Electronic Supplementary Information

Fragment Optimization and Elaboration Strategies- The Discovery of Two Lead Series of PRMT5/MTA Inhibitors from Five Fragment Hits

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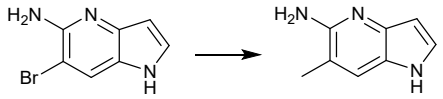
General experimental and chemical procedures

All chemicals were purchased from commercial suppliers and used as received unless otherwise indicated. Nuclear magnetic resonance (NMR) spectroscopy was performed on a Brüker Avance III 400 MHz spectrometer, where proton NMR (^{1}H NMR) spectra, carbon NMR (^{13}C NMR) spectra and fluorine (^{19}F NMR) were acquired at 400, 100 MHz and 376.5 MHz respectively. All spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d_6), deuterated methanol (CD_3OD-d_4) or deuterated chloroform ($CDCl_3$) obtained from Cambridge Isotope Laboratories Inc. Chemical shifts (δ) were measured in parts per million (ppm) and referenced against the internal reference peaks. Coupling constants (*J*), when given, are reported in hertz. Multiplicities are reported using the following abbreviations: s = singlet, d = doublet, dd = doublet of doublets,

t = triplet, q = quartet, m = multiplet (range of multiplet is given), br = broad signal, dt = doublet of triplets. Final Compounds were purified by reverse phase high-performance liquid chromatography (*prep*-HPLC) by either of the following conditions: HCl condition - Phenomenex Luna C18 75 x 30 mm x 3 μm; mobile phase: [water (0.05% HCl)-ACN]; B%: 10%–40%, 6.5 min. Basic condition - Waters Xbridge 150 x 25 mm x 5 μm; mobile phase: [water (0.05% ammonia hydroxide v/v)-acetonitrile]; B%: 3%–40%, 10 min. Ammonium bicarbonate condition - Waters Xbridge 150 x 25 mm x 5; mobile phase: water (10 mM ammonium bicarbonate) - acetonitrile; B: 2%–40%, 10 min. The purity for test compounds was determined by high-performance liquid chromatography (HPLC) on a LC-20AB Shimadzu instrument. HPLC conditions were as follows: Kinetex C18 LC Column 4.6 x 50 mm, 5 μm, 10%–80% ACN (0.0375% TFA) in water (0.01875% TFA), 4 min run, flow rate 1.5 mL/min, UV detection (λ = 220, 215, 254 nm) or XBridge C18, 2.1 x 50 mm, 5 μm, 10%–80% ACN in water buffered with 0.025% ammonia, 4 min run, flow rate 0.8 mL/min, UV detection (λ = 220, 215, 254 nm), or Kinetex EVO C18 100 x 4.6 mm, 2.6 μm, 0%-60% (or 10%–80%) ACN (0.0375% TFA) in water (0.01875% TFA) 10 min run, flow rate 1.0 mL/min, UV detection (λ = 220, 254 nm) or Eclipse plus C18 150 x 4.6 mm, 3.5 μm, 10%–80%) ACN (0.0375% TFA) in water (0.01875% TFA) 15 min run, flow rate 1.0 mL/min, UV detection (λ = 220, 254 nm). The mass spectra were obtained using liquid chromatography mass spectrometry (LCMS) on a LCMS-2020 Shimadzu instrument using electrospray ionization (ESI). LCMS conditions were as follows: Kinetex EVO C18 30 x 2.1 mm, 5 μm, 5%–95% ACN (0.0375% TFA) in water (0.01875% TFA), 1.5 min run, flow rate 1.5 mL/min, UV detection (λ = 220, 254 nm), or Kinetex EVO C18 2.1 x 30 mm, 5 μm， 5%–95% ACN in water buffered with 0.025% ammonia, 1.5 min run, flow rate 1.5 mL/min, UV detection (λ = 220, 254 nm). High resolution mass measurements were carried out on an Agilent 1290LC & 6530Q-TOF series with ESI. All compounds are > 95% pure by HPLC.

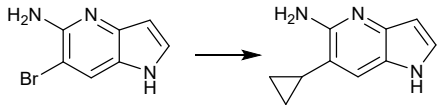
Example 6: 6-methyl-1*H*-pyrrolo[3,2-*b*]pyridin-5-amine.



Example 6

To a solution of 6-bromo-1*H*-pyrrolo[3,2-*b*]pyridin-5-amine (150 mg, 707 µmol, 1.00 *eq.*) and methylboronic acid (212 mg, 3.54 mmol, 5.00 *eq.*) in 1,4-dioxane (2.5 mL) and water (0.5 mL) was added potassium carbonate (293 mg, 2.12 mmol, 3.00 *eq.*) and Pd(dppf)Cl_2 -DCM (58 mg, 70.7 µmol, 0.10 *eq.*). The reaction mixture was stirred at 80 °C for 16 hours under nitrogen atmosphere before being diluted with water (50 mL) and filtered. The filtrate was concentrated. The residue was purified by *prep*-HPLC (basic condition) and lyophilized to afford **6** (21.9 mg, 148 µmol, 21% yield) as a yellow solid. LCMS [M+1] $^+$: 148.3. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.63 (br s, 1H), 7.30 (s, 1H), 7.19 (t, J = 2.8 Hz, 1H), 6.11 (dt, J = 0.8, 2.4 Hz, 1H), 5.06 (s, 2H), 2.13 (s, 3H); HRMS (ESI, +ve ion) *m/z* calcd for $C_8H_9N_3$ 147.0796; found 147.0787; HPLC Rt 1.433 min, 99.7%.

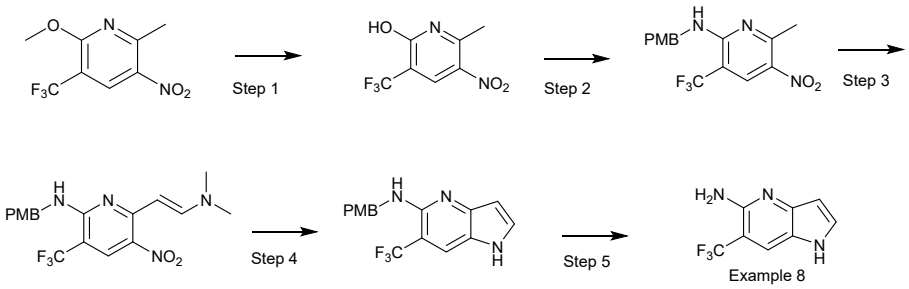
Example 7: 6-cyclopropyl-1*H*-pyrrolo[3,2-*b*]pyridin-5-amine.



Example 7

To a solution of 6-bromo-1*H*-pyrrolo[3,2-*b*]pyridin-5-amine (150 mg, 707 µmol, 1.00 *eq.*) and cyclopropylboronic acid (304 mg, 3.54 mmol, 5.00 *eq.*) in 1,4-dioxane (2.5 mL) and water (0.5 mL) was added potassium carbonate (293 mg, 2.12 mmol, 3.00 *eq.*) and Pd(dppf)Cl_2 -DCM (58 mg, 70.7 µmol, 0.10 *eq.*). The mixture was stirred at 80 °C for 16 hours under nitrogen atmosphere before being diluted with water (50 mL) and filtered. The filtrate was concentrated. The residue was purified by *prep*-HPLC (basic condition) and lyophilized to afford **7** (8.3 mg, 47.4 µmol, 7% yield) as a white solid. LCMS [M+1] $^+$: 174.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.61 (br s, 1H), 7.21 (t, J = 2.8 Hz, 1H), 7.18 (s, 1H), 6.11 (ddd, J = 0.8, 2.0, 2.8 Hz, 1H), 5.20 (s, 2H), 1.78 - 1.69 (m, 1H), 0.92 - 0.85 (m, 2H), 0.54 - 0.47 (m, 2H); HRMS (ESI, +ve ion) *m/z* calcd for $C_{10}H_{11}N_3$ 173.0953; found 173.0941; HPLC Rt 1.319 min, 99.0%.

Example 8: 6-(trifluoromethyl) -1*H*-pyrrolo[3,2-*b*]pyridin-5-amine.



Step 1: To a mixture of 2-methoxy-6-methyl-5-nitro-3-(trifluoromethyl)pyridine (prepared following the method from WO2018215316) (500 mg, 2.12 mmol, 1.00 *eq.*) in acetonitrile (10 mL) was added chlorotrimethylsilane (1.15 g, 10.6 mmol, 1.34 mL, 5.00 *eq.*) and sodium iodide (1.59 g, 10.6 mmol, 5.00 *eq.*). The reaction mixture was stirred at 70 °C for 2 hours. The mixture was concentrated and the residue was purified by reversed-phase HPLC (formic acid condition) to afford 6-methyl-5-nitro-3-(trifluoromethyl)pyridin-2-ol (290 mg, 1.26 mmol, 60% yield) as a black solid. LCMS [M+1] $^{+}$: 223.0. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.23 (s, 1H), 8.46 (s, 1H), 2.70 (s, 3H).

Step 2: To a mixture of 6-methyl-5-nitro-3-(trifluoromethyl)pyridin-2-ol (250 mg, 1.13 mmol, 1.00 *eq.*) in acetonitrile (6 mL) was added DBU (343 mg, 2.25 mmol, 339 µL, 2.00 *eq.*), (4-methoxyphenyl)methanamine (463 mg, 3.38 mmol, 437 µL, 3.00 *eq.*) and BOP (647 mg, 1.46 mmol, 1.30 *eq.*). The mixture was stirred at 30 °C for 2 hours before being concentrated and purified by flash silica gel chromatography (Ethyl acetate / Petroleum ether 0-20%) to afford *N*-[(4-methoxyphenyl)methyl]-6-methyl-5-nitro-3-(trifluoromethyl)pyridin-2-amine (280 mg, 818 µmol, 73% yield) as a yellow oil. LCMS [M+1] $^{+}$: 341.9. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.48 (s, 1H), 7.26 (s, 2H), 6.96 - 6.84 (m, 2H), 5.73 (s, 1H), 4.75 (d, J = 5.2 Hz, 2H), 3.81 (s, 3H), 2.85 (s, 3H).

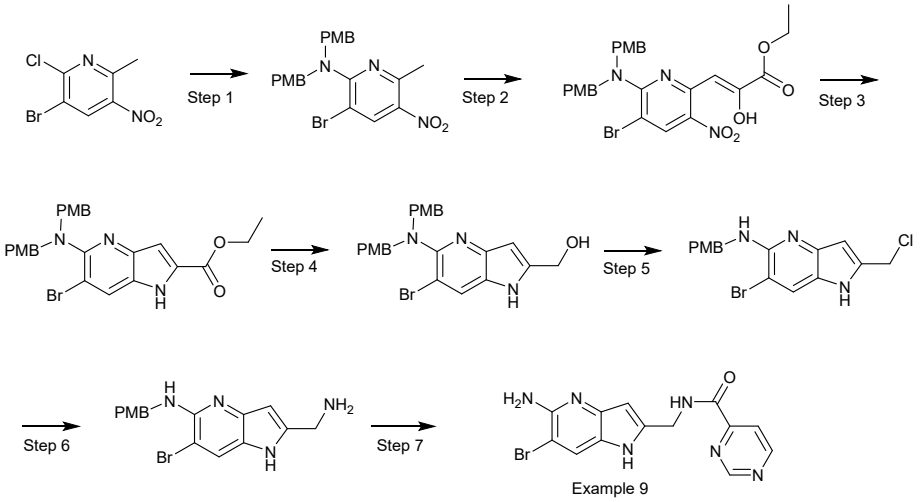
Step 3: To a solution of *N*-[(4-methoxyphenyl)methyl]-6-methyl-5-nitro-3-(trifluoromethyl)pyridin-2-amine (250 mg, 733 µmol, 1.00 *eq.*) in DMF (4.00 mL) was added *N,N*-dimethyl formamide dimethyl acetal (436 mg, 3.66 mmol, 486 µL, 5.00 *eq.*). The mixture was stirred at 90 °C for 3 hours. The reaction mixture was poured into brine (50 mL), extracted with ethyl acetate (10 mL × 3). The combined organic layers were washed with water (30 mL), dried over anhydrous sodium sulfate, filtered and concentrated to afford 6-[(*E*)-2-(dimethylamino)vinyl]-*N*-[(4-methoxyphenyl)methyl]-5-nitro-3-(trifluoromethyl)pyridin-2-

amine (290 mg, 702 µmol, 96% yield) as a brown solid. LCMS [M+1] $^{+}$: 397.2. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.45 (s, 1H), 8.00 (d, J = 2.4 Hz, 1H), 7.24 (d, J = 8.8 Hz, 2H), 6.93 - 6.82 (m, 2H), 6.47 (d, J = 12.0 Hz, 1H), 5.52 (s, 1H), 4.67 (d, J = 5.2 Hz, 2H), 3.79 (s, 3H), 3.03 (d, J = 6.4 Hz, 6H).

Step 4: A suspension of Fe (42.3 mg, 757 µmol, 6.00 *eq.*) in acetic acid (1.0 mL) was stirred at 25 °C for 0.5 hour and then 6-[(*E*)-2-(dimethylamino)vinyl]-*N*-[(4-methoxyphenyl)methyl]-5-nitro-3-(trifluoromethyl)pyridin-2-amine (50.0 mg, 126 µmol, 1.00 *eq.*) was added. The reaction mixture was stirred at 25 °C for 1 hour, filtered and concentrated to afford *N*-[(4-methoxyphenyl)methyl]-6-(trifluoromethyl)-1*H*-pyrrolo[3,2-b]pyridin-5-amine (30 mg, crude, 67% pure by LCMS) as a brown solid. LCMS [M+1] $^{+}$: 322.1.

Step 5: To a mixture of *N*-[(4-methoxyphenyl)methyl]-6-(trifluoromethyl)-1*H*-pyrrolo[3,2-b]pyridin-5-amine (30.0 mg, crude, 67% pure by LCMS) in dichloromethane (3.0 mL) was added trifluoroacetic acid (1.0 mL). The mixture was stirred at 25 °C for 16.5 hours. The mixture was basified to pH 7 with ammonium hydroxide and concentrated. The crude material was purified by *prep*-HPLC (basic condition) to afford **8** (2.5 mg, 12.0 µmol, 10% yield over 2 steps) as an off-white solid. LCMS [M+1] $^{+}$: 202.1. ^{1}H NMR (400 MHz, CD_3OD-d_4) δ = 7.89 (s, 1H), 7.54 (d, J = 3.2 Hz, 1H), 6.32 (dd, J = 0.8, 3.2 Hz, 1H); ^{19}F NMR (376.5 MHz, CD_3OD-d_4) δ -64.4 (s, 1F); HPLC Rt 1.323 min, 95.1%.

Example 9: *N*-[(5-amino-6-bromo-1*H*-pyrrolo[3,2-*b*]pyridin-2-yl)methyl]pyrimidine-4-carboxamide.



Step 1: To a solution of 3-bromo-2-chloro-6-methyl-5-nitro-pyridine (19.0 g, 75.5 mmol, 1.00 *eq*.) and 1-(4-methoxyphenyl)-*N*-[(4-methoxyphenyl)methyl]methanamine (23.3 g, 90.6 mmol, 1.20 *eq*.) in THF (190 mL) was added sodium carbonate (9.61 g, 90.6 mmol, 1.20 *eq*). The mixture was stirred at 75 °C for 16 h. The mixture was concentrated, diluted with ethyl acetate (500 mL), washed with water (200 mL), dried over sodium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography (Ethyl acetate / Petroleum ether 0-50 %) to afford 3-bromo-*N*,*N*-bis[(4-methoxyphenyl)methyl]-6-methyl-5-nitro-pyridin-2-amine (33.0 g, 68.4 mmol, 91% yield) as a yellow solid. LCMS [M+1] $^{+}$: 472.0. ^{1}HNMR (400 MHz, $CDCl_3$) δ = 8.53 (s, 1H), 7.20 (d, J = 8.8 Hz, 4H), 6.94 - 6.80 (m, 4H), 4.75 (s, 4H), 3.82 (s, 6H), 2.76 (s, 3H).

Step 2: To a mixture of 3-bromo-*N*,*N*-bis[(4-methoxyphenyl)methyl]-6-methyl-5-nitro-pyridin-2-amine (10.0 g, 21.1 mmol, 1.00 *eq*.) and diethyl oxalate (9.20 g, 63.5 mmol, 8.60 mL, 3.00 *eq*.) was added DABCO (3.87 g, 25.4 mmol, 3.83 mL, 1.20 *eq*.). The mixture was stirred at 30 °C for 16 h. The mixture was diluted with ethyl acetate (500 mL). Acetic acid (4.0 mL) was added. The resulting solution was washed with water (300 mL), dried over sodium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography (Ethyl acetate / Petroleum ether 0-50 %) to afford ethyl (*Z*)-3-[6-[bis[(4-methoxyphenyl)methyl]amino]-5-bromo-3-nitro-2-pyridyl]-2-hydroxy-prop-2-enoate (7.20 g, 10.6 mmol, 51% yield, 85% purity by LCMS) as a yellow solid. LCMS [M+1] $^{+}$: 572.2. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.70 - 8.63 (m, 1H), 7.53 (s, 1H), 7.07 - 6.98 (m, 4H), 6.88 - 6.83 (m, 4H), 4.64 -4.54 (m, 4H), 4.38 (q, J = 7.2 Hz, 2H), 3.81 (s, 6H), 1.43 - 1.30 (m, 3H).

Step 3: To a solution of ethyl (*Z*)-3-[6-[bis[(4-methoxyphenyl)methyl]amino]-5-bromo-3-nitro-2-pyridyl]-2-hydroxy-prop-2-enoate (5.10 g, 8.91 mmol, 1.00 *eq*.) in THF (15 mL), ethanol (90 mL) and water (10 mL) was added ammonium chloride (571 mg, 10.6 mmol, 1.20 *eq*) followed by iron powder (1.99 g, 35.6 mmol, 4.00 *eq*.) at 25 °C. The mixture was then stirred at 60 °C for 6 hours. After such time the mixture was diluted with dichloromethane (600 mL) and water (600 mL), stirred for 10 min, filtered and the organic layer was separated, dried over sodium sulfate, filtered and concentrated. The residue was purified by silica gel chromatography (Ethyl acetate / Petroleum ether 0-80 %) to afford ethyl 5-[bis [(4-methoxyphenyl) methyl] amino]-6-bromo-1*H*-pyrrolo [3, 2-*b*] pyridine-2-carboxylate (2.10 g, 3.88 mmol, 44% yield) as a yellow solid. LCMS

[M+1] $^{+}$: 526.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.92 (brs, 1H), 7.94 (s, 1H), 7.32 (d, *J* = 8.4 Hz, 4H), 7.20 (d, *J* = 1.2 Hz, 1H),6.89 - 6.78 (m, 4H), 4.44 (q, *J* = 7.2 Hz, 2H), 4.36 (s, 4H), 3.78 (s, 6H), 1.43 (t, *J* = 7.2 Hz, 3H).

Step 4: To a solution of ethyl 5-[bis[(4-methoxyphenyl)methyl]amino]-6-bromo-1*H*-pyrrolo[3,2-*b*]pyridine-2-carboxylate (900 mg, 1.72 mmol, 1.00 *eq*.) in THF (20 mL) was added lithium aluminum hydride (195 mg, 5.15 mmol, 3.00 *eq*.) at 0 °C. The mixture was stirred at 25 °C for 0.5 hour. The mixture was diluted with tetrahydrofuran (100 mL). Sodium sulfate decahydrate (5.0 g) was added. The resulting solution was stirred for 0.5 hour. The resulting mixture was filtered and the filtrate concentrated to afford [5-[bis[(4-methoxyphenyl)methyl]amino]-6-bromo-1*H*-pyrrolo[3,2-*b*]pyridin-2-yl]methanol (827 mg, crude, 76% purity by LCMS) as a brown solid. LCMS [M+1] $^{+}$: 484.2. ^{1}HNMR (400 MHz, $CDCl_3$) δ = 8.53 (br s, 1H), 7.66 - 7.58 (m, 1H), 7.22 (br d, *J* = 8.4 Hz, 4H), 6.91 (s, 1H), 6.71(d, *J* = 8.4 Hz, 4H), 4.62 (s, 2H), 4.20 (s, 4H), 3.66 (s, 6H).

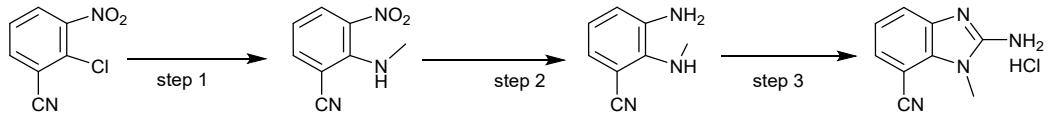
Step 5: To a solution of [5-[bis[(4-methoxyphenyl)methyl]amino]-6-bromo-1*H*-pyrrolo[3,2-*b*]pyridin-2-yl]methanol (827 mg, crude, 76% purity by LCMS) in dichloromethane (20 mL) was added thionyl chloride (1.02 g, 8.57 mmol, 621 µL) and DMF (125 mg, 1.71 mmol, 131 µL) at 0 °C. The mixture was stirred at 25 °C for 0.5 hour. The mixture was concentrated to afford 6-bromo-2-(chloromethyl)-*N*-[(4-methoxyphenyl)methyl]-1*H*-pyrrolo [3,2-*b*] pyridin-5-amine (652 mg, crude) as a black solid. Which was used direclty in the next step.

Step 6: Ammonia gas was passed through ethanol (20 mL) at 0 °C for 10 min. 6-bromo-2-(chloromethyl)-*N*-[(4-methoxyphenyl)methyl]-1*H*-pyrrolo[3,2-*b*]pyridin-5-amine (652 mg, crude) in methanol (15 mL) was added to the ammonia solution. The mixture was stirred at 25 °C for 16 hours. The mixture was concentrated, and the residue was purified by silica gel chromatography (methanol / dichloromethane 0-50%, 5% ammonium hydroxide) to afford 2-(aminomethyl)-6-bromo-*N*-[(4-methoxyphenyl) methyl]-1*H*-pyrrolo [3, 2-*b*] pyridin-5-amine, (75 mg, 159 µmol, 9% yield over 3 steps) as a brown solid. LCMS [M+1] $^{+}$: 363.1.

Step 7: To a solution of 2-(aminomethyl)-6-bromo-*N*-[(4-methoxyphenyl) methyl]-1*H*-pyrrolo [3, 2-*b*] pyridin-5-amine (70 mg, 193 µmol, 1.00 *eq*.) and pyrimidine-4-carboxylic acid (36 mg, 290 µmol, 1.50 *eq*.) in dichloromethane (4.0 mL) was added HATU (110 mg, 290 µmol, 1.50

eq.) and diisopropylethylamine (581 μmol, 101 μL, 3.00 *eq.*) at 25 °C. The mixture was stirred at 25 °C for 1h. The mixture was concentrated and the residue purified by silica gel chromatography (Petroleum ether / Ethyl acetate 0-100 %) to afford *N*-[[6-bromo-5-[(4-methoxyphenyl)methylamino]-1*H*-pyrrolo[3,2-*b*]pyridin-2-yl]methyl]pyrimidine-4-carboxamide (40 mg, 77.0 μmol, 40% yield, 90% purity by LCMS) as brown solid. LCMS [M+1] $^{+}$: 467.1. To a solution of *N*-[[6-bromo-5-[(4-methoxyphenyl)methylamino]-1*H*-pyrrolo[3,2-*b*]pyridin-2-yl]methyl]pyrimidine-4-carboxamide (61.4 μmol, 1.00 *eq*.) in dichloromethane (2.5 mL) was added trifluoroacetic acid (0.5 mL) at 10 °C. The mixture was stirred at 30 °C for 6 hrs. The mixture was basified to pH 8 by addition of saturated sodium bicarbonate, extracted with dichloromethane (100 mL), dried over sodium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography (dichloromethane/ methanol 0-10%) to afford **9** (8.9 mg, 24 μmol, 12% yield over 3 steps) as an off-white solid. LCMS [M+1] $^{+}$: 346.9; ^{1}H NMR (400 MHz, CD_3OD-d_4) δ = 9.30 (d, J = 1.2 Hz, 1H), 9.05 (d, J = 5.2 Hz, 1H), 8.32 (s, 1H), 8.12 (dd, J = 1.2, 5.2 Hz, 1H), 6.43 (s, 1H), 4.81 - 4.77 (m, 2H); HPLC Rt 1.376 min, 95.7%.

Example 13: 2-amino-1-methyl-1*H*-benzo[*d*]imidazole-7-carbonitrile hydrochloride.



Example 13

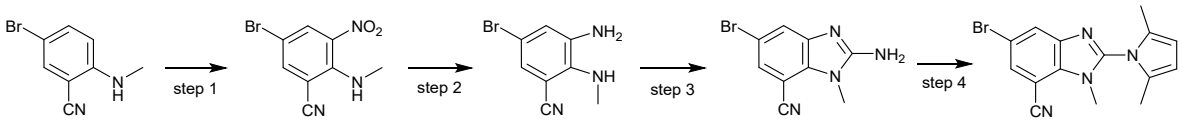
Step 1: To a solution of 2-chloro-3-nitro-benzonitrile (250 mg, 1.37 mmol, 1.0 *eq.*) in ethanol (2.5 mL) was added methylamine in ethanol (42.5 mg, 1.37 mmol, 2.5 M, 10.0 *eq.*). The reaction mixture was stirred at 15 °C for 12 hours then concentrated. The residue was diluted with water (50 mL) and extracted with ethyl acetate (3 x 60 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated to produce 2-(methylamino)-3-nitro-benzonitrile (240 mg, 1.35 mmol, 99% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.51 (s, 1H), 8.39 (d, *J* = 8.8 Hz, 1H), 7.76 (d, *J* = 7.6 Hz, 1H), 6.72 (t, *J* = 8.0 Hz, 1H), 3.49 (d, *J* = 5.2 Hz, 3H).

Step 2: To a solution of 2-(methylamino)-3-nitro-benzonitrile (240 mg, 1.35 mmol, 1.00 *eq.*) in water (3.0 mL) was added iron powder (378 mg, 6.77 mmol, 5.00 *eq.*) and hydrochloric acid (6.00 M, 903 μL, 4.00 *eq.*). The reaction mixture was stirred at 15 °C for 12 hours and after such

time the mixture was concentrated. The residue was diluted with water (50 mL) and extracted with ethyl acetate (2 x 100 mL). The combined organic extracts were dried over sodium sulfate, filtered and concentrated to give 3-amino-2-(methylamino)benzonitrile (140 mg, 0.95 mmol, 70% yield) as a black solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.99 (dd, *J* = 1.6, 7.6 Hz, 1H), 6.91 - 6.85 (m, 1H), 6.84 - 6.77 (m, 1H), 4.06 - 3.42 (m, 2H), 3.32 - 3.08 (m, 1H), 3.01 (s, 3H).

Step 3: To a solution of 3-amino-2-(methylamino)benzonitrile (140 mg, 0.95 mmol, 1.00 *eq.*) in ethanol (3.0 mL) was added cyanogen bromide (202 mg, 1.90 mmol, 2.00 *eq.*). The reaction mixture was stirred at 15 °C for 2 hours. The mixture was then concentrated, and the residue diluted with water (50 mL) and extracted with ethyl acetate (100 x 2 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated. The residue was purified by *prep*-HPLC (HCl condition) to afford **13** (30 mg, 171 μmol, 18% yield) as a white solid. LCMS [M+1]$^+$: 173.0. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.26 (s, 1H), 9.07 (s, 2H), 7.78 - 7.60 (m, 2H), 7.39 (t, *J* = 8.0 Hz, 1H), 3.85 (s, 3H); HPLC Rt 1.283 min, 99.4%.

Compound 19: 5-bromo-2-(2,5-dimethyl-1*H*-pyrrol-1-yl)-1-methyl-1*H*-benzo[*d*]imidazole-7-carbonitrile.



Step 1: To a solution of 5-bromo-2-(methylamino)benzonitrile (15.0 g, 71.1 mmol, 1.00 *eq.*) in acetonitrile (300 mL) was added nitronium tetrafluoroborate (8.78 mL, 85.3 mmol, 1.20 *eq.*) at 0 °C. The reaction was stirred at 20 °C for 14 hours. The reaction mixture was diluted with water (100 mL) and stirred for 5 min. The aqueous phase was extracted with ethyl acetate (150 mL × 3). The combined organic phase was dried with anhydrous sodium sulfate, filtered, and concentrated in vacuum to give a residue. The residue was purified by column chromatography (SiO_2, ethyl acetate in petroleum ether/ 1-33%) to produce 5-bromo-2-(methylamino)-3-nitro-benzonitrile (6.00 g, 23.4 mmol, 33% yield) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.90 - 8.88 (m, 1H), 8.87 - 8.85 (m, 1H), 3.71 (s, 3H).

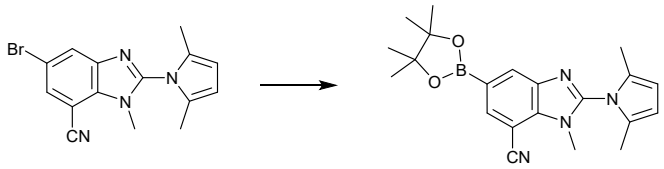
Step 2: To a solution of 5-bromo-2-(methylamino)-3-nitrobenzonitrile (5.00 g, 19.5 mmol, 1.00 *eq.*) in ethyl acetate (50 mL) and water (1.5 mL) was added acetic acid (15 mL) and iron powder

(10.9 g, 195 mmol, 10.0 *eq.*). The reaction was stirred at 60 °C for 1 hour. The reaction mixture was diluted with water (100 mL) and stirred for 5 min. The aqueous phase was extracted with ethyl acetate (200 mL × 3). The combined organic phase was dried over anhydrous sodium sulfate, filtered and concentrated in vacuum to afford 3-amino-5-bromo-2-(methylamino)benzonitrile (4.10 g, 18.1 mmol, 93% yield) as a black brown gum. LCMS [M+1]$^+$: 227.9. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 6.84 (m, 2H), 5.31 (br s, 2H), 4.48 (m, 1H), 3.06 (br d, J = 4.8 Hz, 3H).

Step 3: To a solution of 3-amino-5-bromo-2-(methylamino)benzonitrile (4.10 g, 18.1 mmol, 1.00 *eq.*) in ethyl alcohol (45 mL) was added cyanogen bromide (3.84 g, 36.3 mmol, 2.67 mL, 2.00 *eq.*). The reaction was stirred at 20 °C for 2 hours. The reaction mixture was then diluted with water (50 mL) and stirred for 5 min. The aqueous phase was extracted with ethyl acetate (40 mL × 3). The combined organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuum to give a residue. The residue was purified by column chromatography (SiO_2, ethyl acetate in petroleum ether 30-50%, then methanol in ethyl acetate 10%) to give 2-amino-6-bromo-3-methyl-benzimidazole-4-carbonitrile (1.80 g, 6.77 mmol, 37% yield) as a dark brown solid. LCMS [M+1]$^+$: 252.8. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.54 (d, J = 1.6 Hz, 1H), 7.44 (d, J = 2.0 Hz, 1H), 7.10 (s, 2H), 3.74 (s, 3H).

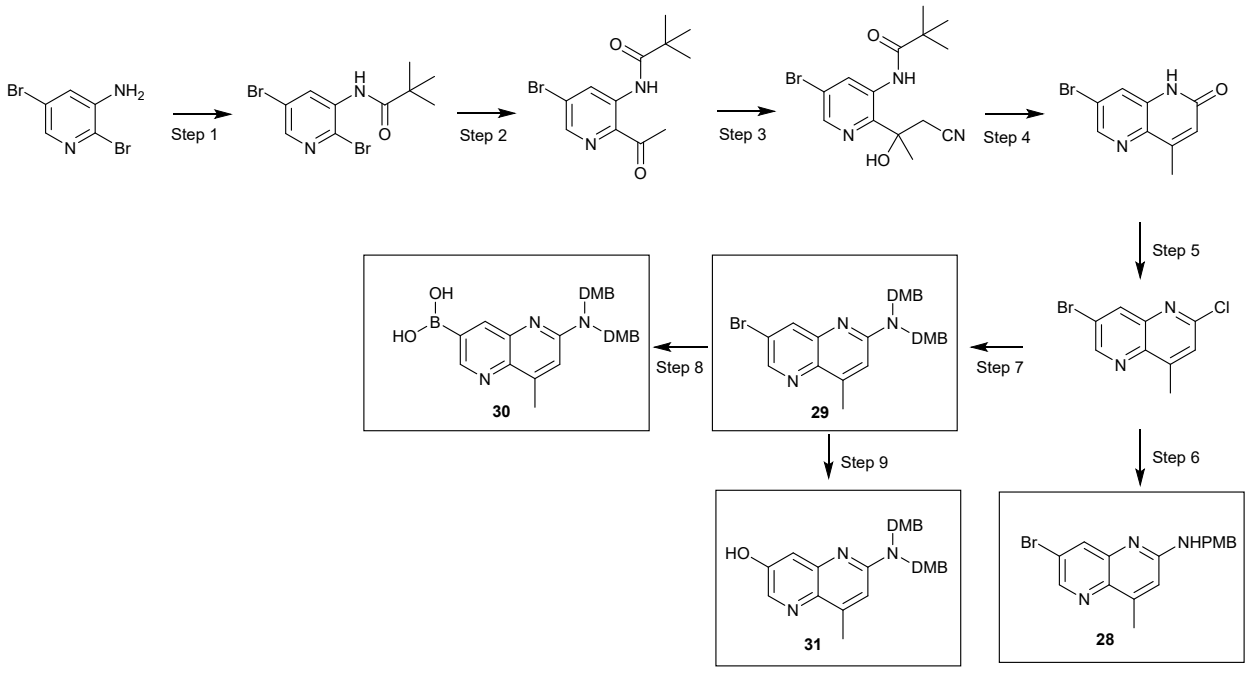
Step 4: To a solution of 2-amino-6-bromo-3-methyl-benzimidazole-4-carbonitrile (300 mg, 1.19 mmol, 1.00 *eq.*) in toluene (18 mL) and *N*,*N*-dimethylformamide (6 mL) was added 4-methylbenzenesulfonic acid hydrate (13.6 mg, 71.7 μmol, 0.06 *eq.*) and hexane-2,5-dione (682 mg, 5.97 mmol, 701 μL, 5.00 *eq.*) at 10 °C. The mixture was stirred at 140 °C for 16 hours fitted with a Dean-Stark trap. The mixture was poured into water (30 mL) and extracted with ethyl acetate (15 mL × 3). The combined organic layers were washed with water (10 mL) and brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuum. The residue was purified by silica gel chromatography (ethyl acetate in petroleum ether 20%) to afford **19** (150 mg, 454 μmol, 38% yield) as a white solid. LCMS [M+1]$^+$: 329.0/331.0. ^{1}H NMR (400 MHz, $CDCl_3$) δ =8.35 (d, J = 2.0 Hz, 1H), 8.13 (d, J = 2.0 Hz, 1H), 5.96 (s, 2H), 3.66 (s, 3H), 1.98 (s, 6H).

Compound 21: 2-(2,5-dimethylpyrrol-1-yl)-3-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzimidazole-4-carbonitrile.



To a solution of **19** (600 mg, 1.82 mmol, 1.00 *eq.*) and 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.39 g, 5.47 mmol, 3.00 *eq.*) in 1,4-dioxane (9 mL) was added potassium acetate (537 mg, 5.47 mmol, 3.00 *eq.*), followed by [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (200 mg, 273 μmol, 0.15 *eq.*) under a nitrogen atmosphere. The mixture was stirred at 80 °C for 16 hours. The mixture was poured into water (20 ml) and extracted with ethyl acetate (20 mL × 3). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuum. The residue was purified by silica gel chromatography (ethyl acetate in petroleum ether 25%) to afford **21** (710 mg, crude) as a white solid that was used in the next steps without further purification. LCMS $[M+1]^+$: 377.2/295.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ =8.40 (s, 1H), 8.06 (s, 1H), 5.90 (s, 2H), 3.67 (s, 3H), 1.97 (s, 6H), 1.31 (s, 12H).

Compounds 28-31



Step 1: To a solution of 2,5-dibromopyridin-3-amine (25.0 g, 99.2 mmol, 1.00 *eq.*) in pyridine (250 mL) was added 2,2-dimethylpropanoyl chloride (18.0 g, 149 mmol, 18.3 mL, 1.50 *eq.*) at 0

°C. The mixture was stirred at 20 °C for 0.5 hr. The reaction mixture was diluted with water (200 mL) and extracted with ethyl acetate (200 mL × 3). The combined organic phases were washed with brine (100 mL), dried over sodium sulfate, filtered, and concentrated to give a residue. The residue was purified by column chromatography (SiO_2, Ethyl acetate / Petroleum ether 2-5%) to give *N*-(2,5-dibromo-3-pyridyl)-2,2-dimethyl-propanamide (33.0 g, 98.2 mmol, 99% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.95 (d, *J* = 2.4 Hz, 1H), 8.14 (d, *J* = 2.4 Hz, 1H), 8.01 (br s, 1H), 1.35 (s, 9H).

Step 2: A mixture of *N*-(2,5-dibromo-3-pyridyl)-2,2-dimethyl-propanamide (33.0 g, 98.2 mmol, 1.00 *eq.*), tri-butyl(1-ethoxyvinyl)stannane (28.4 g, 78.6 mmol, 26.5 mL, 0.80 *eq.*) and $Pd(PPh_3)_4$ (11.4 g, 9.82 mmol, 0.10 *eq.*) in toluene (1.32 L) was degassed and stirred at 80 °C for 12 hours under nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Ethyl acetate / Petroleum ether 2-5%) to give *N*-[5-bromo-2-(1-ethoxyvinyl)-3-pyridyl]-2,2-dimethyl-propanamide (16.0 g, 47.6 mmol, 49% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.19 (br s, 1H), 9.03 - 8.99 (m, 1H), 8.34 - 8.27 (m, 1H), 5.06 (dd, *J* = 2.8, 5.2 Hz, 1H), 4.63 - 4.55 (m, 1H), 4.13 - 4.00 (m, 2H), 1.52 - 1.44 (m, 3H), 1.32 - 1.27 (m, 9H). A mixture of *N*-[5-bromo-2-(1-ethoxyvinyl)-3-pyridyl]-2,2-dimethyl-propanamide (9.50 g, 29.0 mmol, 1.00 *eq.*) in hydrochloric acid/dioxane (4 M, 38.0 mL, 5.24 *eq.*) was stirred at 20 °C for 10 minutes. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was diluted with water (50 mL) and extracted with ethyl acetate (50 mL × 3). The combined organic phases were washed with saturated sodium bicarbonate aqueous solution (50 mL × 2), brine (50 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Ethyl acetate / Petroleum ether 2-5%) to give *N*-(2-acetyl-5-bromo-3-pyridyl)-2,2-dimethyl-propanamide (6.90 g, 17.4 mmol, 60% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.82 (br s, 1H), 9.44 (d, *J* = 2.0 Hz, 1H), 8.39 (d, *J* = 2.0 Hz, 1H), 2.77 (s, 3H), 1.36 (s, 9H).

Step 3: To a solution of acetonitrile (1.84 g, 44.9 mmol, 2.36 mL, 2.10 *eq.*) in THF (45 mL) was added lithium diisopropylamide (2 M, 22.5 mL, 2.10 *eq.*) in a dropwise fashion at -78 °C. After stirring for 0.5 hour, a solution of *N*-(2-acetyl-5-bromo-3-pyridyl)-2,2-dimethyl-propanamide (6.40 g, 21.4 mmol, 1.00 *eq.*) in THF (20 mL) was added to the reaction mixture. The reaction

mixture was stirred at -78 °C for 30 minutes. The reaction mixture was quenched with water (50 mL) and extracted with dichloromethane (50 mL × 3). The combined organic extracts were washed with brine (50 mL), dried over sodium sulfate, filtered, and concentrated to give *N*-[5-bromo-2-(2-cyano-1-hydroxy-1-methyl-ethyl)-3-pyridyl]-2,2-dimethyl-propanamide (8.10 g, crude) as a brown oil, which was used in the next step directly without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.11 (br s, 1H), 8.98 (d, *J* = 2.0 Hz, 1H), 8.23 (d, *J* = 2.0 Hz, 1H), 4.46 (br s, 1H), 3.31 - 3.21 (d, *J* = 16.4 Hz, 1H), 3.17 - 3.06 (d, *J* = 16.4 Hz, 1H), 1.68 (s, 3H), 1.30 (s, 9H).

Step 4: A solution of *N*-[5-bromo-2-(2-cyano-1-hydroxy-1-methyl-ethyl)-3-pyridyl]-2,2-dimethyl-propanamide (0.95 g, crude) in hydrochloric acid (3 M, 3.80 mL) was heated at 160 °C for 5 minutes in a microwave. The resulting mixture was basified to pH 9 with saturated sodium bicarbonate (10 mL). A precipitate formed and the precipitate was filtered and washed with water to give 7-bromo-4-methyl-1*H*-1,5-naphthyridin-2-one (5.00 g, 20.9 mmol) as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.75 (br s, 1H), 8.55 (d, *J* = 2.0 Hz, 1H), 7.82 (d, *J* = 2.0 Hz, 1H), 6.68 (d, *J* = 0.8 Hz, 1H), 2.43 (d, *J* = 0.8 Hz, 3H).

Step 5: A mixture of 7-bromo-4-methyl-1*H*-1,5-naphthyridin-2-one (2.50 g, 10.5 mmol, 1.00 *eq.*) and phosphorus oxychloride (41.3 g, 269 mmol, 25 mL) was stirred at 120 °C for 3 hours. The reaction mixture was then concentrated under reduced pressure to give a residue. The residue was diluted with ethyl acetate (50 mL) and ice water (50 mL). The aqueous phase was separated and extracted with ethyl acetate (50 mL × 3). The combined organic extracts were washed with saturated sodium bicarbonate aqueous solution (50 mL), brine (50 mL), dried over sodium sulfate, filtered, and concentrated to give 7-bromo-2-chloro-4-methyl-1,5-naphthyridine, (2.5 g, 9.71 mmol, 92% yield) as a brown solid which was used in the next step directly without further purification. ^{1}H NMR (400 MHz, DMSO-*d6*) δ = 9.08 (d, *J* = 2.4 Hz, 1H), 8.66 (d, *J* = 2.4 Hz, 1H), 7.79 (d, *J* = 1.2 Hz, 1H), 2.72 (d, *J* = 1.2 Hz, 3H).

Compound 28: 7-bromo-*N*-[(4-methoxyphenyl)methyl]-4-methyl-1,5-naphthyridin-2-amine.

Step 6: To a solution of 7-bromo-2-chloro-4-methyl-1,5-naphthyridine (0.80 g, 3.11 mmol, 1.00 *eq.*) in dimethylsulfoxide (8.0 mL) was added potassium fluoride (541 mg, 9.32 mmol, 218 µL, 3.00 *eq.*) and (4-methoxyphenyl)methanamine (852 mg, 6.21 mmol, 804 µL, 2 *eq.*). The mixture

was stirred at 130 °C for 2 hours. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (10 mL × 3). The combined organic extracts were washed with brine (10 × 2 mL), dried over sodium sulfate, filtered, and concentrated to give **28** (1.30 g, crude, 86% purity) as a yellow oil which was used in the next step directly without further purification. LCMS $[M+1]^+$: 358.1.

Compound 29: 7-bromo-*N,N*-bis[(2,4-dimethoxyphenyl)methyl]-4-methyl-1,5-naphthyridin-2-amine.

Step 7: A mixture of 7-bromo-2-chloro-4-methyl-1,5-naphthyridine (1.00 g, 3.88 mmol, 1.00 *eq.*), 1-(2,4-dimethoxyphenyl)-*N*-[(2,4-dimethoxyphenyl)methyl]methanamine (2.46 g, 7.77 mmol, 2.00 *eq.*), potassium fluoride (677 mg, 11.7 mmol, 273 µL, 3.00 *eq.*) in dimethyl sulfoxide (10 mL) was degassed and stirred at 130 °C for 12 hours under nitrogen atmosphere. The mixture was diluted with brine (10 mL), extracted with ethyl acetate (20 mL × 2) and the combined organic phases were dried over sodium sulfate, filtered, concentrated in vacuo. The residue was purified by column chromatography (SiO_2, ethyl acetate / petroleum ether 5-30%) to give **29** (1.70 g, 3.08 mmol, 79% yield) as a white solid. LCMS $[M+1]^+$: 540.0. ^1H NMR (400 MHz, $CDCl_3$) δ = 8.54 (d, *J* = 2.0 Hz, 1H), 8.12 (d, *J* = 2.4 Hz, 1H), 7.10 (br d, *J* = 7.2 Hz, 2H), 6.85 (d, *J* = 0.8 Hz, 1H), 6.48 (d, *J* = 2.4 Hz, 2H), 6.41 (d, *J* = 2.4 Hz, 1H), 6.39 (d, *J* = 2.0 Hz, 1H), 4.84 (br s, 4H), 3.79 (d, *J* = 2.4 Hz, 12H), 2.58 (s, 3H), 1.60 (s, 1H).

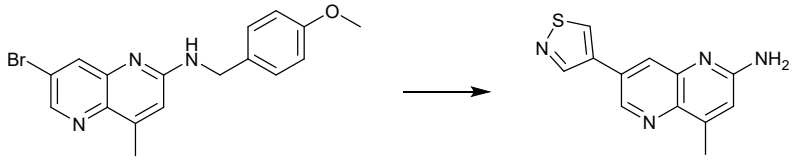
Compound 30: [6-[bis[(2,4-dimethoxyphenyl)methyl]amino]-8-methyl-1,5-naphthyridin-3-yl]boronic acid.

Step 8: A mixture of compound **29** (300 mg, 557 µmol, 1.00 *eq.*), 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (212 mg, 836 µmol, 1.50 *eq.*), potassium acetate (164 mg, 1.67 mmol, 3.00 *eq.*) and $Pd(dppf)_2$-DCM(45.5 mg, 55.7 µmol, 0.10 *eq*) in 1,4-dioxane (5.0 mL) was degassed stirred at 110 °C for 3 hours under a nitrogen atmosphere. The cooled reaction mixture was filtered and concentrated under vacuum to give a residue. The residue was washed with methyl alcohol (20 mL) and filtered to give **30** (450 mg, crude) as a black solid. The material was used directly in the next step without further purification. LCMS $[M+1]^+$: 503.9

Compound 31, 6-[bis[(2,4-dimethoxyphenyl)methyl]amino]-8-methyl-1,5-naphthyridin-3-ol.

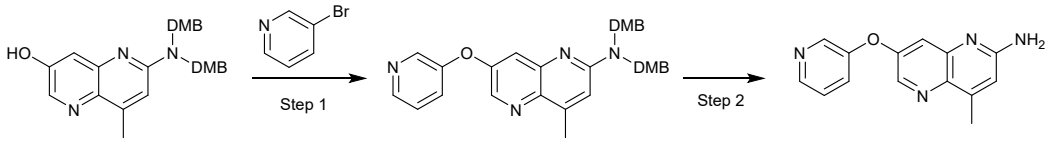
Step 9: A mixture of compound **29** (200 mg, 371 μmol, 1.00 *eq*), $Pd_2(dba)_3$ (34.0 mg, 37.1 μmol, 0.100 *eq*), *t*-BuXphos (31.6 mg, 74.3 μmol, 0.200 *eq*) and potassium hydroxide (208 mg, 3.71 mmol, 10.0 *eq*) in dioxane (1.5 mL) and water (1.5 mL) was degassed and stirred at 100 °C for 12 hours under nitrogen atmosphere. The pH of the reaction mixture was adjusted to pH 7 with HCl (1 N). Then the mixture was poured into water (30 mL) and extracted with ethyl acetate (15 mL × 3). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, ethyl acetate / petroleum ether 10-50%) to give **31** (170 mg, 354 μmol, 95% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.85 - 7.77 (m, 1H), 7.10 – 7.01 (m, 3H), 6.65 (s, 1H), 6.41 - 6.33 (m, 4H), 4.75 (s, 4H), 3.68 (d, *J* = 9.2 Hz, 12H), 2.58 (s, 3H).

Example 32: 7-(isothiazol-4-yl)-4-methyl-1,5-naphthyridin-2-amine.



A mixture of **28** (158 mg, 0.44 mmol, 1.00 eq.), isothiazol-4-ylboronic acid (114 mg, 0.89 mmol, 2.00 eq.), Pd(dppf)Cl_2 (32 mg, 44 μmol, 0.10 eq.) and cesium carbonate (143 mg, 0.89 mmol, 2.00 eq.) in dioxane (2.0 mL) and water (0.40 mL) was degassed and purged with nitrogen atmosphere for 3 times, and then the mixture was stirred at 100 °C for 1 hour under nitrogen atmosphere. The reaction mixture was diluted with water (2.0 mL) and extracted with ethyl acetate (2.0 mL × 3). Combined organic phase was washed with brine (2.0 mL), dried, filtered and concentrated to give a residue which was used into next step directly without further purification. The obtained residue was mixture with TFA (2.0 mL) and stirred at 70 °C for 6 hours. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was basified with ammonium hydroxide (0.10 mL) to pH=9. The solution was purified by prep-HPLC (neutral condition) to give **32** (4.3 mg, 17.7 μmol, 4% yield over 2 steps) was obtained as a white solid. LCMS $[M+1]^+$: 243.2; ^{1}H NMR (400 MHz, CD_3OD-d_4) δ = 9.37 (s, 1H), 9.04 (s, 1H), 8.90 (d, J = 2.0 Hz, 1H), 8.13 (d, J = 2.0 Hz, 1H), 6.94 (d, J = 1.2 Hz, 1H), 2.67 - 2.64 (d, J = 1.2 Hz, 3H).

Example 33: 4-methyl-7-(3-pyridyloxy)-1,5-naphthyridin-2-amine.

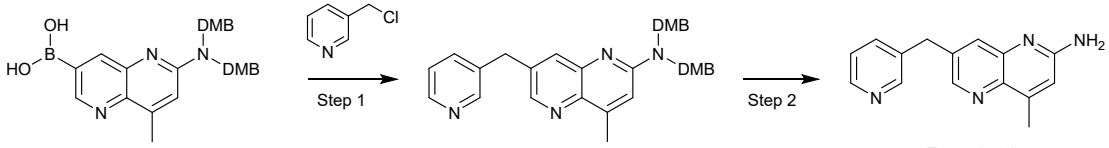


31 Example 33

Step 1: A mixture of 6-[bis[(2,4-dimethoxyphenyl)methyl]amino]-8-methyl-1,5-naphthyridin-3-ol (60 mg, 126 μmol, 1.00 eq.), 3-bromopyridine (60 mg, 379 μmol, 37 μL, 3.0 eq.), CuI (4.8 mg, 25 μmol, 0.20 eq.), 2-(dimethylamino) acetic acid (5.2 mg, 51 μmol, 0.40 eq.) and cesium carbonate (127 mg, 391 μmol, 3.10 eq.) in dioxane (1.0 mL) was degassed and purged with nitrogen 3 times, and then the mixture was stirred at 100 °C for 12 hrs. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO_2, dichloromethane: methyl alcohol = 20:1) to give compound *N,N*-bis[(2,4-dimethoxyphenyl)methyl]-4-methyl-7-(3-pyridyloxy)-1,5-naphthyridin-2-amine (46 mg, 83 μmol, 66% yield) as a yellow solid. LCMS $[M+1]^+$: 553.2.

Step 2: A mixture of *N,N*-bis[(2,4-dimethoxyphenyl)methyl]-4-methyl-7-(3-pyridyloxy)-1,5-naphthyridin-2-amine (46 mg, 83 μmol, 1.00 eq.) and trifluoroacetic acid (1.00 mL) was stirred at 80 °C for 0.5 hrs. The mixture was concentrated under vacuum. Then ammonium hydroxide (2.0 mL) was added, and the mixture was stirred for 0.5 hrs. The mixture was concentrated, and the formed residue was purified by prep-HPLC (ammonia hydroxide conditions) to give **33** (13 mg, 53 μmol, 64% yield) as a white solid. LCMS $[M+1]^+$: 253.2; ^1H NMR (400 MHz, $CDCl_3$) δ = 8.56 (d, J = 2.8 Hz, 1H), 8.53 (d, J = 2.8 Hz, 1H), 8.49 (dd, J = 4.4, 1.2 Hz, 1H), 7.46 - 7.43 (m, 1H), 7.36 - 7.32 (m, 1H), 7.31 (d, J = 2.4 Hz, 1H), 6.73 (d, J = 1.2 Hz, 1H), 4.78 (br, s, 2H), 2.70 (d, J = 0.8 Hz, 3H).

Example 34: 4-methyl-7-(3-pyridylmethyl)-1,5-naphthyridin-2-amine.



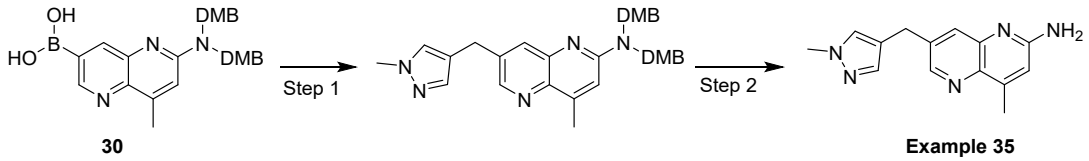
30 Example 34

Step 1: A mixture of **30** (400 mg, 795 μmol, 1. 0 *eq.*), 3-(chloromethyl)pyridine (203 mg, 1.59 mmol, 2.00 *eq.*), cyclopentyl(diphenyl)phosphane;dichloromethane;dichloropalladium;iron (64.9 mg, 80 μmol, 0.1 *eq.*) and potassium carbonate (220 mg, 1.59 mmol, 2.00 *eq.*) in 1,4-dioxane (4.0

mL) and water (0.8 mL) was degassed and purged with nitrogen 3 times, and then stirred at 120 °C for 2 hours. The reaction mixture was filtered and concentrated under reduced pressure and the formed residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate 10/1 to 0/1) to give *N*,*N*-bis[(2,4-dimethoxyphenyl)methyl]-4-methyl-7-(3-pyridylmethyl)-1,5-naphthyridin-2-amine (160 mg, 287 μmol, 36% yield) as a yellow solid. LCMS [M+1] $^+$: 551.5

Step 2: A solution of *N*,*N*-bis[(2,4-dimethoxyphenyl)methyl]-4-methyl-7-(3-pyridylmethyl)-1,5-naphthyridin-2-amine (150 mg, 272 μmol, 1.00 *eq.*) in trifluoroacetic acid (1.00 mL) was stirred at 70 °C for 0.5 hour. The reaction mixture was then diluted with dichloromethane, filtered and the filtrate concentrated under reduced pressure. The residue was purified by *prep*-HPLC (ammonium bicarbonate condition) to give **34** (36 mg, 142 μmol, 52% yield) as a white solid. LCMS [M+1] $^+$: 251.3; ^{1}H NMR (400 MHz, CD_3OD-d_4) δ = 8.52 (d, *J* = 2.0 Hz, 1H), 8.45 (d, *J* = 2.4 Hz, 1H), 8.42 (dd, *J* = 1.6, 4.8 Hz, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.64 (d, *J* = 2.0 Hz, 1H), 7.40 (dd, *J* = 4.8, 8.0 Hz, 1H), 6.87 (d, *J* = 0.8 Hz, 1H), 4.20 (s, 2H), 2.60 (s, 3H); HPLC Rt 1.538 min, 99.5%.

Example 35: 4-methyl-7-[(1-methylpyrazol-4-yl)methyl]-1,5-naphthyridin-2-amine.

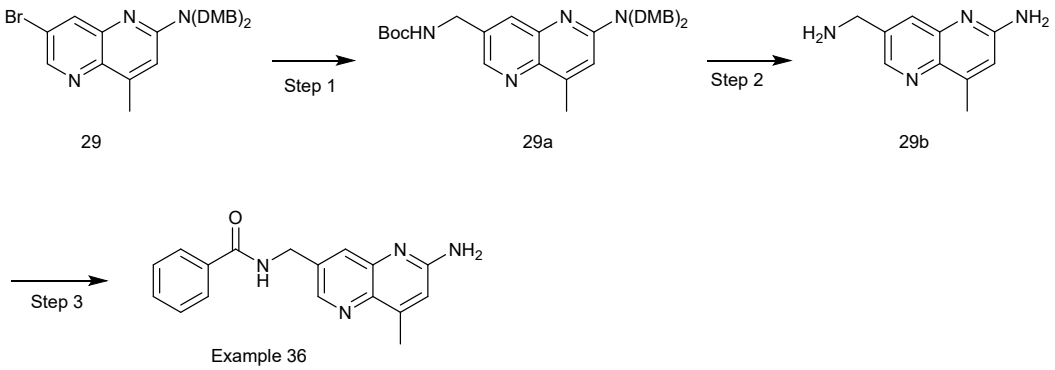


30 **Example 35**

Step 1: A mixture of **30** (60 mg, 119 μmol, 1.00 *eq.*), 4-(chloromethyl)-1-methyl-pyrazole (40 mg, 238 μmol, 2.00 *eq.*), cyclopentyl(diphenyl)phosphane;dichloromethane;dichloropalladium;iron (10 mg, 12 μmol, 0.10 *eq.*) and potassium carbonate (49 mg, 358 μmol, 3.00 *eq.*) in DMF (1.0 mL) was degassed and purged with nitrogen for 3 times. The mixture was then stirred at 100 °C for 2 hours. The reaction mixture was diluted with water (10 mL) and then extracted with ethyl acetate (20 mL × 3). The combined organic layers were washed with brine **(**50 mL × 2**)**, dried with sodium sulfate, filtered, and concentrated under reduced pressure. The formed residue was purified by *prep*-TLC (SiO_2, petroleum ether/ethyl acetate 1/1) to give *N*,*N*-bis[(2,4-dimethoxyphenyl)methyl]-4-methyl-7-[(1-methylpyrazol-4-yl)methyl]-1,5-naphthyridin-2-amine (10 mg, 18 μmol, 15% yield) as a white solid. LCMS [M+1] $^+$: 554.6.

Step 2: A solution of *N*,*N*-bis[(2,4-dimethoxyphenyl)methyl]-4-methyl-7-[(1-methylpyrazol-4-yl)methyl]-1,5-naphthyridin-2-amine (10 mg, 18 μmol, 1.00 *eq.*) in trifluoroacetic acid (0.5 mL) was stirred at 70 °C for 0.5 hour. The reaction mixture was then diluted with dichloromethane, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by prep-HPLC (ammonium bicarbonate condition) to give **35** (1.6 mg, 6.1 μmol, 34% yield) as a white solid. LCMS [M+1] $^{+}$: 254.3; ^{1}H NMR (400 MHz, CD_3OD-d_4) δ = 8.43 (d, *J* = 2.0 Hz, 1H), 7.68 - 7.63 (m, 1H), 7.47 (s, 1H), 7.36 (s, 1H), 6.87 (d, *J* = 0.8 Hz, 1H), 3.99 (s, 2H), 3.85 (s, 3H), 2.60 (d, *J* = 0.8 Hz, 3H); HPLC Rt 1.947 min, 99.6%.

Example 36: *N*-[(6-amino-8-methyl-1,5-naphthyridin-3-yl)methyl]benzamide.



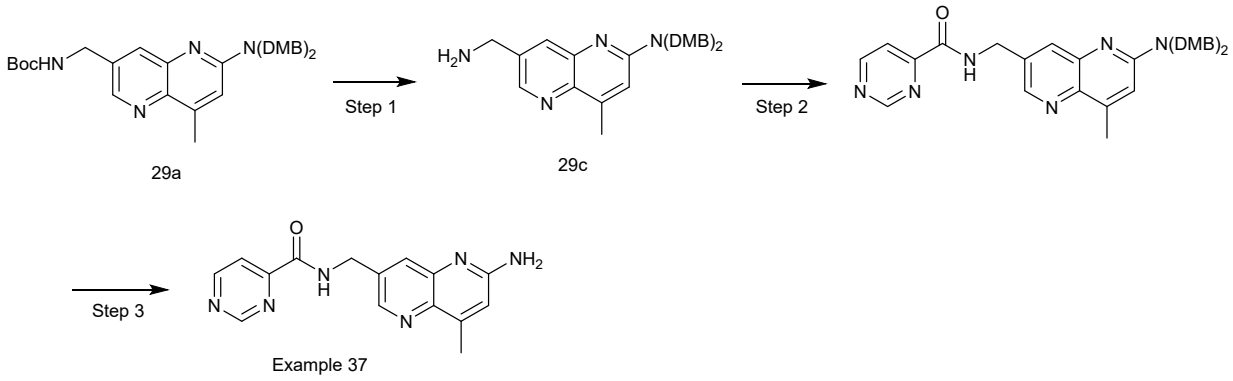
Step 1: A mixture of **29** (1.70 g, 3.16 mmol, 1.00 *eq.*), potassium (*N*-Boc-aminomethyl)trifluoroborate (1.50 g, 6.31 mmol, 2.00 *eq.*), [2-(2-aminophenyl)phenyl]palladium(1+);bis(1-adamantyl)-butyl-phosphane;methanesulfonate (230 mg, 316 μmol, 0.10 *eq.*), sodium carbonate (1.00 g, 9.47 mmol, 3.0 *eq.*) in water (15 mL) and dioxane (75 mL) was degassed and purged with nitrogen 3 times. The mixture was then stirred at 100 °C for 12 hours under nitrogen atmosphere. The mixture was concentrated and the residue was triturated with petroleum ether/ethyl acetate 10/1 (50 mL) for 0.5 hour to give *tert*-butyl *N*-[[6-[bis[(2,4-dimethoxyphenyl)methyl]amino]-8-methyl-1,5-naphthyridin-3-yl]methyl]carbamate, **29a** (1.60 g, 2.72 mmol, 86% yield) as a white solid. LCMS [M+1] $^{+}$: 589.3.

Step 2: A mixture of **29a** (1.50 g, 2.55 mmol, 1.00 *eq.*) in trifluoroacetic acid (5.0 mL) was stirred at 70 °C for 1 hour. The mixture was concentrated under reduced pressure and the residue was diluted with water (10 mL) and washed ethyl acetate (20 mL × 2). The pH of the aqueous phase was then adjusted to pH 9 with sodium bicarbonate and exacted with ethyl acetate (100 mL × 10). The combined organic phases were washed with brine (30 mL × 2), dried over

anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a 7-(aminomethyl)-4-methyl-1,5-naphthyridin-2-amine, **29b** (280 mg, 1.49 mmol, 58% yield) as a yellow solid. The residue was used for the next step without further purification. LCMS [M+1] $^{+}$: 189.1; ^{1}H NMR (400 MHz, CD_3OD-d_4) δ = 8.55 (d, *J* = 2.0 Hz, 1H), 7.85 (d, *J* = 2.0 Hz, 1H), 6.92 (d, *J* = 1.0 Hz, 1H), 4.07 (s, 2H), 2.62 (d, *J* = 0.8 Hz, 3H).

Step 3: To a solution of benzoic acid (16 mg, 128 μmol, 1.00 *eq.*) in DMF (1.0 mL) was added HATU (73 mg, 191 μmol, 1.50 *eq.*) and triethylamine (53 μL, 383 μmol, 3.00 *eq.*). The mixture was stirred at 20 °C for 0.5 hour and then **29b** (31 mg, 166 μmol, 1.30 *eq.*) was added to the mixture and stirred at 20°C for 0.5 hour. The mixture was concentrated under reduced pressure to give a residue. The residue was purified by *prep*-HPLC (HCl condition) to give **36** (7.5 mg, 25 μmol, 19% yield, HCl salt) as a white solid. LCMS [M+1] $^{+}$: 293.2; ^{1}H NMR (400 MHz, CD_3OD-d_4) δ = 8.82 (d, *J* = 2.0 Hz, 1H), 7.98 (br d, *J* = 2.0 Hz, 1H), 7.94 - 7.83 (m, 2H), 7.61 - 7.55 (m, 1H), 7.53 - 7.46 (m, 2H), 7.12 (s, 1H), 4.79 (s, 2H), 2.75 (s, 3H); HPLC Rt 1.667 min, 95.9%.

Example 37: *N*-((6-amino-8-methyl-1,5-naphthyridin-3-yl)methyl)pyrimidine-4-carboxamide.



Step 1: To a solution of **29a** (780 mg, 1.32 mmol, 1.00 *eq.*) in dichloromethane (10 mL) was added zinc bromide (895 mg, 3.97 mmol, 199 μL, 3.00 *eq.*). The mixture was stirred at 25 °C for 12 hours. The reaction mixture was concentrated, and the formed residue was diluted with water (20 mL) and extracted with ethyl acetate (20 mL × 3). The combined organic layers were washed with brine (50 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was triturated with ethyl acetate (10 mL) at for 30 minutes to give

7-(aminomethyl)-*N*,*N*-bis[(2,4-dimethoxyphenyl)methyl]-4-methyl-1,5-naphthyridin-2-amine **29c** (480 mg, 982 μmol, 74% yield) as a yellow solid. LCMS [M+1] $^{+}$: 489.1.

Step 2: To a solution of **29c** (217 mg, 443 μmol, 1.10 *eq.*) and pyrimidine-4-carboxylic acid (50.0 mg, 403 μmol, 1.00 *eq.*) in dichloromethane (2.0 mL) was added T3P (50% in ethyl acetate, 2.01 mmol, 1.20 mL, 5.00 *eq.*) and triethylamine (1.21 mmol, 168 μL, 3.00 *eq.*). The mixture was stirred at 20 °C for 2 hours. The mixture was concentrated under reduced pressure to give a residue. The residue was purified by *prep*-TLC (SiO_2, dichloromethane: methyl alcohol = 10:1) to give *N*-[[6-[bis[(2,4-dimethoxyphenyl)methyl]amino]-8-methyl-1,5-naphthyridin-3-yl]methyl]pyrimidine-4-carboxamide (80 mg, 135 μmol, 33% yield) as a white solid. LCMS [M+1] $^{+}$: 595.2.

Step 3: To a solution of *N*-[[6-[bis[(2,4-dimethoxyphenyl)methyl]amino]-8-methyl-1,5-naphthyridin-3-yl]methyl]pyrimidine-4-carboxamide (80 mg, 135 μmol, 1.00 *eq*) in trifluoroacetic acid (1.50 mL) was stirred at 70 °C for 0.5 hour. The mixture was concentrated under reduced pressure to give a residue. The residue was purified by *prep*-HPLC (HCl condition)) to give **37** (17 mg, 57 μmol, 43% yield) as a white solid. LCMS [M+1] $^{+}$ =295.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 14.68 - 14.08 (m, 1H), 9.92 (br t, *J* = 6.2 Hz, 1H), 9.48 - 9.24 (m, 2H), 9.12 (d, *J* = 5.0 Hz, 1H), 8.80 (d, *J* = 2.0 Hz, 1H), 8.48 - 8.24 (m, 1H), 8.08 – 8.00 (m, 2H), 7.16 (d, *J* = 1.0 Hz, 1H), 4.68 (br d, *J* = 6.2 Hz, 2H), 2.64 (d, *J* = 0.8 Hz, 3H); HPLC Rt 1.440 min, 100%.

PRMT5/MTA X-ray Co-crystallography

PRMT5/MTA protein was expressed, purified, and crystallized as previously described.[1] Crystals were harvested and incubated in a soak solution consisting of reservoir and 5-10 mM fragment for 2-5 hours, dipped in cryoprotectant (soak solution plus 20% ethylene glycol) and flash frozen in pucks for data collection. X-ray diffraction data was collected at the following beamlines: Advanced Photon Source (APS) NE-CAT ID-E, Swiss Light Source (SLS) PXII/X10SA and PXIII/X06DA, and Diamond Light Source (DLS) I03 (Table ESI-1). PRMT5 data sets indexed in the I222 space group and the images were integrated and scaled using HKL2000.[2] The PRMT5 crystals were highly anisotropic and STARANISO server was used to calculate ellipsoidal completeness for several structures.[3] Structures were determined by

molecular replacement using Phaser[4] and refined through iterative rounds of automated refinement using phenix.refine[5] and manual refitting in Coot.[6] Coordinates and structure factors are deposited in the PDB with accession codes: 7UY1, 7UYF, 7ZUP, 7ZUQ, 7ZUU, 7ZUY, 7ZV2, 7ZVL, 7ZVU, 8CSG, 8CTB.

Table ESI-1. X-ray data collection and refinement statistics for PRMT5 crystal structures			
Compound	**Fragment 5**	**Fragment 7**	**Fragment 9**
PDB ID	**7QSG**	**7QVB**	**7QUF**
Data Collection			
Beamline	PXIII/X06DA (SLS)	ID30A-3	PXIII/X06DA (SLS)
Wavelength (Å)	1.000	0.979	0.999
Detector	PILATUS 2M	Eiger2 XE 16M	Eiger2 XE 16M
Resolution (Å)[a]	46 - 2.66 (2.91-2.66)	44.7 -2.61 (2.64-2.61)	108.6 - 2.82 (3.07-2.82)
Space Group	I222	I222	I222
Unit Cell a,b,c (Å)	100.2, 137.2, 178.0	98.5, 138.4, 178.5	102.9, 138.2, 178.1
# Unique Refls	42080 (6721)	28637 (4297)	29243 (4669)
Redundancy	2.8 (2.8)	2.7 (1.9)	5.5 (5.4)
Completeness (%)	95.7 (98.2)	76.1 (23.7)	98.5 (99.5)
$< I/s_i >$	17.4 (3.2)	9.4 (1.5)	16.9 (3.3)
R_{merge}[b]	0.06 (0.44)	0.13 (0.60)	0.09 (0.45)
$CC_{1/2}$[c]	0.95	0.99	0.93
Refinement			
R_{Free} (%)[e]	23.9	28.2	27.2
R (%)[d]	21.0	23.2	22.1
B Average (Å^2)	28.7	75.5	33.2
RMS Deviations			
Bond Lengths(Å)	0.011	0.001	0.007
Bond Angles (°)	1.6	0.38	1.2
Ramachandran (%)			
Favored	99.6	93.1	99.6
Outliers	0.2	0.4	0.1

Table ESI-1 (continued). X-ray data collection and refinement statistics for PRMT5 crystal structures

Compound	**Example 18**	**Example 22**	**Example 25**
PDB ID	**7ZUP**	**7ZV2**	**7ZVU**
Data Collection			
Beamline	DLS I03	DLS I03	DLS I03
Wavelength (Å)	0.919	0.919	0.919
Detector	Eiger2 XE 16M	Eiger2 XE 16M	Eiger2 XE 16M
Resolution (Å)[a]	109.5 - 1.98 (2.26-1.98)	109.2 - 1.94 (2.15-1.94)	109.8 - 1.94 (2.17-1.94)
Space Group	I222	I222	I222
Unit Cell a,b,c (Å)	104.2, 138.6, 178.8	104.5, 138.7, 178.9	105.2, 139.2, 178.6
# Unique Refls	39692 (1984)	49072 (2455)	60214 (3012)
Redundancy	11.9 (14.7)	11.8 (13.1)	12.1 (14.1)
Completeness (%)	93.2[Δ]	93.6[Δ]	94.9[Δ]
< I/σ >	10.7 (1.9)	13.9 (1.7)	13.8 (1.7)
R_{pim}[b]	0.16 (1.5)	0.10 (1.5)	0.10 (1.4)
Outliers	0.2	0.6	0.4
$CC_{1/2}$[c]	0.99	0.99	0.99
Refinement			
R_{Free} (%)[e]	27.0	27.5	26.5
R (%)[d]	22.1	23.4	23.8
B Average (Å^2)	30.7	33.1	30.6
RMS Deviations			
Bond Lengths(Å)	0.007	0.007	0.014
Bond Angles (°)	1.0	1.6	1.3
Ramachandran (%)			
Favored	99.5	99.2	99.6
Outliers	0.2	0.6	0.4

Bond Angles (°)	1.5	1.4	1.8
Ramachandran (%)			
Favored	99.3	99.5	99.2
Outliers	0.7	0.5	0.8

Table ESI-1 (continued). X-ray data collection and refinement statistics for PRMT5 crystal structures.

Compound	**Example 26**	**Example 34**
PDB ID	**7ZUY**	**7ZUU**
Data Collection		
Beamline	DLS I03	DLS I03
Wavelength (Å)	0.919	0.919
Detector	Eiger2 XE 16M	Eiger2 XE 16M
Resolution (Å)[a]	109.7 - 1.97 (2.23-1.97)	109.7 - 1.95 (2.17-1.95)
Space Group	I222	I222
Unit Cell a,b,c (Å)	105.2, 139.1, 178.5	105.2, 139.2, 178.5
# Unique Refls	48889 (2444)	60214 (3012)
Redundancy	11.8 (13.8)	12.1 (14.1)
Completeness (%)	93.7[Δ]	94.6[Δ]
$< I/s_i >$	12.8 (1.7)	13.8 (1.7)
R_{merge}[b]	0.10 (1.5)	0.1 (1.4)
$CC_{1/2}$[c]	0.99	0.99
Refinement		
R_{free} (%)[e]	26.9	28.0
R (%)[d]	22.4	23.0
B Average (Å^2)	31.2	29.5
RMS Deviations		
Bond Lengths(Å)	0.07	0.07
Bond Angles (°)	1.5	1.4
Ramachandran (%)		
Favored	99.7	99.6
Outliers	0.3	0.4

[a]Numbers in parentheses refer to the highest resolution shell. [b]$R_{merge}=\Sigma_{hkl}\Sigma_i |I_{hkl,i} - <I_{hkl}>|/\Sigma_{hkl}\Sigma_i I_{hkl,I}$, where $I_{hkl,i}$ is the scaled intensity of the i^{th} measurement of reflection hkl, $<I_{hkl}>$ is the average intensity for that reflection. [c]$CC_{1/2}$ = Pearson Correlation Coefficient between two random half datasets.

[d]$R = \Sigma_{hkl} | F_o - F_c | / \Sigma_{hkl} | F_o |$ x 100, [e]R_{free} was calculated as for R, but on a test set comprising 5% of the data excluded from refinement.

[Δ]Ellipsoidal completeness was calculated using Staraniso.

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