Design and synthesis of Brain Penetrant Trypanocidal N-Myristoyltransferase Inhibitors

Tracy Bayliss, David A. Robinson, Victoria C. Smith, Stephen Brand, Stuart P. McElroy, Leah S. Torrie, Chido Mpamhanga, Suzanne Norval, Laste Stojanovski, Ruth Brenk, Julie A. Frearson, Kevin D. Read, Ian H. Gilbert, Paul G. Wyatt.*

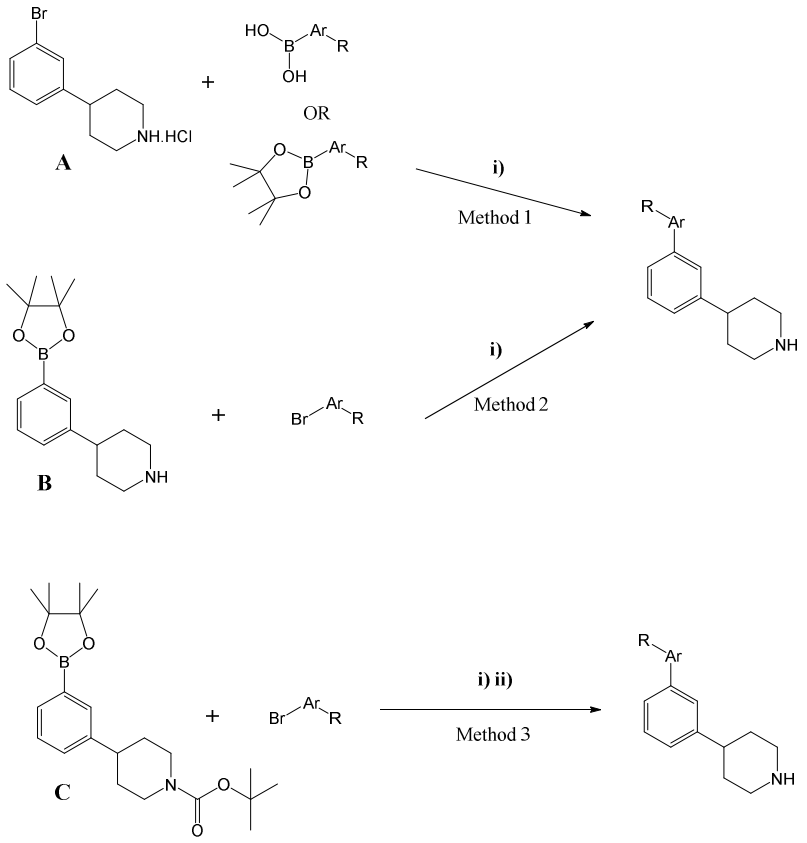
Drug Discovery Unit, College of Life Sciences, University of Dundee, Sir James Black Centre, Dundee, DD1 5EH, U.K.

Supporting Information

S1. Suzuki Array Chemistry

All the boronic acids or boronate esters and aryl bromides used in the Suzuki array are commercially available.

S.1.1 General Suzuki Reaction Scheme

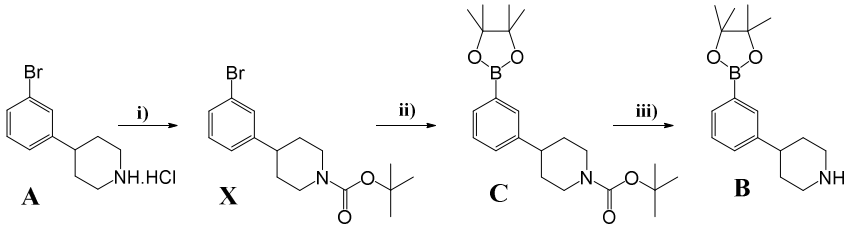


i)$Pd(PPh_3)_4$, Dioxane/1 M aq K_3PO_4, Polystyrene bound-DEAM **ii)** TFA/dichloromethane

Scheme 1. Suzuki Reaction Methods

S.1.2 Experimental

Intermediate B, C Synthesis



i) $BocO_2$, NEt_3, THF **ii)** $Pd(OAc)_2$, bispinacolartodiboron, $Pd(dppf)Cl_2$, Dioxane, **iii)** TFA/Ether

*tert-Butyl 4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)piperidine-1-carboxylate (**C**)*. A solution of 4-(3-bromophenyl)piperidine.hydrochloride (**A**) (5.1 g, 18.4 mmol, 1 eq), Boc_2O (4.4g, 20.2 mmol, 1.1 eq), and triethylamine (3.87 mL, 27.8 mmol, 1.5 eq) in THF (50 mL) was stirred at room temperature for 16 h. The reaction was filtered, the filtrate was washed with dilute 10% citric acid and extracted into ethyl acetate. The ethyl acetate layer was washed with water, and the organic layer dried over $MgSO_4$, filtered and evaporated to give an off-white solid (tert-butyl 4-(3-bromophenyl))piperidine-1-carboxylate **(X)** (6.13 g, 98% yield). ^{1}H NMR, 500MHz, $CDCl_3$ δ1.51 (s, 9H), 1.57-1.66 (m, 2H), 1.81-1.86 (m, 2H), 2.64 (tt, J = 3.70, 12.21, 1H), 2.77-2.85 (m, 2H), 4.22-4.32 (m, 2H), 7.14-7.22 (m, 2H), 7.35-7.39 (m, 2H). $[M+H]^+$ = 388.4

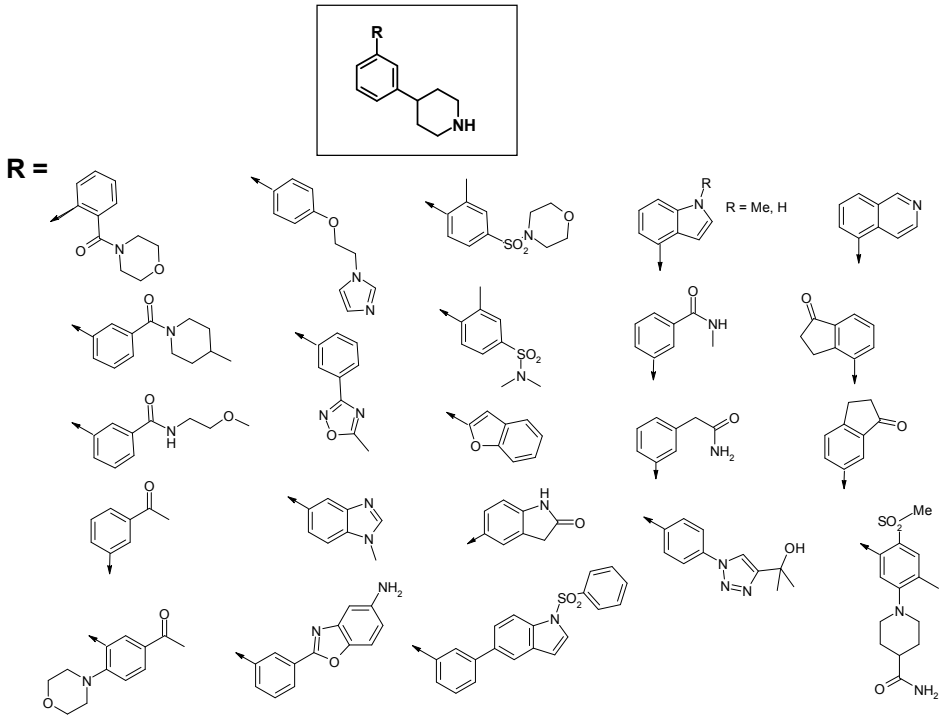
*tert-Butyl 4-(3-bromophenyl)piperidine-1-carboxylate, **X*** (2.9 g, 8.52 mmol, 1 eq), bispinacolartodiboron (2.6 g, 10.2 mmol, 1.2 eq) and potassium acetate (1.84 g, 18.7 mmol, 2.2 eq) were combined in anhydrous dioxane (15 mL) in a microwave vessel and degassed with argon for 5 min before adding $Pd(dppf)Cl_2$ (348 mg, 0.426 mmol, 5 %). The reaction was degassed again before microwaving at 120 ^{o}C for 40 min. The reaction was then partitioned between dichloromethane and aq. $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered and absorbed onto silica before being purified by flash column chromatography, running a gradient from 0% ethyl acetate/hexane to 30% ethyl acetate/hexane. This gave **C** as a white solid (2.4 g, 73%

yield). ^{1}H NMR 500MHz, $CDCl_3$ δ 1.37 (s, 12H), 1.51 (s, 9H), 1.64-1.73 (m, 2H), 1.82-1.87 (m, 2H), 2.68 (tt, *J* = 3.62, 12.25, 1H), 2.76-2.84 (m, 2H), 4.20-4.36 (m, 2H), 7.30-7.36 (m, 2H), 7.67-7.70 (m, 2H).

*4-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)piperidine (**B**)*. To a solution of tert-butyl 4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)piperidine-1-carboxylate (**C**) (2.77 g, 7.15 mmol, 1 eq), in diethylether (15 ml), TFA (4 eq) was added dropwise and the reaction stirred at RT for 96 h. The reaction was evaporated *in vacuo* and passed through an SCX column, eluting with 7 N ammonia in methanol, giving **B** as a white solid (1.9 g, 94% yield). ^{1}H NMR 500MHz, MeOD, δ 1.38 (s, 12H), 1.89-1.98 (m, 2H), 2.08-2.11 (m, 2H), 2.90-2.96 (m, 1H), 3.12-3.18 (m, 2H), 3.49-3.52 (m, 2H), 7.34-7.41 (m, 2H), 7.62-7.70 (m, 2H). $[M+H]^+$ = 288.2138

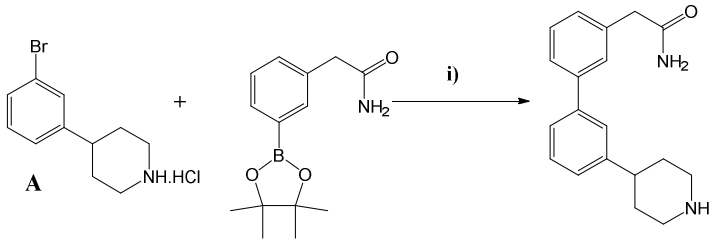
S.1.3 Suzuki Array Examples

No compounds made gave an IC_{50} <10μ M against *Tb*NMT



S.1.4 Representative Suzuki Reaction

S.1.4.1 Method 1

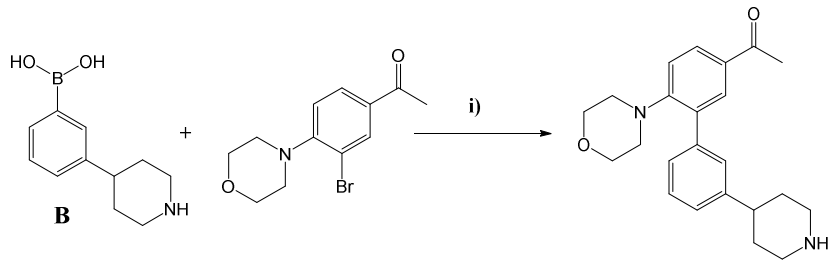


a) $Pd(PPh_3)_4$, Dioxane/1 M aq K_3PO_4, Polystyrene bound-DEAM

2-(3'-(Piperidin-4-yl)-[1,1'-biphenyl]-3-yl)acetamide. A solution of 2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetamide (152 mg, 0.58 mmol, 1.2 eq),

4-(3-bromophenyl)piperidine hydrochloride (**A**) (135 mg, 0.49 mmol, 1 eq), in anhydrous dioxane (3 mL) with 1M aq K_3PO_4 (1 mL), in a microwave vessel was degassed with argon for 5 min, before addition of $Pd(PPh_3)_4$ (0.024mmol, 28 mg, 5%). The reaction was degassed again for a further 5 min, then heated at 140 ^oC for 15 min in a microwave. To the resulting reaction Polystyrene bound-DEAM (polystyrene bound diethanolamine, loading = 1.5-2.2 mmol/g, 1 g, ~5 eq) was added and the reaction microwaved again at 100 ^oC for 10 min. Once cooled, the reaction was loaded onto a pre-washed SCX cartridge. The SCX cartridge was washed with dichloromethane (3 x 10 mL) and methanol (3 x 10 mL) before eluting the product with 7 N ammonia in methanol. This was evaporated to give the title compound (96 mg, 67% yield). ^1H NMR 500MHz, $CDCl_3$ δ 1.68-1.78 (m, 2H), 1.88-1.93 (m, 2H), 2.68-2.76 (m, 1H), 2.76-2.82 (m, 2H), 3.21-3.26 (m, 2H), 3.68 (s, 2H), 5.47 (br.s, 2H), 7.24-7.29 (m, 2H), 7.38-7.48 (m, 4H), 7.51-7.57 (m, 2H). $[M+H]^+$ = 295.2

S.1.4.2 Method 2

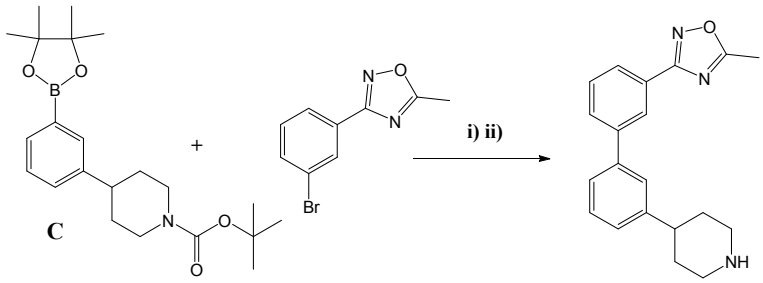


i)$Pd(PPh_3)_4$, Dioxane/1 M aq K_3PO_4, Polystyrene bound-DEAM

1-(6-Morpholino-3'-(piperidin-4-yl)-[1,1'-biphenyl]-3-yl)ethanone. A solution of (3-(piperidin-4-yl)phenyl)boronic acid (**B**) (85 mg, 0.41 mmol, 1.1 eq), 1-(3-bromo-4-morpholinophenyl)ethanone (107 mg, 0.38 mmol, 1 eq), in anhydrous dioxane (3 mL) with 1 M aq K_3PO_4 (1 mL), in a microwave vessel was degassed with argon for 5

min, before addition of $Pd(PPh_3)_4$ (0.021 mmol, 24 mg, 5%). The reaction was degassed again for a further 5 min, then heating at 140 ^oC for 15 min in a microwave. To the resulting reaction polystyrene bound-DEAM (DEAM = diethanolamine, loading = 1.5-2.2 mmol/g, 1 g, ~5 eq) was added and the reaction microwaved again at 100 ^oC for 10 min. Once cooled, the reaction was loaded onto a pre-washed SCX cartridge. The SCX cartridge was washed with dichloromethane (3 x 10 mL) and methanol (3 x 10 mL) before eluting the product with 7 N ammonia in methanol. This was evaporated to give the title compound (128 mg, 93% yield). ^1H NMR 500MHz, $CDCl_3$ δ 1.42-1.52 (m, 2H), 1.64-1.69 (m, 2H), 2.36 (s, 3H), 2.42-2.58 (m, 2H), 2.68-2.70 (m, 4H), 3.37-3.40 (m, 4H), 6.80 (d, *J* = 8.50, 1H), 6.98 (d, *J* = 7.40, 1H), 7.14-7.21 (m, 2H), 7.30 (s, 1H), 7.61 (s, 1H), 7.69 (d, *J* = 8.48, 1H). $[M+H]^+$ = 365.22

S.1.4.3 Method 3



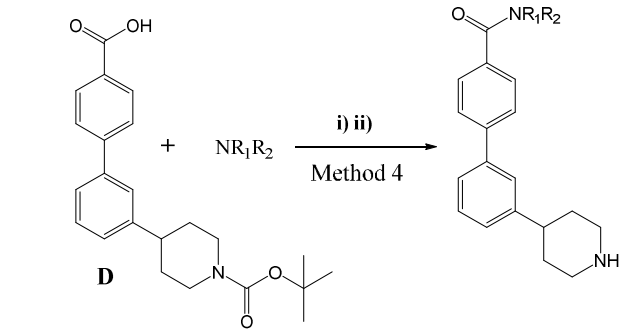
i)$Pd(PPh_3)_4$, Dioxane/1 M aq K_3PO_4, Polystyrene bound-DEAM, ii)TFA/dichloromethane

5-Methyl-3-(3'-(piperidin-4-yl)-[1,1'-biphenyl]-3-yl)-1,2,4-oxadiazole. A solution of tert-butyl 4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)piperidine-1-carboxylate (**C**) (90 mg, 0.23 mmol, 1.1 eq), 3-(3-bromophenyl)-5-methyl-1,2,4-oxadiazole (50 mg, 0.21 mmol, 1 eq), in anhydrous acetonitrile (3 mL) with 1 M aq

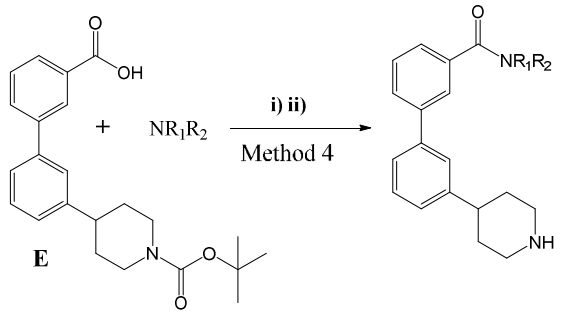
K_3PO_4 (1 mL), in a microwave vessel was degassed with argon for 5 min, before addition of $Pd(PPh_3)_4$ (0.021 mmol, 24 mg, 5%). The reaction was degassed again for a further 5 min, then heated at 140 ^oC for 15 min in a microwave. To the resulting reaction Polystyrene bound-DEAM (loading = 1.5-2.2 mmol/g, 1 g, ~5 eq) was added and the reaction microwaved again at 100 ^oC for 10 min. Reaction was filtered, evaporated *in vacuo*, and the residue dissolved in dichloromethane and treated with triflouroacetic acid (TFA, 1 mL), stirred at RT for 1 h before concentrating *in vacuo*. The resulting residue was loaded onto a pre-washed SCX cartridge. The SCX cartridge was washed with dichloromethane (3 x 10 mL) and methanol (3 x 10 mL) before eluting the product with 7 N ammonia in methanol. This was evaporated to give the title compound (44 mg, 60% yield). ^1H NMR 500MHz, $CDCl_3$ δ 1.70-1.79 (m, 2H), 1.89-1.94 (m, 2H), 2.53 (s, 3H), 2.71-2.75 (m, 1H), 2.77-2.84 (m, 2H), 3.23-3.28 (m, 2H), 3.52 (s, 1H), 7.28-7.31 (m, 1H), 7.42-7.47 (m, 1H), 7.50-7.54 (m, 2H), 7.60-7.64 (m, 1H), 7.827.85 (m, 1H), 8.10-8.12 (m, 1H), 8.36-8.38 (m, 1H). [M+H] = 320.2

S2. Amidation Array Chemistry

S.2.1 Amides (directly linked)



OR

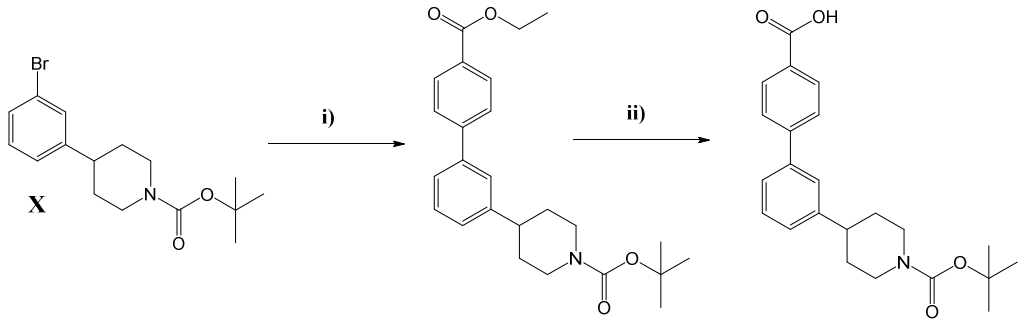


i)PS-CDI, HOBt, MeCN, NR_1R_2 **ii)** TFA, dichloromethane.

Scheme 2. Amide (Directly Linked) Array Chemistry

S.2.2 Experimental (Method 4)

Note: D and E carboxylic acids were synthesised using the same procedure.



i)(4-(ethoxycabonyl)phenyl)boronic acid, dioxane/1 M aq K_3PO_4, $Pd(PPh_3)_4$ **ii)** LiOH, H_2O/methanol, THF

*tert-Butyl 4-(4'-(ethoxycarbonyl)-[1,1'-biphenyl]-3-yl)piperidine-1-carboxylate (**D ethyl ester**)*. tert-Butyl 4-(3-bromophenyl)piperidine-1-carboxylate (**X)** (1.54 g, 4.5 mmol, 1 eq), (4-(ethoxycabonyl)phenyl)boronic acid (1.3 g, 6.75 mmol, 1.5 eq), in anhydrous dioxane (3 mL), and 1 M aq K_3PO_4 (2 mL) were combined in a microwave vessel and argon bubbled through the mixture for 5 min. $Pd(PPh_3)_4$ (260mg, 0.23mmol, 5%), was added and the reaction degassed again for a further 5 min before microwaving at 140^{o}C for 15 min. The resulting solution was extracted into dichloromethane, washing with sat. aq. $NaHCO_3$, and passed through a phase separation cartridge, the filtrate was absorbed onto silica and purified by flash column chromatography running a gradient from 0% ethyl acetate/hexane to 20% ethyl acetate/hexane, to give the named compound as a clear oil (1.7 g, 92% yield). ^{1}H NMR 500MHz, $CDCl_3$ δ 1.45 (t, *J* = 7.36, 3H), 1.52 (s, 9H), 1.67-1.76 (m, 2H), 1.88-1.93 (m, 2H), 2.72-2.79 (m, 2H), 2.82-289 (m, 2H), 4.26 (m, 2H), 4.43 (q, *J* = 7.02,

2H), 7.25-7.28 (m, 1H), 7.42-7.45 (m, 1H), 7.47-7.51 (m, 2H), 7.66-7.68 (m, 2H), 8.12-8.15 (m, 2H). $[M+H]^+$ = 410.2

*3'-(1-(tert-Butoxycarbonyl)piperidin-4-yl)-[1,1'-biphenyl]-4-carboxylic acid (**D**)* . tert-Butyl 4-(4'-(ethoxycarbonyl)-[1,1'-biphenyl]-3-yl)piperidine-1-carboxylate (**D ethyl ester**) (1.7 g, 4.15 mmol, 1 eq) was suspended in a mixture of THF (10 mL), H_2O/methanol (1:1, 10 mL), to this lithium hydroxide (400 mg, 16.6 mmol, 4 eq) was added and the mixture stirred at room temperature for 16 h. The reaction was concentrated *in vacuo*, and acidified to pH 3 with 1 N aq. HCl, then extracted into ethyl acetate (x3), layers separated and the organic layer dried over $MgSO_4$, filtered and evaporated *in vacuo* to give an off-white solid, which was washed with diethyl ether to give **D** as a white solid (1.3 g, 82% yield). ^{1}H NMR 500MHz, MeOD δ 1.50 (s, 9H), 1.64-1.74 (m, 2H), 1.88-1.92 (m, 2H), 2.80-2.96 (m, 3H), 4.23-4.28 (m, 2H), 7.28 (d, *J* = 7.75, 1H), 7.52 (t, *J* = 7.75, 1H), 7.52-7.56 (m, 2H), 7.72-7.75 (m, 2H), 8.10-8.12 (m, 2H). $[M+H]^+$ = 382.2

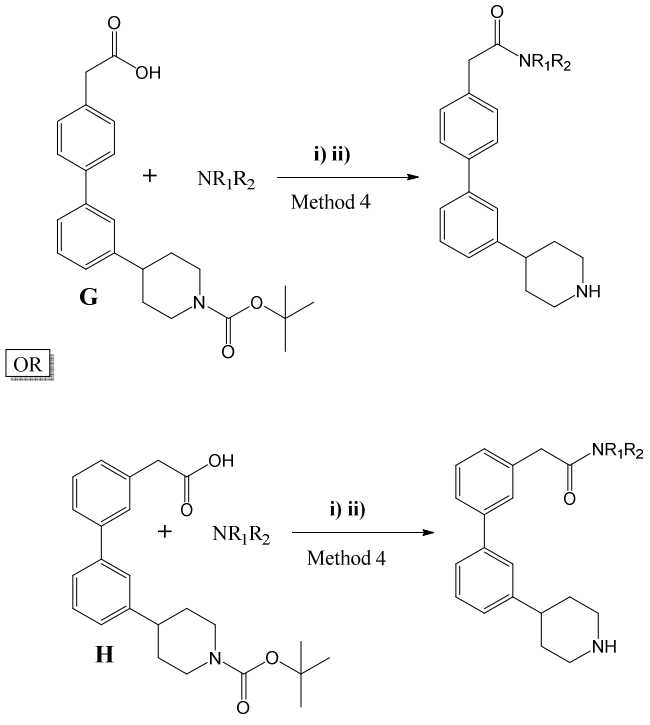
*tert-Butyl 4-(3'-(methoxycarbonyl)-[1,1'-biphenyl]-3-yl)piperidine-1-carboxylate (**E methyl ester**)*. Prepared using tert-butyl 4-(3-bromophenyl)piperidine-1-carboxylate (3 g, 8.8 mmol, 1 eq), and (3-(ethoxycabonyl)phenyl)boronic acid (1.71 g, 8.8 mmol, 1 eq), in DMF:H_2O (1:1, 4 mL), $Pd(PPh_3)_4$ (120 mg) and K_3PO_4 (1.87 g, 8.8 mmol, 1 eq) according to the procedure outlined above to give the title compound as a gum (3.22 g, 89% yield). ^{1}H NMR 500 MHz, $CDCl_3$ δ 1.49-1.45 (m, 3H), 1.59 - 1.57 (s, 9H), 1.76-1.72 (m, 2H), 1.94 - 1.87 (m, 2H), 2.78 - 2.73 (m, 1H), 2.86 - 2.82 (m, 3H), 4.40-4.30 (m, 2H), 4.45 (d, *J* = 6.1 Hz, 2H), 7.43 (dd, *J* = 6.7, 6.7 Hz, 1H), 7.55 - 7.46 (m, 4H), 7.79 (d, *J* = 6.9 Hz, 1H), 8.05 (d, *J* = 7.6 Hz, 1H), 8.28 (s, 1H).

[M+H] = 354.1574 (product – ^{t}Bu)

*3'-(1-(tert-Butoxycarbonyl)piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylic acid (**E**).*

Prepared using tert-butyl 4-(3'-(methoxycarbonyl)-[1,1'-biphenyl]-3-yl)piperidine-1-carboxylate (3.22 g, 7.8 mmol, 1 eq), lithium hydroxide (750 mg, 31.2 mmol, 4 eq), in THF (15 mL) according to the procedure outlined above to give **E** as a white foam. (2.9 g, 97% yield). ^{1}H NMR 500MHz, $CDCl_3$ δ 1.52 (s, 9H), 1.68-1.74 (m, 2H), 1.90-1.93 (m, 2H), 2.72-2.90 (m, 2H), 2.90-2.93 (br. s, 2H), 4.18-4.27 (br. s, 2H), 7.30-7.32 (m, 1H), 7.39-7.52 (m, 4H), 7.92 (d, *J* = 4.5, 1H), 8.16 (d, *J* = 4.6, 1H), 8.40 (s, 1H). $[M+H]^{+}$ = 326.1284 (product-^{t}Bu)

S.2.3 Amides (homologated)

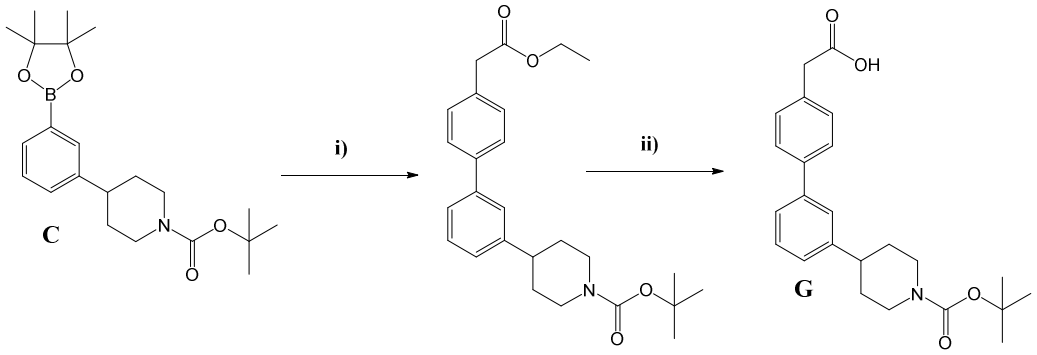


i)Polystyrene bound-CDI, HOBt, MeCN, NR_1R_2 **ii)** TFA, dichloromethane.

Scheme 4. Homologated Amide Array Intermediate Chemistry

S.2.4 Experimental (Method 4)

Note: Intermediates G and H were all made using the same chemistry



i) ethyl 2-(4-bromophenyl)acetate, dioxane/1M aq K_3PO_4, $Pd(PPh_3)_4$ **ii)** LiOH, H_2O/methanol, THF

*tert-Butyl 4-(4'-(2-ethoxy-2-oxoethyl)-[1,1'-biphenyl]-3-yl)piperidine-1-carboxylate **(G ethyl ester)***. tert-Butyl 4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)piperidine-1-carboxylate (**C**) (2 g, 5.17 mmol, 1 eq), ethyl 2-(4-bromophenyl)acetate (1.52 g, 6.24 mmol, 1.2 eq), in anhydrous dioxane (5 mL) were combined and degassed with argon for 5 min in a microwave vessel. $Pd(PPh_3)_4$ (300 mg, 0.26 mmol, 5%) was added and the reaction degassed again before microwaving at 140 ^oC for 15 min. Reaction partitioned between dichloromethane and aq. sat. $NaHCO_3$, and the organic layer dried over $MgSO_4$ before absorbing onto silica and purifying by flash column chromatography, running a gradient from 0% ethyl acetate/hexane to 30% ethyl acetate/hexane to give the title compound as a clear oil (1.92 g, 88% yield). ^1H NMR 500MHz, $CDCl_3$ δ 1.27-1.31 (m, 3H), 1.51 (s, 9H), 1.65-1.75 (m, 2H), 1.86-1.92 (m, 2H), 2.74 (tt, J = 3.53, 12.22, 1H), 2.79-2.89 (m, 2H), 3.68 (s, 2H), 4.20 (q, J = 7.44, 2H), 4.23-4.35 (m, 2H), 7.20-7.22 (m, 1H), 7.37-7.47 (m, 5H), 7.55-7.58 (m, 2H).

*2-(3'-(1-(tert-utoxycarbonyl)piperidin-4-yl)-[1,1'-biphenyl]-4-yl)acetic acid (**G**)* .

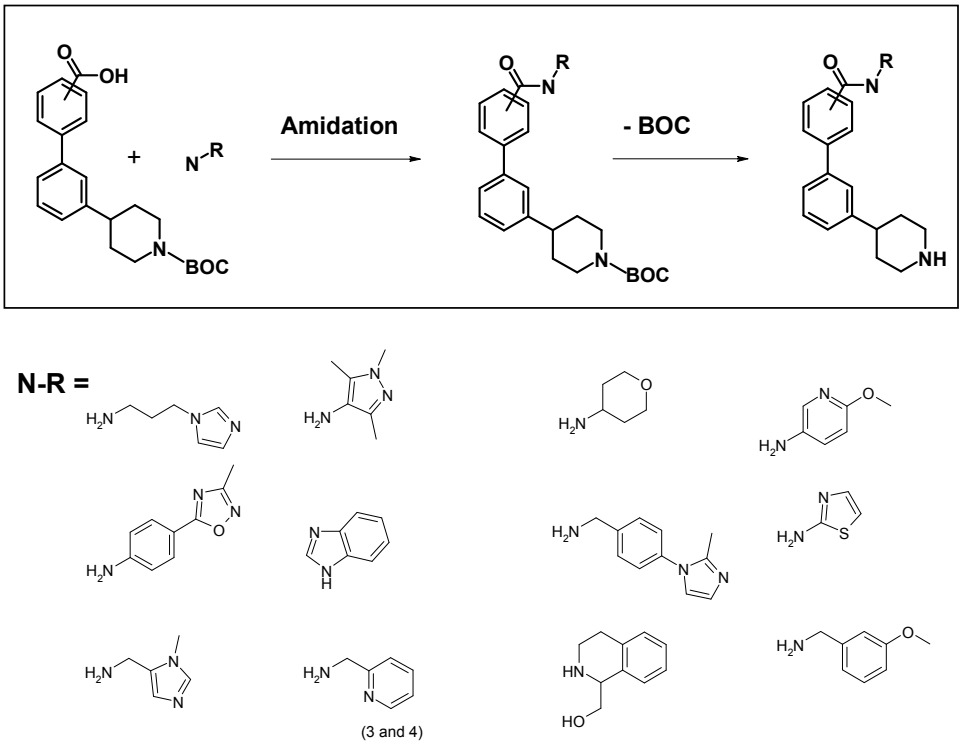
LiOH (435 mg, 18.2 mmol, 4 eq) was added to a solution of tert-butyl 4-(4'-(2-ethoxy-2-oxoethyl)-[1,1'-biphenyl]-3-yl)piperidine-1-carboxylate (1.92 g, 4.54 mmol, 1 eq) in a mixture of THF (10 mL) and H_2O/methanol (1:1, 10 mL), stirring at RT for 16 h. Reaction evaporated *in vacuo*, and the residue acidified to Ph 3 with 1 N HCl before extracting into ethyl acetate. The organic layer was dried over $MgSO_4$, filtered and evaporated to give (**G**) as a white solid (1.5 g, 84% yield). ^{1}H NMR 500MHz, $CDCl_3$ δ 1.52 (s, 9H), 1.65-1.74 (m, 2H), 1.87-1.92 (m, 2H), 2.70 (tt, *J* = 3.64, 12.17, 1H), 2.80-2.89 (m, 2H), 3.73 (s, 2H), 4.23-4.35 (m, 2H), 7.20-7.23 (m, 1H), 7.37-7.46 (m, 5H), 7.56-7.59 (m, 2H).

*tert-Butyl 4-(3'-(2-methoxy-2-oxoethyl)-[1,1'-biphenyl]-3-yl)piperidine-1-carboxylate (**H methyl ester**)*. Prepared using tert-butyl 4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)piperidine-1-carboxylate (**C**) (2 g, 5.17 mmol, 1 eq), methyl 2-(3-bromophenyl)acetate (1.43 g, 6.24 mmol, 1.2 eq), in anhydrous dioxane (5 mL) with $Pd(PPh_3)_4$ (300 mg, 0.26 mmol, 5%), and aq. 1 M K_3PO_4 (5.2 mL) according to the protocol outlined in above, to give the title compound as a clear oil, (1.63 g, 77% yield). ^{1}H NMR 500MHz, $CDCl_3$ δ 1.29 (s, 3H), 1.51 (s, 9H), 1.66-1.76 (m, 2H), 1.87-1.92 (m, 2H), 2.74 (tt, *J* = 3.58, 12.27, 1H), 2.81-2.89 (m, 2H), 3.74 (s, 2H), 4.23-4.36 (m, 2H), 7.20-7.23 (m, 1H), 7.28-7.31 (m, 1H), 7.39-7.47 (m, 4H), 7.50-7.52 (m, 2H).

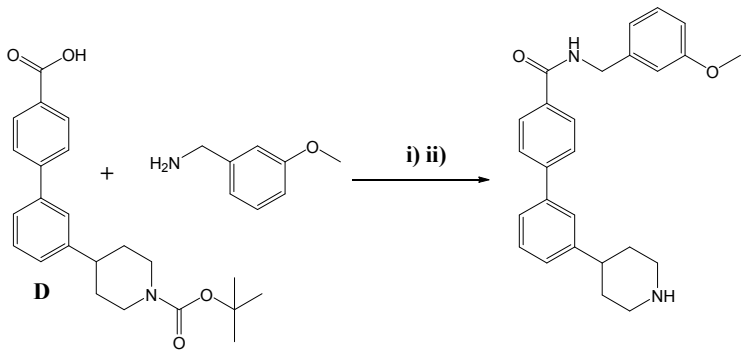
*2-(3'-(1-(tert-Butoxycarbonyl)piperidin-4-yl)-[1,1'-biphenyl]-3-yl)acetic acid (**H**)*. Prepared using tert-butyl 4-(3'-(2-methoxy-2-oxoethyl)-[1,1'-biphenyl]-3-

yl)piperidine-1-carboxylate (1.63 g, 3.98 mmol, 1 eq), and lithium hydroxide (382 mg, 15.9 mmol, 4 eq) in THF and H_2O:methanol according to the procedure above for **G**, to give the title compound as a white solid, (1.47 g, 82% yield). ^{1}H NMR 500MHz, $CDCl_3$ δ 1.51 (s, 9H), 1.66-1.75 (m, 2H), 1.87-1.92 (m, 2H), 2.74 (tt, *J* = 3.42, 12.20, 1H), 2.80-2.88 (m, 2H), 3.75 (s, 2H), 4.24-4.33 (m, 2H), 7.20-7.23 (m, 1H), 7.30-7.32 (m, 1H), 7.37-7.46 (m, 4H), 7.51-7.53 (m, 2H).

S.2.5 Amidation Array Examples



S.2.6 Representative Amidation Reaction



i) Polystyrene bound-CDI, HOBt, MeCN, NR_1R_2, **ii)** TFA, dichloromethane.

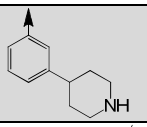
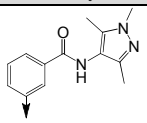
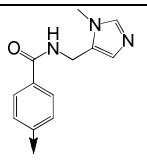
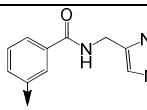
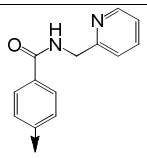
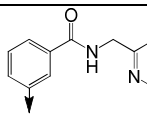
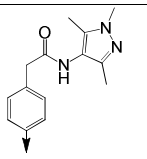
Sauer D.R.; Kalvin D.; PhelanK.M.; Microwave-assisted synthesis utilizing supported reagents; a rapid and efficient acylation procedure, *Org Lett*. 2003, **5**, p4721-4724

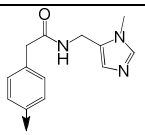
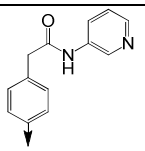
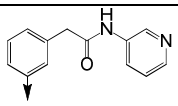
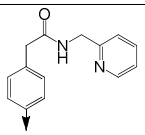
N-(3-methoxybenzyl)-3'-(piperidin-4-yl)-[1,1'-biphenyl]-4-carboxamide. 3'-(1-(tert-butoxycarbonyl)piperidin-4-yl)-[1,1'-biphenyl]-4-carboxylic acid (**D**) (90 mg, 0.24 mmol, 1.1 eq), Polystyrene bound-CDI (CDI = carbodiimide, 1.25 mmol/g loading, 384 mg, 0.48 mmol, 2 eq), HOBt (32 mg, 0.24 mmol, 1eq), (3-methoxyphenyl)methanamine (30 mg, 0.22 mmol, 1 eq) in MeCN (3 mL) was combined in a microwave vessel and heated at 140 ^{o}C for 10 min in a microwave. The reaction was poured onto a SPE cartridge containing 2 g of Si-CO_3 (Silica bound carbonate), washing through with MeCN/methanol (1:1, 15 mL), to gave a pure amide BOC protected intermediate. The filtrate was evaporated *in vacuo,* the residue dissolved in dichloromethane (5 mL) before adding trifluoroacetic acid (10 eq) and allowing the reaction to stir at room temperature for 3 h. The resulting solution was evaporated *in vacuo* before dissolving in dichloromethane (~5 mL) and loading onto a pre-washed SCX cartridge. The SCX cartridge was washed with dichloromethane (3 x 10 mL) and methanol (3 x 10 mL) before eluting the product with 7 N ammonia in methanol. This was evaporated to give (**D**) (73 mg, 76% yield). ^{1}H NMR 500MHz, $CDCl_3$ δ 1.69-1.79 (m, 2H), 1.88-1.94 (m, 2H), 2.69-2.76 (1H), 2.77-2.83 (m, 2H), 3.22-3.27 (m, 2H), 3.52 (s, 2H), 3.84 (s, 3H), 4.68 (s, 2H), 6.45 (br. s 1H), 6.86-6.90 (m, 1H), 6.94-6.96 (m, 2H), 7.27-7.33 (m, 2H), 7.40-7.50 (m, 3H), 7.66-7.70 (m, 2H), 7.87-7.91 (m, 2H). $[M+H]^+$ = 401.22

S.2.7 Yields, NMRs, m/z of directly linked and homologated amide series.

Table 1 of publication

Note: all compounds made using the corresponding directly linked or homologated carboxylic acid and commercially available amines using METHOD 4.

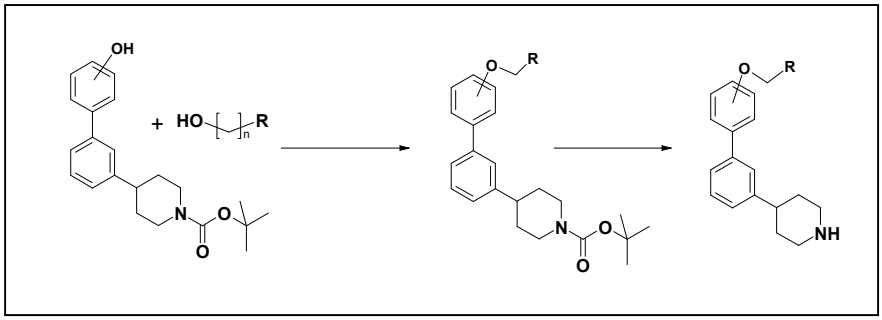
		**NMR and m/z**	**Yield (purity)**
3		^{1}H NMR 500MHz, DMSO, δ 1.61-1.71 (m, 2H), 1.74-1.80 (m, 2H), 2.01 (s, 3H), 2.09 (s, 3H), 2.63-2.74 (m, 3H), 3.06-3.11 (m, 2H), 3.68 (s, 3H), 7.23-7.25 (m, 1H), 7.38-7.41 (m, 1H), 7.56-7.62 (m, 3H), 7.86-7.90 (m, 1H), 7.92-7.96 (m, 1H), 8.20 (s, 1H), 9.65 (s, 1H). [M+H] = 389.2 HRMS [M+H]+ calculated for $C_{24}H_{29}N_4O_1$ = 389.2336, found = 389.2337	82% (96%)
4		^{1}H NMR 500 MHz, $CDCl_3$ δ 1.78 - 1.68 (m, 2H), 1.94 - 1.88 (m, 2H), 2.83 - 2.70 (m, 3H), 3.30-3.25 (m, 2H), 3.69 (s, 3H), 4.74 (d, *J* = 5.5 Hz, 2H), 5.33 (s, 1H), 6.30 (dd, *J* = 5.2, 5.2 Hz, 1H), 7.08 (s, 1H), 7.50 - 7.42 (m, 4H), 7.68 (d, *J* = 8.5 Hz, 2H), 7.86 (d, *J* = 8.5 Hz, 2H). HRMS [M+H]+ calculated for $C_{23}H_{27}N_4O_1$ = 375.2179, found = 375.2192	57% (95%)
5		^{1}H NMR 500MHz, DMSO, δ 1.60-1.70 (m, 2H), 1.75-1.81 (m, 2H), 2.61-2.72 (m, 3H), 3.08-3.11 (m, 2H), 3.62 (s, 3H), 4.5 (d, *J* = 1.8, 2H), 6.85 (s, 1H), 7.23-7.25 (m, 1H), 7.36-7.38 (m, 1H), 7.52-7.58- (m, 4H), 7.78-7.86 (m, 2H), 8.10 (s, 1H), 8.98-9.00 (m, 1H). [M+H] = 375.2. HRMS [M+H]+ calculated for $C_{23}H_{27}N_4O_1$ = 375.2179, found = 375.2187	83% (98%)
6		^{1}H NMR 500MHz, MeOD δ 1.73-1.82 (m, 2H), 1.89-1.94 (m, 2H), 2.77-2.84 (m, 3H), 3.18-3.24 (m, 2H), 4.74 (s, 2H), 7.29-7.37 (m, 2H), 7.41-7.58 (m, 2H), 7.75-7.78 (m, 2H), 7.83-7.87 (m, 1H), 7.99-8.02 (m, 2H), 8.52-8.54 (m, 1H). [M+H] = 372.22	32% (90%)
7		^{1}H NMR 500MHz, DMSO, δ 1.57-1.72 (m, 4H), 2.60-2.69 (m, 3H), 3.01-3.08 (m, 2H), 4.52 (s, 2H), 7.16-7.18 (m, 2H), 7.24-7.27 (m, 1H), 7.31-7.34 (m, 1H), 7.43-7.47 (m, 3H), 7.64-7.67 (m, 1H), 7.72-7.74 (m, 1H), 7.82-7.85 (m, 1H), 8.08 (s, 1H), 8.42 (d, *J* = 1.4, 1H), 9.17-9.19 (m, 1H). [M+H] = 372.4 HRMS [M+H]+ calculated for $C_{24}H_{26}N_3O$ = 372.207039,found = 372.207957	55% (98%)
8		^{1}H NMR 500MHz, DMSO δ 1.68-1.82 (m, 4H), 1.91 (s, 3H), 1.98 (s, 3H), 2.71-2.80 (m, 3H), 3.58 (s, 3H), 3.59 (s, 2H), 7.19 (d, *J* = 7.79, 1H), 7.35 (t, *J* = 7.79, 1H), 7.38 (d, *J* = 8.16, 2H), 7.44-7.48 (m, 2H), 7.60 (d, *J* = 8.16, 2H), 9.23 (s, 1H). [M+H] = 403.28	15% (90%)

9		^{1}H NMR 500MHz, DMSO δ 1.79-1.93 (m, 4H), 2.77-2.84 (m, 1H), 2.84-2.94 (m, 2H), 3.22-3.34 (m, 2H), 3.46 (s, 2H), 3.50 (s, 3H), 4.26 (d, *J* = 4.89, 2H), 6.77 (s, 1H), 7.18-7.21 (d, *J* = 7.17, 1H), 7.32-7.37 (m, 3H), 7.44-7.48 (m, 2H), 7.54 (s, 1H), 7.57 (d, *J* = 7.82, 2H), 8.46 (s, 1H). [M+H] = 389.27	6% (90%)
10		^{1}H NMR 500MHz, $CDCl_3$ δ 1.67-1.77 (m, 2H), 1.89-1.93 (m, 2H), 2.72-2.79 (m, 1H), 2.82-2.89 (m, 2H), 3.85 (s, 2H), 4.26-4.35 (m, 2H), 7.16 (br. s, 1H), 7.23-7.28 (m, 2H), 7.42-7.50 (m, 5H), 7.64-7.67 (m, 2H), 8.13-8.16 (m, 1H), 8.37 (dd, *J* = 1.43, 4.84, 1H), 8.47 (d, *J* = 2.54, 1H). [M+H] = 372.24	5% (90%)
11		^{1}H NMR 500MHz, $CDCl_3$ δ 1.74 (qd, *J* = 4.10, 12.46, 2H), 2.73 (tt, *J* = 3.66, 12.20, 1H), 2.80 (td, *J* = 12.2, 2.44, 2H), 3.22-3.27 (m, 2H), 3.87 (s, 2H), 7.21-7.23 (m, 1H), 7.26-7.28 (m, 1H), 7.34-7.36 (m, 1H), 7.42-7.49 (m, 2H), 7.52 (t, *J* = 7.73, 1H), 7.58-7.63 (m, 2H), 8.13-8.16 (m, 1H), 8.36 (dd, *J* = 1.42, 4.78, 1H), 8.45-8.46 (s, 1H). [M+H] = 372.21	36% (90%)
12		^{1}H NMR 500MHz, $CDCl_3$ δ 1.68-1.73 (m, 4H), 2.48-2.54 (m, 1H), 2.63-2.69 (m, 2H), 3.11-3.15 (m, 2H), 3.51 (s, 2H), 4.38 (d, *J* = 4.97, 2H), 6.74 (s, 1H), 6.98-7.07 (m, 5H), 7.38 (d, *J* = 7.94, 2H), 7.46 (t , *J* = 7.67, 1H), 8.31 (d, *J* = 4.98, 1H). [M+H] = 386.26	16% (85%)

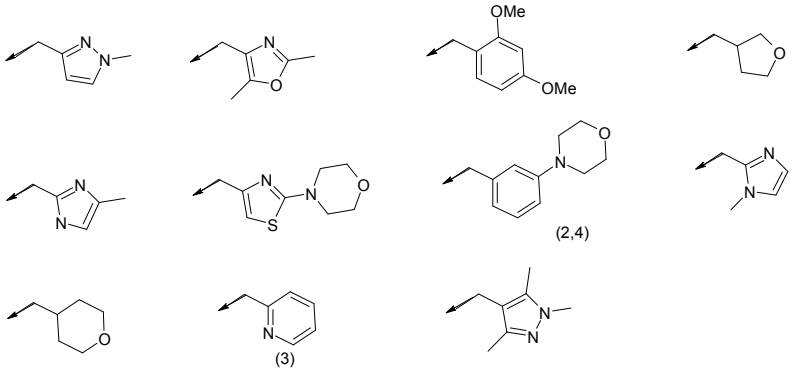
S3. Mitsunobu Array Chemistry

Note: all of the reagents were commercially available and the 2-, 3-, and 4-hydroxy intermediates were made using the same procedure.

S.3.3 Mitsunobu Array Summary



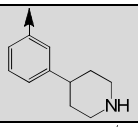
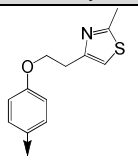
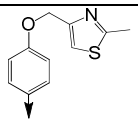
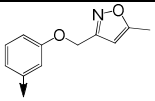
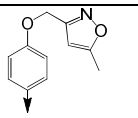
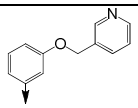
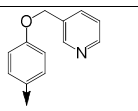
R =

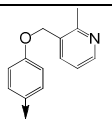


S.3.5 Yields, NMRs, m/z of Mitsunobu Reactions.

Table 1 of publication

Note: all compounds made using the corresponding phenol intermediate and commercially available alcohols using the experimental outlined above.

		NMR and m/z	Yield (purity)
18		^{1}H NMR 500MHz, DMSO, δ 1.88-1.96 (m, 4H), 2.71 (s, 3H), 2.82-3.08 (m, 3H), 3.17 (t, *J* = 8.6, 2H), 3.31-3.38 (m, 2H), 4.31 (t, *J* = 8.8, 2H), 7.06-7.08 (m, 2H), 7.16-7.19 (m, 1H), 7.32-7.48 (m, 4H), 7.56-7.59 (m, 2H). [M+H] = 379.2227 HRMS [M+H]+ calculated for $C_{23}H_{27}N_2OS$ = 379.183861, found = 379.184038	46% (99%)
19		^{1}H NMR 500MHz, DMSO, δ 1.83-1.97 (m, 4H), 2.66 (s, 3H), 2.84-2.91 (m, 1H), 2.92-3.01 (m, 2H), 3.31-3.34 (m, 2H), 5.12 (s, 2H), 7.11-7.18 (m, 3H), 7.39-7.48 (m, 3H), 7.58-7.62 (m, 3H). [M+H] = 365.1629 HRMS [M+H]+ calculated for $C_{22}H_{25}N_2OS$ = 365.168211, found = 365.166674	84% (98%)
20		^{1}H NMR 500MHz, $CDCl_3$ δ 1.70-1.79 (m, 2H), 2.46 (s, 3H), 2.72 (tt, *J* = 3.78, 12.02, 1H), 2.80 (td, *J* = 2.40, 12.37, 2H), 3.24-3.28 (m, 2H), 5.20 (s, 2H), 6.16 (s, 1H), 6.97 (dd, *J* = 0.87, 8.26, 1H), 7.22-7.25 (m, 3H), 7.36-7.46 (m, 4H). [M+H] = 348.2	26% (92%)
21		^{1}H NMR 500MHz, DMSO δ 1.82-1.98 (m, 4H), 2.88-2.91 (m, 1H), 2.92-3.03 (m, 2H), 3.33-3.38 (m, 2H), 5.19 (s, 2H), 3.36 (s, 1H), 7.11-7.13 (m, 2H), 7.16-7.19 (m, 1H), 7.38-7.46 (m, 3H), 7.58-7.61 (2H). [M+H] = 349.1833 HRMS [M+H]+ calculated for $C_{22}H_{25}N_2O_2$ = 349.191054, found = 349.190831	23% (92%)
22		^{1}H NMR 500MHz, $CDCl_3$ δ 1.70-1.81 (m, 2H), 1.89-1.94 (m, 2H), 2.72 (tt, *J* = 12.00, 3.54, 1H), 2.80 (td, *J* = 12.29, 2.39, 2H), 3.23-3.27 (m, 2H), 5.17 (s, 2H), 6.97-7.00 (m, 1H), 7.22-7.26 (m, 3H), 7.36-7.46 (m, 5H), 7.83-7.86 (m, 1H), 8.62 (dd, *J* = 4.91, 1.57, 1H), 8.74 (d, *J* = 1.67, 1H). [M+H] = 345.2 HRMS [M+H]+ calculated for $C_{23}H_{25}N_2O_1$ = 345.1961, found = 345.198	47% (95%)
23		^{1}H NMR 500MHz, MeOD, δ 1.81-1.89 (m, 2H), 1.95-2.01 (m, 2H), 2.79-2.84 (m, 1H), 2.91-2.99 (m, 2H), 3.34-3.38 (m, 2H), 2.51 (s, 2H), 7.08-7.10 (m, 2H), 7.18-7.20 (m, 1H), 7.32-7.48 (m, 4H), 7.52 (m, 2H), 7.95 (d, *J* = 3.2, 1H), 8.52 (d, *J* = 1.8, 1H), 8.68 (s, 1H). [M+H] = 345.2 HRMS [M+H]+ calculated for $C_{23}H_{25}N_2O_1$ = 345.1961, found = 345.197	30% (96%)

24		^{1}H NMR 500MHz, $CDCl_3$, δ 1.68-1.84 (m, 4H), 2.42 (s, 3H), 2.42-2.48 (m, 1H), 2.56-2.64 (m, 2H), 3.05-3.11 (m, 2H), 4.90 (s, 2H), 6.85-6.89 (m, 2H), 6.99-7.04 (m, 2H), 7.12-7.21 (m, 3H), 7.33-7.36 (m, 2H), 7.52-7.56 (m, 1H), 8.31 (s, 1H). [M+H] = 359.2028 HRMS [M+H]+ calculated for $C_{24}H_{27}N_2O_1$ = 359.2118, found = 359.2137	58% (95%)

S.4 X-ray Crystallography Statistics

Data measurement and refinement statistics are shown below.

AfNMT	AfNMT:**24**	AfNMT:**29**	AfNMT:**48**	AfNMT:**49**
PDB code	5T5U	5T6C	5T6E	5T6H
	Data Measurement Statistics			
Source	ID14eh1	ID14eh2	ID14eh1	ID14eh1
Space Group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit Cell Dimensions (Å)	a=51.0, b=58.4, c=152.6	a=51.1, b=58.6, c=152.1	a=51.3, b=58.3, c=152.1	a=50.4, b=58.9, c=152.8
Resolution (Å)[a]	42.4-1.80 (1.84-1.80)	38.5-1.90 (1.94-1.90)	48.6-2.30 (2.39-2.30)	50.0-1.80 (1.86-1.80)
Observations	120004	162085	78514	112546
Unique Observations	42412	36493	20819	40875
Rmerge (%)[a,b]	5.1 (26.2)	8.4 (33.5)	8.8 (47.8)	4.1(13.8)
I/σI[a]	12.9 (3.4)	13.8 (4.3)	9.2 (1.9)	30 (7.5)
Completeness (%)[a]	99.0 (96.2)	99.0 (99.4)	99.4 (100)	94.5 (95.7)
Redundancy[a]	2.8 (2.8)	4.4 (3.7)	3.8 (3.8)	2.8 (2.7)
	Refinement Statistics			
Resolution Range (Å)	76.32 – 1.80	38.5 – 1.90	48.6 – 2.30	19.80-1.90
R-factor (R_{work}/R_{free})[c]	20.9/23.8	22.3/25.6	19.9/24.8	17.5/21.3
Number of atoms[d]	3185/63/27/437	3185/63/29/502	3188/63/30/165	3184/63/30/514
Mean B-factor (Å^2)[e]	18/16/22/26	16/15/16/25	41/34/56/42	19/16/26/29
RMS bond length deviation (Å)	0.011	0.022	0.012	0.016
RMS bond angle deviation (°)	1.19	2.09	1.39	1.47

[a] Values in parentheses are for reflections in the highest resolution shell

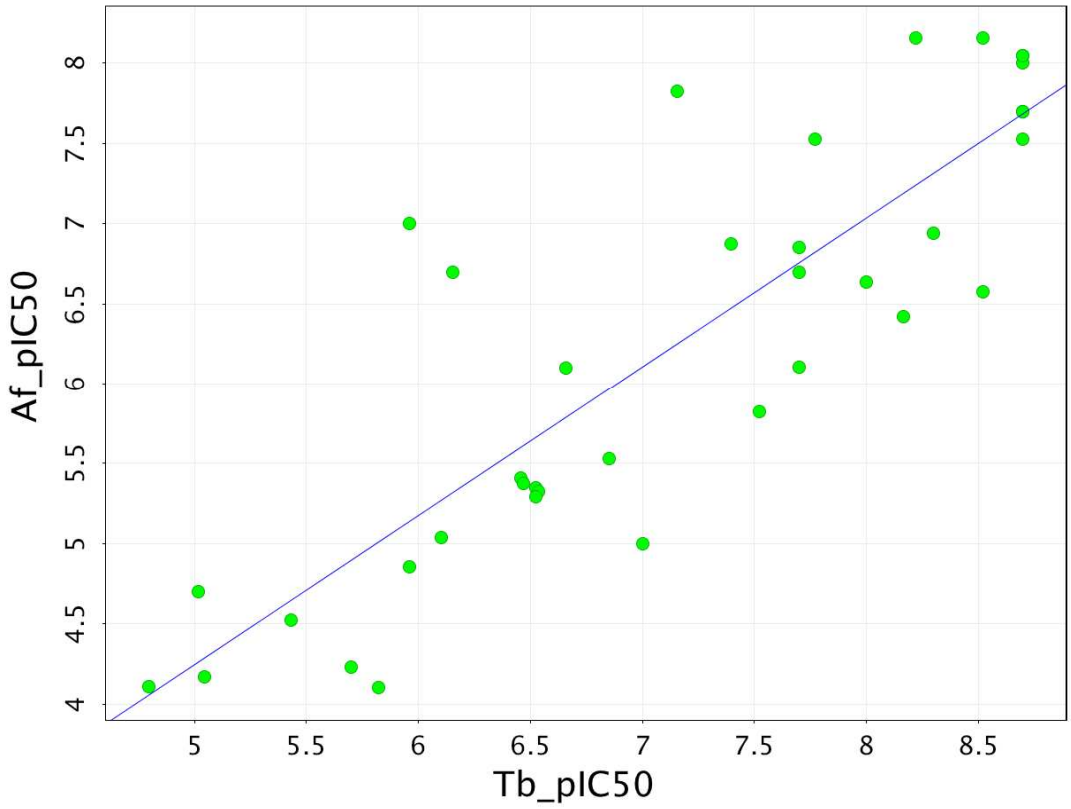
[b] $R_{\text{merge}} = \sum|I - \langle I\rangle|/\sum\langle I\rangle$

[c] R-factor $= \sum|F_{\text{obs}} - F_{\text{calc}}|/\sum|F_{\text{obs}}|$

[d] Number of atoms of protein/cofactor/ligand/water

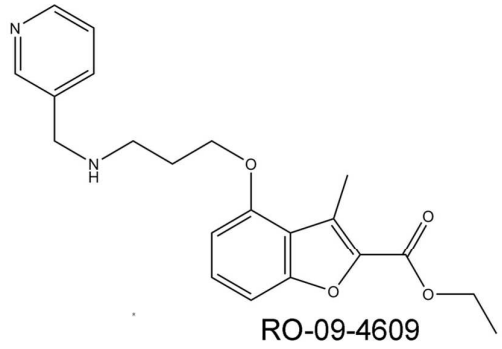
[e] Mean B-factor for protein/cofactor/ligand/water

S.5 Comparison of pIC_{50} data for NMT inhibitors against *Tb*NMT and *Af*NMT

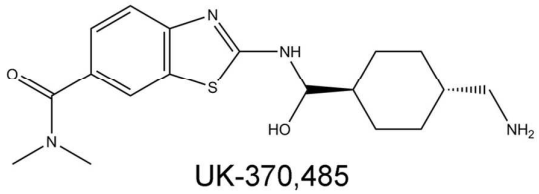


Comparison of pIC_{50} values determined for a selection of NMT inhibitors against *Tb*NMT and *Af*NMT. A linear regression was calculated (blue line) with an R^2 value of 0.73 determined.

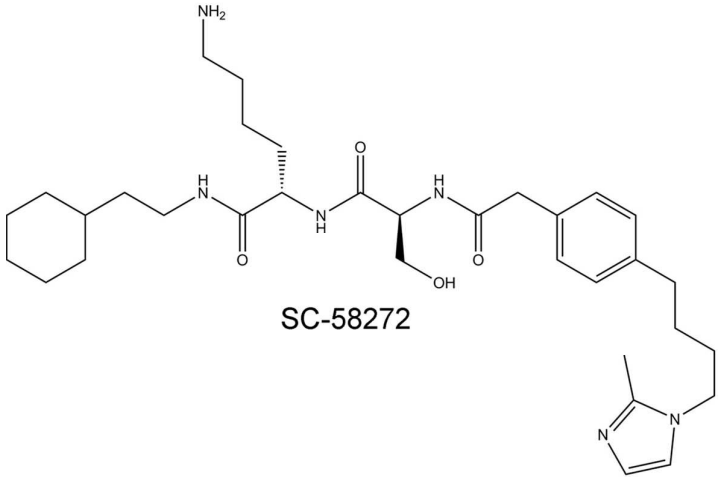
S.6 Molecular structures of known NMT inhibitors



RO-09-4609



UK-370,485



SC-58272