ChemMedChem

Supporting Information

METTL3 Inhibitors for Epitranscriptomic Modulation of Cellular Processes

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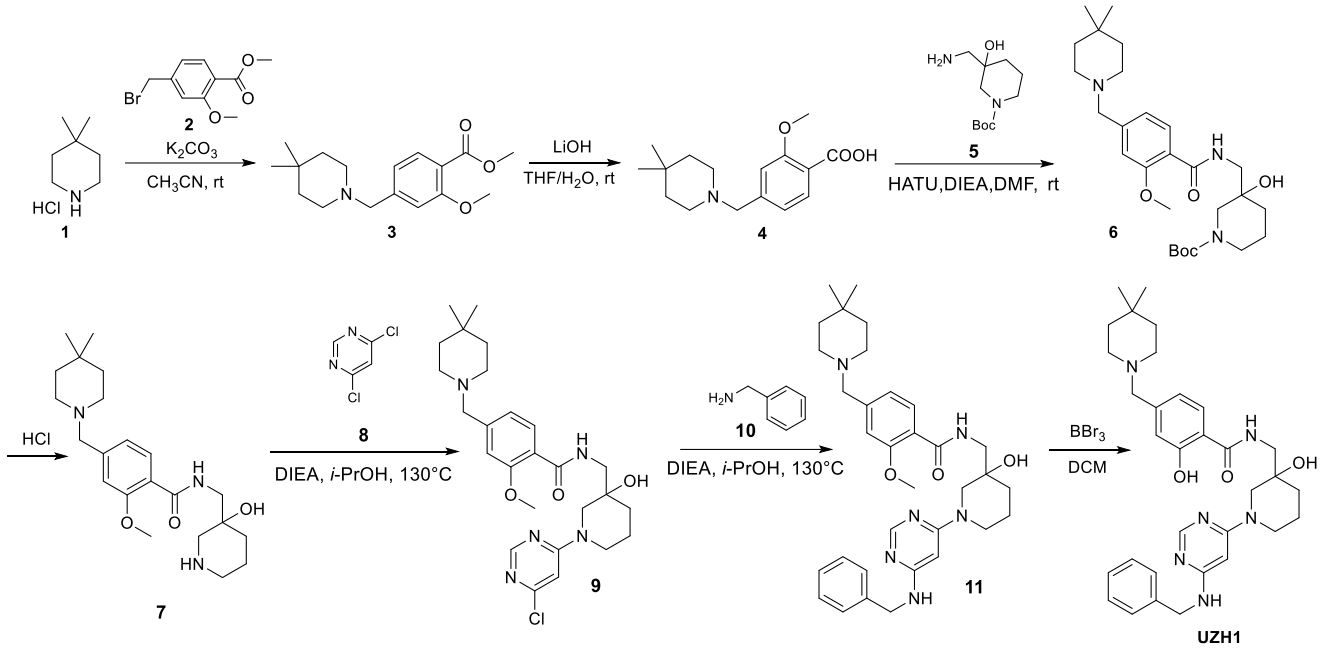
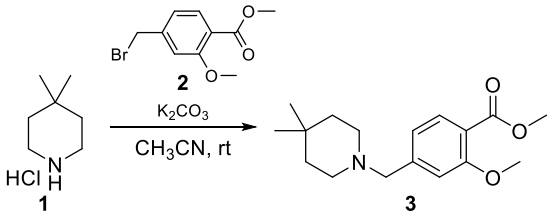
Synthetic procedures

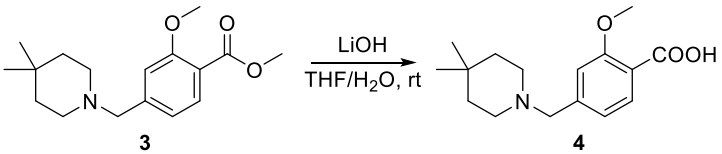


Synthesis of compound **3**



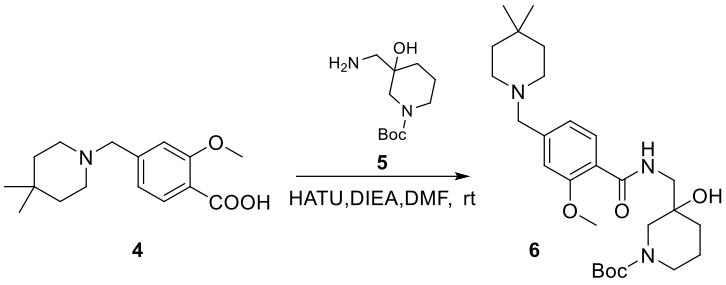
To a solution of 4,4-dimethylpiperidine hydrochloride (350 mg, 2.34 mmol) in CH_3CN (10 ml) was added methyl 4-(bromomethyl)-2-methoxybenzoate (666 mg; 2.57 mmol) and K_2CO_3 (646 mg, 4.68 mmol), then the mixture was stirred at 25 °C for 16 h. The mixture was washed with water (20 mL) and extracted with EtOAc (20 mL × 3), and combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (0-10% EtOAc in petroleum ether) to give methyl 4-((4,4-dimethylpiperidin-1-yl)methyl)-2-methoxybenzoate as a white solid (500 mg, yield: 73%).

Synthesis of compound **4**



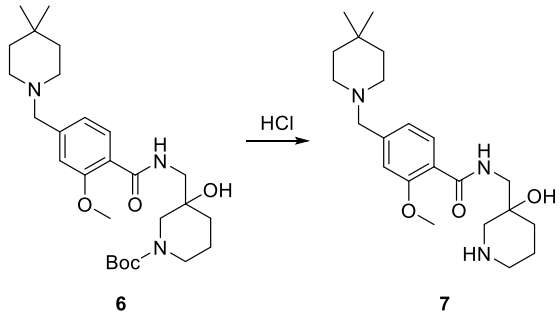
To a solution of methyl-4-((4,4-dimethylpiperidin-1-yl)methyl)-2-methoxybenzoate (500 mg, 1.72 mmol) in THF/H_2O (10 mL; 5/1) was added LiOH (123 mg, 5.15 mmol), the mixture was stirred at 25 °C for 16 h. The mixture was adjusted by 1 M HCl to pH 3, then extracted with EtOAc (20 mL × 3), and combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to give desired product 4-((4,4-dimethylpiperidin-1-yl)methyl)benzoic acid as a white solid (470 mg, crude).

Synthesis of compound **6**



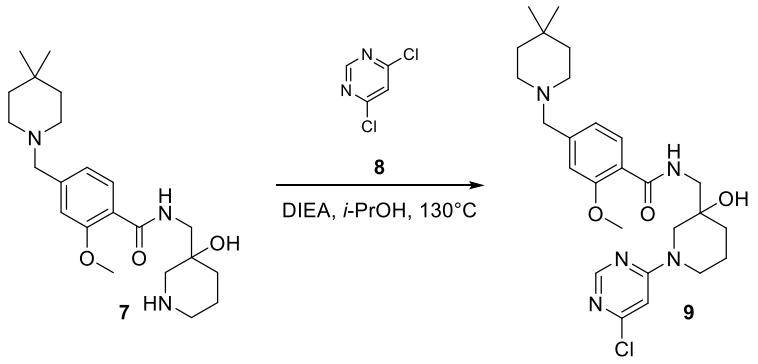
To a solution of 4-((4,4-dimethylpiperidin-1-yl)methyl)-2-methoxybenzoic acid (470 mg, 1.69 mmol) in DMF (10 mL) was added *tert*-butyl 3-(aminomethyl)-3-hydroxypiperidine-1-carboxylate (390 mg, 1.69 mmol) and HATU (773 mg, 2.03 mmol) and DIEA (0.56 mL; 3.39 mmol), then the mixture was stirred at 25 °C for 16 h under N_2. The mixture was concentrated, washed with water (20 mL), extracted with EtOAc (20 mL × 3), and combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (0-5% MeOH in DCM) to give the product *tert*-butyl 3-((4-((4,4-dimethylpiperidin-1-yl)methyl)-2-methoxybenzamido)methyl)-3-hydroxypiperidine-1-carboxylate as yellow oil (560 mg, yield: 67%).

Synthesis of compound **7**



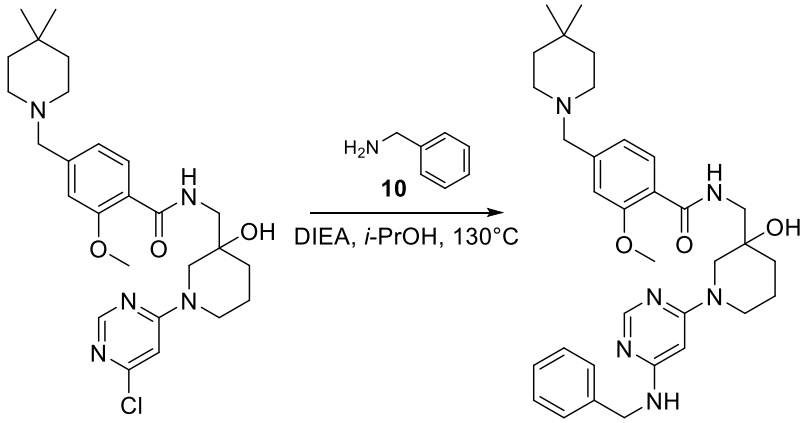
To a solution of *tert*-butyl 3-((4-((4,4-dimethylpiperidin-1-yl)methyl)-2-methoxybenzamido)methyl)-3-hydroxypiperidine-1-carboxylate (560 mg, 1.14 mmol) in DCM (5 mL) was added 3 M HCl in MeOH (1.9 mL; 5.7 mmol), and the mixture was stirred at 25 °C for 16 h. The mixture was concentrated to give desired product 4-((4,4-dimethylpiperidin-1-yl)methyl)-*N*-((3-hydroxypiperidin-3-yl)methyl)-2-methoxybenzamide as yellow oil. (600 mg, crude).

Synthesis of compound **9**



To a solution of 4-((4,4-dimethylpiperidin-1-yl)methyl)-*N*-((3-hydroxypiperidin-3-yl)methyl)-2-methoxybenzamide (680 mg; 1.62 mmol) in *i*-PrOH (10 mL) was added 4,6-dichloropyrimidine (265 mg; 1.78 mmol) and DIEA (0.80 mL; 4.85 mmol). The mixture was stirred at 130°C for 16 h under N_2. The mixture was concentrated, and the residue was purified by column chromatography on silica gel (0-5% MeOH in DCM) to give the product *N*-((1-(6-chloropyrimidin-4-yl)-3-hydroxypiperidin-3-yl)methyl)-4-((4,4-dimeth ylpiperidin-1-yl)methyl)-2-methoxybenzamide as yellow oil (130 mg, yield: 16%).

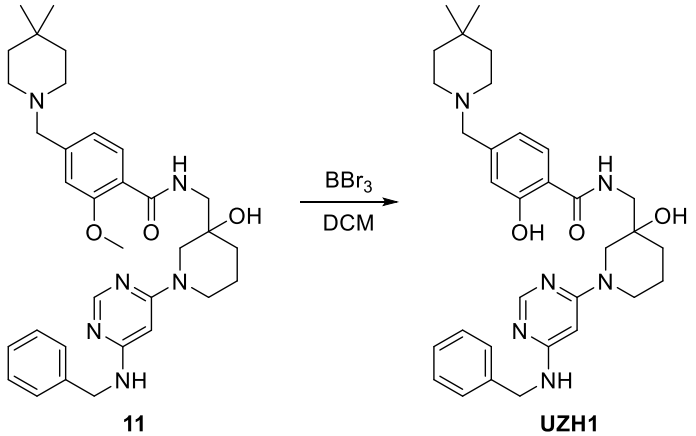
Synthesis of compound **11**



9 **11**

To a solution of *N*-((1-(6-chloropyrimidin-4-yl)-3-hydroxypiperidin-3-yl)methyl)-4-((4,4-dimethylpiperidin-1-yl)methyl)-2-methoxybenzamide (130 mg; 0.26 mmol) in *i*-PrOH (3 mL) was added benzylamine (31 µL; 0.28 mmol) and DIEA (0.12 mL; 0.77 mmol). The mixture was stirred at 130°C for 16 h under N_2. The mixture was concentrated and the residue was purified by column chromatography on silica gel (0-8% MeOH in DCM) to give the product *N*-((1-(6-(benzylamino)pyrimidin-4-yl)-3-hydroxypiperidin-3-yl)methyl)-4-((4,4-dimethylpiperidin-1-yl)methyl)-2-methoxybenzamide as yellow oil (75 mg, yield: 50%).

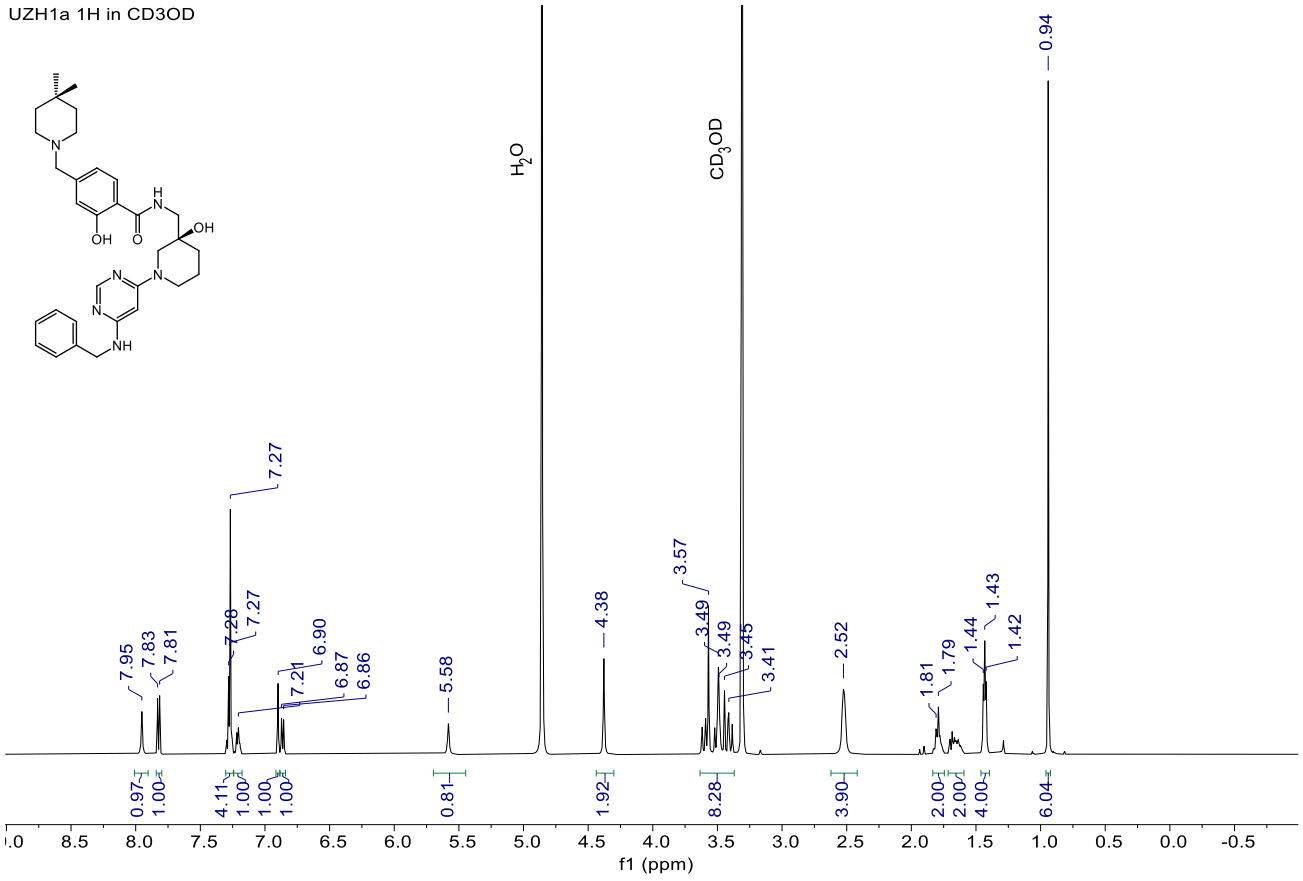
Synthesis of compound **UZH1** (racemic)

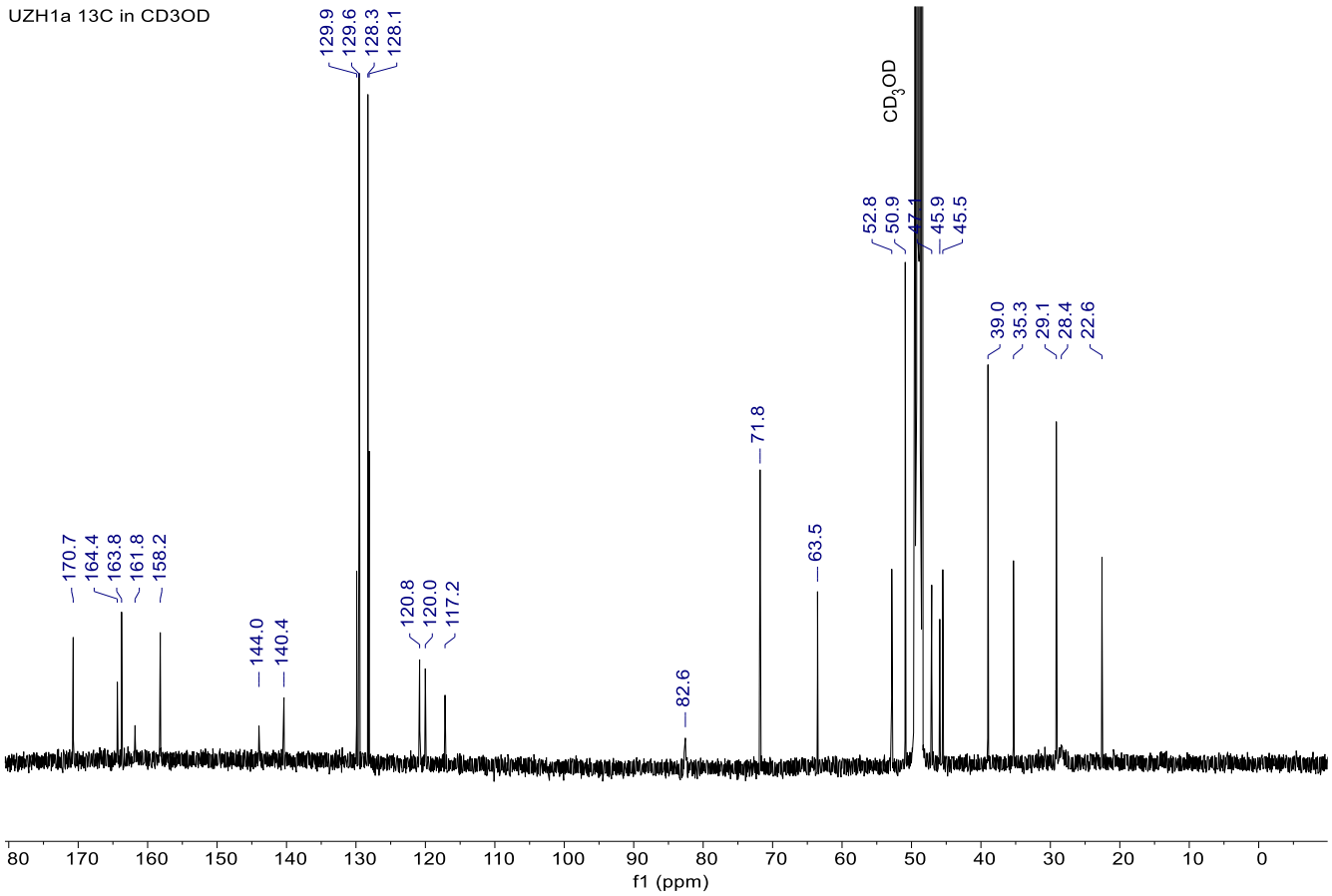


11 **UZH1**

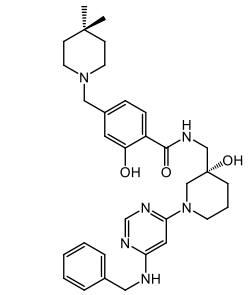
To a solution of *N*-((1-(6-(benzylamino)pyrimidin-4-yl)-3-hydroxypiperidin-3-yl)methyl)-4-((4,4-dimethylpiperidin-1-yl)methyl)-2-methoxybenzamide (75 mg; 0.13 mmol) in DCM (2 mL) was added 1 M BBr_3 in DCM (0.39 mL; 0.39 mmol) at 0°C. The mixture was stirred at 25°C for 16 h under N_2. The mixture was concentrated and purified by

HPLC to give the desired product ***N*-((1-(6-(benzylamino)pyrimidin-4-yl)-3-hydroxypiperidin-3-yl)methyl)-4-((4,4-dimethylpiperidin-1-yl)methyl)-2-hydroxybenzamide** as a white solid (24 mg; yield: 32%). The separation of **UZH1** enantiomers was conducted *via* preparative HPLC on a chiral column to give **UZH1a** and **UZH1b**, both as a white solid (purity over 99.5%). It is important to note that the cellular potency of the compounds was crucially affected by their purity, and high degree of purity was required to observe the difference in cellular toxicity between **UZH1a** and **UZH1b**.

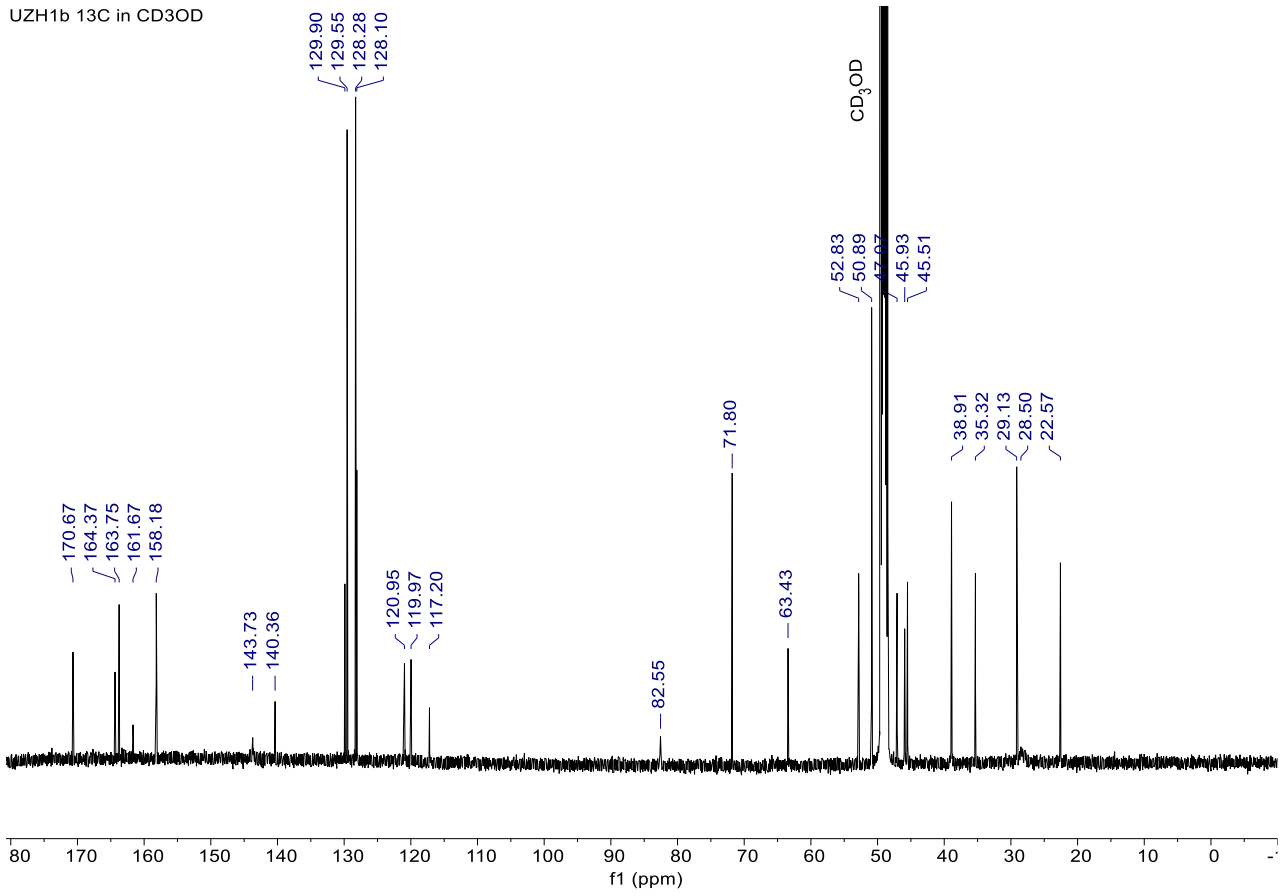


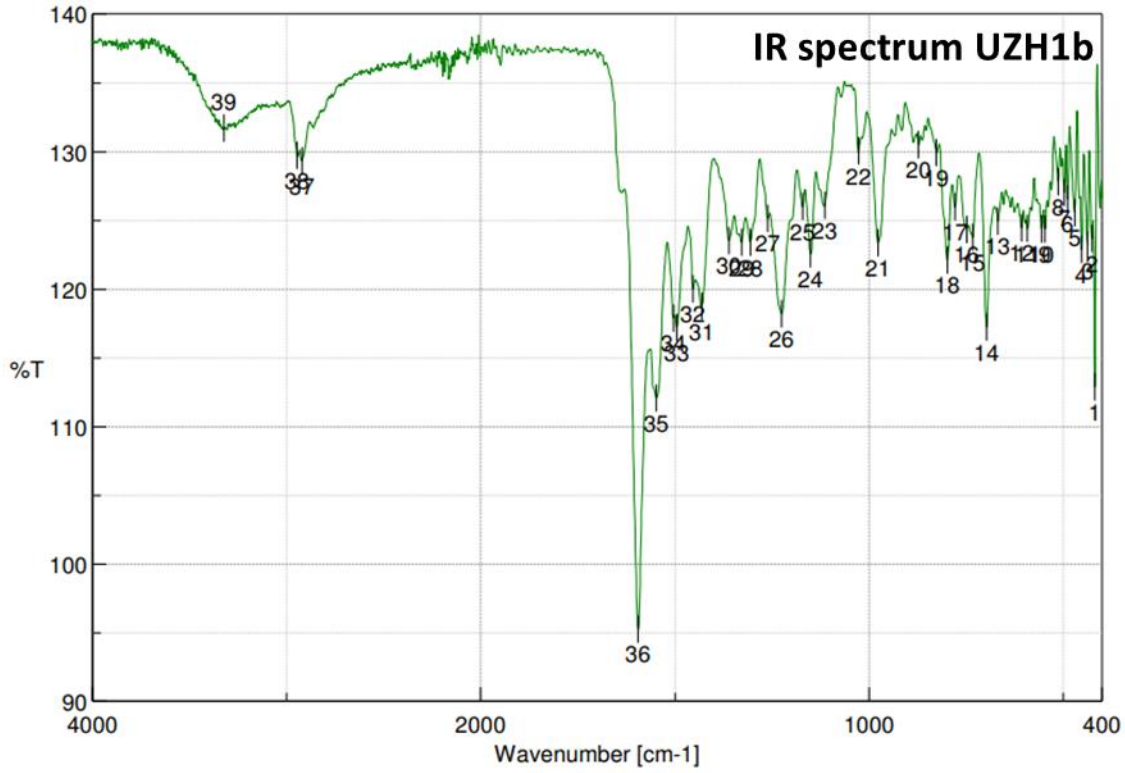

UZH1a 13C in CD3OD
129.9
129.6
128.3
128.1
CD3OD
52.8
50.9
47.1
45.9
45.5
39.0
35.3
29.1
28.4
22.6
71.8
63.5
170.7
164.4
163.8
161.8
158.2
144.0
140.4
120.8
120.0
117.2
82.6
80
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

UZH1b 1H in CD3OD



UZH1b 13C in CD3OD





Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity
1	418.5	112.9	2	426.2	123.7
3	437.8	123.2	4	452.2	122.9
5	470.5	125.5	6	487.9	126.5
7	497.5	127.0	8	513.0	127.8
9	546.7	124.4	10	555.4	124.4
11	592.0	124.4	12	607.5	124.5
13	668.2	124.9	14	697.1	117.2
15	732.8	123.7	16	748.2	124.3
17	778.1	126.0	18	798.4	122.1
19	826.3	129.9	20	872.6	130.5
21	976.8	123.4	22	1026.9	130.0
23	1113.7	126.1	24	1150.3	122.5
25	1170.6	126.0	26	1225.5	118.2
27	1260.3	125.1	28	1305.6	123.4
29	1328.7	123.4	30	1361.5	123.5
31	1430.9	118.8	32	1453.1	120.0
33	1495.5	117.2	34	1504.2	117.9
35	1547.6	112.1	36	1594.8	95.2
37	2921.6	129.3	38	2947.7	129.7
39	3322.7	131.7			

[Comment]

Sample Name	
Comment	
User	
Division	
Company	University of Zurich

[Measurement Information]

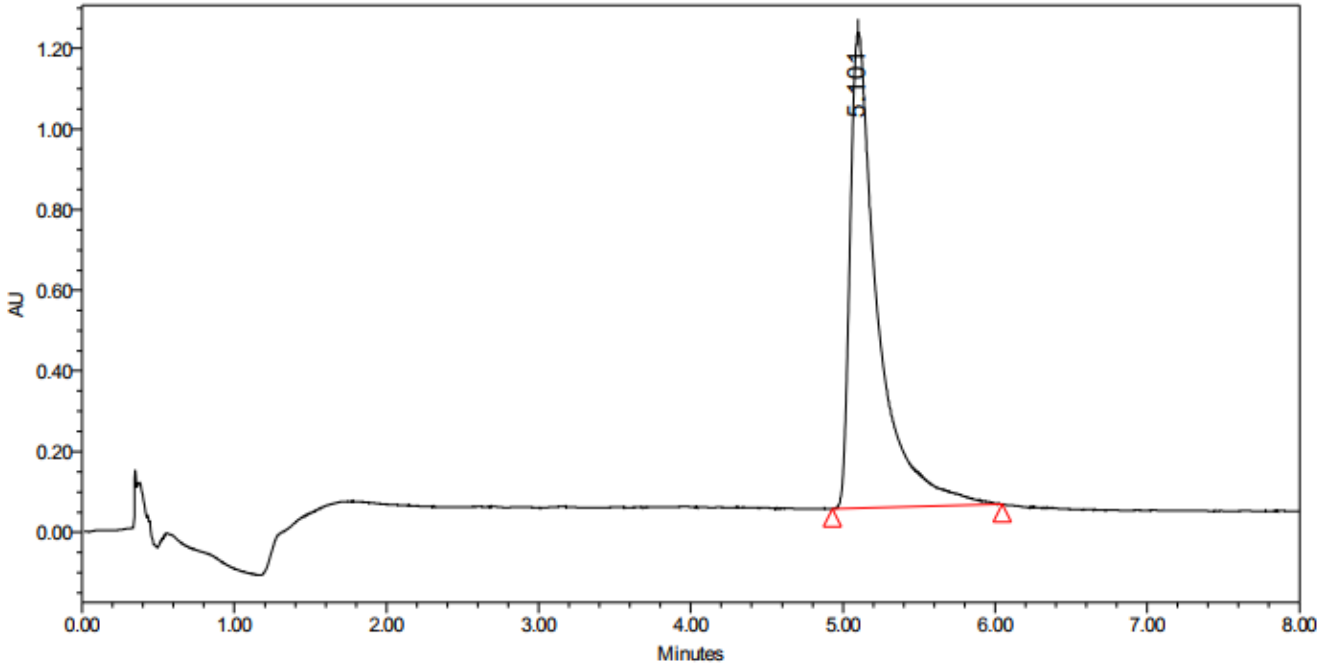
Model Name	FT/IR-4100typeA
Serial Number	B079461016
Accessory	ATR PRO410-S
Accessory S/N	A042261044
Light Source	Standard
Detector	TGS
Accumulation	10
Resolution	4 cm-1
Zero Filling	On
Apodization	Cosine
Gain	Auto (32)
Aperture	Auto (7.1 mm)
Scanning Speed	Auto (2 mm/sec)
Filter	Auto (30000 Hz)

UZH1a chiral chromatogram

SAMPLE INFORMATION

Sample Name:	MC20-381-30-1P1-OM-E-D	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	20210128 1
Vial:	1:D,6	Acq. Method Set:	15 5% 40% ETOH DEA C2 OM
Injection #:	1	Processing Method	chiral sample
Injection Volume:	10.00 ul	Channel Name:	PDA Ch1 214nm@4.8nm
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	PDA Ch1 214nm@4.8nm
Date Acquired:	2021-01-28 11:45:22 CST		
Date Processed:	2021-01-28 12:51:09 CST		

Auto-Scaled Chromatogram



Peak Results

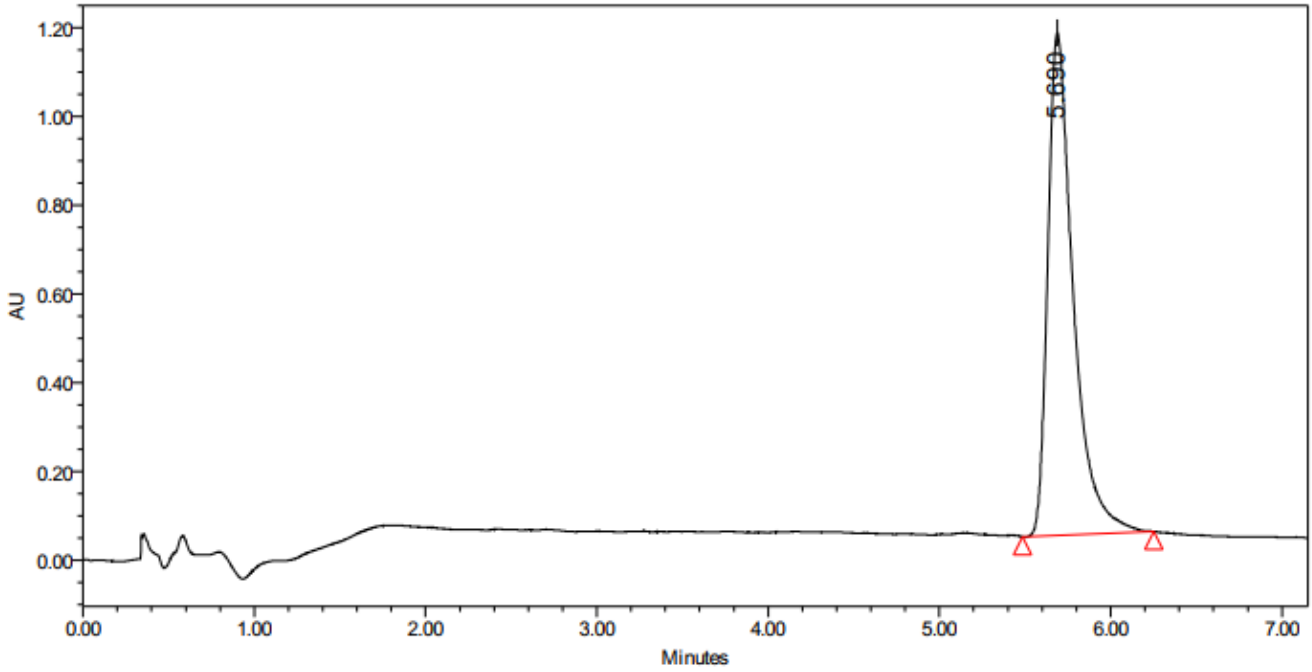
	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)
1	5.101	14857244	100.00	1179895

UZH1b chiral chromatogram

SAMPLE INFORMATION

Sample Name:	MC20-381-30-1P2-OM-E-D	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	20210128 1
Vial:	1:D,7	Acq. Method Set:	15 5% 40% ETOH DEA C2 OM
Injection #:	1	Processing Method	chiral sample
Injection Volume:	1.00 ul	Channel Name:	PDA Ch1 214nm@4.8nm
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	PDA Ch1 214nm@4.8nm
Date Acquired:	2021-01-28 12:43:42 CST		
Date Processed:	2021-01-28 12:51:42 CST		

Auto-Scaled Chromatogram



Peak Results

	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)
1	5.690	11729630	100.00	1132757

Materials

HEK293T cells were obtained from ATCC. MOLM-13 cells were a gift from Prof. W. Wei-Lynn Wong (University of Zurich). U2OS cells were a gift from Prof. Yang Shi (Harvard Medical School). Dulbecco's modified essential medium (DMEM) supplemented with 4.5 g/L glucose and GlutaMAX™, RPMI 1640 medium, Opti-MEM® medium, Dulbecco's phosphate buffered saline (DPBS), Gibco™ Fetal Bovine Serum, Gibco™ Penicillin-Streptomycin, 0.05% Trypsin-EDTA, nuclease-free water, and Lipofectamine® RNAiMAX were purchased from Thermo Fisher Scientific (Waltham, MA). Cell culture treated dishes, flasks, and multiwell plates were obtained from Corning Inc. (Corning, NY). Sera-Mag Oligo(dT)-coated magnetic particles were obtained from GE Healthcare (Chicago, IL). GENEzol™ reagent was purchased from Geneaid Biotech (New Taipei, Taiwan). Human METTL3 specific siRNAs and non-targeting siRNA as a negative control were purchased from Microsynth (Balgach, Switzerland). METTL3-siRNA-1 sense: 5′-GCACAUCCUACUCUUGUAAdTdT-3′, METTL3-siRNA-2 sense: 5′-GGAGAUCCUAGAGCUAUUAdTdT-3′, METTL3-siRNA-3 sense: 5′-GACUGCUCUUUCCUUAAUAdTdT-3′, NC-siRNA sense: 5′-AGGUAGUGUAAUCGCCUUGdTdT-3′.[1,2] NEBuffer™ 2 and nucleoside digestion mix were obtained from NEB (Ipswich, MA). Adenosine, guanosine, 7-methylguanosine (m7G), and SYPRO Orange were purchased from Sigma-Aldrich (St. Louis, MO), N^6-methyladenosine (m^6A) from Chemie Brunschwig (Basel, Switzerland), 1-methyladenosine (m1A) from MedChem Express (NJ, USA), and N^6-methyl-2'-O-methyladenosine (m^6A_m) from Toronto Research Chemicals (Toronto, Canada) as UPLC-MS/MS standards. Anti-METTL3 rabbit and anti-β-actin mouse antibodies were from Abcam (Cambridge, UK), IRDye® 800CW goat anti-rabbit IgG and IRDye® 680RD donkey anti-mouse IgG secondary antibodies were obtained from Li-COR Biosciences (Lincoln, NE). The protease inhibitor cocktail tablets were purchased from Roche (Basel, Switzerland). SurePAGE 12% Bis-Tris polyacrylamide gels were obtained from GenScript (NJ, USA).

X-ray data collection and refinement statistics

PDB ID	7ACD
Compound	**UZH1a**
Data Collection	
Space group	$P3_221$
Cell dimension a, b, c (Å)	63.8, 63.8, 224.92
Cell dimension α, β, γ (°)	90, 90, 120
Resolution (Å)	44.98 (2.5)
Unique reflections*	19248 (3051)
Completeness*	99.9 (99.8)
Redundancy*	9.72 (9.96)
R_{merge}*	19.5 (139.2)
CC (1/2)	99.6 (60.9)
I/σI	11.33 (1.7)
Refinement	
R_{work}/R_{free}	0.1882/0.2259
RMSD bond (Å)	0.009
RMSD angle (°)	1.22
B-factor (Å^2) **	45.26/42.22/44.78
Ramanchandran Favored	97.16
Ramanchandran Allowed	2.61
Ramanchandran Disallowed	0.23

*Statistics for the highest resolution shell is shown in parentheses.
** P/L/W indicate protein, ligand/ion and water molecules, respectively.

X-ray data collection and refinement statistics table