Supplementary Methods for

Small-molecule inhibition of the acyl-lysine reader ENL as a strategy against acute myeloid leukemia

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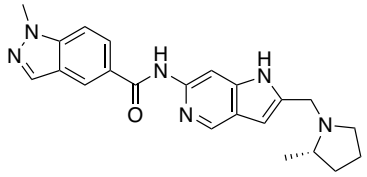
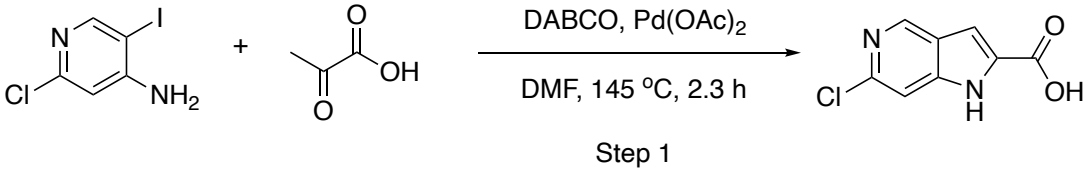
Compound synthesis

SGC-iMLLT is synthesized following reported method (1). TDI-11055 is synthesized following the following steps.



TDI-11055

Step 1



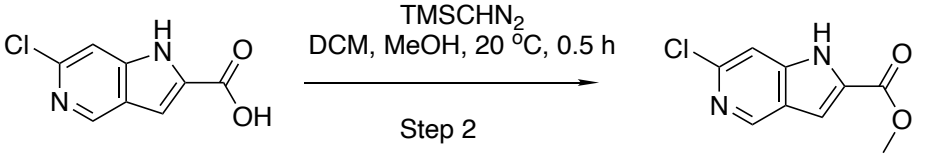
Step 1

6-chloro-1H-pyrrolo[3,2-c]pyridine-2-carboxylic acid, 1

To a solution of 2-chloro-5-iodo-pyridin-4-amine (12.9 g, 50.70 mmol, 1 *eq*), $Pd(OAc)_2$ (682.91 mg, 3.04 mmol, 0.06 *eq*) and DABCO (17.06 g, 152.09 mmol, 16.73 mL, 3 *eq*) in dry DMF (300 mL) was added 2-oxopropanoic acid (13.39 g, 152.09 mmol, 10.71 mL, 3 *eq*) under N_2. The reaction mixture was degassed with N_2 for 20 minutes, then heated to 145 °C for 2 hours. The reaction mixture was concentrated under reudced pressure and the residue taken up in H2O (2.4 L). The mixture was adjust pH to 9-10 by 1N aq. NaOH and filtered through Celite. After washing of the filtrate with EtOAc (500 mL) and MTBE (500 mL), the pH was adjusted to 3 with 1N HCl to give 6-chloro-1H-pyrrolo[3,2-c]pyridine-2-carboxylic acid (3.5 g, 17.80 mmol, 35.11% yield) as a yellow solid.

^{1}H NMR: 1 (400 MHz, DMSO-d6) δ 13.77-13.08 (m, 1H), 12.31 (br s, 1H), 8.78 (s, 1H), 7.39 (s, 1H), 7.26 (s, 1H)

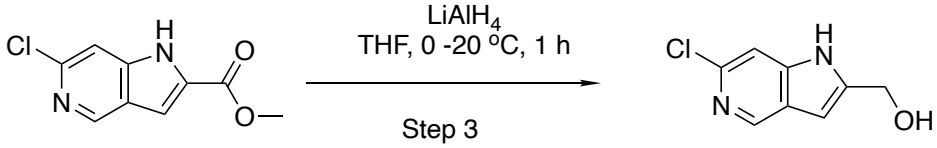
Step 2



Methyl 6-chloro-1H-pyrrolo[3,2-c]pyridine-2-carboxylate, 2

To a solution of 6-chloro-1H-pyrrolo[3,2-c]pyridine-2-carboxylic acid (3.5 g, 17.80 mmol, 1 *eq*) in MeOH (52.5 mL) and DCM (52.5 mL) was added $TMSCHN_2$ (2 M, 35 mL, 3.93 *eq*) dropwise until a persistent yellow color formed, the mixture was stirred at 20 °C for 0.5 hour. The mixture was added 3.5 mL AcOH, then the mixture was concentrated under reduced pressure. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate=1:0 to 3:1) to give methyl 6-chloro-1H-pyrrolo[3,2-c] pyridine-2-carboxylate (2.4 g, 11.40 mmol, 64.00% yield) as a yellow solid. **^{1}H NMR:** 2 (400 MHz, METHANOL-d4) δ 8.57 (s, 1H), 7.30 (s, 1H), 7.17 (s, 1H), 3.79 (s, 3H)

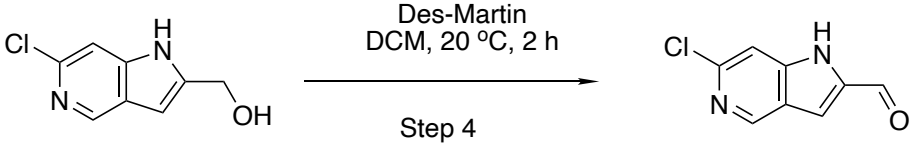
Step 3



(6-chloro-1H-pyrrolo[3,2-c]pyridin-2-yl)methanol, 3

To a solution of LiAlH4 (864.99 mg, 22.79 mmol, 2 *eq*) in dry THF (25 mL) was stirred at 0 °C under N_2. Then methyl 6-chloro-1H-pyrrolo[3,2-c]pyridine-2-carboxylate (2.4 g, 11.40 mmol, 1 *eq*) was dissolved in THF (25 mL) and dropwise to solution of $LiAlH_4$ at 0 °C under N_2. And then the mixture was slowly warmed to 20 °C and stirred at 20 °C for 1 hour under N2. The reaction mixture was cooled to 0 °C, then 10 % aq. NaOH (20 mL) and H_2O (30 mL) were added. The mixture was stirred at 0 °C for 0.5 hour. Then the mixture was filtered and washed with EtOAc (20 mL) and sepatated organic layers were dried over Na_2SO_4, and concentrated under reduced pressure. To give (6-chloro-1H-pyrrolo[3,2-c]pyridin-2-yl) methanol (1.5 g, crude) as a yellow solid. This crude material (3) was used as such for the next reaction without further purification and charecterization.

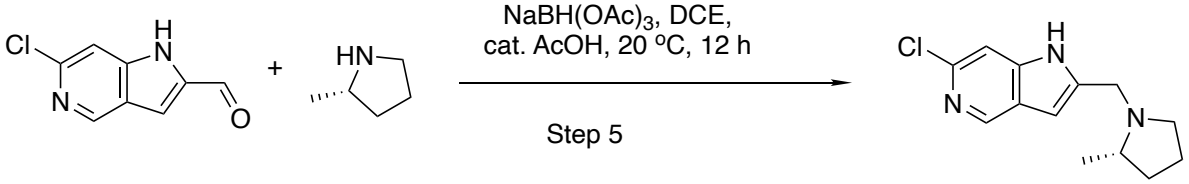
Step 4



6-chloro-1H-pyrrolo[3,2-c]pyridine-2-carbaldehyde, 4

To a solution of (6-chloro-1H-pyrrolo[3,2-c]pyridin-2-yl) methanol (1.5 g, 8.21 mmol, 1 *eq*) in DCM (40 mL) was added Dess-Martin (10.45 g, 24.64 mmol, 7.63 mL, 3 *eq*), the mixture was stirred at 20 °C for 12 hours. The reaction mixture was diluted with Sat. $NaHCO_3$ (50 mL), extracted with DCM (30 mL*3). The combined organic layers were washed with brine (30mL*2), dried over Na_2SO_4, filtered and concentrated under reduced pressure. To give 6-chloro-1H-pyrrolo[3,2-c]pyridine-2-carbaldehyde (1.5 g, crude) as a yellow solid. **^{1}H NMR:** 4 (400 MHz, DMSO-d6) δ 9.91 (s, 1H), 8.88 (s, 1H), 7.57 (s, 1H), 7.42 (s, 1H)

Step 5

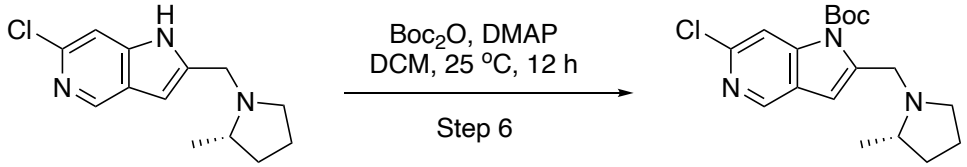


6-chloro-2-[[(2S)-2-methylpyrrolidin-1-yl]methyl]-1H-pyrrolo[3,2-c]pyridine, 5

To a solution of 6-chloro-1H-pyrrolo[3,2-c]pyridine-2-carbaldehyde (1.5 g, 8.31 mmol, 1 *eq*) in DCE (35 mL) was added (2S)-2-methylpyrrolidine (707.24 mg, 8.31 mmol, 1 *eq*) and AcOH (498.78 mg, 8.31 mmol, 475.03 uL, 1 *eq*), the mixture was stirred at 20 °C for 0.5 hour, then $NaBH(OAc)_3$ (3.52 g, 16.61 mmol, 2 *eq*) was added, the result mixture was stirred at 20 °C for 11.5 hours under N_2. The reaction mixture was diluted with sat. $NaHCO_3$ (15 mL) and extracted with DCM (10 mL*3). The combined organic layers were washed with brine (10 mL*3), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, EtOAc/MeOH = 1/0 to 9/1) to give 6-chloro-2-[[(2S)-2-methylpyrrolidin-1-yl]methyl]-1H-pyrrolo[3,2-c] pyridine (0.8 g, 3.20 mmol, 38.57% yield) as a red oil. **^{1}H NMR:** 5 (400 MHz, METHANOL-d4) δ 8.50 (s, 1H), 7.38 (s, 1H), 6.54 (s, 1H), 4.12 (d, *J* = 13.9 Hz, 1H), 3.50 (d, *J* = 13.9 Hz, 1H), 3.04-2.94 (m, 1H), 2.57-2.45 (m, 1H),

2.31 (q, *J* = 9.0 Hz, 1H), 2.03 (dddd, *J* = 5.8, 7.2, 9.2, 12.6 Hz, 1H), 1.80-1.68 (m, 2H), 1.48 (dddd, *J* = 6.3, 8.8, 10.2, 12.5 Hz, 1H), 1.20 (d, *J* = 6.1 Hz, 3H).

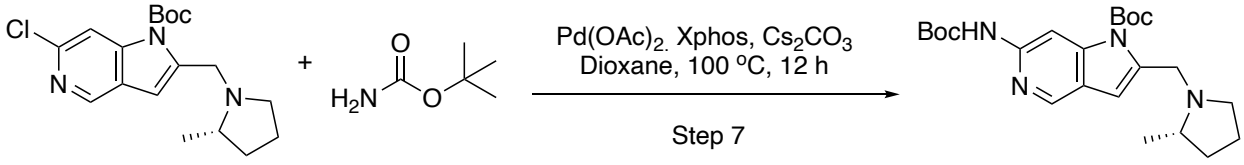
Step 6



Tert-butyl 6-chloro-2-[[(2S)-2-methyl pyrrolidin-1-yl]methyl] pyrrolo[3,2-c] pyridine-1-carboxylate, 6

To a solution of 6-chloro-2-[[(2S)-2-methylpyrrolidin-1-yl]methyl]-1H-pyrrolo[3,2-c] pyridine (0.8 g, 3.20 mmol, 1 *eq*) in DCM (25 mL) was added a solution of tert-butoxycarbonyl tert-butyl carbonate (769.03 mg, 3.52 mmol, 809.51 uL, 1.1 *eq*) in DCM (25 mL), and then DMAP (39.14 mg, 320.33 μmol, 0.1 *eq*) was added, the result mixture was stirred at 25 °C for 12 hours. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 0/1) to give tert-butyl 6-chloro-2-[[(2S)-2-methylpyrrolidin-1-yl] methyl]pyrrolo[3,2-c]pyridine-1-carboxylate, 6 (1 g, 2.86 mmol, 89.23% yield) as a red solid.

Step 7

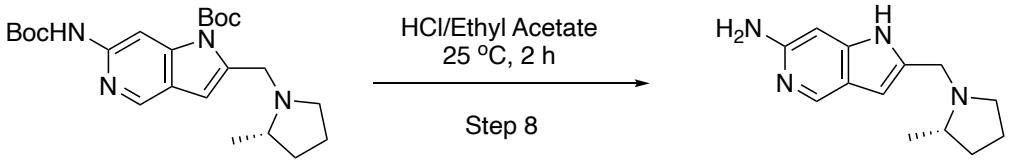


Tert-butyl6-(tert-butoxycarbonylamino)-2-[[(2S)-2-methylpyrrolidin-1-yl] methyl] pyrrolo [3,2-c]pyridine-1-carboxylate, 7

To a solution of tert-butyl 6-chloro-2-[[(2S)-2-methylpyrrolidin-1-yl]methyl]pyrrolo[3,2-c] pyridine-1-carboxylate (1 g, 2.86 mmol, 1 *eq*) and tert-butyl carbamate (502.26 mg, 4.29 mmol, 1.5 *eq*) in dioxane (20 mL) was added XPhos (272.53 mg, 571.67 μmol, 0.2 *eq*), $Pd(OAc)_2$ (64.17 mg, 285.83 μmol, 0.1 *eq*) and Cs_2CO_3 (1.86 g, 5.72 mmol, 2 *eq*), the mixture was stirred at 100 °C for 12 hours under N_2. The reaction mixture was concentrated under reduced pressure

to give a residue. The residue was purified by column chromatography (SiO2, DCM/MeOH = 1/0 to 10/1) to give tert-butyl 6-(tert- butoxycarbonylamino)-2-[[(2S)-2-methylpyrrolidin-1-yl]methyl]pyrrolo [3,2-c]pyridine-1-carboxylate (0.4 g, 929.07 μmol, 32.50% yield as a yellow oil. **^{1}H NMR:**7 (400 MHz, METHANOL-d4) δ 8.45-8.38 (m, 1H), 7.79 (s, 1H), 6.43 (s, 1H), 4.10 (d, *J* = 13.8 Hz, 1H), 3.53-3.43 (m, 1H), 3.06-2.97 (m, 1H), 2.57-2.46 (m, 1H), 2.34 (q, *J* = 9.1 Hz, 1H), 2.11-1.97 (m, 1H), 1.82-1.68 (m, 7H), 1.61-1.42 (m, 11H), 1.25-1.18 (m, 4H).

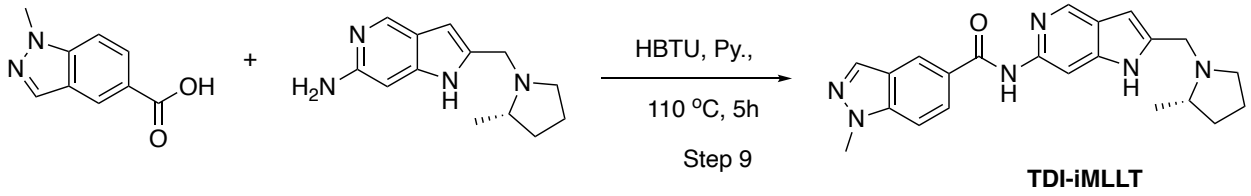
Step 8



2-[[(2S)-2-methylpyrrolidin-1-yl]methyl]-1H-pyrrolo[3,2-c]pyridin-6-amine, 8

A mixture of tert-butyl6-(tert-butoxycarbonylamino)-2-[[(2S)-2-methylpyrrolidin-1-yl]methyl] pyrrolo[3,2-c]pyridine-1-carboxylate (0.4 g, 929.07 μmol, 1 *eq*) in HCl/EtOAc (15 mL) was stirred at 25 °C for 2 hours. The reaction mixture was concentrated under reduced pressure to give 2-[[(2S)-2-methylpyrrolidin-1-yl]methyl]-1H-pyrrolo[3,2-c]pyridine-6-amine, 8 (0.3 g, crude, HCl) as gray solid.

Step 9



TDI-iMLLT

1-methyl-N-[2-[[(2S)-2-methylpyrrolidin-1-yl]methyl]-1H-pyrrolo[3,2-c]pyridin-6-yl]indazole-5-carboxamide, TDI-11055

To a solution of 1-methylindazole-5-carboxylic acid (76.49 mg, 434.20 μmol, 1 *eq*) in Py. (4 mL) was added HBTU (164.67 mg, 434.20 μmol, 1 *eq*), the mixture was stirred at 110 °C for 0.5 hour under N_2. And then 2-[[(2S)-2-methylpyrrolidin-1-yl]methyl]-1H-pyrrolo[3,2-c] pyridin-6-amine (0.1 g, 434.20 μmol, 1 *eq*, HCl) was added, the result mixture was stirred at 110 °C for 4.5 hours under N_2. The reaction mixture was concentrated under pressure. The residue was diluted

with Sat. $NaHCO_3$ (20 mL) and extracted with EtOAc (15 mL*3). The combined organic layers were washed with brine (15 mL*2), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by prep-HPLC (neutral condition) to give 1-methyl-N-[2-[[(2S)-2-methylpyrrolidin-1-yl]methyl]-1H-pyrrolo[3,2-c] pyridin-6-yl]indazole-5-carboxamide (35 mg, 90.10 μmol, 20.75% yield, 100% purity) as a yellow solid.

LCMS: TDI-11055 ($M+H^+$): 389.1@ 1.515 min (10-80 % ACN in H_2O, 4.5 min)

^{1}H NMR: TDI-11055 (400 MHz, METHANOL-d4) δ 8.57-8.45 (m, 2H), 8.18 (d, *J* = 4.2 Hz, 2H), 8.05 (dd, *J* = 1.5, 8.8 Hz, 1H), 7.68 (d, *J* = 9.0 Hz, 1H), 6.49 (s, 1H), 4.18-4.04 (m, 4H), 3.50 (d, *J* = 13.9 Hz, 1H), 3.07-2.96 (m, 1H), 2.56-2.45 (m, 1H), 2.34 (q, *J* = 9.2 Hz, 1H), 2.09-1.95 (m, 1H), 1.81-1.65 (m, 2H), 1.54-1.41 (m, 1H), 1.21 (d, *J* = 6.2 Hz, 3H)

SFC: TDI-11055 (Retention time: 2.62; 100% ee)

References

1. Moustakim M, Christott T, Monteiro OP, Bennett J, Giroud C, Ward J, et al. Discovery of an MLLT1/3 YEATS Domain Chemical Probe. Angewandte Chemie Int Ed. 2018;57:16302–7.