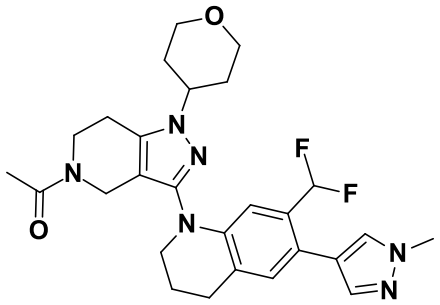
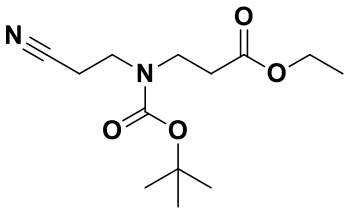
Synthetic Procedure for GNE-049

1-[3-[7-(difluoromethyl)-6-(1-methylpyrazol-4-yl)-3,4-dihydro-2*H*-quinolin-1-yl]-1-tetrahydrofuran-3-yl-6,7-dihydro-4*H*-pyrazolo[4,3-*c*]pyridin-5-yl]ethanone



Step 1:

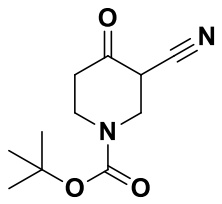
ethyl 3-((*tert*-butoxycarbonyl)(2-cyanoethyl)amino)propanoate



To a solution of ethyl 3-aminopropanoate hydrochloride (366.5 g, 2.39 mol) in MeOH (1.2 L) at room temperature was added NaOH (95.6 g, 2.39 mol) in portion-wise. The mixture was heated to 70 ^{o}C, acrylonitrile (158 g, 2.98 mol) was added dropwise and the reaction mixture stirred for 6 h. The solution was cooled to 0 ^{o}C before di-*tert*-butyl dicarbonate (521 g, 2.39 mol) was added. The reaction was stirred at room temperature for 6 h, filtered, and washed with MeOH (200 mL). The filtrate was concentrated in vacuo to give a yellow oily residue that was re-dissolved in EtOAc and water (500 mL). The aqueous layer was extracted with EtOAc (800 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give the title compound (638 g) as light yellow oil that required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (q, *J* = 7.2 Hz, 2H), 3.68 – 3.62 (m, 4H), 2.57 – 2.53 (m, 4H), 1.49 (s, 9H), 1.29 (t, *J* = 7.2 Hz, 3H).

Step 2:

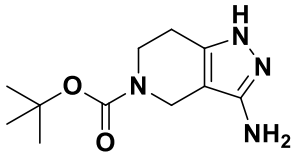
***tert*-butyl 3-cyano-4-oxopiperidine-1-carboxylate**



To toluene (2.7 L) at 25 °C was added NaH (80 g, 2.0 mol) portion-wise and the suspension was heated to 80 °C. Ethyl 3-((*tert*-butoxycarbonyl)(2-cyanoethyl)amino)propanoate (270 g, crude) in anhydrous toluene (270 mL) was added dropwise. The mixture was heated to 100 ^{o}C and stirred for 5 h. The mixture was cooled to room temperature, quenched with sat. aq. ammonium chloride (800 mL) and washed with hexanes (800 mL). The aqueous phase was acidified with HCl (2 N) to pH 6 and the mixture was extracted with EtOAc (1 L x 2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give the title compound (310 g) as yellow oil that required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 – 4.14 (m, 1H), 3.59 – 3.56 (m, 2H), 3.43 – 3.41 (m, 2H), 2.70 – 2.66 (m, 2H), 1.51 (s, 9H).

Step 3:

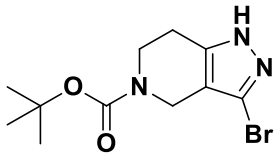
***tert*-butyl 3-amino-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxylate**



A mixture of *tert*-butyl 3-cyano-4-oxopiperidine-1-carboxylate (310 g, 1.38 mol) and hydrazine mono-hydrate (140 mL, 2.08 mol) in EtOH (1.5 L) was heated to 60 °C for 2 h. The mixture was concentrated in vacuo to give the crude product that was dissolved in EtOAc (1 L) and washed with water (1 L x 2). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to afford the title compound (230 g, 70%) as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD) δ 4.28 (s, 2H), 3.66 – 3.63 (m, 2H), 2.62 – 2.59 (m, 2H), 1.49 (s, 9H).

Step 4:

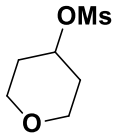
***tert*-butyl 3-bromo-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxylate**



To a stirred mixture of *tert*-butyl 3-amino-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxylate (120 g, 503.6 mmol), $CuBr_2$ (112.5 g, 503.6 mmol) and MeCN (1.2 L) at 0 °C was added isopentyl nitrite (76.7 g, 654.7 mmol) and the reaction mixture stirred for 20 min. The temperature was raised to 60 °C and the reaction mixture was stirred for an additional 5 h. After cooling the reaction to room temperature, the reaction mixture was quenched with water (1 L) and the mixture was extracted with EtOAc (1 L x 2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude residue was purified by silica gel chromatography (petroleum ether/EtOAc = 4: 1) to afford the title compound (52 g, 34%) as light yellow solid. LCMS M/Z (M+H) 302.

Step 5:

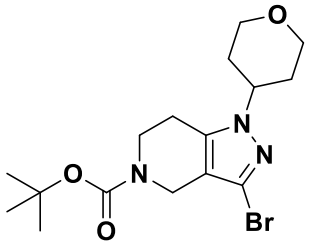
tetrahydro-2*H*-pyran-4-yl methanesulfonate



To a solution of tetrahydro-2*H*-pyran-4-ol (5 g, 49.0 mmol) and triethylamine (5.94 g, 58.7 mmol) in DCM (100 mL) was added mesyl chloride (16.8 g, 146.9 mmol) dropwise at 0 °C under a nitrogen atmosphere. The mixture was stirred at room temperature for 5 h. Water (100 mL) was added and washed with brine (100 mL x 2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give the title compound (4 g, 45%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.85 – 4.81 (m 1H), 3.90 – 3.87 (m, 2H), 3.52 – 3.46 (m, 2H), 2.99 (s, 3H), 2.01 – 1.97 (m, 2H), 1.83 – 1.80 (m, 2H).

Step 6:

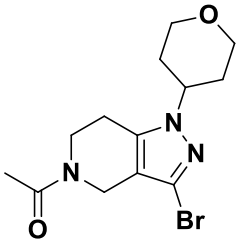
***tert*-butyl 3-bromo-1-(tetrahydro-2*H*-pyran-4-yl)-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxylate**



To a solution of *tert*-butyl 3-bromo-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxylate (6 g, 19.8 mmol) in DMF (40 mL) was added Cs_2CO_3 (19.5 g, 59.6 mmol) and tetrahydro-2*H*-pyran-4-yl methanesulfonate (3.9 g, 21.8 mmol). The mixture was heated to 80 °C for 12 h under a nitrogen atmosphere. After cooling the reaction to room temperature, the mixture was filtered. The mixture was diluted with EtOAc (100 mL) and washed with brine (100 mL x 2). The organic layer was concentrated in vacuo. The crude residue was purified by silica gel chromatography (petroleum ether : *tert*-butyl methyl ether : THF = from 10 : 1 : 1 to 2 : 1 : 1) to give the title compound (3.2 g, 47%) as a clear oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.35 – 4.25 (m, 1H), 4.17 (s, 2H), 3.95 – 3.93 (m, 2H), 3.62 – 3.57 (m, 2H), 3.42 (t, *J* = 11.2 Hz, 2H), 2.74 – 2.73 (m, 2H), 1.98 – 1.89 (m, 2H), 1.80 – 1.77 (m, 2H), 1.41 (s, 9H).

Step 7:

1-(3-bromo-1-(tetrahydro-2*H*-pyran-4-yl)-6,7-dihydro-1*H*-pyrazolo[4,3-c]pyridin-5(4*H*)-yl)ethanone

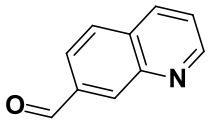


To a solution of *tert*-butyl 3-bromo-1-(tetrahydro-2*H*-pyran-4-yl)-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxylate (3.2 g, 8.3 mmol) in DCM (20 mL) at 0 ^{o}C was added trifluoroacetic acid (20 mL) dropwise. The mixture was stirred at

room temperature for 2 h. The mixture was concentrated in vacuo and the residue was re-dissolved in DCM (30 mL). The mixture was cooled to 0 °C before triethylamine (2.1 g, 21 mmol) and acetic anhydride (0.93 g, 9.1 mmol) were added dropwise. The mixture was stirred at room temperature for an additional 0.5 h. The reaction was quenched with water (60 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude residue was purified by silica gel chromatography (DCM / MeOH = 50 : 1) to give the title compound (2.1 g, 77%) as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.33 – 4.29 (m, 1H), 4.28 (s 2H), 3.95 – 3.92 (m, 2H), 3.70 – 3.67 (m, 2H), 3.43 – 3.36 (m, 2H), 2.84 – 2.69 (m, 2H), 2.09 – 2.08 (m, 3H), 1.96 – 1.91 (m, 2H), 1.80 – 1.76 (m, 2H).

Step 8:

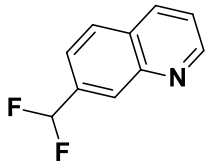
quinoline-7-carbaldehyde



To a solution of 7-methylquinoline (27.0 g, 189 mmol) at 160 ^{o}C was added SeO_2 (21.0 g, 189 mmol) portion-wise over 5 min. The mixture was stirred at 160 ^{o}C for 8 h. After cooling the reaction to room temperature, DCM (400 mL) was added and the mixture was filtered through celite. The organic layer was concentrated in vacuo. The crude residue was purified by silica gel chromatography (petroleum ether / EtOAc = 10 : 1) to give the title compound (14.0 g, 47%) as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.23 (s, 1H), 9.03 (d, *J* = 2.8 Hz, 1H), 8.56 (s, 1H), 8.22 (d, *J* = 8.4 Hz, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.55 – 7.52 (m, 1H).

Step 9:

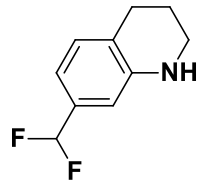
7-(difluoromethyl)quinoline



To a solution of 7-(difluoromethyl)quinoline (14.0 g, 89.2 mmol) in DCM (150 mL) 0 ^{o}C was added diethylaminosulfurtrifluoride (65.0 g, 446 mmol) dropwise over 20 min. The mixture was stirred at room temperature for 16 h. The mixture was poured into sat. aq. $NaHCO_3$ (1 L) at 0 ^{o}C and extracted with DCM (200 mL x 2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude residue was purified by silica gel chromatography (petroleum ether / EtOAc = 5 : 1) to give the title compound (13.0 g, 81%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (d, *J* = 2.8 Hz, 1H), 8.15 (d, *J* = 8.4 Hz, 2H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.63 (d, *J* = 8.8 Hz, 1H), 7.44 – 7.41 (m, 1H), 6.78 (t, *J* = 56.0 Hz, 1H).

Step 10:

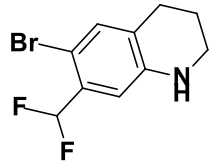
7-(difluoromethyl)-1,2,3,4-tetrahydroquinoline



To a solution of 7-(difluoromethyl)quinoline (13.0 g, 72.6 mmol) and $NaBH_3CN$ (23.0 g, 363 mmol) in MeOH (150 mL) at 0 ^{o}C was added boron trifluoride diethyl etherate (17.9 mL, 145 mmol) dropwise over 20 min. The mixture was heated to 90 ^{o}C for 24 h. After cooling the reaction to room temperature, the mixture was poured into sat. aq. $NaHCO_3$ (1 L) at 0 ^{o}C and extracted with DCM (200 mL x 2). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The crude residue was purified by silica gel chromatography (petroleum ether / EtOAc = 20 : 1) to give the title compound (8.0 g, 56%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (d, *J* = 7.2 Hz, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.59 (s, 1H), 6.50 (t, *J* = 56.8 Hz, 1H), 3.33 (t, *J* = 5.6 Hz, 2H), 2.79 (t, *J* = 6.4 Hz, 2H), 1.98 – 1.92 (m, 2H).

Step 11:

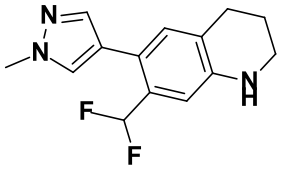
6-bromo-7-(difluoromethyl)-1,2,3,4-tetrahydroquinoline



To a solution of 7-(difluoromethyl)-1,2,3,4-tetrahydroquinoline (7.0 g, 38.3 mmol) in DCM (100 mL) at 0 ^{o}C was added *N*-bromosuccinimide (6.9 g, 38.3 mmol) portion-wise over 20 min. The mixture was stirred at room temperature for 16 h. The mixture was poured into water (100 mL) and extracted with DCM (200 mL x 2). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The crude residue was purified by silica gel chromatography (petroleum ether / EtOAc = 300 : 1) to give the title compound (6.0 g, 60%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 1H), 6.78 (t, *J* = 55.2 Hz, 1H), 6.72 (s, 1H), 3.31 (t, *J* = 5.2 Hz, 2H), 2.74 (t, *J* = 6.0 Hz, 2H), 1.95 – 1.87 (m, 2H).

Step 12:

7-(difluoromethyl)-6-(1-methyl-1*H*-pyrazol-4-yl)-1,2,3,4-tetrahydroquinoline

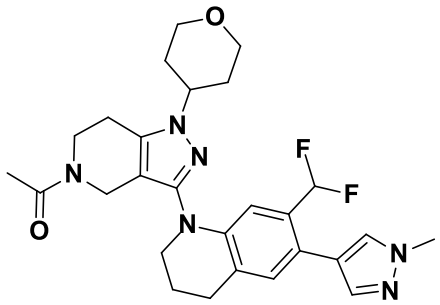


To a solution of 6-bromo-7-(difluoromethyl)-1,2,3,4-tetrahydroquinoline (600 mg, 2.3 mmol) in dioxane (8 mL) and H_2O (2 mL) was added K_2CO_3 (635 mg, 4.6 mmol), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (169 mg, 0.23 mmol) and 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (478 mg, 2.3 mmol). The mixture was heated to 110 ^{o}C for 18 h under a nitrogen atmosphere. After cooling the reaction to room temperature, the mixture was concentrated in vacuo. The crude residue was purified by silica gel

chromatography (petroleum ether / EtOAc = 40 : 1) to give the title compound (520 mg, 86%) as a yellow oil. LCMS M/Z (M+H) 264.

Step 13:

1-[3-[7-(difluoromethyl)-6-(1-methylpyrazol-4-yl)-3,4-dihydro-2*H*-quinolin-1-yl]-1-tetrahydrofuran-3-yl-6,7-dihydro-4*H*-pyrazolo[4,3-*c*]pyridin-5-yl]ethanone



To a solution of 7-(difluoromethyl)-6-(1-methyl-1*H*-pyrazol-4-yl)-1,2,3,4-tetrahydroquinoline (251 mg, 0.95 mmol) in 2-methyl-2-butanol (8 mL) was added 1-(3-bromo-1-(tetrahydro-2*H*-pyran-4-yl)-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridin-5(4*H*)-yl)ethanone (500 mg, 1.52 mmol), (2-dicyclohexylphosphino-2′,4′,6′-triisopropyl-1,1′-biphenyl)[2-(2′-amino-1,1′-biphenyl)]palladium(II) methanesulfonate (129 mg, 0.15 mmol) and K_3PO_4 (971 mg, 4.6 mmol). The mixture was heated to 95 ^oC for 42 h under an argon atmosphere. After cooling the reaction to room temperature, the mixture was concentrated in vacuo. The crude residue was purified by silica gel chromatography (DCM / MeOH = 50 : 1) to give the title compound (360 mg, 74%) as a yellow solid. ^1H NMR (400 MHz, DMSO-d_6) δ 7.75 (s, 1H), 7.50 (s, 1H), 7.10 (s, 1H), 6.96 – 6.63 (m, 2H), 4.33 – 4.25 (m, 1H), 4.20 – 4.09 (m, 2H), 3.99 – 3.90 (m, 2H), 3.86 (s, 3H), 3.78 – 3.66 (m, 2H), 3.63 – 3.55 (m, 2H), 3.49 – 3.41 (m, 2H), 2.89 – 2.66 (m, 4H), 2.11 – 1.90 (m, 7H), 1.85 – 1.80 (m, 2H); ^{13}C NMR (100 MHz, 360K, DMSO-d_6) δ 169.28, 148.41, 142.72, 138.33, 137.69, 131.14, 129.71, 129.21 (t, J = 20.8 Hz), 126.38, 121.35, 119.13, 114.30 (t, J = 235.3 Hz), 110.65, 106.49, 66.51, 54.49, 49.62, 43.40, 38.96, 38.70, 32.81, 27.42, 22.46, 22.28, 21.72; HRMS *m/z* 511.2628 ($M + H^+$, $C_{27}H_{33}F_2N_6O_2$, requires 511.2633).