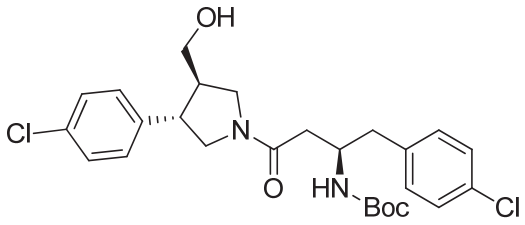
(3*S*,4*R*)-methyl 1-((*R*)-3-((tert-butoxycarbonyl)amino)-4-(4-chlorophenyl)butanoyl)-4-(4-chlorophenyl)pyrrolidine-3-carboxylate 13b



13b

As for synthesis of **13a**, starting with (3*S*,4*R*)-methyl 4-(4-chlorophenyl)pyrrolidine-3-carboxylate hydrochloride **11b** (103.5 mg, 0.375 mmol). The product **13b** was isolated as a beige oil (155 mg, 77%); Amide rotamers were observed by ^{1}H NMR. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 – 7.23 (m, 4H), 7.20 – 7.10 (m, 4H), 5.66 (br s, 1H), 4.10 – 3.97 (m, 2H), 3.73 – 3.45 (m, 6H), 3.40 – 2.95 (m, 2H), 2.95 – 2.80 (m, 1H), 2.55 – 2.36 (dd, 2H, J = 19.1, 5.0 Hz), 1.40 (s, 9H).

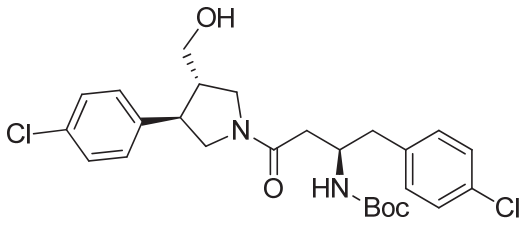
***tert*-butyl ((*R*)-1-(4-chlorophenyl)-4-((3*S*,4*R*)-3-(4-chlorophenyl)-4-(hydroxymethyl)pyrrolidin-1-yl)-4-oxobutan-2-yl)carbamate 14a**



14a

To a solution of **13a** (100 mg, 0.187 mmol) in dry THF (8 mL) was added $LiBH_4$ (16.3 mg, 0.747 mmol). The suspension was stirred at rt for 5 h under N_2. Water (20 mL) and ethyl acetate (20 mL) were added and the phases were separated . The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to give **14a** as a colorless oil , which was used in the next step without further purication (92.3 mg, yield : 97%); ^{1}H NMR (300 MHz, CDCl3) δ 7.35-7.23 (m, 4H), 7.20 – 7.10 (m, 4H), 4.13 – 3.90 (m, 2H), 3.75 – 3.61 (m, 2H), 3.59 – 3.46 (m, 1H), 3.45 – 3.33 (m, 1H), 3.32 – 3.08 (m, 2H), 3.07 – 2.95 (m, 1H), 2.92 – 2.83 (m, 1H), 2.55 – 2.35 (m, 3H), 1.40 (m, 9H).

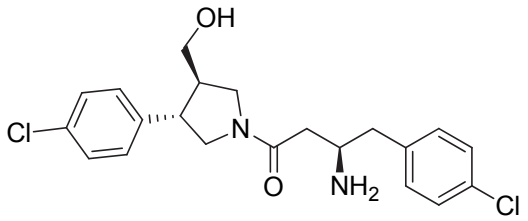
***tert*-butyl ((*R*)-1-(4-chlorophenyl)-4-((3*R*,4*S*)-3-(4-chlorophenyl)-4-(hydroxymethyl)pyrrolidin-1-yl)-4-oxobutan-2-yl)carbamate 14b**



14b

To a solution of **13b** (100 mg, 0.187 mmol) in dry THF (8 mL) was added LiBH4 (16.3 mg, 0.747 mmol). The suspension was stirred at rt for 5 h under N_2.Water (20 mL) and ethyl acetate (20 mL) were added and the layers were separated. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to give **14b** as a colourless oil, which was used in the next step without further purification (93 mg, 98%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 – 7.23 (m, 4H), 7.20 – 7.10 (m, 4H), 4.06 – 3.88 (m, 2H), 3.74 – 3.29 (m, 5H), 3.29 – 3.08 (m, 1H), 3.07 – 2.95 (m, 1H), 2.94 – 2.81 (m, 1H), 2.53 – 2.27 (m, 3H), 1.40 (m, 9H).

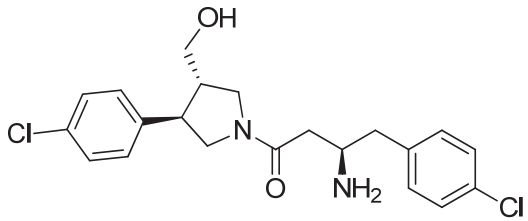
(*R*)-3-amino-4-(4-chlorophenyl)-1-((3*S*,4*R*)-3-(4-chlorophenyl)-4-(hydroxymethyl)pyrrolidin-1-yl)butan-1-one 2a



2a

To a stirred solution of **14a** (57.9 mg, 0.114 mmol) in DCM (4 mL) was added TFA (1 mL) at 0 ° C. The ice bath was removed and the solution was stirred at RT for 2.5h. Volatiles were removed. The residue was dissolved in water/methanol and purified by LCMS (Method B) to give **2a** as white fluffy powder (16.2 mg, 35%); ^{1}H NMR (300 MHz, MeOD) δ 7.44 – 7.33 (m, 4H), 7.32 – 7.24 (m, 4H), 4.02 – 3.73 (m, 3H), 3.56 (dt, J = 11.2, 3.5 Hz, 1H), 3.50 – 3.24 (m, 3H), 3.19 – 3.10 (m, 1H), 3.08 – 2.90 (m, 2H), 2.72 (m, 1H), 2.58 – 2.42 (m, 2H), m/z 407 ($[M+H]^+$) HRMS found 407.1305, $C_{21}H_{25}N_2O_2Cl_2$requires 407.1293; LCMS Rt = 12.45 min

(*R*)-3-amino-4-(4-chlorophenyl)-1-((3*R*,4*S*)-3-(4-chlorophenyl)-4-(hydroxymethyl)pyrrolidin-1-yl)butan-1-one 2b

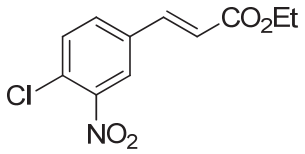


2b

To a stirred solution of **14b** (53.5 mg, 0.105 mmol) in DCM (4 mL) was added TFA (1 mL) at 0 °C. The ice bath was removed and the solution was stirred at RT for 2.5h. Volatiles were removed. The residue was dissolved in water/methanol and purified by LCMS (Method B) to give **2b** as white fluffy

powder (10.2 mg, 22%); ^{1}H NMR (300 MHz, MeOD) δ 7.43 – 7.33 (m, 4H), 7.32 – 7.24 (m, 4H), 3.96 – 3.73 (m, 3H), 3.56 (dt, J = 11.2, 3.5 Hz, 1H), 3.49 – 3.24 (m, 3H), 3.21 – 3.10 (m, 1H), 3.04 – 2.85 (m, 2H), 2.64 (m, 1H), 2.59 – 2.38 (m, 2H); m/z 407 ([M+H]$^+$) HRMS found 407.1289, $C_{21}H_{25}N_2O_2Cl_2$requires 407.1293; LCMS Rt = 12.47 min

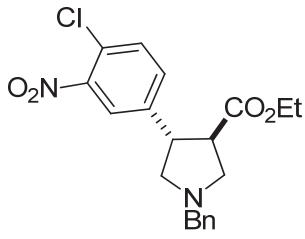
(*E*)-ethyl 3-(4-chloro-3-nitrophenyl)acrylate 21



21

Sodium hydride (155 mg, 6.47 mmol) was added to a solution of 4-chloro-3-nitrobenzaldehyde (1.00g, 6.47 mmol) and triethyl phosphonoacetate (1.28 mL, 6.47 mmol) in DMF (9 mL) at 0 °C. The red suspension was stirred at 0 °C for 3 hours. Water was added (10 mL) and the yellow precipitate formed was isolated by filtration and washed with water and hexane to give the product **21** as a yellow solid (923 mg, 67%) which was used without further purification; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 2.0 Hz, 1H), 7.67 – 7.56 (m, 3H), 6.50 (d, J = 16.0 Hz, 1H), 4.28 (q, J = 7.1 Hz, 2H), 1.34 (t, J = 7.1 Hz, 3H).

(±) (3*R*,4*S*)-ethyl 1-benzyl-4-(4-chloro-3-nitrophenyl)pyrrolidine-3-carboxylate 22

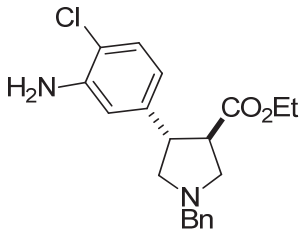


22

TFA (30 µL, 0.20 mmol) was added to a solution of benzyl-(methyoxymethyl) [(trimethylsilyl)methyl]amine **9** (1.00 mL, 3.90 mmol) and *trans*-ethyl 4-chloro 3-nitrocinnamate (499 mg, 1.95 mmol) in DCM (20 mL) at 0 °C and the solution was stirred at room temperature for 24 hours. Saturated aqueous $NaHCO_3$ (20 mL) was added and the phases were separated. The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude residue was

purified by column chromatography (1:4 EtOAc- hexane, R_f 0.3) to give the product **22** as a colourless oil (550 mg, 79%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 2.0 Hz, 1H), 7.57 – 7.45 (m, 2H), 7.43 – 7.34 (m, 4H), 7.34 – 7.27 (m, 1H), 4.18 (qd, J = 7.1, 1.7 Hz, 2H), 3.78 – 3.63 (m, 3H), 3.24 (app t, J = 8.8 Hz, 1H), 3.03 (app q, J = 7.5 Hz, 1H), 2.92 (app t, J = 8.8 Hz, 1H), 2.87 – 2.80 (m, 1H), 2.76 (app t, J = 8.6 Hz, 1H), 1.27 (t, J = 7.1 Hz, 3H).

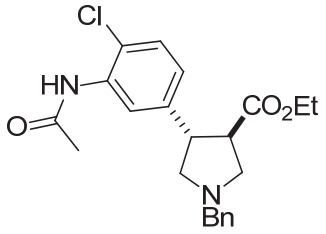
(±) (3*R*,4*S*)-ethyl 4-(3-amino-4-chlorophenyl)-1-benzylpyrrolidine-3-carboxylate 23



23

$SnCl_2 \cdot 2H_2O$ (870 mg, 3.86 mmol) was added to a solution of **22** (300 mg, 0.77 mmol) in ethanol (10 mL) and the suspension was stirred under reflux for 2 hours. The reaction was cooled to room temperature and the ethanol removed under reduced pressure. EtOAc (10 mL) was added and the solution washed with 4M NaOH solution. The organic layer was dried over MgSO4 and the solvent removed under reduced pressure to give the product **23** as a yellow oil (241 mg, 87%) which was used without further purification; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.29 (m, 4H), 7.29 – 7.23 (m, 1H), 7.14 (d, J = 8.2 Hz, 1H), 6.75 (d, J = 2.0 Hz, 1H), 6.66 (dd, J = 8.2, 2.0 Hz, 1H), 4.19 – 4.07 (m, 2H), 4.01 (br s, 2H), 3.73 – 3.62 (m, 2H), 3.60 – 3.46 (m, 1H), 3.10 – 2.99 (m, 2H), 2.96 (app t, J = 8.8 Hz, 1H), 2.82 (dd, J = 8.2, 6.2 Hz, 1H), 2.75 (dd, J = 9.5, 6.2 Hz, 1H), 1.21 (t, J = 7.1 Hz, 3H).

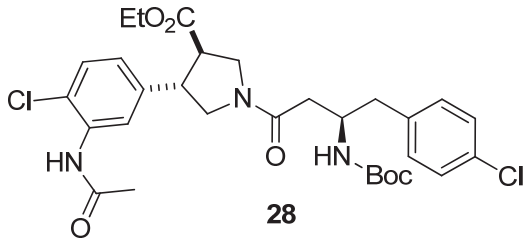
(3*R*,4*S*)-ethyl 4-(3-acetamido-4-chlorophenyl)-1-benzylpyrrolidine-3-carboxylate 24



24

Acetic anhydride (36 µL, 0.377 mmol) was added to a solution of **23** (123 mg, 0.343 mmol) and Et_3N (53 µL, 0.377 mmol) in DCM (3 mL) and the solution was stirred at room temperature for 2 hours. Saturated aqueous $NaHCO_3$ (3 mL) was added and the phases were separated. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude residue was purified by column chromatography (1:1 EtOAc - hexane, R_f 0.4) to give the product **24** as a yellow oil (90 mg, 65%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.57 (s, 1H), 7.42 – 7.21 (m, 5H), 7.05 (d, J = 8.3 Hz, 1H), 4.16 – 4.06 (m, 2H), 3.77 – 3.58 (m, 3H), 3.12 – 2.95 (m, 3H), 2.88 (br s, 1H), 2.75 (br s, 1H), 2.24 (s, 3H), 1.64 (br s, 1H), 1.21 (t, J = 7.1 Hz, 3H).

(3*R*,4*S*)-ethyl 4-(3-acetamido-4-chlorophenyl)-1-((*R*)-3-((tert-butoxycarbonyl)amino)-4-(4-chlorophenyl)butanoyl)pyrrolidine-3-carboxylate 28

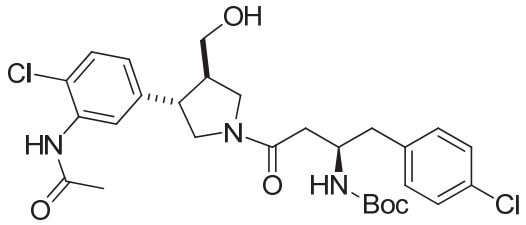


28

1-Chloroethyl chloroformate (46 µL, 0.42 mmol) was added to a solution of **24** (85 mg, 0.21 mmol) in toluene (5 mL) and the solution was stirred at 110 °C for 3 hours. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The residue was dissolved in methanol (5 mL) and the solution was heated at reflux for 30 minutes before cooling to room temperature. The solvent was removed under reduced pressure to give 70 mg crude yellow oil. 56 mg of this oil was dissolved in DMF (3 mL) and acid **12** (62 mg, 0.198 mmol) was added followed by EDCI (38 mg, 0.198 mmol), HOBt (27 mg, 0.198 mmol) and DIPEA (35 µL, 0.198 mmol). The solution was stirred at room temperature for 4 hours. EtOAc (5 mL) was added and the mixture was washed with saturated aqueous $NaHCO_3$ (5 mL), water (3 x 5 mL) and brine (5 mL). The solvent was removed under reduced pressure and the residue purified by column chromatography (2:1 EtOAc - hexane, R_f 0.32) to give the product **28** as a colourless oil (45 mg, 41% over 2 steps) as a mixture of diastereoisomers and as a mixture of amide rotamers by ^{1}H NMR at room temperature; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46

– 8.36 (m, 1H), 7.69 – 7.61 (m, 1H), 7.37 – 7.33 (m, 1H), 7.32 – 7.26 (m, 1H), 7.21 – 7.14 (m, 2H), 6.97 – 6.89 (m, 1H), 5.79 (br s, 1H), 4.21 – 3.98 (m, 4H), 3.91 – 3.36 (m, 4H), 3.34 – 3.11 (m, 1H), 3.11 – 3.00 (m, 1H), 3.00 – 2.85 (m, 1H), 2.57 – 2.37 (m, 2H), 2.31 – 2.23 (m, 3H), 1.46 – 1.39 (m, 9H), 1.25 – 1.16 (m, 3H).

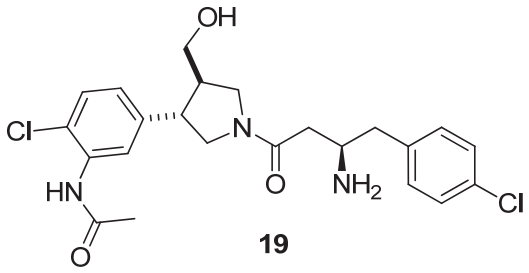
***tert*-butyl ((*R*)-4-((3*S*,4*R*)-3-(3-acetamido-4-chlorophenyl)-4-(hydroxymethyl)pyrrolidin-1-yl)-1-(4-chlorophenyl)-4-oxobutan-2-yl)carbamate 30**



30

$LiBH_4$ (7 mg, 0.30 mmol) was added to a solution of **28** (45 mg, 0.07 mmol) in dry THF (2 mL)**.** The solution was stirred at room temperature for 3 hours. Water was added (2 mL) followed by DCM (2mL) and the phases were separated. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure to give the product **30** as a colourless oil as a mixture of diastereoiosmers (38 mg, 93%). Amide rotamers were also seen by ^{1}H NMR; 1H NMR (400 MHz, CDCl3) δ 8.39 – 8.29 (m, 1H), 7.68 (s, 1H), 7.38 – 7.33 (m, 1H), 7.31 – 7.26 (m, 1H), 7.21 – 7.14 (m, 2H), 6.98 – 6.89 (m, 1H), 5.94 – 5.69 (m, 1H), 4.20 – 3.89 (m, 2H), 3.80 – 3.33 (m, 5H), 3.32 – 3.23 (m, 1H), 3.21 – 3.11 (m, 1H), 3.09 – 3.01 (m, 1H), 2.96 – 2.84 (m, 1H), 2.59 – 2.39 (m, 3H), 2.30 – 2.24 (m, 3H), 1.43 – 1.41 (m, 9H).

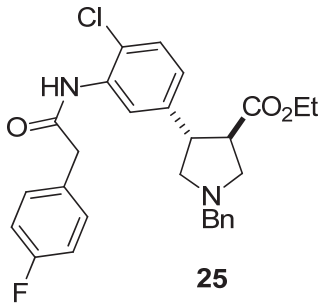
***N*-(5-((3*S*,4*R*)-1-((*R*)-3-amino-4-(4-chlorophenyl)butanoyl)-4-(hydroxymethyl)pyrrolidin-3-yl)-2-chlorophenyl)acetamide 19**



19

TFA (26 µL, 0.34 mmol) was added to a solution of **30** (38 mg, 0.067 mmol) in DCM (3 mL). The reaction was stirred at room temperature for 4 hours. Solvent was removed under reduced pressure and the residue was dissolved in methanol/water and purified by LCMS (method A) to give the product **19** as a colourless oil as a mixture of diastereoiosmers (19 mg, 61%); Amide rotamers were also observed by ^{1}H NMR . ^{1}H NMR (400 MHz, MeOD) δ 8.39 (s, 1H), 7.80 – 7.73 (m, 1H), 7.46 – 7.37 (m, 3H), 7.34 – 7.26 (m, 2H), 7.18 – 7.10 (m, 1H), 4.02 – 3.71 (m, 3H), 3.64 – 3.56 (m, 1H), 3.42 (m, 3H), 3.29 – 3.14 (m, 1H), 3.12 – 2.92 (m, 2H), 2.81 – 2.41 (m, 3H), 2.23 – 2.18 (m, 3H); m/z 464 ($[M+H]^+$) HRMS found 464.1505, $C_{23}H_{28}N_3O_3Cl_2$ requires 464.1508; LCMS Rt = 11.22 min.

(3*R*,4*S*)-ethyl 1-benzyl-4-(4-chloro-3-(2-(4-fluorophenyl)acetamido)phenyl)pyrrolidine-3-carboxylate 25

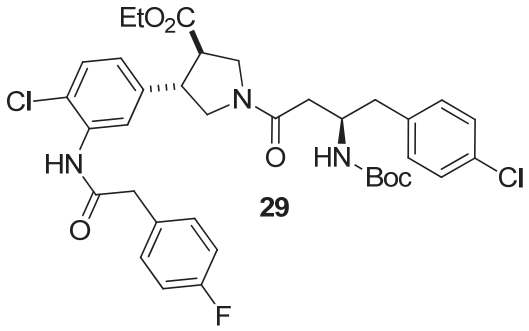


25

Para-Fluorophenylacetylchloride (50 µL, 0.37 mmol) was added to a solution of **23** (120 mg, 0.33 mmol) and triethylamine (51 µL, 0.37 mmol) in DCM (3 mL) at 0 °C and the solution was stirred at 0 °C for 2 hours. Saturated aqueous $NaHCO_3$ (3 mL) was added and the layers were separated. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude

residue was purified by column chromatography (1:3 EtOAC - Hexane, R_f 0.35) to give the product **25** as a yellow oil (69 mg, 42%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, J = 2.2 Hz, 1H), 7.64 (s, 1H), 7.43 – 7.31 (m, 6H), 7.30 – 7.22 (m, 1H), 7.18 – 7.10 (m, 2H), 7.05 (dd, J = 8.3, 2.2 Hz, 1H), 4.15 (m, 2H), 3.80 (s, 2H), 3.75 – 3.62 (m, 3H), 3.12 – 3.04 (m, 2H), 3.03 – 2.97 (m, 1H), 2.94 – 2.84 (m, 1H), 2.75 (dd, J = 9.3, 6.2 Hz, 1H), 1.24 (t, J = 7.2 Hz, 3H).

(3*R*,4*S*)-ethyl 1-((*R*)-3-((tert-butoxycarbonyl)amino)-4-(4-chlorophenyl)butanoyl)-4-(4-chloro-3-(2-(4-fluorophenyl)acetamido)phenyl)pyrrolidine-3-carboxylate 29

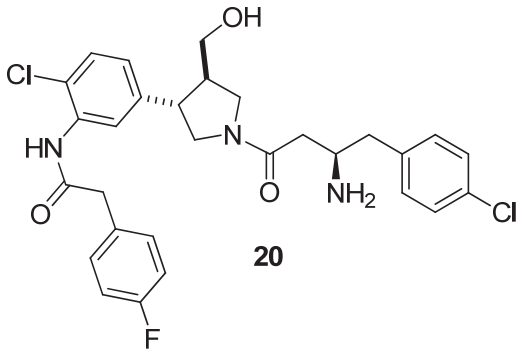


29

1-Chloroethyl chloroformate (30 µL, 0.23 mmol) was added to a solution of **25** (69 mg, 0.14 mmol) in toluene (3 mL) and the solution was stirred at 110 °C for 3 hours. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The residue was dissolved in methanol (3 mL) and the solution was heated at reflux for 30 minutes before cooling to room temperature. The solvent was removed under reduced pressure to give 60 mg crude yellow oil which was dissolved in DMF (3 mL). Acid **12** (51 mg, 0.16 mmol) was added followed by EDCI (43 mg, 0.22 mmol), HOBt (30 mg, 0.22 mmol) and DIPEA (39 µL, 0.22 mmol). The solution was stirred at room temperature for 3 hours. EtOAc (5 mL) was added and the mixture was washed with saturated aqueous $NaHCO_3$ (5 mL), water (3 x 5 mL) and brine (5 mL). The solvent was removed under reduced pressure and the residue purified by column chromatography (2:1 EtOAc - hexane, R_f 0.20) to give the product **29** as a colourless oil (45 mg, 43% over 2 steps) as a mixture of diastereoisomers and as a mixture of amide rotamers by ^{1}H NMR at room temperature; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 – 8.34 (m, 1H), 7.72 – 7.66 (m, 1H), 7.35 (m, 2H), 7.32 – 7.26 (m, 2H), 7.22 – 7.11 (m, 4H), 6.95 – 6.87 (m, 1H), 5.79 (br s, 1H), 4.20 – 3.99 (m, 4H), 3.82 – 3.77 (m, 2H), 3.75 – 3.49 (m, 3H), 3.46 – 3.12 (m, 2H), 3.06 (m,

1H), 2.98 – 2.85 (m, 1H), 2.56 – 2.37 (m, 2H), 1.75 (br s, 1H), 1.45 – 1.38 (m, 9H), 1.25 – 1.17 (m, 3H).

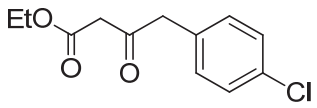
***N*-(5-((3*S*,4*R*)-1-((*R*)-3-amino-4-(4-chlorophenyl)butanoyl)-4-(hydroxymethyl)pyrrolidin-3-yl)-2-chlorophenyl)-2-(4-fluorophenyl)acetamide 20**



20

$LiBH_4$ (4 mg, 0.2 mmol) was added to a solution of **29** (35 mg, 0.05 mmol) in dry THF (2 mL)**.** The solution was stirred at room temperature for 5 hours. Water was added (2 mL) followed by DCM (2mL) and the phases were separated. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure to give 31 mg yellow oil. The oil was dissolved in DCM (3 mL) and treated with TFA (18 μL, 0.24 mmol). The reaction was stirred for 4 hours and the solvent was removed under reduced pressure. The residue was purified by LCMS (method A) to give the product **20** as a colourless oil as a mixture of diastereoisomers and as a mixture of amide rotamers by ^{1}H NMR at room temperature (9 mg, 34%); ^{1}H NMR (400 MHz, MeOD) δ 8.45 (s, 1H), 7.78 (dd, J = 8.4, 2.2 Hz, 1H), 7.46 – 7.36 (m, 5H), 7.33 – 7.25 (m, 2H), 7.16 – 7.06 (m, 3H), 4.01 – 3.70 (m, 5H), 3.58 (dd, J = 11.2, 3.9 Hz, 1H), 3.48 – 3.34 (m, 3H), 3.29 – 3.12 (m, 1H), 3.10 – 2.89 (m, 2H), 2.80 – 2.39 (m, 3H); m/z 558 ($[M+H]^+$) HRMS found 558.1721, $C_{29}H_{31}N_3O_3Cl_2F$ requires 558.1727; LCMS Rt = 12.67 min

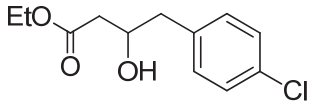
ethyl 4-(4-chlorophenyl)-3-oxobutanoate 33



33

4-chlorophenylacetyl chloride **32** (641 µL, 4.38 mmol) was added dropwise to a solution of Meldrum's acid (500 mg, 4.38 mmol) and pyridine (718 µL, 8.88 mmol) in DCM (8 mL) at 0 °C. The solution was stirred at 0 °C for 30 minutes and then at room temperature overnight. The solution was washed with 10% HCl (10 mL) and water (10 mL) and the organic layer dried over $MgSO_4$. Solvent was removed under reduced pressure and the residue dissolved in ethanol (8 mL). The solution was stirred at reflux for 2.5 hours. Ethanol was removed under reduced pressure and the residue purified by column chromatography (1:8 EtOAc - hexane; R_f 0.4) to give the product **33** as an orange oil (740 mg, 70%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.28 (m, 3H), 7.20 – 7.15 (m, 3H), 4.21 (q, J = 7.1 Hz, 2H), 3.85 (s, 2H), 3.49 (s, 2H), 1.30 (t, J = 7.1 Hz, 3H).

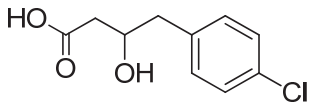
ethyl 4-(4-chlorophenyl)-3-hydroxybutanoate 34



34

$NaBH_4$ (31 mg, 0.831 mmol) was added portion-wise to a solution of **33** (200 mg, 0.831 mmol) in methanol (3 mL) at 0 °C. The solution was allowed to stir at room temperature for 1.5 hours. Water was added (3 mL) followed by EtOAc (3 mL) and the layers were separated. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. The residue was purified by column chromatography (1:4 EtOAc - hexane R_f 0.30) to give the product **34** as a colourless oil (80 mg, 40%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.26 (m, 2H), 7.20 – 7.15 (m, 2H), 4.29 – 4.22 (m, 1H), 4.18 (q, J = 7.1 Hz, 2H), 2.84 (dd, J = 13.7, 7.2 Hz, 1H), 2.76 (dd, J = 13.7, 5.8 Hz, 1H), 2.51 (dd, J = 16.5, 3.6 Hz, 1H), 2.43 (dd, J = 16.5, 8.7 Hz, 1H), 1.28 (t, J = 7.1 Hz, 3H).

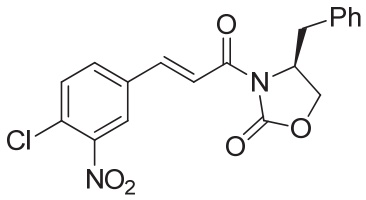
4-(4-chlorophenyl)-3-hydroxybutanoic acid 35



35

A solution of LiOH (41 mg, 0.99 mmol) in water (1 mL) was added to a solution of **34** (80 mg, 0.33 mmol) in methanol (1 mL) and the reaction was stirred at room temperature for 1 hour. 2M HCl was added until pH=2. EtOAc (3 mL) was added and the phases were separated. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure to give the product **35** as a colourless oil (69 mg, 99%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.24 (m, 2H), 7.19 – 7.12 (m, 2H), 4.48 (br s, 1H), 4.29 – 4.20 (m, 1H), 2.86 – 2.74 (m, 2H), 2.58 (dd, J = 16.7, 3.7 Hz, 1H), 2.51 (dd, J = 16.7, 8.5 Hz, 1H).

(*S,E*)-4-benzyl-3-(3-(4-chloro-3-nitrophenyl)acryloyl)oxazolidin-2-one 37

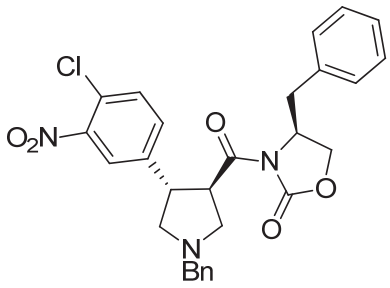


37

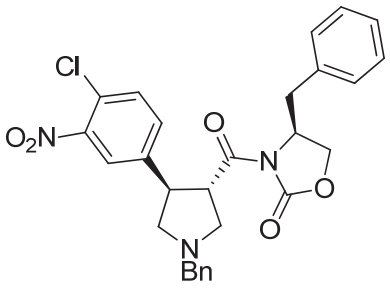
A solution of oxalyl chloride (3.73 mL, 43.9 mmol) in dichloromethane (10 mL) was added dropwise over 30 min to a suspension of *trans*-4-Chloro-3-nitrocinnamic acid **36** (5.0 g, 21.9 mmol) in dichloromethane (40 mL) and *N,N*-dimethylformamide (5 μL) at 0 °C. The reaction mixture was stirred for 24 hours at room temperature and a yellow solution was obtained. The solution was added dropwise to a cooled solution of (4S)-4-benzyl-1,3-oxazolidin-2-one (3.38 g, 19.1 mmol), triethylamine (12.24 mL, 87.9 mmol) and lithium chloride (3.79 g, 87.9 mmol) in dichloromethane (40 mL) at 5 °C. The reaction mixture was stirred for 15 min at 5 °C. Then, the ice bath was removed and the mixture was stirred for 1 hour at RT. Water (40 mL) and 5% citric acid solution (50 mL) were added. The mixture was extracted 3 times with dichloromethane. The phases were separated and the

organic phase was dried over magnesium sulfate, filtered and concentrated in vacuo. The residue was purified by column chromatography (1:3 EtOAc - Hexane, R_f 0.2) to give the product **37** as a colourless oil (4.0 g, 45%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 2.1 Hz, 1H), 8.00 (d, J = 15.7 Hz, 1H), 7.84 (d, J = 15.7 Hz, 1H), 7.79 (dd, J = 8.4, 2.1 Hz, 1H), 7.62 (d, J = 8.4 Hz, 1H), 7.40 – 7.30 (m, 3H), 7.27 – 7.24 (m, 1H), 4.87 – 4.78 (m, 1H), 4.31 – 4.26 (m, 2H), 3.39 (dd, J = 13.5, 3.4 Hz, 1*H), 2.89 (dd, J = 13.4, 9.4 Hz, 1H).*

(*S*)-4-benzyl-3-((3*R*,4*S*)-1-benzyl-4-(4-chloro-3-nitrophenyl)pyrrolidine-3-carbonyl)oxazolidin-2-one 38a and (*S*)-4-benzyl-3-((3*S*,4*R*)-1-benzyl-4-(4-chloro-3-nitrophenyl)pyrrolidine-3-carbonyl)oxazolidin-2-one 38b



38a

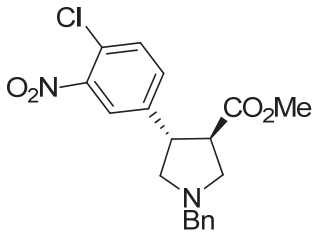


38b

To a cooled solution of **37** (4.0 g, 10.3 mmol) and *N*-benzyl-1-methoxy-*N*-[(trimethylsilyl)methyl]methanamine **9** (5.29 mL, 20.6 mmol) in dichloromethane (60 mL) was added trifluoroacetic acid (79 uL, 1.03 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 20 minutes and then warmed to room temperature and stirred for 24 hours. Saturated aqueous $NaHCO_3$ (30 mL) was added and the reaction mixture was stirred for 20 minutes (pH=7). The phases were separated and the organic phase was dried over magnesium sulfate and the solvent was removed in vacuo to give a yellow oil. Purification by flash column chromatography on silica gel (1:3 to 1:2 EtOAc - hexane) **38a** (R_f 0.35, 2.8 g, 40%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, J = 2.1 Hz, 1H), 7.54 (dd, J = 8.4, 2.1 Hz, 1H), 7.47 (d, J = 8.3 Hz, 1H), 7.41 – 7.26 (m, 8H), 7.20 (m, 2H), 4.74 – 4.66 (m, 1H), 4.36 – 4.05 (m, 4H), 3.79 (d, J = 13.0 Hz, 1H), 3.67 (d, J = 13.0 Hz, 1H), 3.42 (t, J = 9.2 Hz, 1H), 3.26 (dd, J = 13.3, 3.3 Hz, 1H), 3.12 (dd, J = 9.3, 7.1 Hz, 1H), 2.87 – 2.71 (m, 3H) and **38b** (R_f 0.2, 2.0g, 36%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 2.1 Hz, 1H), 7.53 (dd, J = 8.4, 2.1 Hz,

1H), 7.47 (d, J = 8.3 Hz, 1H), 7.37 – 7.27 (m, 8H), 7.15 – 7.09 (m, 2H), 4.67 (m, 1H), 4.23 – 4.14 (m, 3H), 4.06 – 3.98 (m, 1H), 3.74 (d, J = 13.0 Hz, 1H), 3.64 (d, J = 13.0 Hz, 1H), 3.33 (t, J = 9.3 Hz, 1H), 3.25 (dd, J = 13.5, 3.4 Hz, 1H), 3.06 (dd, J = 9.4, 7.3 Hz, 1H), 2.80 – 2.67 (m, 3H).

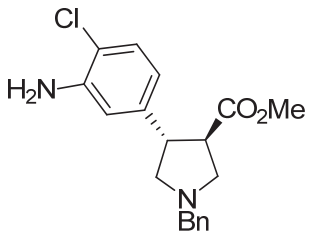
(3*R*,4*S*)-methyl 1-benzyl-4-(4-chloro-3-nitrophenyl)pyrrolidine-3-carboxylate 39



39

Sodium methoxide (25% solution in methanol, 2.52 mL, 11.7 mmol) was added to a solution of **38a** (2.5 g, 4.68 mmol) and dimethylcarbonate (986 µL, 11.7 mmol) in DCM (30 mL) and the solution was stirred at room temperature for 15 hours. Saturated aqueous NH_4Cl solution (30 mL) was added and the phases were separated. The organic layer was washed with water, dried over $MgSO_4$ and the solvent removed under reduced pressure. The residue was purified by flash column chromatography (10 g SNAP) using n-hexane/EtOAc as eluent (gradient100% hexane – 70/30) to give the product **39** as a colourless oil (639 mg, 36%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 2.0 Hz, 1H), 7.53 (dd, J = 8.4, 2.1 Hz, 1H), 7.47 (d, J = 8.3 Hz, 1H), 7.39 – 7.26 (m, 5H), 3.76 – 3.69 (m, 5H), 3.66 (d, J = 13.0 Hz, 1H), 3.23 (app t, J = 8.8 Hz, 1H), 3.04 (td, J = 8.1, 6.2 Hz, 1H), 2.91 (dd, J = 9.6, 7.9 Hz, 1H), 2.82 (dd, J = 9.6, 4.6 Hz, 1H), 2.75 (dd, J = 9.3, 7.9 Hz, 1H).

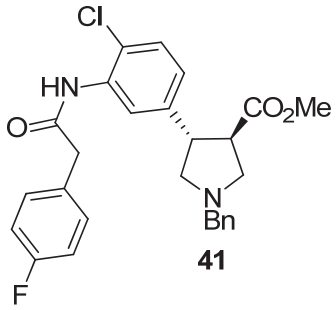
(3*R*,4*S*)-methyl 4-(3-amino-4-chlorophenyl)-1-benzylpyrrolidine-3-carboxylate 40



40

$SnCl_2 \cdot 2H_2O$ (1.89 mg, 8.40 mmol) was added to a solution of **39** (630 mg, 1.68 mmol) in ethanol (20 mL) and the suspension was stirred under reflux for 2 hours. The reaction was cooled to room temperature and the ethanol removed under reduced pressure. EtOAc (20 mL) was added and the solution washed with 4M NaOH solution (20 mL). The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure to give the product **40** as a yellow oil (580 mg, quantitative) which was used without further purification; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.31 (m, 4H), 7.30 – 7.26 (m, 1H), 7.17 (d, J = 8.2 Hz, 1H), 6.76 (d, J = 2.1 Hz, 1H), 6.68 (dd, J = 8.2, 2.1 Hz, 1H), 4.03 (br s, 2H), 3.72 – 3.64 (m, 5H), 3.60 – 3.53 (m, 1H), 3.07 (dd, J = 6.0, 3.1 Hz, 2H), 2.96 (dd, J = 9.4, 8.2 Hz, 1H), 2.87 – 2.81 (m, 1H), 2.74 (dd, J = 9.4, 6.0 Hz, 1H).

(3*R*,4*S*)-methyl 1-benzyl-4-(4-chloro-3-(2-(4-fluorophenyl)acetamido)phenyl)pyrrolidine-3-carboxylate 41

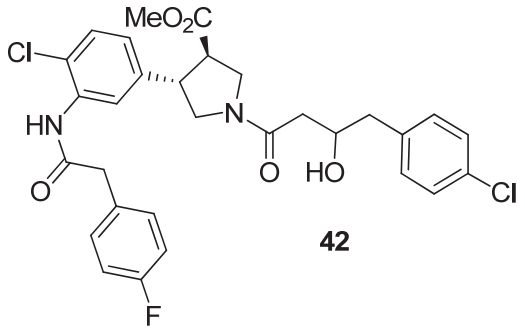


41

Para-Fluorophenylacetylchloride (435 mg, 2.52 mmol) was added to a solution of **40** (580 mg, 1.68 mmol) and triethylamine (352 µL, 2.52 mmol) in DCM (10 mL) at 0 °C and the solution was stirred at 0 °C for 2 hours. Saturated aqueous $NaHCO_3$ (10 mL) was added and the layers were separated. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude

residue was purified by column chromatography (25 g SNAP) using n-hexane/EtOAc as eluent (gradient100% hexane – 70/30), R_f 0.30 (1:3 EtOAC- hexane) to give the product **41** as a yellow oil (448 mg, 55%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.62 (s, 1H), 7.42 – 7.30 (m, 6H), 7.27 – 7.21 (m, 1H), 7.16 – 7.09 (m, 2H), 7.07 – 7.00 (m, 1H), 3.78 (s, 2H), 3.73 – 3.57 (m, 6H), 3.14 – 2.83 (m, 4H), 2.74 (br s, 1H).

(3*R*,4*S*)-methyl 4-(4-chloro-3-(2-(4-fluorophenyl)acetamido)phenyl)-1-(4-(4-chlorophenyl)-3-hydroxybutanoyl)pyrrolidine-3-carboxylate 42

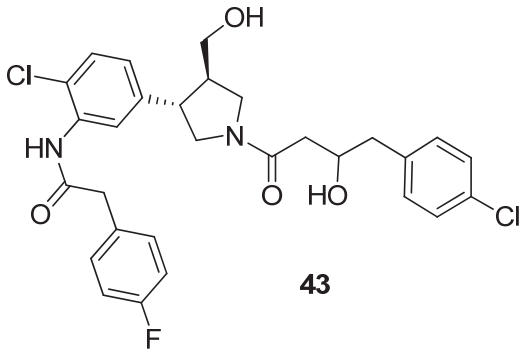


42

1-Chloroethyl chloroformate (197 µL, 1.83 mmol) was added to a solution of **41** (440 mg, 0.196 mmol) in toluene (20 mL) and the solution was stirred at 110 °C for 3 hours. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The residue was dissolved in methanol (20 mL) and the solution was heated at reflux for 30 minutes before cooling to room temperature. The solvent was removed under reduced pressure to give 480 mg crude yellow oil. 50 mg of this oil was dissolved in DCM (2 mL) and DMA (300 uL). Acid **35** (20 mg, 0.153 mmol) was added followed by triethylamine (39 µL, 0.281 mmol) and DMC (22 mg, 0.128 mmol). They yellow solution was allowed to stir at room temperature overnight. Saturated aqueous $NaHCO_3$ was added (3 mL) followed by DCM (3 mL) and the phases were separated. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by column chromatography (5 g SNAP) using EtOAC/hexane as eluent (gradient 20/80 to 100% EtOAc) to give the product **42** as a colourless oil as a mixture of diastereoisomers (20 mg, 27%), R_f 0.25 (2:1 EtOAc – hexane) Amide rotamers were also seen by 1H NMR at room temperature; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 – 8.35 (m, 1H), 7.73 – 7.67 (m, 1H), 7.38 – 7.32 (m, 3H), 7.29 – 7.26 (m, 1H), 7.24 –

7.10 (m, 4H), 6.91 (m, 1H), 4.38 – 4.25 (m, 1H), 4.09 – 3.99 (m, 1H), 3.90 – 3.81 (m, 1H), 3.80 – 3.77 (m, 2H), 3.74 – 3.64 (m, 5H), 3.27 – 3.14 (m, 1H), 3.07 – 2.85 (m, 3H), 2.79 – 2.70 (m, 1H), 2.52 – 2.26 (m, 2H).

***N*-(2-chloro-5-((3*S*,4*R*)-1-(4-(4-chlorophenyl)-3-hydroxybutanoyl)-4-(hydroxymethyl)pyrrolidin-3-yl)phenyl)-2-(4-fluorophenyl)acetamide 43**



43

$LiBH_4$ (3 mg, 0.14 mmol) was added to a solution of **42** (20 mg, 0.03 mmol) in THF (1 mL) and the solution was stirred at room temperature for 2 hours. Water was added (2 mL) followed by EtOAc (2 mL). The phases were separated and the organic layer was dried over $MgSO_4$. Solvent was removed under reduced pressure and the residue purified by prep LCMS (method B) to give the product **43** as a colourless oil (4.8 mg, 25%); ^{1}H NMR (400 MHz, MeOD) δ 7.78 – 7.71 (m, 1H), 7.45 – 7.37 (m, 3H), 7.33 – 7.21 (m, 4H), 7.18 – 7.05 (m, 3H), 4.29 (m, 1H), 4.04 – 3.81 (m, 2H), 3.80 – 3.75 (m, 2H), 3.63 – 3.36 (m, 4H), 3.27 – 3.12 (m, 1H), 2.87 – 2.74 (m, 2H), 2.61 – 2.37 (m, 3H); m/z 559 ($[M+H]^+$) HRMS found 559.1570 $C_{29}H_{30}N_2O_4FCl_2$ requires 559.1567; LCMS Rt = 14.41 min.

Enzyme assay. All IC_{50} determinations were carried out using a 7-diethylamine-3-(4'maleimidylphenyl)-4-methylcoumarin (CPM) fluorescence assay, as described previously.[1-3] The HsNMT1 assays are as described in the literature, for LdNMT and LmNMT the final enzyme concentration and peptide substrates are: 400 ng/mL; Peptide Substrate: *Homo sapiens* $p60^{src}$ (2-16) with sequence GSNKSKPKDASQRRR-NH2 and the final concentration was 4.0 μM. IC_{50} of an inhibitor was calculated by a nonlinear regression analysis using GraFit 7.0.1 version (Erithacus

Software Limited, UK).The values are the mean value of two determinations; standard deviation is within 20% of the IC_{50} unless otherwise specified.

K_i Calculation. K_i values were determined using the following equation:[4]

$$K_i = \frac{\left(\mathrm{IC}_{50} - \frac{[E]}{2}\right)}{\left(1 + \frac{[S]}{K_m}\right)}$$

[E] = enzyme concentration; [LdNMT] = 8.2 nM; [HsNMT1] = 8.0 nM.

[S] = substrate concentration; 4 μM

LdNMT K_m = 19.40 μM

HsNMT1 K_m = 3.29 μM

L. donovani ex-vivo amastigotes inhibition assay.[5] *L. donovani* amastigotes (4 x 10^5/well of 96-well plate) were incubated with test compounds, with the concentrations ranging from 90 μM to 0.034 μM in 200 μL RPMI 1640 medium supplemented with 20% heat-inactivated FCS, 100 μM adenine, 20 mM 2-[*N*-morpholino]ethanesulphonic acid (pH 5.5), 5 μM hemin, 3 μM biopterin, 1 μM biotin, 100 U penicillin and 100 μg streptomycin. After 72 hour incubation at 26 ˚C, alamar blue (10% v/v) was added to each well and the resulting mixture was incubated for additional 4 hours prior to measuring the fluorescence (excitation wavelength was 544 nm and emission was recorded with a 590 ±10 nm bandpass filter, POLARstar Optima). Parasites cultured in medium alone were used as a positive control and medium without cells containing corresponding concentration of a compound was used as a medium control. Growth inhibition at each concentration was calculated as % inhibition of normalized data = [1-(readout-medium)/(positive-medium)] x 100%. Assays were carried out in triplicate.

Macrophage toxicity test. Bone marrow-derived macrophage (BMDM, 4.2 × 10^4/well of 96-well plate) were incubated with the same concentrations of test compounds as mentioned above in DMEM medium containing 4% L929 cell and 10% FCS (fetal calf serum). Cells were incubated at 37˚ C and 5% CO_2 for 72 hours. Aalamar blue (10% v/v) was added to each well and the resulting

mixture was incubated for additional 6 hours prior to measuring the fluorescence. Medium without cells containing the corresponding concentration of a compound was used as a medium control. Assays were performed in duplicate.

X-ray crystallography. Protein production and crystallisation of compounds **1** (PDB code 4cgn) and **2a** (PDB code 4cgl) with LmNMT and MyrCoA have been previously described along with X-ray diffraction data and refinement statistics.[6] Compounds **2b**, **20** and **43** were crystallised according to the same method using ligand compounds (25 mM stocks in 50 % DMSO) in stabilisation solution (33 % PEG 1500, 0.22 M NaCl, 0.11 M Na cacodylate, pH 5.5) at a final ligand concentration of 2.5 mM to soak LmNMT-MyrCoA crystals.

X-ray diffraction data were collected on synchrotron beamlines at the Diamond Light Source and processed using XDS[7] and SCALA[8] implemented within xia2.[9] Data collection and refinement statistics are summarised in Table S1. For Rfree calculations, 5% of the data were excluded. Cycles of refinement using maximum likelihood methods implemented in REFMAC[10] were interspersed with model building and adjustment using COOT.[11] The final refined protein structure model displays good geometry with only amino acid residue His347 outside preferred regions of the Ramachandran plot. The coordinates and structure factor files have been deposited in the Protein Data Bank with accession codes 4cyn (LmNMT-MyrCoA-**2b**), 4cyo (LmNMT-MyrCoA-**20**), 4cyq (LmNMT-MyrCoA-**43**).

Table S1. X-ray diffraction data and refinement statistics.

	LmNMT-MYA-**2b**	LmNMT-MYA-**20**	LmNMT-MYA-**43**
PDB accession code	**4cyn**	**4cyo**	**4cyq**
Cell dimensions *a*, *b*, *c* (Å)	47.67, 91.64, 53.16	48.55, 92.73, 53.64	48.00, 92.03, 53.27
Cell angles α, β, γ (°)	90.0, 111.9, 90.0	90.0, 113.9, 90.0	90.0, 113.1, 90.0
Space Group	$P2_1$	$P2_1$	$P2_1$
Data collection			
Beamline / Wavelength (Å)	DLS i04-1 / 0.9200	DLS i04-1 / 0.9200	DLS i03 / 0.9763
Detector type	CMOS Pilatus 2M	CMOS Pilatus 2M	CMOS Pilatus 2M
Images x oscillation (°)	900 x 0.2	900 x 0.2	900 x 0.2
Resolution (Å)	34–1.40 (1.42–1.40)	22–1.50 (1.53–1.50)	21–1.65 (1.68–1.65)
R_{sym} (%) [b]	5.2 (76.4)	4.9 (75.9)	4.9 (70.4)
$I/\sigma I$	11.0 (1.2)	12.6 (1.5)	10.3 (1.9)
Completeness (%)	98.7 (98.8)	98.9 (99.5)	99.9 (99.9)
Redundancy	3.5 (3.3)	3.5 (3.5)	3.4 (3.4)
Refinement			
No. unique reflections	82099	68546	51112
R_{work} / R_{free} [c]	17.4 / 21.2	17.8 / 21.6	18.3 / 23.4
No. atoms	4205	4052	3981
protein	3559	3495	3535
ligand	27	38	38
co-factor	63	63	63
water	555	425	345
B-factors (Å^2)			
all atoms	17.5	22.0	26.7
protein	15.8	21.0	25.7
ligand	17.5	14.4	24.0
co-factor	12.0	14.3	18.6
water	28.8	30.9	36.5
R.m.s.deviations [d]			
bond lengths (Å)	0.026	0.026	0.022
bond angles (°)	2.420	2.418	2.284

[a] Highest resolution shell is shown in parentheses.
[b] $R_{sym} = \Sigma_{\mathbf{h}}\Sigma_l |I_{\mathbf{h}l} - \langle I_{\mathbf{h}}\rangle| / \Sigma_{\mathbf{h}}\Sigma_l \langle I_{\mathbf{h}}\rangle$, where I_l is the l^{th} observation of reflection h and $\langle I_{\mathbf{h}}\rangle$ is the weighted average intensity for all observations l of reflection h.
[c] $R_{cryst} = \sum||F_o| - |F_c||/\sum|F_o|$ where F_o and F_c are the observed and calculated structure factor amplitudes, respectively. R_{free} is the R_{cryst} calculated with 5% of the reflections omitted from refinement.
[d] Root-mean-square deviation of bond lengths or bond angles from ideal geometry.

Figure S1. Compound **20** bound to LmNMT showing conserved interactions when compared with compounds **1** and **2** (Figures 1 and 2).

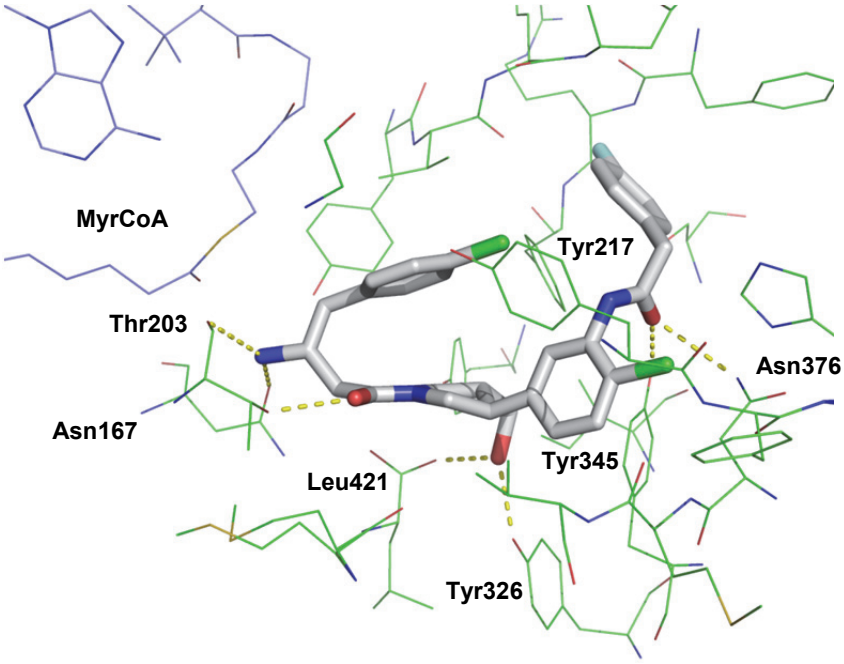


Figure S2. a) Overlay of amine **20** (white) and alcohol **43** (yellow) bound to LmNMT obtained by alignment of the protein main chain atoms.

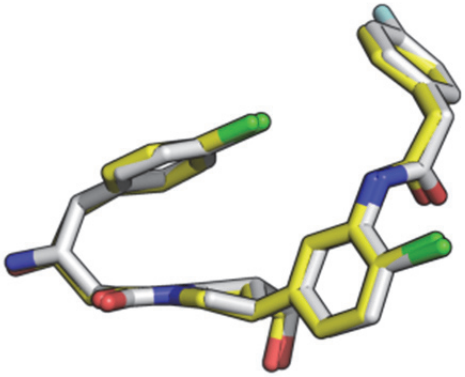
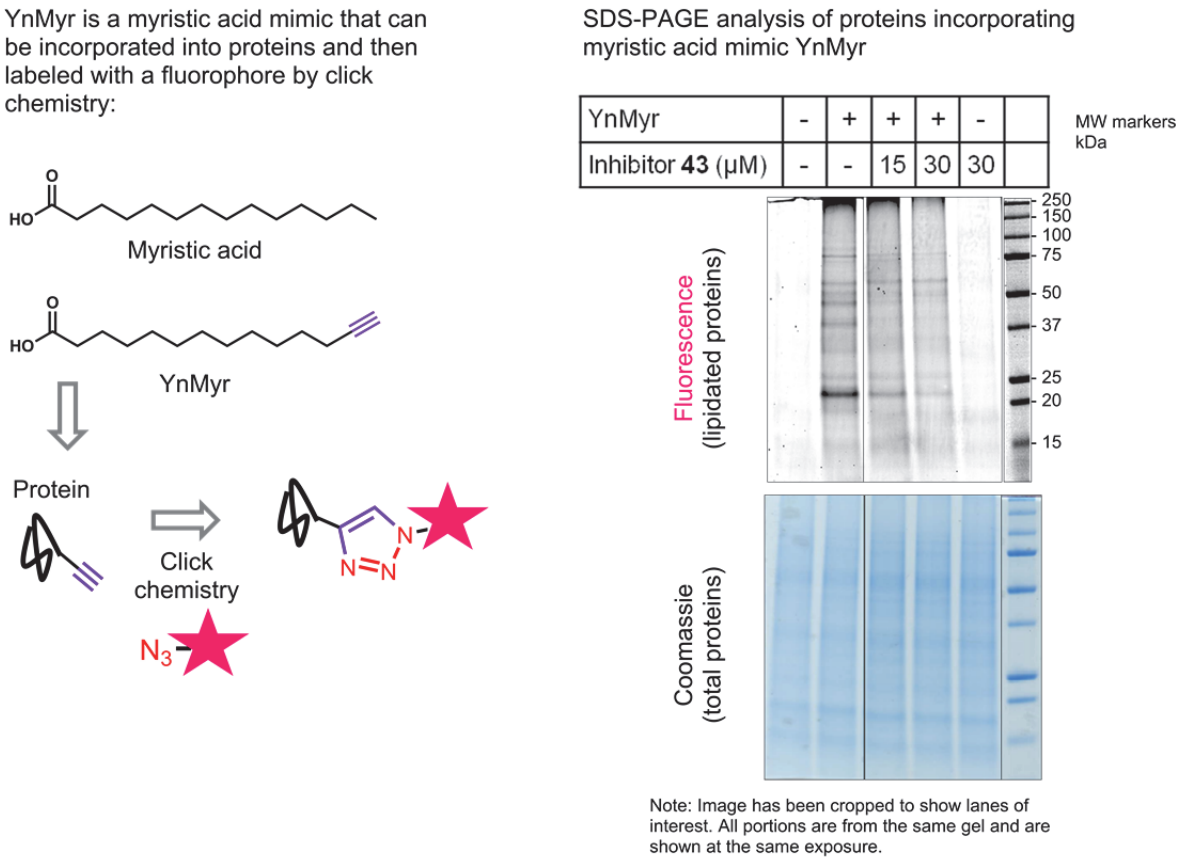


Figure S3. Metabolic chemical tagging[12]



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