Synthesis of MPOL_B_1: 13-(1-acetylpiperidine-4-carbonyl)-5-phenyl-1,9,13-triazatricyclo[9.4.0.03,8]pentadeca-3(8),4,6-triene-2,10-dione

Scheme 1: Preparation of compound 3

To a stirred solution of **1** (500 mg, 2.03 mmol) in anhydrous N,N-dimethylformamide (DMF, 21.0 mL) under an argon atmosphere, N,N,N′,N′-tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate (HATU, 927 mg, 2.44 mmol) and triethylamine (Et_3N, 0.85 mL, 6.10 mmol) were added. The reaction mixture was stirred at room temperature for 30 min, after which compound **2** (496 mg, 2.03 mmol) was added. The resulting mixture was stirred at 25 °C for 6 h. The reaction was quenched with water, and the mixture was extracted with dichloromethane (DCM). The combined organic layers were dried over anhydrous sodium sulfate (Na_2SO4), filtered, and concentrated under vacuum. The crude yellow solid (1.0 g) was purified by reverse-phase preparative HPLC on a C18 column using a gradient of water with 0.2% ammonium bicarbonate and acetonitrile. Fractions containing the desired product were combined and evaporated under reduced pressure to yield compound **3** as a white solid (500 mg, 1.06 mmol, 53% yield).

Scheme 1: Preparation of Compound 4

To a solution of **3** (400 mg, 0.85 mmol) in a mixture of methanol (MeOH, 5.60 mL) and water (H_2O, 2.41 mL), iron powder (473 mg, 8.47 mmol) and ammonium chloride (NH_4Cl, 2.27 mg, 0.042 mmol) were added. The reaction mixture was stirred at room temperature for 3 days. The mixture was filtered through a pad of Celite and concentrated under vacuum to yield compound **4** as a white solid (320 mg, 0.78 mmol, 93% yield).

Scheme 1: Preparation of Compound 5

Under nitrogen, a solution of **4** (300 mg, 0.73 mmol) in a mixture of 1,4-dioxane (1.5 mL) and water (1.5 mL) was treated with tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$ (42.25 mg, 0.037 mmol) and sodium carbonate (Na_2CO_3, 310 mg, 2.92 mmol). The reaction mixture was stirred at room temperature for 30 m, followed by the addition of phenylboronic acid (98.0 mg, 0.80 mmol). The mixture was then heated at 80 °C for 6 h. The reaction was quenched with water, and the mixture was extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO4, filtered, and concentrated to afford the crude product (300 mg). The crude material was purified by normal-phase flash chromatography (Biotage) using a gradient of cyclohexane and ethyl acetate. Fractions containing the desired product were combined and concentrated under reduced pressure to yield compound **5** (200 mg, 0.49 mmol, 67% yield).

Scheme 1: Preparation of Compound 6

To a solution of **5** (200 mg, 0.49 mmol) in 1,4-dioxane (2.0 mL), 4 M HCl in 1,4-dioxane (0.36 mL, 1.44 mmol) was added. The reaction mixture was stirred at 25 °C for 6 h. The solvent was removed under vacuum, and the crude residue was triturated with diethyl ether (Et_2O) and filtered to yield compound **6** as a white solid (150 mg, 0.49 mmol, 100% yield).

Scheme 1: Preparation of final product [MPOL_B_1]

To a stirred solution of **6** (150 mg, 0.44 mmol) in anhydrous dimethylformamide (DMF) (4.50 mL) under argon, HATU (200 mg, 0.52 mmol) and Et_3N (0.18 mL, 1.31 mmol) were added. The reaction mixture was stirred at room temperature for 30 m, after which compound **7** (75.0 mg, 0.44 mmol) was added. The resulting mixture was stirred at 25 °C for 6 h. The reaction was quenched with water, and the mixture was extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated to afford the crude product (100 mg). The crude material was purified by reverse-phase preparative HPLC on a C18 column using a gradient of water with 0.2% ammonium bicarbonate and acetonitrile. Fractions containing the desired product were combined and evaporated under reduced pressure to yield a solid (60 mg), which was triturated with a mixture of DCM and Et_2O to yield the final product, MPOL_B_1, as a white powder (20.0 mg, 0.043 mmol, 10% yield).

Analytical data for MPOL_B_1

Raw spectroscopic data is in Supplementary File 1. LC-MS (ESI): m/z 461.2 $[M+H]^+$, 459.1 $[M–H]^-$; calculated for $C_{26}H_{28}N_4O_4$ 460.21. Purity (LC/UV, 220 nm): 99.3%. LC-MS was done with UV detection at 220 nm, using a Poroshell 120 EC-C18 column. Elution was with a gradient from water containing 0.2 wt% ammonium bicarbonate to acetonitrile, starting at 98:2 and ending at 0:100. The main peak has a retention time of 7.95 min. The synthesized compound was verified using proton nuclear magnetic resonance (^1H NMR) spectroscopy. ^1H NMR (500 MHz, DMSO-d6) δ 10.65 (br s, 1H, NH), 7.87–7.85 (m, 2H), 7.69–7.67 (m, 2H), 7.49–7.47 (m, 2H), 7.23–7.21 (m, 1H), 4.38–4.32 (m, 1H), 4.22–4.18 (m, 2H), 3.87–3.83 (m, 2H), 3.65–3.60 (m, 2H), 3.51–3.49 (m, 2H), 3.07–2.97 (m, 2H), 2.01 (s, 3H), 1.85–1.82 (m, 2H), 1.66–1.64 (m, 2H), 1.53–1.50 (m, 1H), 1.36–1.33 (m, 2H).

Synthesis of MPOL_D_1: (6S)-6-[3-(2H-1,3-benzodioxol-5-yl)-1,2,4oxadiazol-5-yl]-N-(4-methylphenyl)3H,4H,5H,6H,7H-imidazo[4,5-c]pyridine-5-carboxamide

All reactions were carried out under nitrogen and anhydrous conditions. Reaction progress was monitored by liquid chromatography-mass spectrometry (LC-MS) and thin-layer chromatography (TLC). Solvents were removed using a rotary evaporator.

Scheme 2: Preparation of Compound 3

To a solution of **1** (1.11 mmol, 200 mg, 1.0 equiv.) in dichloromethane (DCM), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (1.44 mmol, 224 mg, 1.3 equiv.) and 1-Hydroxybenzotriazole (HOBt) (2.22 mmol, 300 mg, 2.0 equiv.) were added. The resulting mixture was stirred at room temperature for 30 m, after which **2** (0.62 mmol, 167 mg, 0.56 equiv.) was introduced. The reaction mixture was heated at 25 °C for 6 h. The residue was diluted with DCM and washed twice with water. The combined aqueous phases were extracted with DCM. The combined organic phases were dried over anhydrous Na_2SO_4 and concentrated to afford 3 (100 mg, 0.23 mmol, 37%) as an off-white solid.

Scheme 2: Preparation of Compound 4

A solution of **3** (0.23 mmol, 100 mg) in pyridine (100 mL) was stirred at 120 °C for 12 h. Upon cooling to room temperature, the reaction mixture was evaporated under vacuum. The crude product was purified by normal phase preparative liquid chromatography (LC) using a Biotage system with an Interchim IR_50SI_F0040 column and solid loading with SiO_2. A gradient mobile phase of cyclohexane/ethyl acetate (10:90 to 90:10) was employed. Fractions containing the desired compound were combined and evaporated under reduced pressure to yield **4** (50.0 mg, 0.12 mmol, 52%) as a pale yellow solid.

Scheme 2: Preparation of Compound 5

To a solution of **4** (0.12 mmol, 50.0 mg, 1.0 equiv.) in dioxane (0.31 mL) was added HCl in dioxane (4 M, 0.18 mL, 0.73 mmol, 6.0 equiv.) at 25 °C for 3 h. The reaction mixture was concentrated under reduced pressure to give **5** (79.0 mg, 0.96 mmol, 30%) as a white solid.

Scheme 2: Preparation of final product [MPOL_D_1]

To a solution of **5** (0.096 mmol, 30 mg, 1.0 equiv.) and **6** (0.096 mmol, 12.8 mg, 1.0 equiv.) in DCM (0.310 mL) was added Et_3N (0.29 mmol, 29.26 mg, 0.04 mL, 3.0 equiv.) at 25 °C for 3 h. The crude product was purified by reverse phase preparative LC using a YMC_C18_40G_25µm column. A gradient mobile phase of H_2O (0.2 wt% NH_4CO_3)/acetonitrile (ACN) (90:10 to 00:100) was used. Fractions containing the pure compound were combined and evaporated under reduced pressure to afford MPOL_D_1 (10.0 mg, 0.022 mmol, 23%) as a white powder.

Analytical data for MPOL_D_1

Raw spectroscopic data is in Supplementary File 2. LC-MS (ESI): m/z 445.1 $[M+H]^+$, 443.1 $[M-H]^-$; calculated for $C_{23}H_{20}N_6O_4$ 444.15. Purity (LC/UV, 220 nm): 97.7%. LC-MS was done as above for MPOL_B_1. The main peak had a retention time of 9.29 min. ^1H NMR (500 MHz, DMSO-d6) δ 11.98 (s, 1H, NH), 8.88 (s, 1H), 8.78 (s, 1H), 8.38 (s, 1H), 8.02 (s, 1H), 7.53 (s, 1H), 7.49 (s, 1H), 7.48 (s, 1H), 7.43 (s, 1H), 7.41 (s, 1H), 7.36 (s, 1H), 7.27 (s, 1H), 7.26 (s, 1H), 7.09 (s, 1H), 7.07 (s, 1H), 7.05 (s, 1H), 7.02 (s, 1H), 6.29 (s, 1H), 6.12 (s, 1H), 5.75 (s, 1H), 4.97 (s, 1H), 4.94 (s, 1H), 4.91 (s, 1H), 4.44 (s, 1H), 4.41 (s, 1H), 4.34 (s, 1H), 4.31 (s, 1H), 4.08 (s, 1H), 3.64 (s, 1H), 3.51 (s, 1H), 3.34 (s, 1H), 3.30 (s, 1H), 3.26 (s, 1H), 2.50 (s, 1H), 2.25 (s, 1H), 2.21 (s, 1H), 1.24 (s, 1H), 1.07 (s, 1H), 0.85 (s, 1H).

Synthesis of MPOL_D_2: (6S)-N-(3-fluorophenyl)-6-[3-(3-methylphenyl)1,2,4-oxadiazol-5-yl]-3H,4H,5H,6H,7H-imidazo[4,5c]pyridine-5-carboxamide

Scheme 3: Preparation of Compound 3

To a solution of **1** (1.12 mmol, 169 mg) in DCM was added HATU (1.46 mmol, 556 mg, 1.3 equiv.) and N,N-Diisopropylethylamine (DIPEA) (3.37 mmol, 435 mg, 3.0 equiv.). After stirring at room temperature for 30 m, **2** (1.12 mmol, 300 mg, 1.0 equiv.) was added. The reaction was heated at 25 °C for 18 h. The solvent was evaporated under vacuum to yield **3** (258 mg, 0.65 mmol, 58% yield).

Scheme 3: Preparation of Compound 4

A solution of **3** (0.65 mmol, 550 mg) in pyridine (0.80 mL) was stirred at 120 °C for 12 h. The mixture was cooled and evaporated under vacuum to afford **4** (232 mg, 0.61 mmol, 52% yield).

Scheme 3: Preparation of Compound 5

To a solution of **4** (0.608 mmol, 400 mg) in dioxane (1.20 mL) was added HCl in dioxane (4 M, 1.42 mL, 5.69 mmol, 10 equiv.). The mixture was stirred at 25 °C for 6 h. After concentration under vacuum, the crude was triturated with Et_2O (2 x 5.0 mL) and filtered to give **5** (99 mg, 0.31 mmol, 51.22% yield).

Scheme 3: Preparation of final product [MPOL_D_2]

To a solution of **5** (0.31 mmol, 99 mg) and **6** (0.31 mmol, 43.0 mg) in DCM (4.50 mL) was added Et3N (3.15 mmol, 0.44 mL, 10.0 equiv.). After stirring at room temperature for 3 h, the mixture was concentrated under vacuum. The crude product was purified by reverse-phase chromatography as in Scheme 2, using a gradient of H_2O (0.2 wt% NH_4CO_3)/ACN (from 70:30 to 00:100). Fractions containing the pure product were collected, evaporated under vacuum, and triturated with Et_2O to produce MPOL_D_2 (50.0 mg, 0.12 mmol, 38% yield).

Analytical data for MPOL_D_2

Raw spectroscopic data is in Supplementary File 3. LC-MS (ESI): m/z 419.1 $[M+H]^+$; calculated for $C_{22}H_{19}FN_6O_2$ 418.16. Purity (LC/UV, 220 nm): 98.9%. LC-MS was done as above for MPOL_B_1, except that elution was with a gradient from water containing 0.1 wt% trifluoroacetic acid (TFA) and acetonitrile. The main peak had a retention time of 8.99 min. ^1H NMR (500 MHz, DMSO-d6) δ 12.02 (br s, 1H, NH), 11.95 (br s, 1H, NH), 9.21 (s, 1H), 9.09 (s, 1H), 7.74–7.72 (m, 2H), 7.54–7.51 (m, 2H), 7.44–7.41 (m, 3H), 7.37 (s, 1H), 7.33–7.29 (m, 3H), 6.84–6.82 (m, 2H), 6.80 (s, 1H), 6.34–6.33 (m, 2H), 6.25 (s, 1H), 4.99–4.94 (m, 3H), 4.46–4.43 (m, 2H), 4.37–4.34 (m, 2H), 3.43–3.29 (m, 4H), 2.50 (s, 1H, solvent), 1.10 (t, J = 7.1 Hz, 3H), 1.09 (s, 1H), 1.07 (s, 1H).