Supporting Information

Development of inhibitors against *Mycobacterium abscessus* tRNA (m^1G37) methyltransferase (TrmD) using fragment-based approaches

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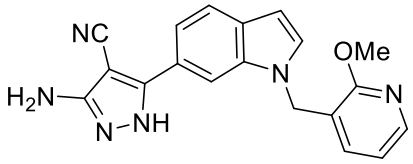
Synthetic Chemistry

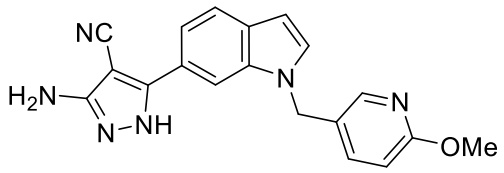
3-Amino-5-(1-((2-methoxypyridin-3-yl)methyl)-1H-indol-6-yl)-1H-pyrazole-4-carbonitrile (26h).



Trichloroacetonitrile (0.122 mL, 1.22 mmol) was added to a suspension of 3-(1-((2-methoxypyridin-3-yl)methyl)-1H-indol-6-yl)-3-oxopropanenitrile **55h** (0.124 g, 0.406 mmol) and sodium acetate (0.167 g, 2.03 mmol) in ethanol (5 mL). The reaction mixture was stirred over 15 hours, then concentrated *in vacuo*, diluted with $NaHCO_3$ solution (25 mL) and extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. The crude residue was dissolved in ethanol (10 mL) and hydrazine monohydrate (0.198 mL, 4.06 mmol) was added. The reaction mixture was heated under reflux for 18 hours, then quenched with excess acetone at room temperature and concentrated *in vacuo*. Purification by flash column chromatography (0 – 70% ethyl acetate in petroleum ether) afforded **26h** (85 mg, 61% yield).

LCMS (ESI+): m/z 345.2 $[M + H]^+$, (ESI-): m/z 343.1 $[M - H]^-$, rt 1.83 minutes, 97%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 12.00 (1H, br s), 8.07 (1H, dd, J = 5.0, 1.6 Hz), 7.93-7.79 (1H, m), 7.76-7.42 (3H, m), 7.15 (1H, dd, J = 7.3, 1.8 Hz), 6.88 (1H, dd, J = 7.2, 5.0 Hz), 6.62-6.48 (1H, m), 6.39 (1H, br s), 5.37 (2H, s), 3.95 (3H, s); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 160.7, 154.6, 151.2, 145.9, 136.8, 135.6, 130.8, 128.5, 125.6, 120.6, 120.1, 117.5, 117.2, 116.7, 107.3, 101.2, 69.6, 53.4, 44.2; ν_{max}/cm^{-1} 3200 (br, N-H), 2918, 2210 (C≡N), 1622, 1599, 1584, 1526, 1500; HRMS (ESI+): m/z calculated for $[C_{19}H_{16}N_6O + Na]^+$ = 367.1278, observed 367.1277.

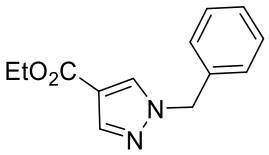
3-Amino-5-(1-((6-methoxypyridin-3-yl)methyl)-1H-indol-6-yl)-1H-pyrazole-4-carbonitrile (26i).



Trichloroacetonitrile (0.114 mL, 1.14 mmol) was added to a suspension of 3-(1-((6-methoxypyridin-3-yl)methyl)-1H-indol-6-yl)-3-oxopropanenitrile **55i** (0.121 g, 0.380 mmol) and sodium acetate (0.156 g, 1.90 mmol) in ethanol (5 mL). The reaction mixture was stirred over 18 hours, then concentrated *in vacuo*, diluted with $NaHCO_3$ solution (20 mL) and extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. The crude residue was dissolved in ethanol (10 mL) and hydrazine monohydrate (0.185 mL, 3.80 mmol) was added. The reaction mixture was heated under reflux for 7 hours, then quenched with excess acetone at room temperature and concentrated *in vacuo*. Purification by flash column chromatography (0 – 100% ethyl acetate in petroleum ether) afforded **26i** (62 mg, 47% yield).

LCMS (ESI+): m/z 345.2 $[M + H]^+$, (ESI-): m/z 343.1 $[M - H]^-$, rt 1.75 minutes, >99%; ^{1}H NMR (500 MHz, CD_3OD) 8.07 (1H, d, J = 2.5 Hz), 7.91-7.87 (1H, m), 7.64 (1H, dd, J = 8.3, 0.5 Hz), 7.55 (1H, dd, J = 8.8, 2.5 Hz), 7.51 (1H, dd, J = 8.2, 1.5 Hz), 7.40 (1H, d, J = 3.2 Hz), 6.72 (1H, dd, J = 8.6, 0.5 Hz), 6.55 (1H, dd, J = 3.1, 0.8 Hz), 5.36 (2H, s), 3.85 (3H, s); ^{13}C NMR (125 MHz, CD_3OD) 165.3, 157.9, 152.6, 146.4, 139.8, 137.3, 131.4, 131.2, 127.9, 124.3, 122.3, 118.9, 117.0, 111.9, 108.9, 103.0, 74.0, 54.2, 47.9; ν_{max}/cm^{-1} 2942, 2211 (C≡N), 1676, 1608, 1573; HRMS (ESI)+: m/z calculated for $[C_{19}H_{16}N_6O + Na]^+$ = 367.1278, observed 367.1278.

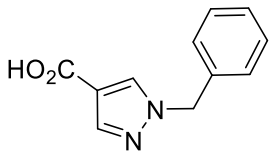
Ethyl 1-benzyl-1H-pyrazole-4-carboxylate (32).



Benzyl bromide (0.85 mL, 7.1 mmol) was added to a suspension of ethyl 4-pyrazolecarboxylate **1** (1.00 g, 7.14 mmol) and potassium carbonate (4.93 g, 35.7 mmol) in acetone (20 mL). The reaction mixture was heated under reflux for 7 hours, then concentrated *in vacuo*. The crude residue was dissolved in water (15 mL) and extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo* to afford **32** (1.63 g, 93% yield).

LCMS (ESI+): m/z 231.3 $[M + H]^+$, rt 2.02 minutes, 94%; ^{1}H NMR (500 MHz, $CDCl_3$) 7.94 (1H, s), 7.85 (1H, s), 7.39-7.31 (3H, m), 7.27-7.22 (2H, m), 5.30 (2H, s), 4.27 (2H, q, J = 7.1 Hz), 1.32 (3H, t, J = 7.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) 163.1, 141.4, 135.4, 132.7, 129.2, 128.7, 128.1, 115.7, 60.3, 56.6, 14.5; spectroscopic data consistent with literature.[1]

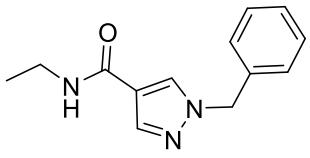
1-Benzyl-1H-pyrazole-4-carboxylic acid (33).



A solution of NaOH (0.287 g, 7.18 mmol) in water (5 mL) was added to a solution of ethyl 1-benzyl-1H-pyrazole-4-carboxylate **32** (1.60 g, 6.53 mmol) in THF (5 mL). The reaction mixture was stirred over 14 hours, then heated to 70 °C over 150 minutes. The reaction mixture was concentrated *in vacuo*, with water (10 mL) and aqueous HCl (3 M, 2.5 mL) added to the resultant residue. The product was extracted into DCM (3 x 30 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo* to afford **33** (1.30 g, 94% yield).

LCMS (ESI-): m/z 201.2 $[M - H]^-$, rt 1.61 minutes, 95%; ^1H NMR (400 MHz, $CDCl_3$) 7.99 (1H, s), 7.90 (1H, s), 7.41-7.30 (3H, m), 7.29-7.22 (2H, m), 5.32 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 168.2, 142.1, 135.1, 133.6, 129.2, 128.8, 128.2, 114.7, 56.7; ^1H NMR spectroscopic data consistent with literature.[2]

1-Benzyl-N-ethyl-1H-pyrazole-4-carboxamide (34).

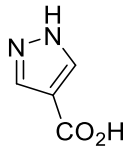


N,*N*-Diisopropylethylamine (0.123 mL, 0.705 mmol) and DMAP (9 mg, 0.07 mmol) were added to a suspension of ethylamine hydrochloride (46 mg, 0.56 mmol) and 1-benzyl-1H-pyrazole-4-carboxylic acid **33** (0.100 g, 0.470 mmol) in DCM (3 mL). The suspension was stirred over 5 minutes, then EDC.HCl (0.135 g, 0.705 mmol) was added. The reaction mixture was stirred over 15 hours, then diluted with $NaHCO_3$ solution (15 mL). The product

was extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 10% DCM in methanol) afforded **34** (90 mg, 84% yield).

LCMS (ESI+): m/z 230.2 $[M + H]^+$, rt 1.66 minutes, >99%; ^1H NMR (400 MHz, CD_3OD) 8.09 (1H, s), 7.90 (1H, s), 7.39-7.22 (5H, m), 5.35 (2H, s), 3.33 (2H, q, J = 7.3 Hz), 1.18 (3H, t, J = 7.3 Hz); ^{13}C NMR (100 MHz, CD_3OD) 164.8, 140.0, 137.5, 132.7, 129.9, 129.3, 128.9, 120.0, 56.9, 35.2, 14.9; ν_{max}/cm^{-1} 3287 (N-H), 3110, 2975, 2934, 1716, 1621 (C=O), 1566, 1531; HRMS (ESI)+: m/z calculated for $[C_{13}H_{15}N_3O + H]^+$ = 230.1288, observed 230.1285.

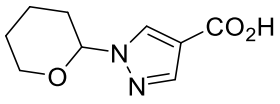
1H-Pyrazole-4-carboxylic acid (35).



Ethyl 4-pyrazolecarboxylate **1** (2.00 g, 14.3 mmol) was added to a solution of NaOH (0.570 g, 14.3 mmol) in water (20 mL). The reaction mixture was heated under reflux for 2 h, then aqueous HCl (37.5% w/v, 2.4 mL) was added dropwise at 0 °C. The resultant precipitate was collected by filtration and washed with water at 0 °C to afford **35** (1.54 g, 96% yield).

^1H NMR (400 MHz, CD_3OD) 8.03 (2H, s); ^{13}C NMR (100 MHz, CD_3OD) 165.3, 136.2, 114.5; ^1H NMR spectroscopic data consistent with literature. [3]

1-(Tetrahydro-2H-pyran-2-yl)-1H-pyrazole-4-carboxylic acid (36).

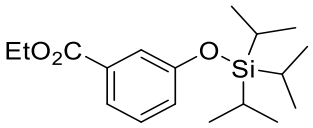


3,4-Dihydro-2H-pyran (0.82 mL, 8.9 mmol) and *p*-toluenesulfonic acid monohydrate (85 mg, 0.45 mmol) were added to a suspension of 1H-pyrazole-4-carboxylic acid **35** (0.500 g,

4.46 mmol) in ethyl acetate (25 mL) and DMF (2.5 mL). The reaction mixture was stirred overnight, then diluted with ethyl acetate (200 mL), washed with LiCl solution (2 x 100 mL) and brine (2 x 200 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 20% methanol in DCM) afforded **36** (0.380 g, 43% yield).

^{1}H NMR (400 MHz, CD_3OD) 8.26 (1H, s), 7.89 (1H, s), 5.43 (1H, dd, J = 9.6, 2.8 Hz), 4.07-4.02 (1H, m), 3.72 (1H, td, J = 10.8, 2.8 Hz), 2.13-2.00 (3H, m), 1.80-1.60 (3H, m); ^{13}C NMR (100 MHz, CD_3OD) 166.3, 142.0, 133.4, 116.7, 89.1, 69.0, 31.6, 26.0, 23.2; ν_{max}/cm^{-1} 3120, 2918, 2861, 1662 (C=O), 1561; HRMS (ESI)-: m/z calculated for $[C_9H_{12}N_2O_3 - H]^-$ = 195.0775, observed 195.0768.

Ethyl 3-((triisopropylsilyl)oxy)benzoate (38).

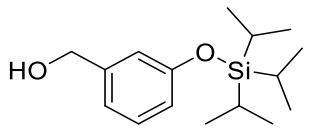


Imidazole (0.82 g, 12 mmol) and triisopropylsilyl chloride (1.9 mL, 9.0 mmol) were added to a solution of ethyl-3-hydroxybenzoate **37** (1.00 g, 6.02 mmol) in DMF (5.5 mL). The reaction mixture was stirred over 2 hours then diluted with ethyl acetate (150 mL), washed with LiCl solution (2 x 200 mL) and brine (2 x 200 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 5% ethyl acetate in petroleum ether) afforded **38** (1.57 g, 81% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 7.63 (1H, dt, J = 7.7, 1.1 Hz), 7.55 (1H, t, J = 1.5 Hz), 7.26 (1H, t, J = 8.0 Hz), 7.07 (1H, ddd, J = 8.0, 2.6, 1.0 Hz), 4.37 (2H, q, J = 7.2 Hz), 1.40 (3H, t, J = 7.2 Hz), 1.29 (3H, sep, J = 7.3 Hz), 1.11 (18H, d, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 166.7, 156.2, 131.9, 129.4, 124.6, 122.3, 120.9, 61.1, 18.0, 14.4, 12.8; ν_{max}/cm^{-1} 2945, 2868,

1721 (C=O), 1601, 1583; HRMS (ESI)+: m/z calculated for $[C_{18}H_{30}O_3Si + H]^+$ = 323.2037, observed 323.2048.

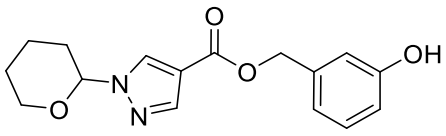
(3-((Triisopropylsilyl)oxy)phenyl)methanol (39).



Lithium aluminium hydride (2.4 M in THF, 4 mL, 9.4 mmol) was added dropwise to a solution of ethyl 3-((triisopropylsilyl)oxy)benzoate **38** (1.52 g, 4.71 mmol) in diethyl ether (40 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred over 3 hours. Isopropanol (1 mL) was added dropwise at 0 °C to the reaction mixture, followed by aqueous potassium sodium tartrate (10% w/v, 50 mL). The product was extracted into ethyl acetate (3 x 50 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo* to afford **39** (1.08 g, 83% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 7.21 (1H, t, J = 7.8 Hz), 6.94-6.91 (2H, m), 6.81 (1H, dd, J = 8.2, 2.0 Hz), 4.65 (2H, s), 1.31-1.22 (3H, m), 1.11 (18H, d, J = 7.2 Hz); spectroscopic data consistent with literature.[4]

3-Hydroxybenzyl 1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole-4-carboxylate (40).

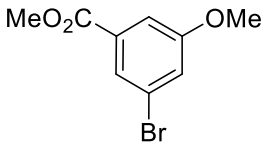


(3-((Triisopropylsilyl)oxy)phenyl)methanol **39** (1.08 g, 3.85 mmol), DMAP (71 mg, 0.58 mmol), EDC.HCl (1.11 g, 5.78 mmol) and *N*,*N*-diisopropylethylamine (0.84 mL, 5.8 mmol) were added to a solution of 1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole-4-carboxylic acid **36** (0.755 g, 3.85 mmol) in DCM (17 mL). The reaction mixture was stirred overnight,

then tetra-*n*-butylammonium fluoride (1 M in THF, 9.6 mL, 9.6 mmol) was added. The reaction mixture was stirred over 10 minutes, then diluted with water (50 mL) and extracted into DCM (3 x 30 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 60% ethyl acetate in petroleum ether) afforded **40** (0.386 g, 28% yield).

LCMS (ESI-): m/z 301.1 $[M - H]^-$, rt 1.74 minutes, >99%; ^{1}H NMR (500 MHz, $CDCl_3$) 8.15 (1H, s), 7.99 (1H, s), 7.24 (1H, t, J = 8.0 Hz), 6.95 (1H, d, J = 8.0 Hz), 6.91-6.87 (1H, m), 6.81 (1H, dd, J = 8.1, 2.8 Hz), 5.70 (1H, br s), 5.39 (1H, dd, J = 9.2, 3.0 Hz), 5.24 (2H, s), 4.08-4.04 (1H, m), 3.70 (1H, td, J = 10.4, 3.0 Hz), 2.13-2.00 (3H, m), 1.74-1.60 (3H, m); ^{13}C NMR (125 MHz, $CDCl_3$) 163.0, 156.2, 141.2, 138.0, 131.6, 130.0, 120.3, 115.4, 115.1, 115.0, 88.0, 68.0, 65.8, 30.8, 25.0, 22.2; ν_{max}/cm^{-1} 3131, 2945, 1700 (C=O), 1588, 1558; HRMS (ESI)-: m/z calculated for $[C_{16}H_{18}N_2O_4 - H]^-$ = 301.1194, observed 301.1190.

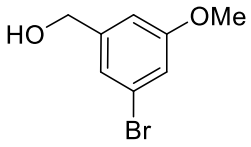
Methyl 3-bromo-5-methoxybenzoate (42).



Methyl iodide (0.90 mL, 12 mmol) was added to a solution of 3-bromo-5-hydroxybenzoic acid **41** (1.00 g, 4.61 mmol) and potassium carbonate (1.59 g, 11.5 mmol) in DMF (5 mL). The reaction mixture was stirred over 72 hours, then diluted with ethyl acetate (100 mL), washed with LiCl solution (2 x 100 mL) and brine (2 x 100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 35% ethyl acetate in petroleum ether) afforded **42** (1.10 g, 97% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 7.76-7.72 (1H, m), 7.49-7.45 (1H, m), 7.24 (1H, t, J = 2.0 Hz), 3.92 (3H, s), 3.84 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 165.8, 160.4, 132.8, 125.1, 122.8, 122.3, 113.6, 55.9, 52.6; spectroscopic data consistent with literature.[5]

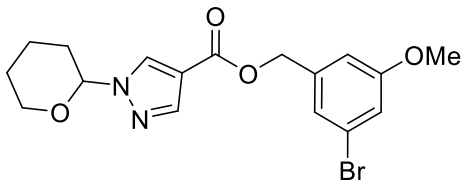
(3-Bromo-5-methoxyphenyl)methanol (43).



Lithium aluminium hydride (2.4 M in THF, 2.2 mL, 5.3 mmol) was added dropwise at 0 °C to a solution of methyl 3-bromo-5-methoxybenzoate **42** (1.07 g, 4.39 mmol) in diethyl ether (40 mL). The reaction mixture was warmed to room temperature and stirred overnight. Isopropanol (3 mL) was added dropwise at 0 °C to the reaction mixture, followed by aqueous potassium sodium tartrate (10% w/v, 50 mL). The product was extracted into ethyl acetate (3 x 50 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 35% ethyl acetate in petroleum ether) afforded **43** (0.680 g, 71% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 7.11 (1H, br s), 6.96 (1H, br s), 6.86 (1H, br s), 4.66 (2H, d, J = 6.0 Hz), 3.81 (3H, s), 1.76 (1H, t, J = 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 160.7, 144.2, 123.0, 122.2, 116.4, 111.4, 64.7, 55.7; ^{1}H NMR spectroscopic data consistent with literature.[6]

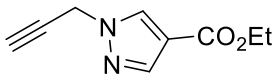
3-Bromo-5-methoxybenzyl-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole-4-carboxylate (44).



(3-Bromo-5-methoxyphenyl)methanol **43** (0.664 g, 3.09 mmol), *N*,*N*-diisopropylethylamine (0.81 mL, 4.6 mmol), DMAP (75 mg, 0.45 mmol) and EDC.HCl (0.879 mg, 4.63 mmol) were added to a solution of 1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole-4-carboxylic acid **36** (0.605 g, 3.09 mmol) in DCM (17 mL). The reaction mixture was stirred overnight, then diluted with water (70 mL) and extracted into DCM (3 x 80 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in* vacuo. Purification by flash column chromatography (0 – 7% methanol in DCM) afforded **44** (0.772 g, 63% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 8.14 (1H, s), 7.97 (1H, s), 7.15-7.11 (1H, m), 7.01 (1H, t, J = 2.0 Hz), 6.87-6.84 (1H, m), 5.39 (1H, dd, J = 8.8, 3.2 Hz), 5.20 (2H, s), 4.11-4.02 (1H, m), 3.80 (3H, s), 3.75-3.66 (1H, m), 2.15-1.97 (3H, m), 1.74-1.59 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) 162.7, 160.6, 141.2, 139.3, 131.5, 123.3, 123.0, 116.9, 114.9, 113.0, 88.0, 67.9, 65.1, 55.7, 30.8, 24.9, 22.1; ν_{max}/cm^{-1} 3119, 2965, 2940, 1706 (C=O), 1607, 1570, 1559; HRMS (ESI)+: m/z calculated for $[C_{17}H_{19}BrN_2O_4 + Na]^+$ = 417.0420, observed 417.0425.

Ethyl 1-(prop-2-yn-1-yl)-1H-pyrazole-4-carboxylate (45).

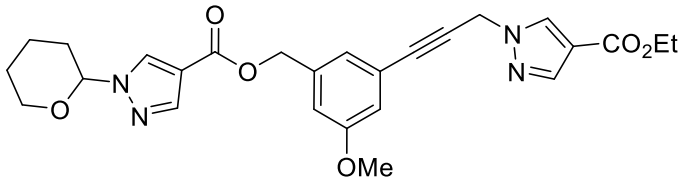


Propargyl bromide (80 wt. % solution in toluene, 0.60 mL, 5.4 mmol) was added to a suspension of ethyl 4-pyrazolecarboxylate **1** (0.500 g, 3.57 mmol) and potassium carbonate (0.740 g, 5.35 mmol) in DMF (4.5 mL). The reaction mixture was stirred over 72 hours, then diluted with ethyl acetate (100 mL), washed with LiCl solution (2 x 100 mL) and brine (2 x 100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 35% ethyl acetate in petroleum ether) afforded **45** (0.515 g, 81% yield).

LCMS (ESI+): m/z 179.1 $[M + H]^+$, rt 1.54 minutes, >99%; ^{1}H NMR (400 MHz, $CDCl_3$) 8.14 (1H, s), 7.94 (1H, s), 4.96 (2H, d, J = 2.5 Hz), 4.31 (2H, q, J = 7.1 Hz), 2.58 (1H, t, J =

2.5 Hz), 1.35 (3H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 162.9, 141.8, 132.4, 115.9, 75.9, 75.7, 60.4, 42.1, 14.5; ν_{max}/cm^{-1} 3244 (C≡C), 3130, 2977, 2935, 2133, 1697 (C=O), 1551; HRMS (ESI)+: m/z calculated for $[C_9H_{10}N_2O_2 + H]^+$ = 179.0815, observed 179.0822.

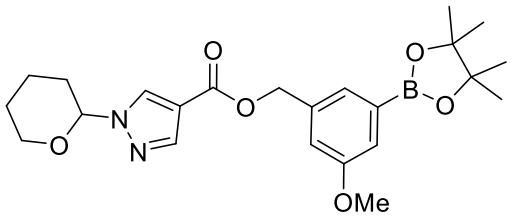
3-(3-(4-(Ethoxycarbonyl)-1H-pyrazol-1-yl)prop-1-yn-1-yl)-5-methoxybenzyl-1-(tetrahydro-2H-pyran-2-yl) -1H-pyrazole-4-carboxylate (46).



Ethyl 1-(prop-2-yn-1-yl)-1H-pyrazole-4-carboxylate **45** (0.135 g, 0.759 mmol), $Pd(dppf)Cl_2$ (28 mg, 0.038 mmol) and CuI (15 mg, 0.076 mmol) were added to a solution of 3-bromo-5-methoxybenzyl 1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole-4-carboxylate **44** (0.150 g, 0.380 mmol) in DMF (3 mL) and triethylamine (3 mL). The reaction mixture was stirred at 100 °C over 4 hours, then diluted with ethyl acetate (200 mL), washed with LiCl solution (2 x 200 mL) and brine (2 x 200 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 100% ethyl acetate in petroleum ether) afforded **46** (81 mg, 43% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 8.18 (1H, s), 8.14 (1H, s), 7.98 (1H, s), 7.95 (1H, s), 7.11 (1H, s), 6.95 (2H, s), 5.39 (1H, dd, J = 8.8, 3.1 Hz), 5.22 (2H, s), 5.17 (2H, s), 4.30 (2H, q, J = 7.1 Hz), 4.10-4.01 (1H, m), 3.81 (3H, s), 3.76-3.65 (1H, m), 2.16-1.94 (3H, m), 1.77-1.61 (3H, m), 1.34 (3H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 163.0, 162.7, 159.7, 141.7, 141.2, 138.2, 132.4, 131.5, 124.0, 123.0, 116.5, 115.8, 115.5, 114.9, 88.0, 86.8, 81.0, 67.9, 65.3, 60.4, 55.6, 43.0, 30.8, 24.9, 22.1, 14.5; ν_{max}/cm^{-1} 2944, 1710 (C=O), 1591, 1553; HRMS (ESI)+: m/z calculated for $[C_{26}H_{28}N_4O_6 + Na]^+$ = 515.1901, observed 515.1890.

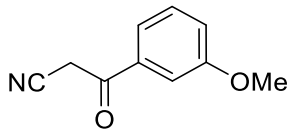
3-Methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole-4-carboxylate (47).



Dioxane (8 mL) was added to a mixture of 3-bromo-5-methoxybenzyl 1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole-4-carboxylate **44** (0.363 g, 0.790 mmol), bis(pinacolato)diboron (0.401 g, 1.58 mmol), Pd(dppf)Cl_2 (29 mg, 0.040 mmol) and potassium acetate (0.194 g, 1.97 mmol). The reaction mixture was heated to 80 °C over 3 hours, then diluted with water (15 mL) and extracted into ethyl acetate (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 40% ethyl acetate in petroleum ether) afforded **47** (0.192 g, 44% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 8.13 (1H, s), 7.97 (1H, s), 7.43 (1H, s), 7.29 (1H, d, J = 2.4 Hz), 7.06 (1H, t, J = 1.9 Hz), 5.38 (1H, dd, J = 8.5, 3.4 Hz), 5.24 (2H, s), 4.09-4.00 (1H, m), 3.84 (3H, s), 3.74-3.64 (1H, m), 2.14-1.95 (3H, m), 1.76-1.54 (3H, m), 1.35 (12H, s).

3-(3-Methoxyphenyl)-3-oxopropanenitrile (49).

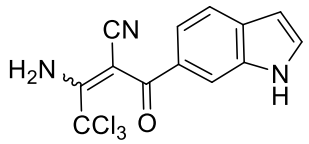


n-Butyllithium (1.6 M in hexanes, 5.2 mL, 8.3 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (0.87 mL, 17 mmol) and THF (10 mL). The reaction mixture was stirred at -78 °C over 30 minutes. Ethyl 3-methoxybenzoate **48** (0.600 g, 3.33 mmol) was added dropwise at -78 °C, and the reaction mixture stirred at -78 °C over 30 minutes. Water

(10 mL) and aqueous HCl (1 M, 20 mL) were added dropwise at 0 °C. The product was extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 50% ethyl acetate in petroleum ether) afforded **49** (0.599 g, 99% yield).

LCMS (ESI-): m/z 174.1 $[M - H]^-$, rt 1.56 minutes, >99%; ^{1}H NMR (400 MHz, $CDCl_3$) 7.49-7.39 (3H, m), 7.23-7.17 (1H, m), 4.07 (2H, s), 3.87 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 187.1, 160.3, 135.7, 130.3, 121.4, 121.1, 113.9, 112.8, 55.7, 29.6; spectroscopic data consistent with literature.[7]

3-Amino-4,4,4-trichloro-2-(1H-indole-6-carbonyl)but-2-enenitrile (53).

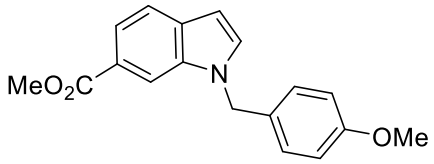


Trichloroacetonitrile (0.738 mL, 7.36 mmol) was added to a suspension of 3-(1H-indol-6-yl)-3-oxopropanenitrile **52** (0.491 g, 2.45 mmol) and sodium acetate (1.01 g, 12.3 mmol) in ethanol (15 mL). The reaction mixture was stirred over 90 minutes. The reaction mixture was concentrated *in vacuo*, then $NaHCO_3$ solution (20 mL) was added. The product was extracted into DCM/methanol (10:1, 3 x 50 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 8% methanol in DCM) afforded **53** (0.717 g, 87% yield).

^{1}H NMR (500 MHz, $(CD_3)_2SO$) 11.88 (1H, br s), 11.49 (1H, s), 9.72 (1H, br s), 7.92-7.88 (1H, m), 7.62 (1H, d, J = 8.3 Hz), 7.58 (1H, t, J = 2.8 Hz), 7.38 (1H, dd, J = 8.3, 1.6 Hz), 6.55-6.51 (1H, m); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) 193.6, 167.9, 134.5, 131.1, 130.4, 129.0, 119.5, 119.0, 118.8, 112.5, 101.5, 91.3, 77.2; ν_{max}/cm^{-1} 3389 (N-H), 3280 (N-H), 2205

(C≡N), 1597 (C=O), 1508; HRMS (ESI)+: m/z calculated for $[C_{13}H_8Cl_3N_3O + H]^+$ = 327.9806, observed 327.9812.

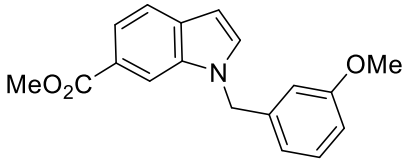
Methyl 1-(4-methoxybenzyl)-1H-indole-6-carboxylate (54a).



4-Methoxybenzyl chloride (0.255 mL, 1.88 mmol) was added to a suspension of methyl 1H-indole-6-carboxylate **50** (0.300 g, 1.71 mmol) and caesium carbonate (1.12 g, 3.42 mmol) in acetonitrile (10 mL). The reaction mixture was heated under reflux for 1 hour, then concentrated *in vacuo*. Water (25 mL) was added to the resultant residue and the product extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 30% ethyl acetate in petroleum ether) afforded **54a** (0.445 g, 88% yield).

LCMS (ESI+): m/z 296.2 $[M + H]^+$, rt 2.16 minutes, >99%; ^{1}H NMR (400 MHz, $CDCl_3$) 8.12 (1H, s), 7.81 (1H, dd, J = 8.3, 1.3 Hz), 7.65 (1H, d, J = 8.4 Hz), 7.24 (1H, d, J = 3.1 Hz), 7.12-7.04 (2H, m), 6.87-6.81 (2H, m), 6.57 (1H, dd, J = 3.1, 0.6 Hz), 5.31 (2H, s), 3.92 (3H, s), 3.77 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 168.3, 159.4, 135.8, 132.5, 131.3, 129.1, 128.5, 123.5, 120.7, 120.6, 114.4, 112.1, 102.1, 55.4, 52.1, 49.7; ν_{max}/cm^{-1} 2951, 2835, 1697 (C=O), 1614, 1585, 1511; HRMS (ESI)+: m/z calculated for $[C_{18}H_{17}NO_3 + Na]^+$ = 318.1101, observed 318.1107.

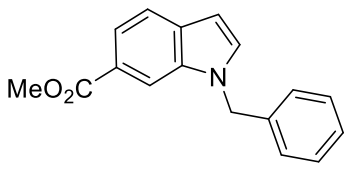
Methyl 1-(3-methoxybenzyl)-1H-indole-6-carboxylate (54b).



3-Methoxybenzyl chloride (0.299 mL, 2.05 mmol) was added to a suspension of methyl 1H-indole-6-carboxylate **50** (0.300 g, 1.71 mmol) and caesium carbonate (1.12 g, 3.42 mmol) in acetonitrile (10 mL). The reaction mixture was heated under reflux for 1 hour, then concentrated *in vacuo*. Water (20 mL) was added to the resultant residue and the product extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 25% ethyl acetate in petroleum ether) afforded **54b** (0.471 g, 93% yield).

LCMS (ESI+): m/z 296.2 $[M + H]^+$, rt 2.18 minutes, 98%; ^1H NMR (500 MHz, $CDCl_3$) 8.10 (1H, s), 7.81 (1H, dd, J = 8.3, 1.4 Hz), 7.66 (1H, dd, J = 8.4, 0.6 Hz), 7.27 (1H, d, J = 3.2 Hz), 7.22 (1H, t, J = 7.9 Hz), 6.81 (1H, dd, J = 8.1, 2.4 Hz), 6.72-6.68 (1H, m), 6.65-6.62 (1H, m), 6.59 (1H, dd, J = 3.1, 0.9 Hz), 5.35 (2H, s), 3.91 (3H, s), 3.73 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) 168.3, 160.2, 138.8, 135.9, 132.4, 131.6, 130.1, 123.6, 120.8, 120.7, 119.2, 113.1, 112.8, 112.1, 102.4, 55.3, 52.1, 50.1; ν_{max}/cm^{-1} 2913, 1703 (C=O), 1602, 1505; HRMS (ESI)+: m/z calculated for $[C_{18}H_{17}NO_3 + H]^+$ = 296.1281, observed 296.1277.

Methyl 1-benzyl-1H-indole-6-carboxylate (54c).

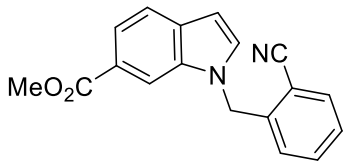


Benzyl bromide (0.244 mL, 2.05 mmol) was added to a suspension of methyl 1H-indole-6-carboxylate **50** (0.300 g, 1.71 mmol) and caesium carbonate (1.12 g, 3.42 mmol) in acetonitrile (15 mL). The reaction mixture was heated under reflux for 2 hours, then concentrated *in vacuo*. Water (15 mL) was added to the resultant residue and the product extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried

($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 25% ethyl acetate in petroleum ether) afforded **54c** (0.436 g, 91% yield).

LCMS (ESI+): m/z 266.2 $[M + H]^+$, rt 2.16 minutes, 95%; ^{1}H NMR (400 MHz, $CDCl_3$) 8.09 (1H, s), 7.80 (1H, dd, J = 8.3, 1.4 Hz), 7.65 (1H, d, J = 8.3 Hz), 7.34-7.23 (4H, m), 7.13-7.07 (2H, m), 6.58 (1H, dd, J = 3.1, 0.7 Hz), 5.38 (2H, s), 3.90 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 168.3, 137.2, 135.9, 132.4, 131.5, 129.0, 128.0, 126.9, 123.6, 120.8, 120.7, 112.1, 102.3, 52.1, 50.2; ν_{max}/cm^{-1} 2949, 1704 (C=O), 1613; HRMS (ESI)+: m/z calculated for $[C_{17}H_{15}NO_2 + H]^+$ = 266.1176, observed 266.1182.

Methyl 1-(2-cyanobenzyl)-1H-indole-6-carboxylate (54d).

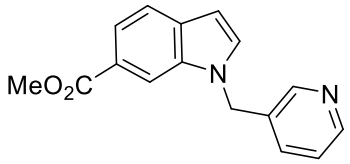


2-(Bromomethyl)benzonitrile (0.403 g, 2.05 mmol) was added to a suspension of methyl 1H-indole-6-carboxylate **50** (0.300 g, 1.71 mmol) and caesium carbonate (1.12 g, 3.42 mmol) in acetonitrile (10 mL). The reaction mixture was heated under reflux for 4 hours, then concentrated *in vacuo*. Water (20 mL) was added to the resultant residue and the product extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 25% ethyl acetate in petroleum ether) afforded **54d** (0.399 g, 80% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 8.04 (1H, s), 7.84 (1H, dd, J = 8.4, 1.4 Hz), 7.73 (1H, dd, J = 7.5, 1.4 Hz), 7.69 (1H, d, J = 8.3 Hz), 7.44 (1H, td, J = 7.7, 1.4 Hz), 7.38 (1H, td, J = 7.6, 1.2 Hz), 7.33 (1H, d, J = 3.2 Hz), 6.78 (1H, dd, J = 7.7, 0.7 Hz), 6.65 (1H, dd, J = 3.2, 0.8 Hz), 5.62 (2H, s), 3.91 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 168.1, 140.9, 135.8, 133.6, 133.3, 132.5, 131.6, 128.5, 127.4, 124.1, 121.2, 121.0, 117.2, 111.8, 110.9, 103.2, 52.2, 48.3;

ν_{max}/cm^{-1} 2227 (C≡N), 1703 (C=O), 1616, 1601, 1502; HRMS (ESI)+: m/z calculated for $[C_{18}H_{14}N_2O_2 + H]^+$ = 291.1128, observed 291.1134.

Methyl 1-(pyridin-3-ylmethyl)-1H-indole-6-carboxylate (54g).

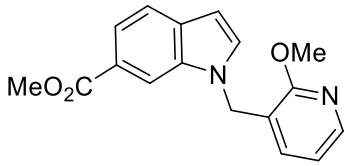


Methyl 1H-indole-6-carboxylate **50** (0.600 g, 3.42 mmol) was added portionwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.685 g, 17.1 mmol) in DMF (5 mL). The reaction mixture was warmed to room temperature and stirred over 1 hour. 3-(Chloromethyl)pyridine hydrochloride (0.674 g, 4.11 mmol) was added portionwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 45 minutes. Methanol (150 mL) was added dropwise at 0 °C, and the reaction mixture adjusted to pH 1 using sulfuric acid dropwise at 0 °C. The reaction mixture was heated under reflux for 14 hours, then further sulfuric acid (10 mL) was added dropwise at 0 °C. The reaction mixture was heated under reflux for 2 hours, then concentrated *in vacuo*. $NaHCO_3$ solution (200 mL) was added dropwise at 0 °C. The product was extracted into DCM (3 x 150 mL). The combined organic extracts were washed with $NaHCO_3$ solution (100 mL) and brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (70% ethyl acetate in petroleum ether) afforded **54g** (0.732 g, 75% yield).

LCMS (ESI+): m/z 267.2 $[M + H]^+$, rt 1.61 minutes, 96%; ^1H NMR (400 MHz, $CDCl_3$) 8.54 (1H, dd, J = 4.8, 1.4 Hz), 8.51 (1H, d, J = 1.9 Hz), 8.06 (1H, s), 7.82 (1H, dd, J = 8.3, 1.4 Hz), 7.67 (1H, d, J = 8.3 Hz), 7.33 (1H, dt, J = 7.8, 1.9 Hz), 7.27 (1H, d, J = 3.2 Hz), 7.22 (1H, dd, J = 7.8, 4.9 Hz), 6.62 (1H, dd, J = 3.2, 0.8 Hz), 5.41 (2H, s), 3.91 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 168.1, 149.4, 148.3, 135.7, 134.7, 132.9, 132.5, 131.2, 124.0, 123.9, 121.1,

120.9, 111.8, 103.0, 52.1, 47.8; ν_{max}/cm^{-1} 3093, 2941, 1696 (C=O), 1613, 1577, 1506; HRMS (ESI)+: m/z calculated for $[C_{16}H_{14}N_2O_2 + H]^+$ = 267.1128, observed 267.1122.

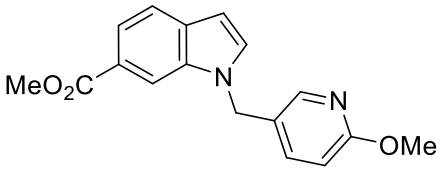
Methyl 1-((2-methoxypyridin-3-yl)methyl)-1H-indole-6-carboxylate (54h).



p-Toluenesulfonyl chloride (1.11 g, 5.80 mmol) and DMAP (6 mg, 0.05 mmol) were added to a solution of (2-methoxypyridin-3-yl)methanol **60** (0.673 g, 4.84 mmol) and triethylamine (1.35 mL, 9.67 mmol) in DCM (7 mL). The reaction mixture was stirred over 2 hours, then diluted with $NaHCO_3$ solution (25 mL) and extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 15% ethyl acetate in petroleum ether) was attempted, affording a crude residue. A solution of methyl 1H-indole-6-carboxylate **50** (0.710 g, 4.05 mmol) in DMF (2 mL) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.405 g, 10.1 mmol) in DMF (2 mL). The reaction mixture was warmed to room temperature and stirred over 30 minutes. A solution of the crude residue in DMF (1 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 30 minutes. Methanol (200 mL) was added dropwise at 0 °C to the reaction mixture followed by sulfuric acid (15 mL). The reaction mixture was heated under reflux for 90 minutes, then concentrated *in vacuo*. The reaction mixture was diluted with ethyl acetate (200 mL), washed with $NaHCO_3$ solution (2 x 200 mL) and brine (200 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (10% ethyl acetate in petroleum ether) afforded **54h** (0.455 g, 31% yield).

LCMS (ESI+): m/z 297.2 $[M + H]^+$, rt 2.14 minutes, 97%; ^{1}H NMR (400 MHz, $CDCl_3$) 8.10-8.05 (2H, m), 7.81 (1H, dd, J = 8.3, 1.4 Hz), 7.66 (1H, dd, J = 8.4, 0.6 Hz), 7.31 (1H, d, J = 3.2 Hz), 6.89-6.84 (1H, m), 6.73 (1H, dd, J = 7.3, 5.1 Hz), 6.60 (1H, dd, J = 3.1, 0.9 Hz), 5.34 (2H, s), 4.06 (3H, s), 3.91 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 168.3, 161.1, 146.1, 136.1, 135.8, 132.4, 131.8, 123.7, 120.8, 120.7, 120.1, 117.1, 112.1, 102.4, 53.7, 52.1, 45.1; ν_{max}/cm^{-1} 2948, 1711 (C=O), 1617, 1595, 1584, 1501; HRMS (ESI)+: m/z calculated for $[C_{17}H_{16}N_2O_3 + Na]^+$ = 319.1053, observed 319.1056.

1-((6-Methoxypyridin-3-yl)methyl)-1H-indole-6-carboxylate (54i).

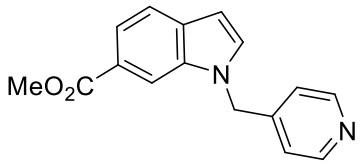


A solution of methyl 1H-indole-6-carboxylate **50** (0.237 g, 1.36 mmol) in DMF (1 mL) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.108 g, 2.71 mmol) in DMF (1 mL). The reaction mixture was warmed to room temperature and stirred over 30 minutes. A solution of 5-(chloromethyl)-2-methoxypyridine **58** (0.160 g, 0.904 mmol) in DMF (1 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 1 hour. Methanol (150 mL) was added dropwise at 0 °C to the reaction mixture followed by sulfuric acid (5 mL). The reaction mixture was heated under reflux for 1 hour, then concentrated *in vacuo*. The reaction mixture was diluted with ethyl acetate (100 mL), washed with $NaHCO_3$ solution (2 x 100 mL) and brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 15% ethyl acetate in petroleum ether) afforded **54i** (0.227 g, 85% yield).

LCMS (ESI+): m/z 297.2 $[M + H]^+$, rt 2.09 minutes, >99%; ^{1}H NMR (400 MHz, $CDCl_3$) 8.13-8.10 (1H, m), 8.05 (1H, dd, J = 2.5, 0.6 Hz), 7.81 (1H, dd, J = 8.4, 1.4 Hz), 7.65 (1H, dd,

J = 8.3, 0.6 Hz), 7.31 (1H, dd, J = 8.5, 2.5 Hz), 7.23 (1H, d, J = 3.2 Hz), 6.67 (1H, d, J = 8.5 Hz), 6.58 (1H, dd, J = 3.2, 0.8 Hz), 5.29 (2H, s), 3.92 (3H, s), 3.91 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 168.2, 164.1, 145.6, 137.8, 135.6, 132.5, 131.0, 125.4, 123.7, 120.9, 120.8, 111.9, 111.5, 102.6, 53.7, 52.1, 47.3; ν_{max}/cm^{-1} 2948, 1704 (C=O), 1610, 1572; HRMS (ESI)+: m/z calculated for $[C_{17}H_{16}N_2O_3 + H]^+$ = 297.1234, observed 297.1235.

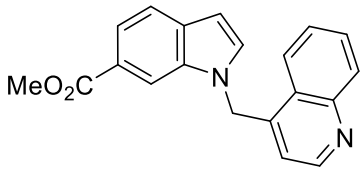
Methyl 1-(pyridin-4-ylmethyl)-1H-indole-6-carboxylate (54l).



Methyl 1H-indole-6-carboxylate **50** (0.400 g, 2.28 mmol) was added portionwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.457 g, 11.4 mmol) in DMF (5 mL). The reaction mixture was warmed to room temperature and stirred over 1 hour. 4-(Bromomethyl)pyridine hydrobromide (0.635 g, 2.51 mmol) was added portionwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 45 minutes. Methanol (150 mL) was added dropwise at 0 °C, and the reaction mixture adjusted to pH 1 using sulfuric acid dropwise at 0 °C. The reaction mixture was heated under reflux for 14 hours, then further sulfuric acid (10 mL) was added dropwise at 0 °C. The reaction mixture was heated under reflux for 2 hours, then concentrated *in vacuo*. $NaHCO_3$ solution (200 mL) was added dropwise at 0 °C. The product was extracted into DCM (3 x 150 mL). The combined organic extracts were washed with $NaHCO_3$ solution (100 mL) and brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (20 – 80% ethyl acetate in petroleum ether) afforded **54l** (0.474 g, 71% yield).

LCMS (ESI+): m/z 267.2 $[M + H]^+$, rt 1.53 minutes, 96%; ^{1}H NMR (400 MHz, $CDCl_3$) 8.56-8.49 (2H, m), 7.97 (1H, s), 7.83 (1H, dd, J = 8.4, 1.4 Hz), 7.69 (1H, dd, J = 8.3, 0.5 Hz), 7.28 (1H, d, J = 3.1 Hz), 6.96-6.91 (2H, m), 6.65 (1H, dd, J = 3.2, 0.8 Hz), 5.41 (2H, s), 3.90 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 168.0, 150.3, 146.5, 135.7, 132.4, 131.5, 124.1, 121.4, 121.2, 121.0, 111.7, 103.1, 52.1, 49.1; ν_{max}/cm^{-1} 2948, 1699 (C=O), 1602, 1562, 1504; HRMS (ESI)+: m/z calculated for $[C_{16}H_{14}N_2O_2 + Na]^+$ = 289.0947, observed 289.0940.

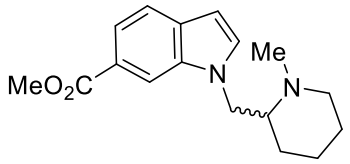
Methyl 1-(quinolin-4-ylmethyl)-1H-indole-6-carboxylate (54m).



A suspension of quinolin-4-ylmethanol **62** (0.378 g, 2.37 mmol) in aqueous HBr (48%, 5 mL) was heated under reflux for 90 minutes, then concentrated *in vacuo* to afford a crude residue. A solution of methyl 1H-indole-6-carboxylate **50** (0.400 g, 2.28 mmol) in DMF (2 mL) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.457 g, 11.4 mmol) in DMF (5 mL). The reaction mixture was warmed to room temperature and stirred over 30 minutes. The crude residue was added portionwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 15 minutes. Methanol (150 mL) was added dropwise at 0 °C to the reaction mixture followed by sulfuric acid (10 mL). The reaction mixture was heated under reflux for 2 hours, then concentrated *in vacuo*. The reaction mixture was diluted with ethyl acetate (200 mL), washed with $NaHCO_3$ solution (2 x 100 mL), water (2 x 200 mL) and brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 30% ethyl acetate in petroleum ether) afforded **54m** (0.497 g, 65% yield).

LCMS (ESI+): m/z 317.2 $[M + H]^+$, rt 1.83 minutes, 94%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 8.69 (1H, d, J = 4.4 Hz), 8.30 (1H, d, J = 8.4 Hz), 8.12-8.04 (2H, m), 7.85 (1H, ddd, J = 8.3, 7.0, 1.2 Hz), 7.79-7.67 (4H, m), 6.72 (1H, dd, J = 3.1, 0.7 Hz), 6.34 (1H, d, J = 4.4 Hz), 6.20 (2H, s), 3.77 (3H, s); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 167.0, 150.5, 147.5, 144.0, 135.5, 133.4, 132.0, 129.7, 129.6, 126.9, 125.4, 123.7, 122.7, 120.7, 120.2, 117.4, 112.0, 102.2, 51.8, 46.3; ν_{max}/cm^{-1} 3112, 3095, 1700 (C=O), 1618, 1599, 1570, 1508; HRMS (ESI)+: m/z calculated for $[C_{20}H_{16}N_2O_2 + Na]^+$ = 339.1104, observed 339.1100.

Methyl 1-((1-methylpiperidin-2-yl)methyl)-1H-indole-6-carboxylate (54n).

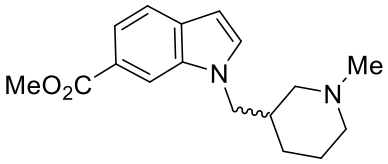


A solution of methyl 1H-indole-6-carboxylate **50** (0.392 g, 2.24 mmol) in DMF (2 mL) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.122 g, 3.05 mmol) in DMF (2 mL). The reaction mixture was warmed to room temperature and stirred over 30 minutes. A solution of 2-(chloromethyl)-1-methylpiperidine **64** (0.300 g, 2.03 mmol) in DMF (2 mL) was added at 0 °C to the reaction mixture. The reaction mixture was heated to 60 °C over 1 hour. Methanol (150 mL) was added dropwise at 0 °C to the reaction mixture followed by sulfuric acid (10 mL). The reaction mixture was heated under reflux for 1 hour, then concentrated *in vacuo*. The reaction mixture was diluted with ethyl acetate (200 mL), washed with $NaHCO_3$ solution (2 x 200 mL) and brine (200 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 100% ethyl acetate in petroleum ether, 0 – 10% methanol in DCM) afforded **54n** (0.172 g, 27% yield).

LCMS (ESI+): m/z 287.3 $[M + H]^+$, rt 1.32 minutes, 90%; ^{1}H NMR (400 MHz, $CDCl_3$) 8.11 (1H, s), 7.78 (1H, dd, J = 8.3, 1.4 Hz), 7.61 (1H, d, J = 8.3 Hz), 7.24 (1H, d, J = 3.1 Hz), 6.52

(1H, dd, J = 3.0, 0.8 Hz), 4.56 (1H, dd, J = 14.2, 4.4 Hz), 4.01-3.90 (4H, m), 2.95-2.83 (1H, m), 2.47 (3H, s), 2.44-2.34 (1H, m), 2.14 (1H, td, J = 11.3, 3.6 Hz), 1.67-1.46 (3H, m), 1.28-1.02 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) 168.3, 135.9, 132.2, 132.0, 123.3, 120.5, 120.4, 112.1, 101.8, 63.4, 57.3, 52.0, 49.4, 43.6, 29.5, 25.7, 23.6; ν_{max}/cm^{-1} 2938, 2855, 2784, 1707 (C=O), 1614, 1505; HRMS (ESI)+: m/z calculated for $[C_{17}H_{22}N_2O_2 + H]^+$ = 287.1754, observed 287.1751.

Methyl 1-((1-methylpiperidin-3-yl)methyl)-1H-indole-6-carboxylate (54o).

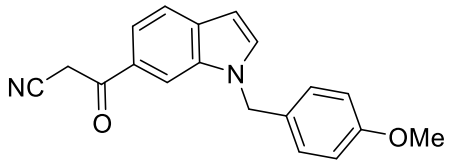


A solution of methyl 1H-indole-6-carboxylate **50** (0.568 g, 3.24 mmol) in DMF (2 mL) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.151 g, 3.78 mmol) in DMF (2 mL). The reaction mixture was warmed to room temperature and stirred over 30 minutes. A solution of (1-methylpiperidin-3-yl)methyl 4-methylbenzenesulfonate **66** (0.797 g, 2.70 mmol) in DMF (2 mL) was added at 0 °C to the reaction mixture, followed by NaI (81 mg, 0.54 mmol). The reaction mixture was heated to 60 °C over 1 hour, then diluted with ethyl acetate (200 mL), washed with $NaHCO_3$ solution (2 x 200 mL) and brine (200 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 8% methanol in DCM), followed by reverse phase chromatography (40 – 60% acetonitrile in water (+ 0.1% NH_3)), afforded **54o** (0.348 g, 45% yield).

LCMS (ESI+): m/z 287.3 $[M + H]^+$, rt 1.49 minutes, >99%; ^{1}H NMR (400 MHz, $CDCl_3$) 8.10 (1H, s), 7.78 (1H, d, J = 8.3 Hz), 7.62 (1H, d, J = 8.4 Hz), 7.22 (1H, d, J = 3.0 Hz), 6.52

(1H, d, J = 2.9 Hz), 4.12 (1H, dd, J = 14.3, 7.9 Hz), 4.04 (1H, dd, J = 14.2, 7.2 Hz), 3.94 (3H, s), 2.73-2.61 (1H, m), 2.56 (1H, d, J = 10.2 Hz), 2.30-2.16 (4H, m), 2.06-1.93 (1H, m), 1.78 (1H, t, J = 10.1 Hz), 1.74-1.47 (3H, m), 1.12-0.97 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) 168.4, 135.8, 132.2, 131.6, 123.3, 120.6, 120.5, 112.1, 101.7, 59.6, 56.3, 52.1, 50.3, 46.8, 37.5, 28.1, 24.7; ν_{max}/cm^{-1} 2934, 2780, 1707 (C=O), 1615, 1504; HRMS (ESI)+: m/z calculated for $[C_{17}H_{22}N_2O_2 + H]^+$ = 287.1754, observed 287.1748.

3-(1-(4-Methoxybenzyl)-1H-indol-6-yl)-3-oxopropanenitrile (55a).

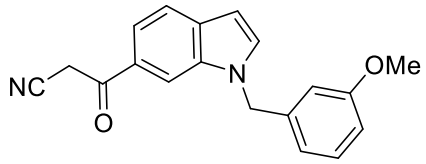


n-Butyllithium (1.6 M in hexanes, 1.8 mL, 2.9 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (0.23 mL, 4.3 mmol) and THF (4 mL). The reaction mixture was stirred at -78 °C over 30 minutes. A solution of methyl 1-(4-methoxybenzyl)-1H-indole-6-carboxylate **54a** (0.425 g, 1.44 mmol) in THF (2 mL) was added dropwise at -78 °C over 30 minutes. The reaction mixture was stirred at -78 °C over 15 minutes. Aqueous HCl (1 M, 25 mL) was added dropwise at 0 °C. The product was extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 50% ethyl acetate in petroleum ether) afforded **55a** (0.387 g, 88% yield).

LCMS (ESI+): m/z 327.2 $[M + Na]^+$, (ESI-): m/z 303.1 $[M - H]^-$, rt 2.01 minutes, >99%; (400 MHz, $(CD_3)_2SO$) 8.22 (1H, s), 7.79 (1H, d, J = 3.0 Hz), 7.67 (1H, d, J = 8.3 Hz), 7.60 (1H, dd, J = 8.3, 1.5 Hz), 7.25-7.19 (2H, m), 6.91-6.84 (2H, m), 6.59 (1H, d, J = 3.1 Hz), 5.45 (2H, s), 4.77 (2H, s), 3.69 (3H, s); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 189.2, 158.7, 135.0, 133.8, 132.7, 129.8, 128.7, 128.0, 120.5, 119.1, 116.3, 114.0, 111.9, 101.8, 55.1, 48.6, 29.9; ν_{max}/cm^{-}

[1] 2943, 1679 (C=O), 1607, 1511; HRMS (ESI)+: m/z calculated for $[C_{19}H_{16}N_2O_2 + Na]^+$ = 327.1104, observed 327.1096.

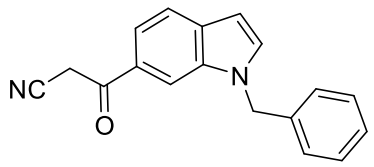
3-(1-(3-Methoxybenzyl)-1H-indol-6-yl)-3-oxopropanenitrile (55b).



n-Butyllithium (1.6 M in hexanes, 2.9 mL, 4.6 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (0.40 mL, 7.6 mmol) and THF (5 mL). The reaction mixture was stirred at -78 °C over 45 minutes. A solution of methyl 1-(3-methoxybenzyl)-1H-indole-6-carboxylate **54b** (0.450 g, 1.52 mmol) in THF (5 mL) was added dropwise at -78 °C over 30 minutes. The reaction mixture was stirred at -78 °C over 1 hour. Aqueous HCl (1 M, 15 mL) was added dropwise at 0 °C. The product was extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 50% ethyl acetate in petroleum ether) afforded **55b** (0.465 g, 99% yield).

LCMS (ESI-): m/z 303.2 $[M - H]^-$, rt 2.01 minutes, >99%; ^{1}H NMR (400 MHz, $CDCl_3$) 7.97 (1H, s), 7.70 (1H, d, J = 8.4 Hz), 7.59 (1H, dd, J = 8.4, 1.6 Hz), 7.37 (1H, d, J = 3.1 Hz), 7.24 (1H, t, J = 7.9 Hz), 6.81 (1H, dd, J = 8.3, 2.5 Hz), 6.73-6.67 (1H, m), 6.64-6.60 (2H, m), 5.37 (2H, s), 4.09 (2H, s), 3.74 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 187.1, 160.2, 138.2, 136.0, 133.8, 133.4, 130.2, 128.1, 121.3, 119.7, 119.2, 114.5, 113.2, 112.9, 111.3, 102.7, 55.4, 50.4, 29.6; ν_{max}/cm^{-1} 2942, 1677 (C=O), 1606, 1585; HRMS (ESI)+: m/z calculated for $[C_{19}H_{16}N_2O_2 + Na]^+$ = 327.1104, observed 327.1089.

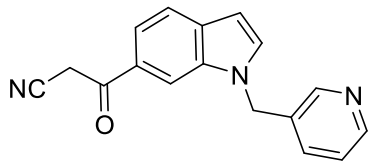
3-(1-Benzyl-1H-indol-6-yl)-3-oxopropanenitrile (55c).



n-Butyllithium (1.6 M in hexanes, 4.7 mL, 7.5 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (0.78 mL, 15 mmol) and toluene (7 mL). The reaction mixture was stirred at -78 °C over 30 minutes. A solution of methyl 1-benzyl-1H-indole-6-carboxylate **54c** (0.418 g, 1.50 mmol) in toluene (5 mL) was added dropwise at -78 °C over 30 minutes. The reaction mixture was stirred at -78 °C over 1 hour, then warmed to 0 °C and stirred over 20 minutes. Aqueous HCl (3 M, 15 mL) was added dropwise at 0 °C. The product was extracted into ethyl acetate (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 30% ethyl acetate in petroleum ether, 0 – 20% methanol in DCM) afforded **55c** (0.384 g, 84% yield).

LCMS (ESI-): m/z 273.2 $[M - H]^-$, rt 1.99 minutes, >99%; ^1H NMR (400 MHz, $(CD_3)_2SO$) 8.21 (1H, s), 7.81 (1H, d, J = 3.0 Hz), 7.69 (1H, d, J = 8.3 Hz), 7.61 (1H, dd, J = 8.4, 1.2 Hz), 7.39-7.18 (5H, m), 6.63 (1H, d, J = 2.9 Hz), 5.55 (2H, s), 4.76 (2H, s); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 189.2, 138.0, 135.1, 134.0, 132.7, 128.7, 128.1, 127.5, 127.1, 120.6, 119.2, 116.3, 111.8, 101.9, 49.1, 29.9; ν_{max}/cm^{-1} 1679, 1663, 1606, 1504; HRMS (ESI)+: m/z calculated for $[C_{18}H_{14}N_2O + H]^+$ = 275.1179, observed 275.1179.

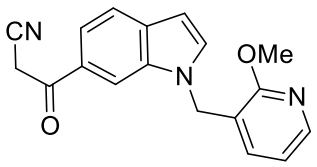
3-Oxo-3-(1-(pyridin-3-ylmethyl)-1H-indol-6-yl)propanenitrile (55g).



n-Butyllithium (1.6 M in hexanes, 5.1 mL, 8.1 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (0.71 mL, 14 mmol) and THF (6 mL). The reaction mixture was stirred at -78 °C over 30 minutes. A solution of methyl 1-(pyridin-3-ylmethyl)-1H-indole-6-carboxylate **54g** (0.721 g, 2.71 mmol) in THF (4 mL) was added dropwise at -78 °C over 30 minutes. The reaction mixture was stirred at -78 °C over 45 minutes. Water (20 mL) was added dropwise at 0 °C, and the reaction mixture adjusted to pH 7. The product was extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 4% methanol in DCM) afforded **55g** (0.612 g, 82% yield).

LCMS (ESI+): m/z 276.2 $[M + H]^+$, (ESI-): m/z 274.1 $[M - H]^-$, rt 1.41 minutes, >99%; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) 8.55 (1H, d, J = 1.9 Hz), 8.47 (1H, dd, J = 4.8, 1.6 Hz), 8.25 (1H, s), 7.85 (1H, d, J = 3.1 Hz), 7.69 (1H, d, J = 8.2 Hz), 7.64-7.59 (2H, m), 7.34 (1H, ddd, J = 7.8, 4.8, 0.8 Hz), 6.64 (1H, d, J = 3.0 Hz), 5.60 (2H, s), 4.76 (2H, s); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) 189.2, 148.9, 148.5, 135.0, 134.9, 133.8, 133.5, 132.7, 128.2, 123.8, 120.7, 119.3, 116.3, 111.8, 102.3, 46.6, 29.9; ν_{max}/cm^{-1} 3089, 2952, 2926, 2267 (C≡N), 1677 (C=O), 1611, 1577, 1561, 1502; HRMS (ESI-): m/z calculated for $[C_{17}H_{13}N_3O - H]^-$ = 274.0986, observed 274.0976.

3-(1-((2-Methoxypyridin-3-yl)methyl)-1H-indol-6-yl)-3-oxopropanenitrile (55h).

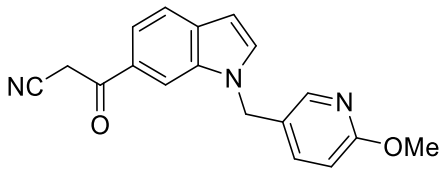


n-Butyllithium (1.6 M in hexanes, 1.1 mL, 1.7 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (0.15 mL, 2.9 mmol) and THF (4 mL). The reaction mixture was stirred at -78 °C over 30 minutes. A solution of methyl 1-((2-methoxypyridin-3-yl)methyl)-

1H-indole-6-carboxylate **54h** (0.177 g, 0.579 mmol) in THF (1 mL) was added dropwise at -78 °C. The reaction mixture was stirred at -78 °C over 1 hour, then NH_4Cl solution (25 mL) was added dropwise at 0 °C. The product was extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 50% ethyl acetate in petroleum ether) afforded **55h** (0.134 g, 76% yield).

LCMS (ESI+): m/z 306.2 $[M + H]^+$, (ESI-): m/z 304.1 $[M - H]^-$, rt 1.94 minutes, >99%; ^1H NMR (400 MHz, $CDCl_3$) 8.09 (1H, dd, J = 5.0, 1.8 Hz), 8.02 (1H, s), 7.70 (1H, d, J = 8.3 Hz), 7.59 (1H, dd, J = 8.5, 1.5 Hz), 7.41 (1H, d, J = 3.1 Hz), 6.97-6.92 (1H, m), 6.76 (1H, dd, J = 7.3, 5.0 Hz), 6.63 (1H, dd, J = 3.1, 0.6 Hz), 5.35 (2H, s), 4.12 (2H, s), 4.04 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 187.1, 161.2, 146.5, 136.3, 135.9, 133.7, 133.6, 128.2, 121.4, 119.8, 119.5, 117.1, 114.4, 111.2, 102.7, 53.8, 45.3, 29.6; ν_{max}/cm^{-1} 2953, 2261 (C≡N), 1683 (C=O), 1589; HRMS (ESI)+: m/z calculated for $[C_{18}H_{15}N_3O_2 + H]^+$ = 306.1237, observed 306.1237.

3-(1-((6-Methoxypyridin-3-yl)methyl)-1H-indol-6-yl)-3-oxopropanenitrile (55i).

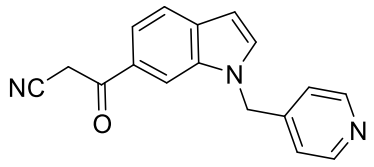


n-Butyllithium (1.6 M in hexanes, 1.3 mL, 2.1 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (0.18 mL, 3.5 mmol) and THF (4 mL). The reaction mixture was stirred at -78 °C over 20 minutes. A solution of methyl 1-((6-methoxypyridin-3-yl)methyl)-1H-indole-6-carboxylate **54i** (0.206 g, 0.695 mmol) in THF (2 mL) was added dropwise at -78 °C over 20 minutes. The reaction mixture was stirred at -78 °C over 30 minutes. NH_4Cl solution (25 mL) was added dropwise at 0 °C. The product was extracted into DCM (3 x 25

mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 50% ethyl acetate in petroleum ether) afforded **55i** (0.137 g, 62% yield).

LCMS (ESI+): m/z 306.2 $[M + H]^+$, (ESI-): m/z 304.1 $[M - H]^-$, rt 1.86 minutes, 96%; ^1H NMR (400 MHz, $CDCl_3$) 8.06-8.00 (2H, m), 7.69 (1H, d, J = 8.4 Hz), 7.58 (1H, dd, J = 8.3, 1.0 Hz), 7.36-7.30 (2H, m), 6.69 (1H, d, J = 8.6 Hz), 6.61 (1H, d, J = 3.0 Hz), 5.32 (2H, s), 4.11 (2H, s), 3.90 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 187.1, 164.3, 145.7, 137.8, 135.8, 133.9, 132.9, 128.2, 124.8, 121.4, 119.9, 114.5, 111.6, 111.0, 103.0, 53.7, 47.5, 29.6; ν_{max}/cm^{-1} 2940, 2249 (C≡N), 1676 (C=O), 1607, 1572; HRMS (ESI)+: m/z calculated for $[C_{18}H_{15}N_3O_2 + H]^+$ = 306.1237, observed 306.1234.

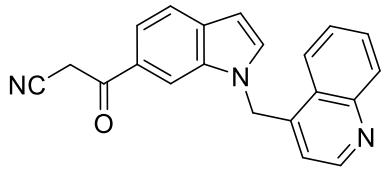
3-Oxo-3-(1-(pyridin-4-ylmethyl)-1H-indol-6-yl)propanenitrile (55l).



n-Butyllithium (1.6 M in hexanes, 2.9 mL, 4.7 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (0.41 mL, 7.8 mmol) and THF (6 mL). The reaction mixture was stirred at -78 °C over 30 minutes. A solution of methyl 1-(pyridin-4-ylmethyl)-1H-indole-6-carboxylate **54l** (0.454 g, 1.55 mmol) in THF (4 mL) was added dropwise at -78 °C over 30 minutes. The reaction mixture was stirred at -78 °C over 45 minutes. Water (20 mL) was added dropwise at 0 °C, and the reaction mixture adjusted to pH 7. The product was extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 4% methanol in DCM) afforded **55l** (0.303 g, 71% yield).

LCMS (ESI+): m/z 276.2 $[M + H]^+$, (ESI-): m/z 274.2 $[M - H]^-$, rt 1.37 minutes, >99%; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) 8.51-8.47 (2H, m), 8.15 (1H, s), 7.83 (1H, d, J = 3.1 Hz), 7.72 (1H, d, J = 8.4 Hz), 7.63 (1H, dd, J = 8.4, 1.4 Hz), 7.11-7.06 (2H, m), 6.68 (1H, d, J = 2.9 Hz), 5.63 (2H, s), 4.74 (2H, s); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) 189.2, 149.9, 146.9, 135.2, 134.1, 132.6, 128.3, 121.7, 120.7, 119.4, 116.2, 111.7, 102.3, 47.9, 29.8; ν_{max}/cm^{-1} 3076, 2918, 2258 (C≡N), 1664 (C=O), 1599, 1560; HRMS (ESI-): m/z calculated for $[C_{17}H_{13}N_3O - H]^-$ = 274.0986, observed 274.0976.

3-Oxo-3-(1-(quinolin-4-ylmethyl)-1H-indol-6-yl)propanenitrile (55m).

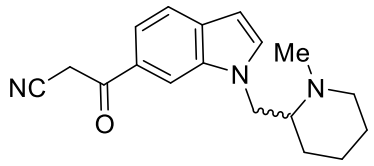


n-Butyllithium (1.6 M in hexanes, 1.7 mL, 2.8 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (0.22 mL, 4.2 mmol) and toluene (3 mL). The reaction mixture was stirred at -78 °C over 30 minutes. A suspension of methyl 1-(quinolin-4-ylmethyl)-1H-indole-6-carboxylate **54m** (0.467 g, 1.39 mmol) in toluene/THF (3:1, 12 mL) was added dropwise at -78 °C. The reaction mixture was stirred at -78 °C over 30 minutes. Water (50 mL) was added dropwise at 0 °C, and the reaction mixture adjusted to pH 8. The product was extracted into ethyl acetate (50 mL) and DCM/methanol (9:1, 3 x 50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 60% ethyl acetate in petroleum ether) afforded **55m** (0.119 g, 26% yield).

LCMS (ESI+): m/z 326.2 $[M + H]^+$, (ESI-): m/z 324.1 $[M - H]^-$, rt 1.65 minutes, >99%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 8.70 (1H, d, J = 4.4 Hz), 8.31 (1H, d, J = 8.2 Hz), 8.20 (1H, s), 8.09 (1H, d, J = 8.2 Hz), 7.90-7.71 (4H, m), 7.67 (1H, d, J = 8.3 Hz), 6.75 (1H, d, J = 2.7 Hz),

6.36 (1H, d, J = 4.4 Hz), 6.21 (2H, s), 4.70 (2H, s); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 189.2, 150.5, 147.5, 144.0, 135.6, 134.3, 132.6, 129.66, 129.64, 128.4, 126.9, 125.4, 123.6, 120.8, 119.4, 117.4, 116.2, 111.9, 102.5, 46.3, 29.8; ν_{max}/cm^{-1} 2948, 2845, 2252 (C≡N), 1673 (C=O), 1610, 1597, 1570, 1560, 1504; HRMS (ESI)+: m/z calculated for $[C_{21}H_{15}N_3O + Na]^+$ = 348.1107, observed 348.1106.

3-(1-((1-Methylpiperidin-2-yl)methyl)-1H-indol-6-yl)-3-oxopropanenitrile (55n).

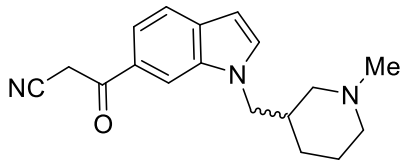


n-Butyllithium (1.6 M in hexanes, 0.62 mL, 0.99 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (78 µL, 1.5 mmol) and THF (3 mL). The reaction mixture was stirred at -78 °C over 30 minutes. A solution of methyl 1-((1-methylpiperidin-2-yl)methyl)-1H-indole-6-carboxylate **54n** (0.158 g, 0.497 mmol) in THF (3 mL) was added dropwise at -78 °C over 30 minutes. The reaction mixture was stirred at -78 °C over 1 hour. Water (50 mL) was added dropwise at 0 °C, and the reaction mixture adjusted to pH 10. The product was extracted into DCM/methanol (10:1, 4 x 50 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 5% methanol in DCM), followed by reverse phase chromatography (0 – 50% acetonitrile in water (+ 0.1% NH_3)), afforded **55n** (48 mg, 32% yield).

LCMS (ESI+): m/z 296.3 $[M + H]^+$, (ESI-): m/z 294.2 $[M - H]^-$, rt 1.21 minutes, 97%; ^{1}H NMR (400 MHz, $CDCl_3$) 8.02 (1H, s), 7.65 (1H, d, J = 8.5 Hz), 7.55 (1H, dd, J = 8.4, 1.1 Hz), 7.35 (1H, d, J = 3.1 Hz), 6.55 (1H, d, J = 2.7 Hz), 4.55 (1H, dd, J = 14.2, 4.3 Hz), 4.12 (2H, br s), 3.99 (1H, dd, J = 14.3, 8.7 Hz), 2.88 (1H, d, J = 11.6 Hz), 2.46 (3H, s), 2.42-2.32

(1H, m), 2.15 (1H, td, J = 11.5, 3.2 Hz), 1.72-1.42 (3H, m), 1.31-1.01 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) 187.1, 136.1, 133.8, 133.4, 127.9, 121.1, 119.4, 114.6, 111.2, 102.2, 63.3, 57.2, 49.3, 43.6, 29.6, 29.4, 25.5, 23.5; ν_{max}/cm^{-1} 2939, 2855, 2787, 2168 (C≡N), 1676 (C=O), 1606, 1500; HRMS (ESI)+: m/z calculated for $[C_{18}H_{21}N_3O + H]^+$ = 296.1757, observed 296.1747.

3-(1-((1-Methylpiperidin-3-yl)methyl)-1H-indol-6-yl)-3-oxopropanenitrile (55o).

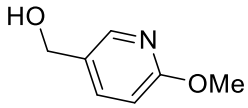


n-Butyllithium (1.6 M in hexanes, 1.4 mL, 2.2 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (0.17 mL, 3.3 mmol) and THF (3 mL). The reaction mixture was stirred at -78 °C over 30 minutes. A suspension of methyl 1-((1-methylpiperidin-3-yl)methyl)-1H-indole-6-carboxylate **54o** (0.318 g, 1.11 mmol) in THF (10 mL) was added dropwise at -78 °C. The reaction mixture was stirred at -78 °C over 30 minutes. Water (50 mL) was added dropwise at 0 °C, and the reaction mixture adjusted to pH 8. The product was extracted into DCM/methanol (10:1, 4 x 50 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 10% methanol in DCM) afforded **55o** (0.221 g, 67% yield).

LCMS (ESI+): m/z 296.3 $[M + H]^+$, (ESI-): m/z 294.2 $[M - H]^-$, rt 1.37 minutes, >99%; ^{1}H NMR (400 MHz, $CDCl_3$) 8.03 (1H, s), 7.66 (1H, d, J = 8.4 Hz), 7.57 (1H, d, J = 8.4 Hz), 7.31 (1H, d, J = 3.1 Hz), 6.56 (1H, d, J = 2.8 Hz), 4.34-3.96 (4H, m), 2.74-2.61 (1H, m), 2.55 (1H, d, J = 10.5 Hz), 2.31-2.14 (4H, m), 2.13-1.99 (1H, m), 1.83 (1H, t, J = 10.1 Hz), 1.78-1.68 (1H, m), 1.67-1.50 (2H, m), 1.15-1.01 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) 187.2, 136.0,

133.5, 133.4, 127.9, 121.2, 119.5, 114.6, 111.2, 102.2, 59.3, 56.1, 50.1, 46.6, 37.3, 29.7, 27.8, 24.4; ν_{max}/cm^{-1} 2936, 2782, 2166 (C≡N), 1675 (C=O), 1607, 1564; HRMS (ESI)+: m/z calculated for $[C_{18}H_{21}N_3O + H]^+$ = 296.1757, observed 296.1753.

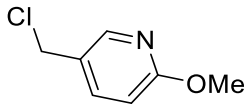
(6-Methoxypyridin-3-yl)methanol (57).



Lithium aluminium hydride (2.4 M in THF, 1.25 mL, 2.99 mmol) was added dropwise at 0 °C to a solution of methyl 6-methoxynicotinate **56** (1.00 g, 5.98 mmol) in THF (10 mL). The reaction mixture was stirred at 0 °C over 1 hour. Aqueous potassium sodium tartrate (1 M, 25 mL) was added dropwise at 0 °C to the reaction mixture. The product was extracted into ethyl acetate (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 5% methanol in DCM) afforded **57** (0.759 g, 91% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 8.07 (1H, d, J = 2.3 Hz), 7.60 (1H, dd, J = 8.5, 2.3 Hz), 6.73 (1H, d, J = 8.5 Hz), 4.59 (2H, s), 3.91 (3H, s), 2.38 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 164.0, 145.7, 138.7, 129.2, 111.1, 62.5, 53.7; spectroscopic data consistent with literature.[8]

5-(Chloromethyl)-2-methoxypyridine (58).

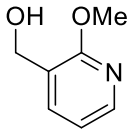


p-Toluenesulfonyl chloride (1.21 g, 6.37 mmol) and DMAP (6 mg, 0.05 mmol) were added to a solution of (6-methoxypyridin-3-yl)methanol **57** (0.739 g, 5.31 mmol) and triethylamine (1.48 mL, 10.6 mmol) in DCM (10 mL). The reaction mixture was stirred over 18 hours, then diluted with water (20 mL). The product was extracted into DCM (3 x 25 mL). The combined

organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 30% ethyl acetate in petroleum ether) afforded **58** (0.160 g, 17% yield).

LCMS (ESI+): m/z 158.2 $[M + H]^+$, rt 1.71 minutes, 89%; ^1H NMR (400 MHz, $CDCl_3$) 8.15 (1H, d, J = 2.4 Hz), 7.62 (1H, dd, J = 8.6, 2.5 Hz), 6.75 (1H, d, J = 8.5 Hz), 4.55 (2H, s), 3.94 (3H, s); spectroscopic data consistent with literature.[8]

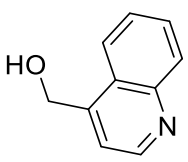
(2-Methoxypyridin-3-yl)methanol (60).



Sodium borohydride (1.02 g, 26.9 mmol) was added portionwise at 0 °C to a solution of methyl 2-methoxynicotinate **59** (1.50 g, 8.97 mmol) in ethanol (24 mL). The reaction mixture was warmed to room temperature and stirred over 24 hours, then diluted with $NaHCO_3$ solution (50 mL) and extracted into DCM (4 x 50 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 10% methanol in DCM) afforded **60** (0.840 g, 67% yield).

^1H NMR (400 MHz, $CDCl_3$) 8.09 (1H, dd, J = 5.1, 2.0 Hz), 7.62-7.55 (1H, m), 6.88 (1H, dd, J = 7.2, 5.1 Hz), 4.64 (2H, s), 3.99 (3H, s), 2.41 (1H, br s); ^{13}C NMR (100 MHz, $CDCl_3$) 161.7, 145.8, 136.7, 123.5, 117.0, 61.1, 53.6; spectroscopic data consistent with literature.[9]

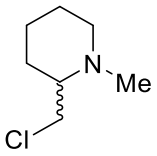
Quinolin-4-ylmethanol (62).[10]



Sodium borohydride (0.144 g, 3.82 mmol) was added at 0 °C to a solution of 4-quinolinecarboxaldehyde **61** (0.500 g, 3.18 mmol) in methanol (10 mL). The reaction mixture was stirred at 0 °C over 90 minutes, then diluted with water (50 mL) and extracted into DCM (3 x 50 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 7% methanol in DCM) afforded **62** (0.398 g, 79% yield).

^{1}H NMR (400 MHz, CD_3OD) 8.82 (1H, d, J = 4.4 Hz), 8.11-7.99 (2H, m), 7.77 (1H, ddd, J = 8.4, 7.0, 1.4 Hz), 7.69-7.59 (2H, m), 5.17 (2H, s); ^{13}C NMR (100 MHz, CD_3OD) 151.2, 150.0, 148.3, 130.8, 129.6, 128.0, 127.3, 124.4, 119.3, 61.5; spectroscopic data consistent with literature.[10]

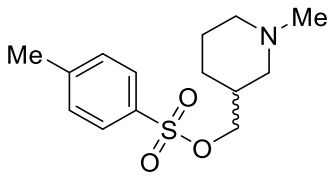
2-(Chloromethyl)-1-methylpiperidine (64).



Thionyl chloride (3 mL) was added dropwise to a solution of (1-methylpiperidin-2-yl)methanol **63** (1.02 mL, 7.74 mmol) in DCM (15 mL). The reaction mixture was heated under reflux for 7 hours, then diluted with $NaHCO_3$ solution (100 mL) and extracted into DCM (3 x 100 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo* to afford **64** (0.658 g, 58% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 3.63 (1H, dd, J = 11.5, 5.2 Hz), 3.50 (1H, dd, J = 11.5, 2.6 Hz), 2.84 (1H, dtd, J = 11.5, 3.5, 1.5 Hz), 2.27 (3H, s), 2.14-2.02 (2H, m), 1.78-1.48 (5H, m), 1.34-1.18 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) 64.2, 57.0, 46.9, 43.0, 29.6, 25.8, 24.0; ν_{max}/cm^{-1} 2936, 2855, 2780, 2712.

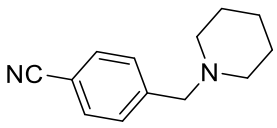
(1-Methylpiperidin-3-yl)methyl 4-methylbenzenesulfonate (66).



DMAP (47 mg, 0.39 mmol), triethylamine (2.2 mL, 16 mmol) and *p*-toluenesulfonyl chloride (1.62 g, 8.51 mmol) were added to a solution of (1-methylpiperidin-3-yl)methanol **65** (0.987 mL, 7.74 mmol) in DCM (15 mL). The reaction mixture was stirred over 4 hours, then diluted with water (25 mL) and extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 20% methanol in DCM) afforded **66** (0.815 g, 36% yield).

LCMS (ESI+): m/z 284.2 $[M + H]^+$, rt 1.36 minutes, 96%; ^1H NMR (500 MHz, $CDCl_3$) 7.80-7.75 (2H, m), 7.36-7.31 (2H, m), 3.91 (1H, dd, J = 9.7, 5.8 Hz), 3.86 (1H, dd, J = 9.6, 7.1 Hz), 2.74 (1H, d, J = 10.2 Hz), 2.68 (1H, d, J = 10.8 Hz), 2.44 (3H, s), 2.22 (3H, s), 2.02-1.93 (1H, m), 1.90 (1H, t, J = 11.3 Hz), 1.72 (1H, t, J = 10.5 Hz), 1.67-1.48 (3H, m), 1.03-0.90 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$) 144.9, 133.1, 130.0, 128.0, 73.0, 58.3, 56.0, 46.6, 35.9, 26.1, 24.4, 21.8; ν_{max}/cm^{-1} 2938, 2783, 1598; HRMS (ESI)+: m/z calculated for $[C_{14}H_{21}NO_3S + H]^+$ = 284.1315, observed 284.1315.

4-(Piperidin-1-ylmethyl)benzonitrile (77b).

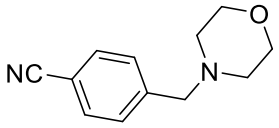


Sodium triacetoxyborohydride (2.59 g, 12.2 mmol) was added to a solution of 4-formylbenzonitrile **76** (1.00 g, 7.63 mmol), piperidine (0.753 mL, 7.63 mmol) and acetic acid (0.524 mL, 9.15 mmol) in DCM (10 mL). The reaction mixture was stirred over 5 hours, then

diluted with water (10 mL), adjusted to pH 14 and extracted into DCM (3 x 25 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 25% ethyl acetate in petroleum ether) afforded **77b** (1.21 g, 79% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 7.62-7.55 (2H, m), 7.44 (2H, d, J = 8.3 Hz), 3.49 (2H, s), 2.43-2.27 (4H, m), 1.57 (4H, quin, J = 5.6 Hz), 1.48-1.38 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) 145.0, 132.1, 129.6, 119.2, 110.7, 63.4, 54.7, 26.1, 24.3; spectroscopic data consistent with literature. [11]

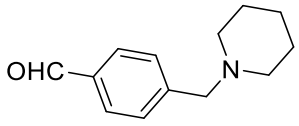
4-(Morpholinomethyl)benzonitrile (77c).



Sodium triacetoxyborohydride (1.42 g, 6.71 mmol) was added to a solution of 4-formylbenzonitrile **76** (0.550 g, 4.19 mmol), morpholine (0.367 mL, 4.19 mmol) and acetic acid (0.288 mL, 5.03 mmol) in DCM (6 mL). The reaction mixture was stirred over 90 minutes, then diluted with water (10 mL), adjusted to pH 14 and extracted into DCM (3 x 25 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 60% ethyl acetate in petroleum ether) afforded **77c** (0.738 g, 77% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 7.62-7.57 (2H, m), 7.45 (2H, d, J = 8.2 Hz), 3.70 (4H, t, J = 4.6 Hz), 3.53 (2H, s), 2.43 (4H, t, J = 4.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 143.9, 132.3, 129.6, 119.0, 111.1, 67.0, 62.9, 53.7; spectroscopic data consistent with literature. [12]

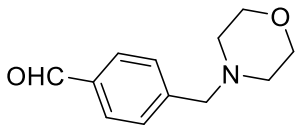
4-(Piperidin-1-ylmethyl)benzaldehyde (78b).



Diisobutylaluminium hydride (1 M in THF, 5.9 mL, 5.9 mmol) was added dropwise at 0 °C to a solution of 4-(piperidin-1-ylmethyl)benzonitrile **77b** (1.19 g, 5.94 mmol) in THF (10 mL). The reaction mixture was warmed to room temperature and stirred over 90 minutes, then $NaHCO_3$ solution (50 mL) was added dropwise at 0 °C. The product was extracted into DCM/methanol (10:1, 3 x 50 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 18% ethyl acetate in petroleum ether) afforded **78b** (0.877 g, 58% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 9.97 (1H, s), 7.80 (2H, d, J = 8.1 Hz), 7.48 (2H, d, J = 8.1 Hz), 3.53 (2H, s), 2.48-2.26 (4H, m), 1.64-1.51 (4H, m), 1.47-1.37 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) 192.1, 146.3, 135.5, 129.8, 129.6, 63.5, 54.6, 26.0, 24.3; spectroscopic data consistent with literature. [13]

4-(Morpholinomethyl)benzaldehyde (78c).

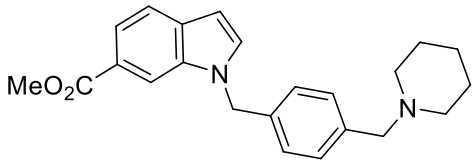


Diisobutylaluminium hydride (1 M in THF, 3.1 mL, 3.1 mmol) was added dropwise at 0 °C to a solution of 4-(morpholinomethyl)benzonitrile **77c** (0.717 g, 3.12 mmol) in THF (6 mL). The reaction mixture was warmed to room temperature and stirred over 30 minutes, then further diisobutylaluminium hydride (1 M in THF, 3.1 mL, 3.1 mmol) was added dropwise at 0 °C. The reaction mixture was warmed to room temperature and stirred over 30 minutes, then $NaHCO_3$ solution (50 mL) was added dropwise at 0 °C. The product was extracted into

DCM/methanol (10:1, 3 x 50 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 50% ethyl acetate in petroleum ether) afforded **78c** (0.297 g, 46% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 9.99 (1H, s), 7.87-7.81 (2H, m), 7.51 (2H, d, J = 8.1 Hz), 3.71 (4H, t, J = 4.6 Hz), 3.57 (2H, s), 2.45 (4H, t, J = 4.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 192.1, 145.5, 135.7, 129.9, 129.6, 67.1, 63.2, 53.8; spectroscopic data consistent with literature.[13]

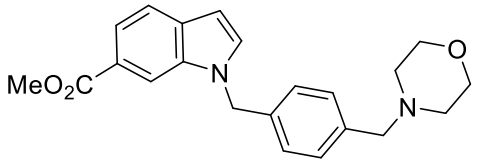
Methyl 1-(4-(piperidin-1-ylmethyl)benzyl)-1H-indole-6-carboxylate (79b).



A solution of 4-(piperidin-1-ylmethyl)benzaldehyde **78b** (0.400 g, 1.57 mmol) in toluene (3 mL) was added to a mixture of methyl indoline-6-carboxylate **75** (0.360 g, 1.89 mmol) and benzoic acid (38 mg, 0.32 mmol). The reaction mixture was heated to 200 °C by microwave for 30 minutes, then diluted with ethyl acetate (25 mL), washed with $NaHCO_3$ solution (3 x 15 mL) and brine (15 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (20 – 41% ethyl acetate in petroleum ether) afforded **79b** (0.393 g, 67% yield).

LCMS (ESI+): m/z 363.4 $[M + H]^+$, rt 1.50 minutes, 97%; ^{1}H NMR (400 MHz, $CDCl_3$) 8.10 (1H, s), 7.83-7.77 (1H, m), 7.65 (1H, d, J = 8.4 Hz), 7.29-7.22 (3H, m), 7.05 (2H, d, J = 7.6 Hz), 6.61-6.55 (1H, m), 5.36 (2H, s), 3.91 (3H, s), 3.42 (2H, s), 2.34 (4H, br s), 1.62-1.50 (4H, m), 1.46-1.36 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) 168.3, 138.6, 135.9, 135.6, 132.4, 131.5, 129.8, 126.8, 123.5, 120.72, 120.66, 112.2, 102.2, 63.6, 54.6, 52.1, 50.0, 26.1, 24.5; ν_{max}/cm^{-1} 2933, 2851, 2793, 2754, 1707 (C=O), 1615, 1505; HRMS (ESI)+: m/z calculated for $[C_{23}H_{26}N_2O_2 + H]^+$ = 363.2067, observed 363.2051.

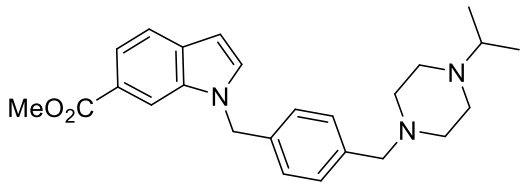
Methyl 1-(4-(morpholinomethyl)benzyl)-1H-indole-6-carboxylate (79c).



Toluene (3 mL) was added to a mixture of 4-(morpholinomethyl)benzaldehyde **78c** (0.282 g, 1.37 mmol), methyl indoline-6-carboxylate **75** (0.325 g, 1.65 mmol) and benzoic acid (34 mg, 0.28 mmol). The reaction mixture was heated to 200 °C by microwave for 30 minutes, then diluted with ethyl acetate (25 mL), washed with $NaHCO_3$ solution (3 x 15 mL) and brine (15 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 55% ethyl acetate in petroleum ether) afforded **79c** (0.330 g, 59% yield).

LCMS (ESI+): m/z 365.3 $[M + H]^+$, rt 1.45 minutes, >99%; ^1H NMR (400 MHz, $CDCl_3$) 8.10 (1H, s), 7.81 (1H, dd, J = 8.3, 1.4 Hz), 7.66 (1H, d, J = 8.3 Hz), 7.31-7.23 (3H, m), 7.06 (2H, d, J = 7.9 Hz), 6.59 (1H, dd, J = 3.1, 0.7 Hz), 5.37 (2H, s), 3.91 (3H, s), 3.69 (4H, t, J = 4.7 Hz), 3.45 (2H, s), 2.41 (4H, t, J = 4.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 168.3, 137.7, 136.0, 135.9, 132.4, 131.5, 129.8, 126.9, 123.6, 120.73, 120.68, 112.1, 102.3, 67.1, 63.1, 53.7, 52.1, 50.0; ν_{max}/cm^{-1} 2950, 2854, 2808, 1706 (C=O), 1615, 1504; HRMS (ESI)+: m/z calculated for $[C_{22}H_{24}N_2O_3 + H]^+$ = 365.1860, observed 365.1848.

Methyl 1-(4-((4-isopropylpiperazin-1-yl)methyl)benzyl)-1H-indole-6-carboxylate (79e).

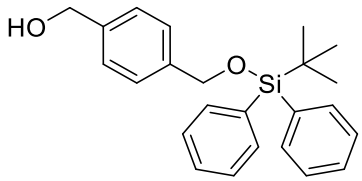


Methanesulfonyl chloride (0.126 mL, 1.63 mmol) was added dropwise at 0 °C to a solution of methyl 1-(4-(hydroxymethyl)benzyl)-1H-indole-6-carboxylate **82** (0.400 g, 1.35 mmol)

and triethylamine (0.245 mL, 1.76 mmol) in DCM (4 mL). The reaction mixture was warmed to room temperature and stirred over 90 minutes. Water (15 mL) was added dropwise at 0 °C. The intermediate was extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. The crude residue was dissolved in DMF (4 mL), then 1-isopropylpiperazine (0.233 mL, 1.63 mmol) and caesium carbonate (0.574 g, 1.76 mmol) were added. The reaction mixture was stirred over 11 hours, then further 1-isopropylpiperazine (0.233 mL, 1.63 mmol) and caesium carbonate (0.574 g, 1.76 mmol) were added. The reaction mixture was stirred over 1 hour, then diluted with ethyl acetate (100 mL), washed with water (3 x 100 mL) and brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether) afforded **79e** (0.254 g, 43% yield).

LCMS (ESI+): m/z 406.3 $[M + H]^+$, rt 1.54 minutes, 92%; ^1H NMR (400 MHz, $CDCl_3$) 8.10 (1H, s), 7.80 (1H, dd, J = 8.4, 1.4 Hz), 7.65 (1H, d, J = 8.4 Hz), 7.29-7.22 (3H, m), 7.06 (2H, d, J = 8.1 Hz), 6.58 (1H, dd, J = 3.2, 0.8 Hz), 5.36 (2H, s), 3.91 (3H, s), 3.47 (2H, s), 2.62 (1H, sept, J = 6.5 Hz), 2.62-2.32 (8H, m), 1.03 (6H, d, J = 6.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 168.3, 138.1, 135.9, 135.8, 132.4, 131.5, 129.8, 126.9, 123.6, 120.74, 120.67, 112.1, 102.3, 62.8, 54.6, 53.6, 52.1, 50.0, 48.8, 18.8; ν_{max}/cm^{-1} 2964, 2929, 2875, 2808, 1709 (C=O), 1618, 1571, 1505; HRMS (ESI)+: m/z calculated for $[C_{25}H_{31}N_3O_2 + H]^+$ = 406.2489, observed 406.2481.

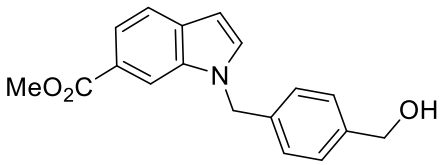
(4-(((Tert-butyldiphenylsilyl)oxy)methyl)phenyl)methanol (81).



tert-Butyldiphenylchlorosilane (3.8 mL, 15 mmol) was added to a solution of 1,4-benzenedimethanol **80** (4.02 g, 29.1 mmol) and imidazole (1.09 g, 16.0 mmol) in DMF (20 mL). The reaction mixture was stirred over 21 hours. The reaction was diluted with ethyl acetate (100 mL), washed with water (3 x 100 mL) and brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 20% ethyl acetate in petroleum ether) afforded **81** (4.14 g, 38% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 7.73-7.68 (4H, m), 7.47-7.32 (10H, m), 4.78 (2H, s), 4.70 (2H, d, J = 5.7 Hz), 1.66 (1H, t, J = 5.9 Hz), 1.10 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 140.8, 139.6, 135.7, 133.6, 129.8, 127.9, 127.2, 126.4, 65.5, 65.4, 27.0, 19.5; spectroscopic data consistent with literature.[14]

Methyl 1-(4-(hydroxymethyl)benzyl)-1H-indole-6-carboxylate (82).

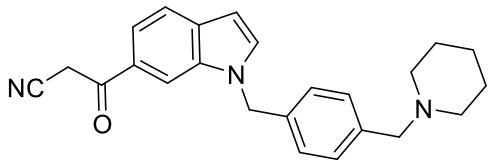


Methanesulfonyl chloride (1.0 mL, 13 mmol) was added dropwise at 0 °C to a solution of (4-(((*tert*-butyldiphenylsilyl)oxy)methyl)phenyl)methanol **81** (4.12 g, 11.0 mmol) and triethylamine (2.0 mL, 14 mmol) in DCM (20 mL). The reaction mixture was warmed to room temperature and stirred over 1 hour. Water (1 mL) was added dropwise at 0 °C. The mixture was diluted with DCM (30 mL), washed with water (3 x 50 mL) and brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford a crude residue. A solution of methyl 1H-indole-6-carboxylate **50** (1.88 g, 10.7 mmol) in DMF (6 mL) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.514 g, 12.8 mmol) in DMF (4 mL). The reaction mixture was warmed to room temperature over 30 minutes. A solution of the crude residue in DMF (10 mL) was added dropwise at 0 °C to the reaction mixture. The reaction

mixture was warmed to room temperature and stirred over 45 minutes. Water (10 mL) was added dropwise at 0 °C. The mixture was diluted with ethyl acetate (200 mL), washed with water (3 x 200 mL) and brine (200 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Tetra-*n*-butylammonium fluoride (1 M in THF, 14 mL, 14 mmol) was added to the resultant residue. The mixture was stirred over 30 minutes, then water (50 mL) was added. The product was extracted into DCM (3 x 50 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (10 – 50% ethyl acetate in petroleum ether) afforded **82** (2.33 g, 72% yield).

LCMS (ESI+): m/z 296.2 $[M + H]^+$, rt 1.96 minutes, 100%; ^1H NMR (400 MHz, $CDCl_3$) 8.08 (1H, s), 7.80 (1H, dd, J = 8.4, 1.4 Hz), 7.66 (1H, d, J = 8.4 Hz), 7.30 (2H, d, J = 8.2 Hz), 7.26 (1H, d, J = 3.2 Hz), 7.10 (2H, d, J = 8.2 Hz), 6.59 (1H, dd, J = 3.1, 0.8 Hz), 5.37 (2H, s), 4.65 (2H, d, J = 2.8 Hz), 3.90 (3H, s), 1.82-1.72 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) 168.3, 140.7, 136.5, 135.8, 132.5, 131.5, 127.6, 127.2, 123.6, 120.8, 120.7, 112.1, 102.4, 65.0, 52.1, 50.0; ν_{max}/cm^{-1} 3295 (br, O-H), 2949, 1705 (C=O), 1618, 1500; HRMS (ESI)+: m/z calculated for $[C_{18}H_{17}NO_3 + Na]^+$ = 318.1101, observed 318.1094.

3-Oxo-3-(1-(4-(piperidin-1-ylmethyl)benzyl)-1H-indol-6-yl)propanenitrile (83b).

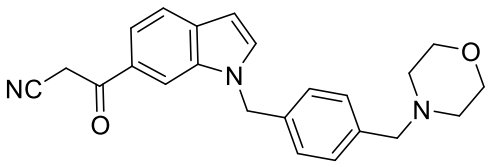


n-Butyllithium (1.6 M in hexanes, 1.3 mL, 2.0 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (0.16 mL, 3.0 mmol) and THF (3 mL). The reaction mixture was stirred at -78 °C over 30 minutes. A solution of methyl 1-(4-(piperidin-1-ylmethyl)benzyl)-1H-indole-6-carboxylate **79b** (0.377 g, 1.01 mmol) in THF (3 mL) was added dropwise at -78 °C over 30 minutes. The reaction mixture was stirred at -78 °C over 1 hour. Water (20 mL)

was added dropwise at 0 °C, and the reaction mixture adjusted to pH 8. The product was extracted into DCM (4 x 50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 90% ethyl acetate in petroleum ether) afforded **83b** (0.289 g, 77% yield).

LCMS (ESI+): m/z 372.4 $[M + H]^+$, rt 1.43 minutes, >99%; ^1H NMR (400 MHz, $CDCl_3$) 7.99 (1H, s), 7.70 (1H, d, J = 8.4 Hz), 7.60 (1H, dd, J = 8.3, 1.5 Hz), 7.37 (1H, d, J = 3.2 Hz), 7.27 (2H, d, J = 7.5 Hz), 7.06 (2H, d, J = 8.2 Hz), 6.62 (1H, d, J = 3.1 Hz), 5.38 (2H, s), 4.09 (2H, s), 3.43 (2H, s), 2.34 (4H, br s), 1.55 (4H, quin, J = 5.6 Hz), 1.47-1.36 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) 187.0, 138.9, 136.0, 135.1, 133.8, 133.4, 129.9, 128.1, 126.8, 121.3, 119.7, 114.5, 111.3, 102.6, 63.5, 54.6, 50.3, 29.6, 26.1, 24.5; ν_{max}/cm^{-1} 2914, 2805, 2252 (C≡N), 1682 (C=O), 1608, 1503; HRMS (ESI)+: m/z calculated for $[C_{24}H_{25}N_3O + H]^+$ = 372.2070, observed 372.2053.

3-(1-(4-(Morpholinomethyl)benzyl)-1H-indol-6-yl)-3-oxopropanenitrile (83c).

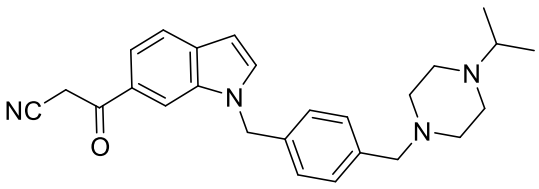


n-Butyllithium (1.6 M in hexanes, 1.5 mL, 2.3 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (0.20 mL, 3.9 mmol) and THF (3 mL). The reaction mixture was stirred at -78 °C over 30 minutes. A solution of methyl 1-(4-(morpholinomethyl)benzyl)-1H-indole-6-carboxylate **79c** (0.315 g, 0.778 mmol) in THF (3 mL) was added dropwise at -78 °C over 30 minutes. The reaction mixture was stirred at -78 °C over 20 minutes. Water (10 mL) was added dropwise at 0 °C, and the reaction mixture adjusted to pH 8. The product was extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried

($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (20 – 80% ethyl acetate in petroleum ether) afforded **83c** (0.272 g, 87% yield).

LCMS (ESI+): m/z 374.2 $[M + H]^+$, rt 1.36 minutes, 93%; ^{1}H NMR (400 MHz, $CDCl_3$) 7.99 (1H, s), 7.70 (1H, d, J = 8.3 Hz), 7.59 (1H, dd, J = 8.4, 1.5 Hz), 7.37 (1H, d, J = 3.2 Hz), 7.28 (2H, d, J = 8.1 Hz), 7.07 (2H, d, J = 7.9 Hz), 6.62 (1H, d, J = 3.2 Hz), 5.38 (2H, s), 4.09 (2H, s), 3.68 (4H, t, J = 4.6 Hz), 3.45 (2H, s), 2.45-2.36 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$) 187.1, 138.0, 136.0, 135.5, 133.9, 133.4, 129.9, 128.1, 126.9, 121.3, 119.8, 114.4, 111.3, 102.7, 67.1, 63.1, 53.7, 50.3, 29.6; ν_{max}/cm^{-1} 2940, 2846, 2251 (C≡N), 1681 (C=O), 1607, 1503; HRMS (ESI)-: m/z calculated for $[C_{23}H_{23}N_3O_2 - H]^-$ = 372.1718, observed 372.1723.

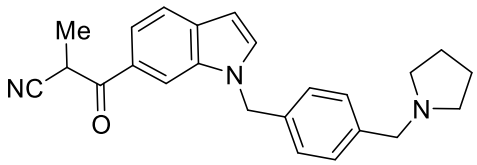
3-(1-(4-((4-Isopropylpiperazin-1-yl)methyl)benzyl)-1H-indol-6-yl)-3-oxopropanenitrile (83e).



n-Butyllithium (1.6 M in hexanes, 1.0 mL, 1.6 mmol) was added dropwise at -78 °C to a mixture of acetonitrile (0.14 mL, 2.7 mmol) and THF (2 mL). The reaction mixture was stirred at -78 °C over 30 minutes. A solution of methyl 1-(4-((4-isopropylpiperazin-1-yl)methyl)benzyl)-1H-indole-6-carboxylate **79e** (0.241 g, 0.547 mmol) in THF (4 mL) was added dropwise at -78 °C over 30 minutes. The reaction mixture was stirred at -78 °C over 30 minutes. Water (15 mL) was added dropwise at 0 °C, and the reaction mixture adjusted to pH 8. The product was extracted into DCM/methanol (9:1, 3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 8% methanol in DCM) afforded **83e** (0.187 g, 83% yield).

LCMS (ESI+): m/z 415.3 $[M + H]^+$, (ESI-): m/z 413.2 $[M - H]^-$, rt 1.45 minutes, >99%; ^{1}H NMR (400 MHz, $CDCl_3$) 7.98 (1H, s), 7.70 (1H, d, J = 8.4 Hz), 7.59 (1H, dd, J = 8.4, 1.5 Hz), 7.37 (1H, d, J = 3.2 Hz), 7.26 (2H, d, J = 8.2 Hz), 7.06 (2H, d, J = 8.1 Hz), 6.62 (1H, d, J = 3.2 Hz), 5.38 (2H, s), 4.10 (2H, s), 3.48 (2H, s), 2.76-2.41 (9H, m), 1.06 (6H, d, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 187.0, 138.2, 136.0, 135.4, 133.9, 133.4, 130.0, 128.1, 126.9, 121.3, 119.7, 114.5, 111.3, 102.6, 62.6, 54.8, 53.1, 50.3, 48.7, 29.6, 18.6; ν_{max}/cm^{-1} 2934, 2810, 2162 (C≡N), 1681 (C=O), 1607, 1502; HRMS (ESI)+: m/z calculated for $[C_{26}H_{30}N_4O + H]^+$ = 415.2492, observed 415.2479.

2-Methyl-3-oxo-3-(1-(4-(pyrrolidin-1-ylmethyl)benzyl)-1H-indol-6-yl)propanenitrile (84a).



n-Butyllithium (1.6 M in hexanes, 1.1 mL, 1.7 mmol) was added dropwise at -78 °C to a mixture of propionitrile (0.123 mL, 1.72 mmol) and toluene (4 mL). The reaction mixture was stirred at -78 °C over 30 minutes. A solution of methyl 1-(4-(pyrrolidin-1-ylmethyl)benzyl)-1H-indole-6-carboxylate **79a** (0.300 g, 0.861 mmol) in toluene (2 mL) was added dropwise at -78 °C over 30 minutes. The reaction mixture was stirred at -78 °C over 1 hour, then warmed to 0 °C and stirred over 1 hour. Water (15 mL) was added dropwise at 0 °C, and the reaction mixture adjusted to pH 8. The product was extracted into ethyl acetate (3 x 25 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether) afforded **84a** (0.140 g, 35% yield).

^{1}H NMR (400 MHz, $CDCl_3$) 8.03 (1H, s), 7.70 (2H, s), 7.36 (1H, d, J = 3.1 Hz), 7.29 (2H, d, J = 8.1 Hz), 7.09 (2H, d, J = 7.9 Hz), 6.61 (1H, d, J = 2.9 Hz), 5.38 (2H, s), 4.42 (1H, q, J = 7.2 Hz), 3.58 (2H, s), 2.54-2.40 (4H, m), 1.80-1.70 (4H, m), 1.64 (3H, d, J = 7.2 Hz).

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X-ray Crystallography

Figure S1: X-ray crystal structures of *Mab* TrmD in complex with a) **3** (PDB code 6QRF), b) **8** (PDB code 6QRG), and c) **14** (PDB code 6QQQ), illustrating one of the active sites.

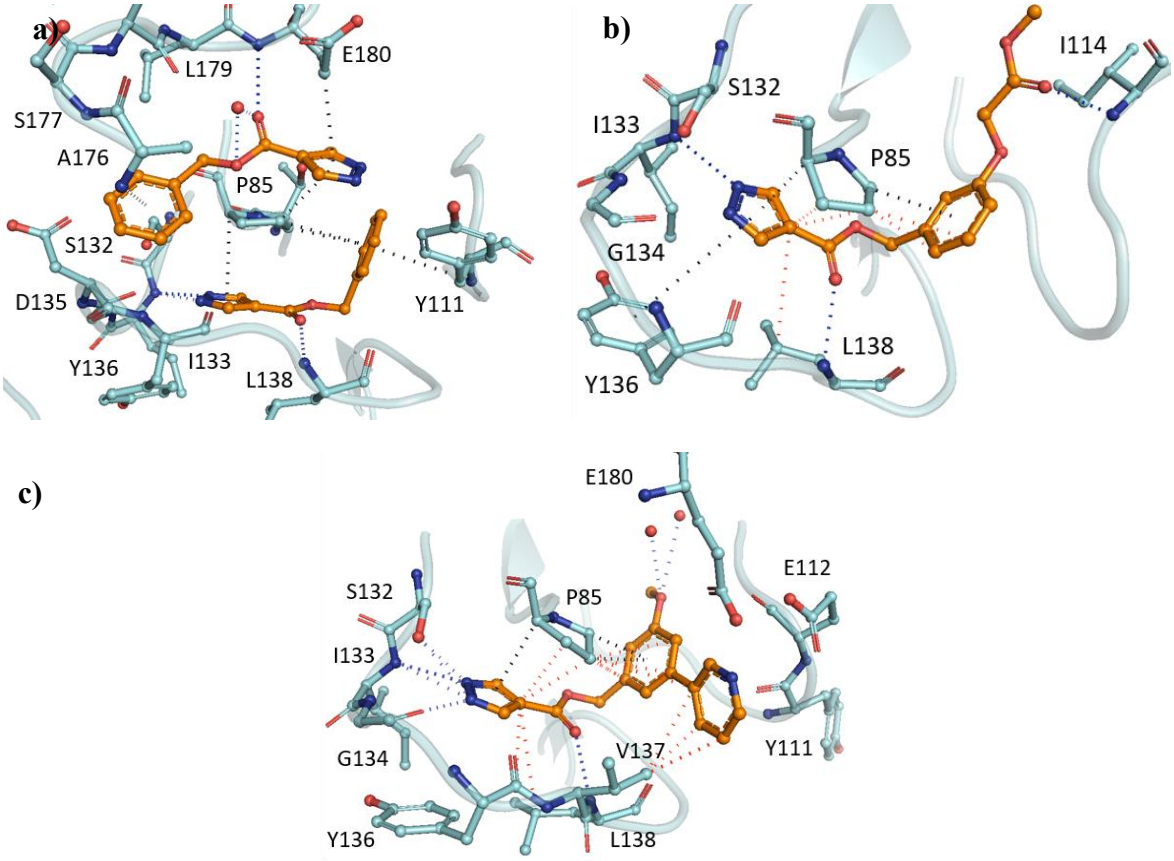

Figure S2: X-ray crystal structures of *Mab* TrmD in complex with a) **22** (PDB code 6QQR), b) **24d** (PDB code 6QQV), c) **26n** (PDB code 6QR4), and d) **31b** (PDB code 6QRA), illustrating one of the active sites.

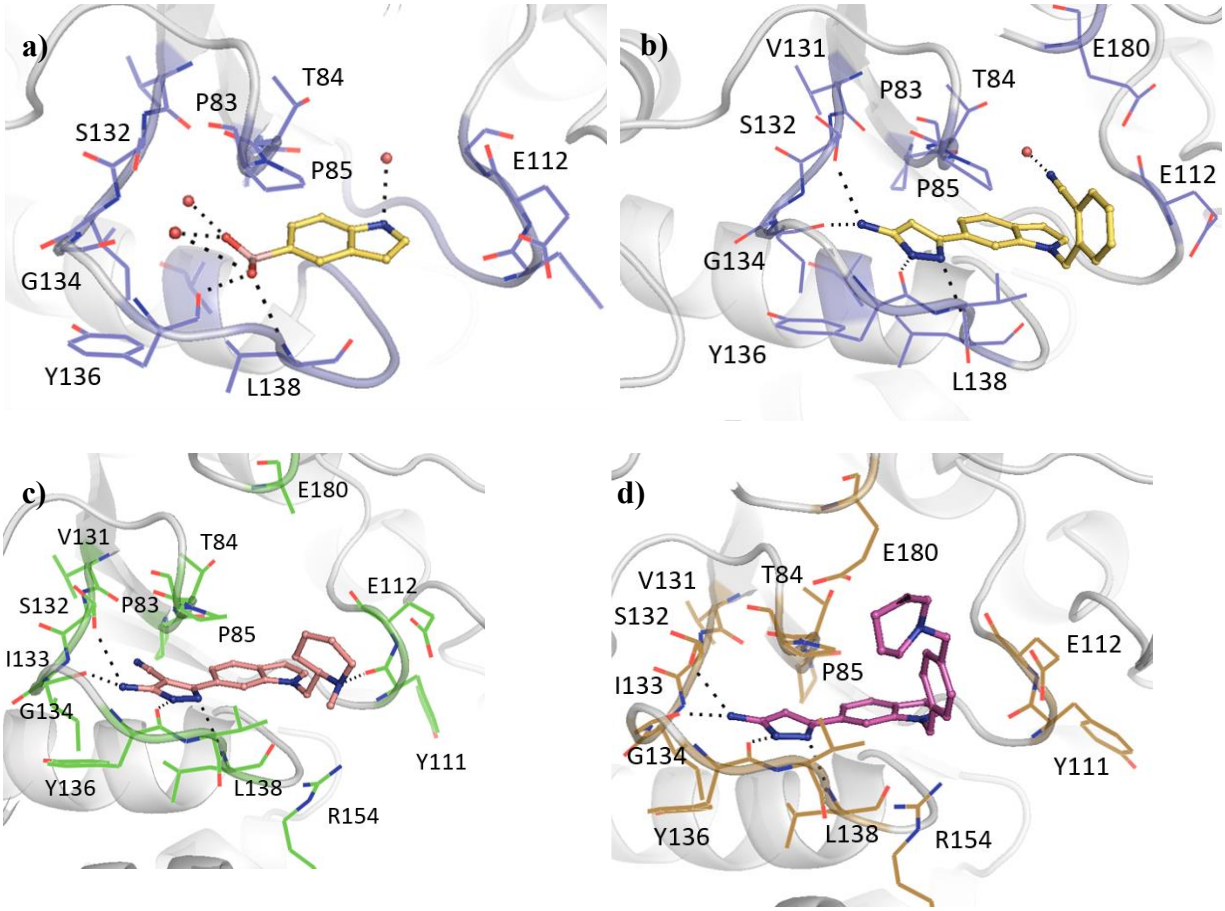

Table S1: X-ray crystallographic data collection and refinement statistics for crystal structures described in this study.

Ligand	3	7	8
PDB Codes	6QRF	6QRE	6QRG
Resolution range (Å)	45.44 - 1.86 (1.926 - 1.86)	57.92 - 1.926 (1.995 - 1.926)	38.93 - 1.838 (1.904 - 1.838)
Space group	P 21 21 21	P 21 21 21	P 21 21 21
Unit cell	73.82 78.61 84.83 90 90 90	73.7 79.2 84.918 90 90 90	74.273 77.867 86.49 90 90 90
Total reflections	84240 (8326)	68177 (6220)	88620 (8733)
Unique reflections	42137 (4164)	34257 (3168)	44337 (4369)
Multiplicity	2.0 (2.0)	2.0 (2.0)	2.0 (2.0)
Completeness (%)	99.98 (100.00)	89.41 (83.26)	99.96 (100.00)
Mean I/sigma(I)	18.67 (1.51)	23.24 (2.28)	21.81 (2.18)
Wilson B-factor	35.77	38.8	37.1
R-merge	0.01902 (0.5364)	0.01362 (0.2915)	0.01299 (0.2857)
R-meas	0.02689	0.01926	0.01838
CC1/2	1 (0.713)	1 (0.87)	1 (0.81)
CC*	1 (0.912)	1 (0.965)	1 (0.946)
R-work	0.1860 (0.3223)	0.1870 (0.2811)	0.1770 (0.2463)
R-free	0.2171 (0.3781)	0.2361 (0.3157)	0.1962 (0.2848)
Number of non-hydrogen atoms	3426	3382	3385
macromolecules	3184	3223	3215
ligands	65	68	47
water	177	91	123
Protein residues	417	425	426
RMS(bonds)	0.007	0.007	0.007
RMS(angles)	0.99	1.03	1.02
Ramachandran favored (%)	98	98	98
Ramachandran outliers (%)	0.72	0.71	0
Clashscore	1.57	3.27	1.41
Average B-factor	46.6	49.2	45.4
macromolecules	46.2	49	45
ligands	58.9	57.7	61.8
solvent	49.4	47.1	48.1

Ligand	14	22	24a
PDB Codes	6QQQ	6QQR	6QQT
Resolution range (Å)	57.64 - 1.848 (1.914 - 1.848)	58.17 - 1.559 (1.615 - 1.559)	45.74 - 1.673 (1.733 - 1.673)
Space group	P 21 21 21	P 21 21 21	P 21 21 21
Unit cell	74.517 77.662 85.999 90 90 90	73.78 78.711 86.357 90 90 90	74.591 77.862 86.636 90 90 90
Total reflections	84133 (8541)	144417 (14230)	117821 (11682)
Unique reflections	42260 (4288)	72301 (7121)	58926 (5841)
Multiplicity	2.0 (2.0)	2.0 (2.0)	2.0 (2.0)
Completeness (%)	97.27 (99.74)	99.93 (99.99)	99.99 (100.00)
Mean I/sigma(I)	14.12 (2.40)	27.04 (2.27)	21.36 (2.18)
Wilson B-factor	33.5	26.37	30.4
R-merge	0.02202 (0.3002)	0.01254 (0.3206)	0.01445 (0.323)
R-meas	0.03114	0.01773	0.02043
CC1/2	0.999 (0.829)	0.999 (0.778)	1 (0.764)
CC*	1 (0.952)	1 (0.936)	1 (0.931)
R-work	0.1906 (0.2531)	0.1812 (0.2414)	0.1712 (0.2422)
R-free	0.2281 (0.2739)	0.2030 (0.2539)	0.2052 (0.2673)
Number of non-hydrogen atoms	3410	3547	3491
macromolecules	3200	3241	3202
ligands	51	12	58
water	159	294	231
Protein residues	417	427	418
RMS(bonds)	0.007	0.006	0.006
RMS(angles)	0.97	1.04	0.98
Ramachandran favored (%)	99	99	99
Ramachandran outliers (%)	0	0.48	0
Clashscore	1.88	0.94	0.94
Average B-factor	38.6	34.2	38.7
macromolecules	38.4	33.2	38.1
ligands	37.5	32.1	42.2
solvent	43	45.2	47

Ligand	24b	24c	24d
PDB Codes	6QRC	6QQW	6QQV
Resolution range (Å)	57.73 - 1.73 (1.792 - 1.73)	31.34 - 1.8 (1.864 - 1.8)	57.73 - 1.712 (1.773 - 1.712)
Space group	P 21 21 21	P 21 21 21	P 21 21 21
Unit cell	74.459 77.412 86.648 90 90 90	74.52 77.97 86.5 90 90 90	74.556 77.749 86.182 90 90 90
Total reflections	105878 (10419)	94652 (9297)	109252 (10769)
Unique reflections	52953 (5211)	47361 (4652)	54642 (5391)
Multiplicity	2.0 (2.0)	2.0 (2.0)	2.0 (2.0)
Completeness (%)	99.99 (100.00)	99.98 (100.00)	99.99 (100.00)
Mean I/sigma(I)	15.71 (2.46)	18.22 (1.35)	15.20 (2.53)
Wilson B-factor	31.09	33.98	28.71
R-merge	0.02128 (0.2311)	0.02003 (0.5149)	0.02158 (0.2462)
R-meas	0.03009	0.02833	0.03053
CC1/2	0.999 (0.89)	1 (0.593)	0.999 (0.864)
CC*	1 (0.97)	1 (0.863)	1 (0.963)
R-work	0.1876 (0.2529)	0.1774 (0.3011)	0.1758 (0.2486)
R-free	0.2136 (0.2835)	0.2109 (0.3489)	0.2019 (0.2881)
Number of non-hydrogen atoms	3545	3417	3539
macromolecules	3251	3168	3206
ligands	53	49	58
water	241	200	275
Protein residues	421	411	420
RMS(bonds)	0.007	0.007	0.007
RMS(angles)	1.03	1	1.03
Ramachandran favored (%)	99	99	99
Ramachandran outliers (%)	0	0.25	0
Clashscore	1.08	0.95	1.25
Average B-factor	35	40	33.7
macromolecules	34.5	39.6	32.9
ligands	34.5	40.5	34.3
solvent	41.4	45.9	42.7
Ligand	25	26g	26j
PDB Codes	6QQU	6QQZ	6QR2
Resolution range (Å)	54.06 - 1.59 (1.647 - 1.59)	45.74 - 1.702 (1.763 - 1.702)	38.36 - 1.545 (1.6 - 1.545)
Space group	P 21 21 21	P 21 21 21	P 21 21 21
Unit cell	74.41 78.66 85.91 90 90 90	74.779 77.775 86.438 90 90 90	74.881 76.728 86.588 90 90 90
Total reflections	136750 (13512)	102804 (11037)	146213 (14565)
Unique reflections	68402 (6759)	51585 (5520)	73212 (7285)
Multiplicity	2.0 (2.0)	2.0 (2.0)	2.0 (2.0)
Completeness (%)	99.99 (100.00)	92.27 (100.00)	99.36 (99.97)
Mean I/sigma(I)	19.53 (1.44)	23.54 (2.20)	22.72 (2.33)
Wilson B-factor	27.51	29.12	26.83
R-merge	0.01701 (0.4995)	0.01437 (0.3218)	0.01326 (0.2743)
R-meas	0.02405	0.02032	0.01875
CC1/2	1 (0.618)	0.999 (0.776)	1 (0.812)
CC*	1 (0.874)	1 (0.935)	1 (0.947)
R-work	0.1801 (0.2660)	0.1730 (0.2312)	0.1687 (0.2349)
R-free	0.1992 (0.2959)	0.1961 (0.2809)	0.1821 (0.2626)
Number of non-hydrogen atoms	3490	3433	3537
macromolecules	3207	3146	3172
ligands	34	53	65
water	249	234	300
Protein residues	420	412	413
RMS(bonds)	0.007	0.007	0.006
RMS(angles)	1.07	1.04	1.05
Ramachandran favored (%)	99	99	99
Ramachandran outliers (%)	0	0	0
Clashscore	1.57	1.43	2.2
Average B-factor	34.7	35.8	34.4
macromolecules	34.1	35.2	33.1
ligands	24.4	32.4	40.3
solvent	44.3	45.3	46.3

Ligand	26l	26n	26o
PDB Codes	6QR0	6QR4	6QR3
Resolution range (Å)	56.81 - 1.59 (1.647 - 1.59)	57.89 - 1.517 (1.571 - 1.517)	57.61 - 1.614 (1.671 - 1.614)
Space group	P 21 21 21	P 21 21 21	P 21 21 21
Unit cell	74.92 77.17 87.15 90 90 90	74.79 77.652 86.845 90 90 90	74.87 77.106 86.663 90 90 90
Total reflections	136698 (13510)	157659 (15579)	130143 (12828)
Unique reflections	68515 (6762)	78858 (7791)	65146 (6417)
Multiplicity	2.0 (2.0)	2.0 (2.0)	2.0 (2.0)
Completeness (%)	99.96 (100.00)	99.98 (100.00)	99.98 (99.95)
Mean I/sigma(I)	19.62 (1.46)	23.67 (2.43)	13.17 (2.33)
Wilson B-factor	27.22	26.85	22.64
R-merge	0.01669 (0.5086)	0.01434 (0.272)	0.02737 (0.3382)
R-meas	0.0236	0.02028	0.03871
CC1/2	1 (0.619)	0.999 (0.832)	0.997 (0.754)
CC*	1 (0.875)	1 (0.953)	0.999 (0.927)
R-work	0.1737 (0.2815)	0.1852 (0.2344)	0.1801 (0.2446)
R-free	0.1987 (0.2809)	0.2060 (0.2563)	0.2060 (0.2979)
Number of non-hydrogen atoms	3553	3531	3558
macromolecules	3198	3211	3178
ligands	53	50	60
water	302	270	320
Protein residues	416	421	412
RMS(bonds)	0.006	0.006	0.006
RMS(angles)	1.05	1.05	1.07
Ramachandran favored (%)	99	99	99
Ramachandran outliers (%)	0	0	0
Clashscore	1.72	3.13	2.2
Average B-factor	34.6	32.9	28.7
macromolecules	33.8	32.3	27.4
ligands	27	27.2	28.2
solvent	44.6	41.2	41
Ligand	27g	29b	29c
PDB Codes	6QR1	6QR7	6QR9
Resolution range (Å)	56.48 - 1.669 (1.728 - 1.669)	57.97 - 2.028 (2.101 - 2.028)	54.04 - 2.417 (2.503 - 2.417)
Space group	P 21 21 21	P 21 21 21	P 21 21 21
Unit cell	74.432 77.484 86.716 90 90 90	75.011 77.787 86.943 90 90 90	75.679 77.179 87.556 90 90 90
Total reflections	521348 (53444)	66985 (6531)	170262 (16902)
Unique reflections	59008 (5843)	33528 (3268)	20227 (2006)
Multiplicity	8.8 (9.1)	2.0 (2.0)	8.4 (8.4)
Completeness (%)	99.97 (100.00)	99.69 (98.58)	99.94 (100.00)
Mean I/sigma(I)	24.79 (2.40)	9.25 (1.99)	8.81 (2.21)
Wilson B-factor	29.99	28.46	37.7
R-merge	0.04071 (0.8134)	0.06422 (0.5302)	0.2269 (1.712)
R-meas	0.04327	0.09082	0.2418
CC1/2	1 (0.807)	0.995 (0.482)	0.993 (0.626)
CC*	1 (0.945)	0.999 (0.807)	0.998 (0.877)
R-work	0.1797 (0.2375)	0.1882 (0.2550)	0.1818 (0.2296)
R-free	0.2051 (0.2573)	0.2258 (0.3018)	0.2461 (0.3030)
Number of non-hydrogen atoms	3484	3413	3299
macromolecules	3164	3174	3174
ligands	50	62	62
water	270	177	63
Protein residues	414	417	417
RMS(bonds)	0.006	0.007	0.008
RMS(angles)	1.02	1.07	1.15
Ramachandran favored (%)	99	99	98
Ramachandran outliers (%)	0	0.24	0
Clashscore	2.06	2.21	3.8
Average B-factor	35.8	31.7	42.2
macromolecules	35.1	31.3	42.2
ligands	31.8	31.8	41.6
solvent	44.6	39.4	43.5

Ligand	29e	31b
PDB Codes	**6QRD**	**6QRA**
Resolution range (Å)	53.51 - 1.749 (1.812 - 1.749)	37.67 - 1.705 (1.766 - 1.705)
Space group	P 21 21 21	P 21 21 21
Unit cell	74.532 76.876 86.787 90 90 90	74.134 79.218 85.635 90 90 90
Total reflections	102001 (10020)	111220 (11036)
Unique reflections	51013 (5017)	55632 (5519)
Multiplicity	2.0 (2.0)	2.0 (2.0)
Completeness (%)	99.97 (99.78)	99.96 (99.95)
Mean I/sigma(I)	28.07 (4.09)	16.93 (2.39)
Wilson B-factor	28.29	28.77
R-merge	0.01128 (0.1372)	0.01907 (0.2622)
R-meas	0.01595	0.02697
CC1/2	1 (0.953)	0.999 (0.869)
CC*	1 (0.988)	1 (0.964)
R-work	0.1687 (0.1854)	0.1836 (0.2263)
R-free	0.2062 (0.2245)	0.2133 (0.2782)
Number of non-hydrogen atoms	3535	3501
macromolecules	3186	3183
ligands	83	63
water	266	255
Protein residues	414	418
RMS(bonds)	0.007	0.006
RMS(angles)	1.09	1.04
Ramachandran favored (%)	99	99
Ramachandran outliers (%)	0	0
Clashscore	0.78	2.04
Average B-factor	34.6	33.3
macromolecules	33.6	32.6
ligands	45.3	35
solvent	43.3	40.9

Isothermal Titration Calorimetry (ITC)

Figure S3: ITC traces with *Mab* TrmD (n = 1) for a) **1**, b) **3**, c) **6**, and d) **7**.

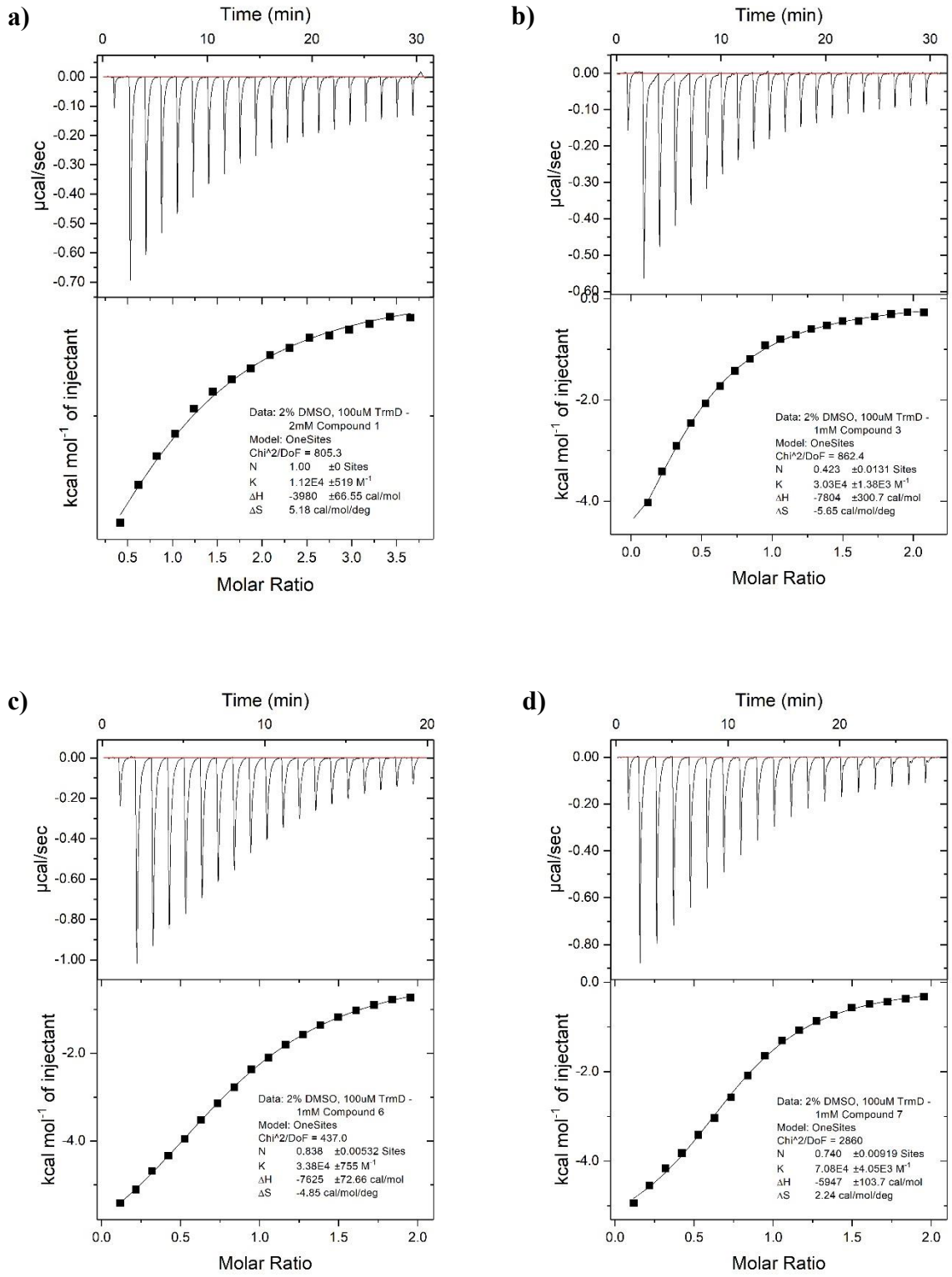

Figure S4: ITC traces with *Mab* TrmD (n = 1) for a) **8**, b) **10**, c) **12**, and d) **13**.

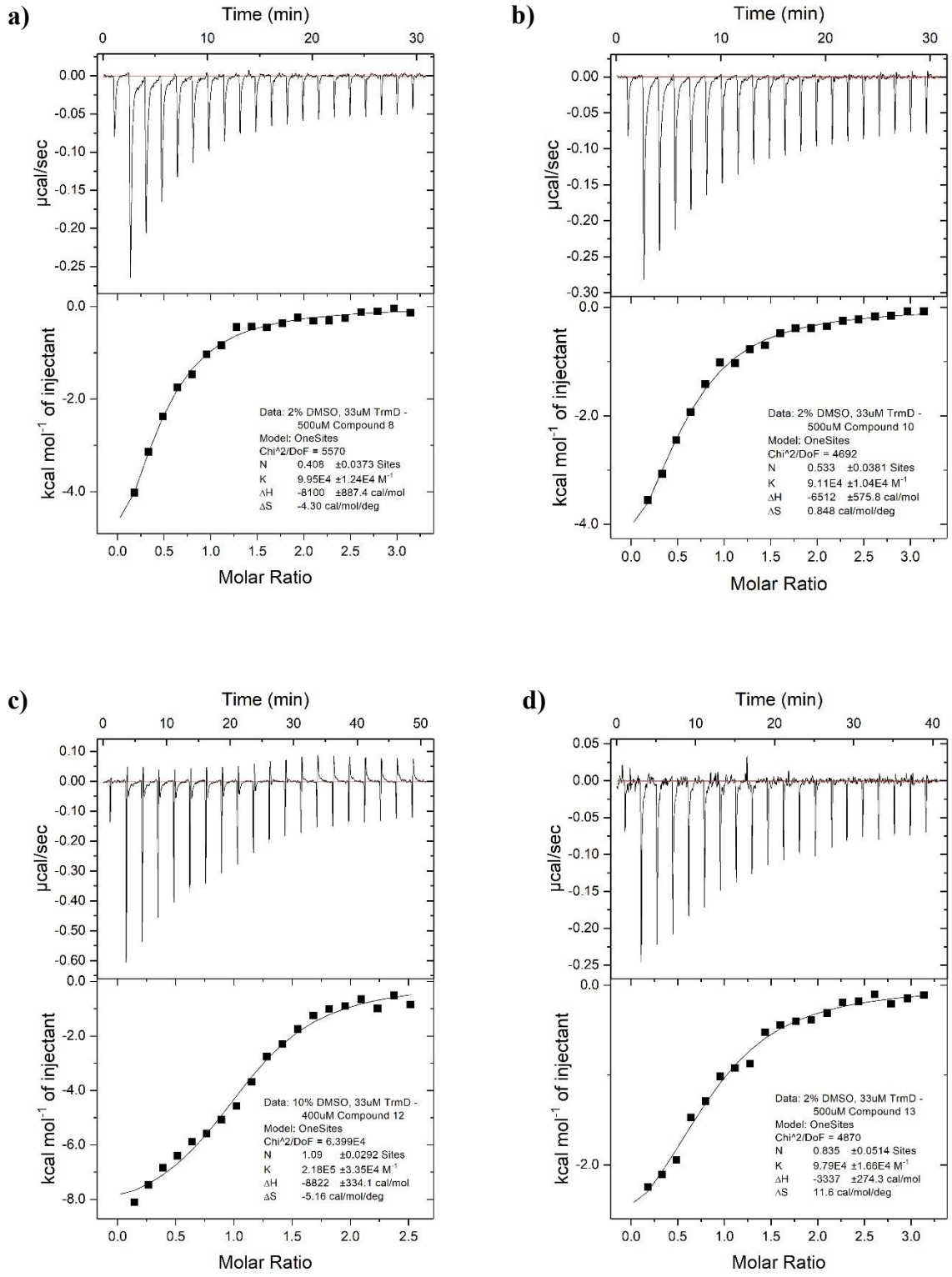

Figure S5: ITC traces with *Mab* TrmD (n = 1) for a) **14**, b) **15**, c) **24a**, and d) **24b**.

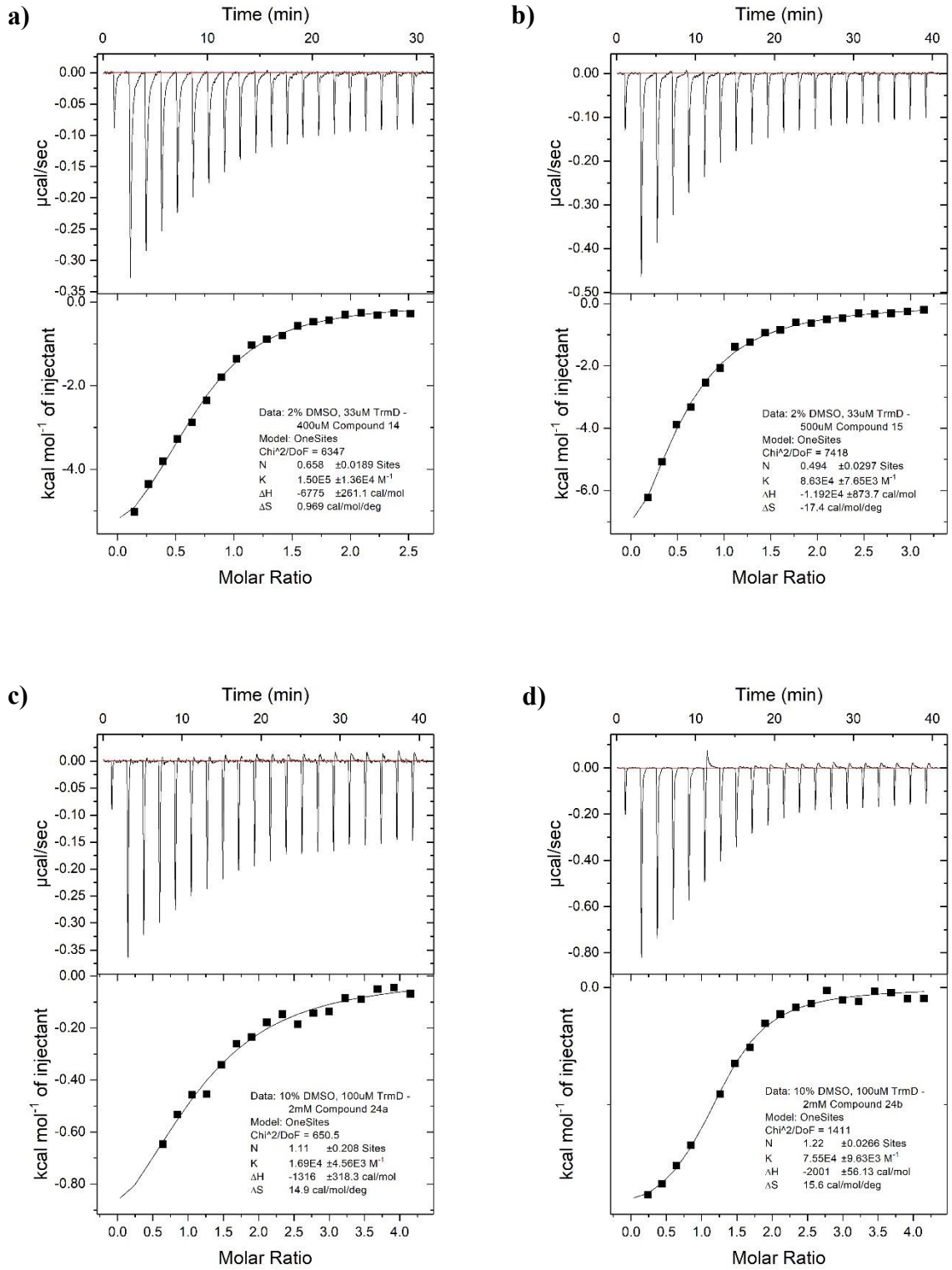

Figure S6: ITC traces with *Mab* TrmD for a) **24c** (n = 1), b) **24d** (n = 1), c) **25** (n = 2), and d) **26g** (n = 3).

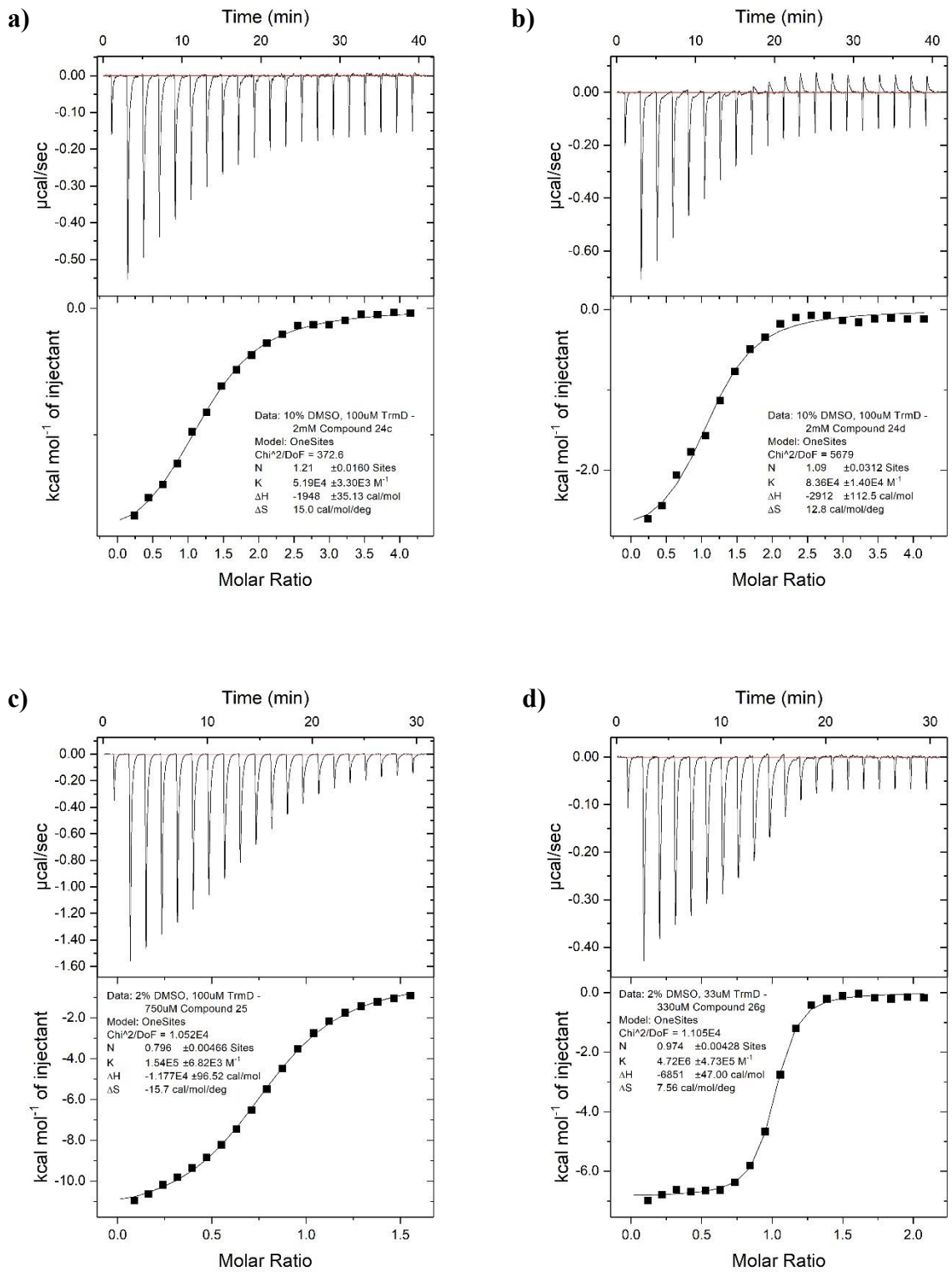

Figure S7: ITC traces with *Mab* TrmD for a) **26j** (n = 1), b) **26k** (n = 1), c) **26l** (n = 3), and d) **26n** (n = 2).

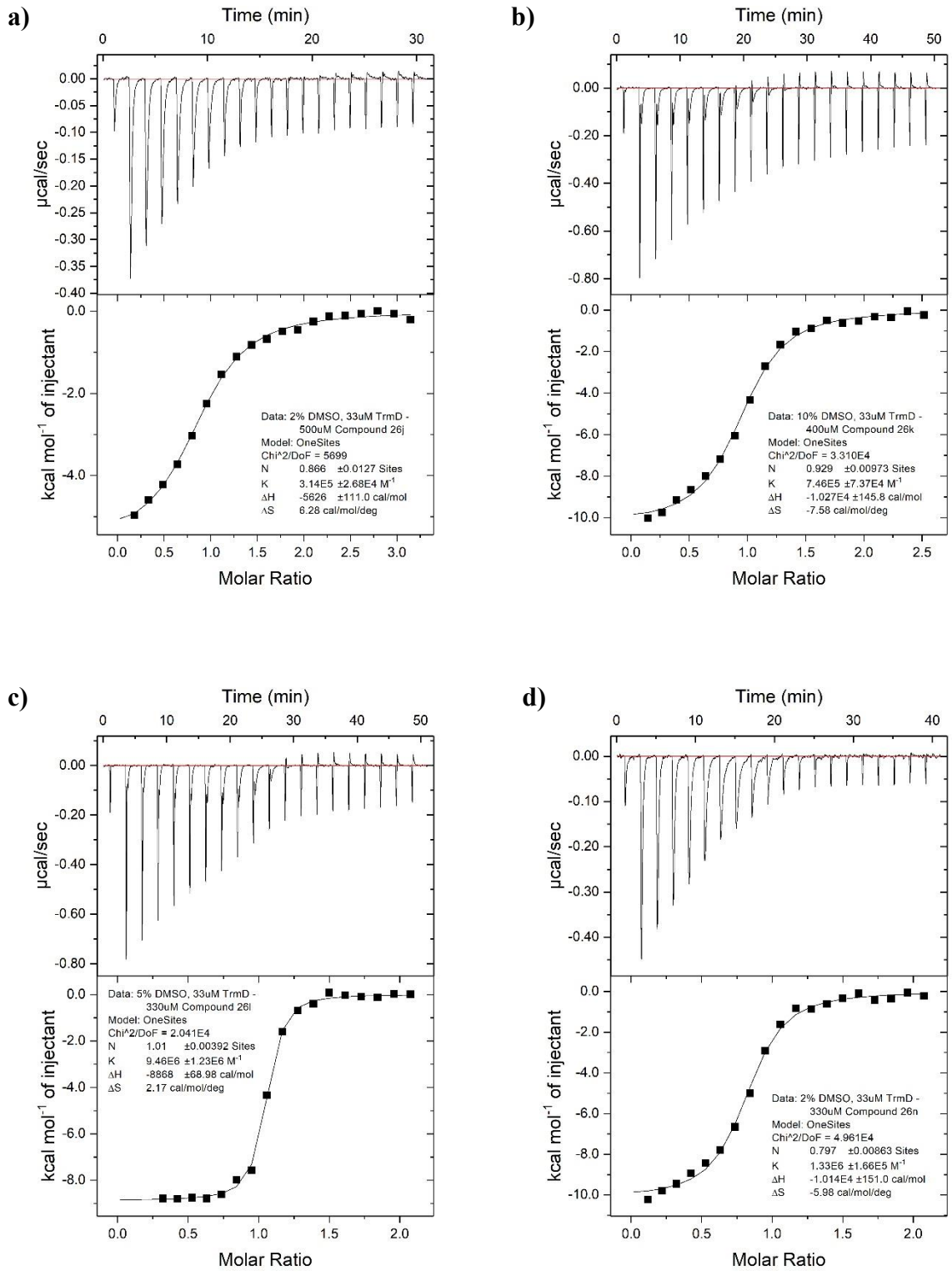

Figure S8: ITC traces with *Mab* TrmD for a) **26o** (n = 3), b) **26o** (Reverse titration, n = 1), c) **27g** (n = 1), and d) **29b** (n = 3).

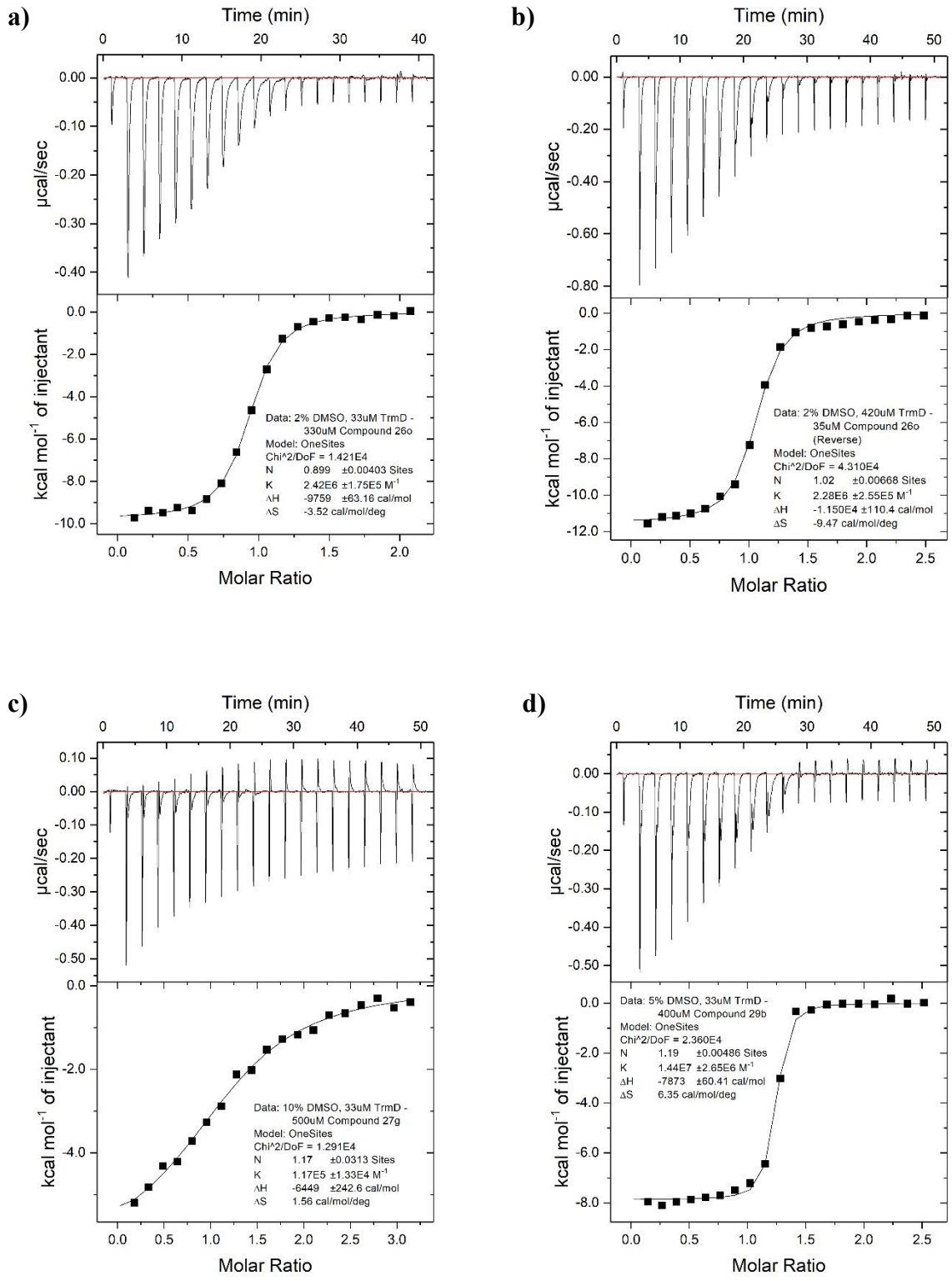

Figure S9: ITC traces with *Mab* TrmD for a) **29c** (n = 1), b) **29e** (n = 3), c) **30a** (n = 1), and d) **31b** (n = 2).