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Supporting Information

Design of the First-in-Class, Highly Potent Irreversible Inhibitor Targeting the Menin-MLL Protein–Protein Interaction

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Author Contributions

S.W. Conceptualization: Lead; Formal analysis: Lead; Funding acquisition: Lead; Investigation: Lead; Project administration: Lead; Supervision: Lead; Writing—original draft: Lead; Writing—review & editing: Lead
J.S. Investigation: Equal; Supervision: Equal; Writing—review & editing: Supporting.

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Protein Expression and Purification

Menin (residues 2-610 containing a deletion from 460-519) was cloned into an N-terminally His_6-Sumo tagged expression construct. Transformed cells were grown in Terrific Broth at 37 °C to an $O.D._{600}$ of 1.0, then induced with 0.4 mM IPTG overnight at 20 °C. Freeze-thawed cell pellets were resuspended in 25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM DTT with protease inhibitors, then sonicated. The supernatant was cleared of debris through centrifugation. The supernatant was incubated for 1 h at 4 °C with Ni-NTA (Qiagen) equilibrated with 25 mM Tris-HCl, pH 8.0 and 150 mM NaCl (Buffer A). The Ni-NTA was washed extensively with Buffer A and the protein eluded with Buffer A plus 250 mM imidazole. Eluent was dialyzed overnight at 4 °C in Buffer A containing 1 mM DTT and Sumo protease for tag cleavage. The cleaved protein was reapplied to the Ni-NTA column to remove the His_6-Sumo tag, then concentrated and loaded onto a Superdex 200 (GE Healthcare) size exclusion column equilibrated with Buffer A and 5 mM DTT. The purified protein was concentrated to 25 mg/mL and stored at -80 °C.

Fluorescence Polarization (FP)-Based Binding Assay

We used the assay conditions described previously.[1]

Cell Growth Inhibition Assay

MV4;11, K562, HL-60, and RS4;11 cells were purchased from ATCC and SEM, MOLM-13, MOLM-14, MOLM-16, SKM-1, and MONO-MAC-6 were purchased from DSMZ. Cells were cultured in either Iscove's Modified Dulbecco's Medium or RPMI 1640 medium (ATCC) supplemented with 10% fetal bovine serum and 100 U/L penicillin-streptomycin and incubated at 37 °C under 5% CO_2, according to the cell supplier's instructions.

For cell growth experiments, cells were seeded into 96-well plates at a density of 100,000 cells per well (4-day testing) or 2,000-3,000 cells per well (7-day testing) in 200 µL culture medium and treated with either vehicle control (DMSO, 0.1%) or compounds for indicated time. At the end of an experiment, Cell Count Kit (Dojindo) was used to measure cell viability. Data were analyzed using PRISM software.

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Detailed Method of Modeling of Compounds 6 and 7 Bound to Menin

The crystal structure of the complex between Menin and (*R*)-MIV-6 (PDB ID: 4OG8[2]) was used to build the binding models of designed molecules with Menin. Menin protein from the crystal structure was extracted and the missing side chains in Menin were added using the MOE program.[3] The "protonate 3D" module in MOE was then used to add hydrogen atoms to Menin at the physiological condition. The designed compounds were prepared using the MOE program and the GOLD program[4] (version 5.1) was used to perform the docking simulations. The binding site was centered at Y323 in Menin with a radius of 15 Å. Default parameters for the genetic algorithm (GA) run were adopted. The selection of docking poses were assessed by GoldScore (the fitness function) implemented in Gold 5.1. The top ranked conformations based on GoldScore were analyzed and compare with the available crystal structures to determine the binding modes of the compounds. Figures were prepared using the PyMOL program.

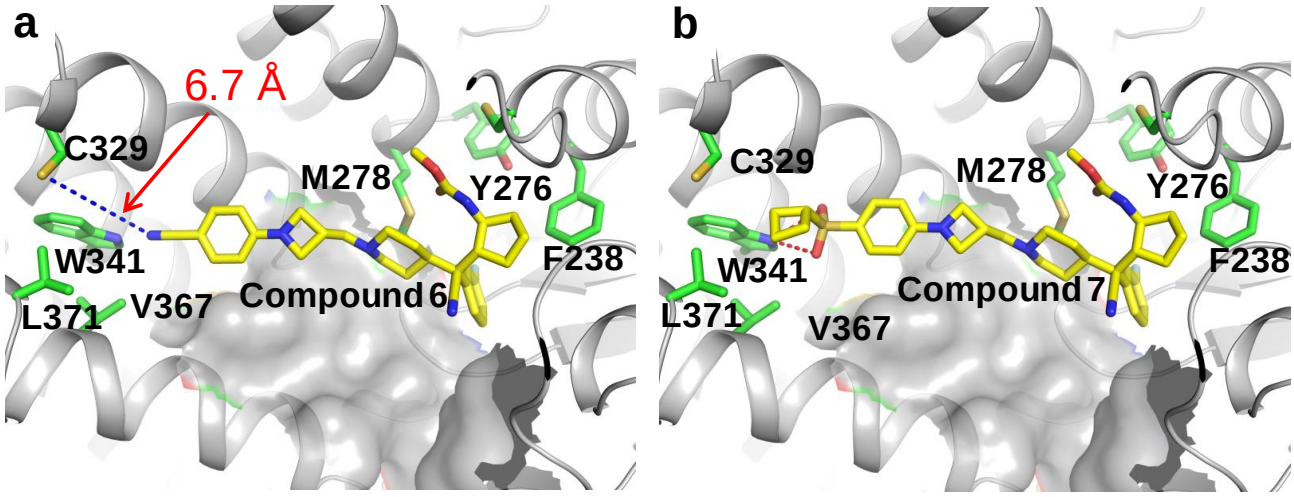


Figure S1. a), b) Modeling of compounds **6** or **7** bound to Menin. (Protein pocket is shown in gray cartoon model and the residues close to inhibitor are highlighted in stick model. Inhibitors are shown in stick representation with yellow carbon atoms. Oxygen atoms are shown in red; nitrogens in blue and sulfurs in yellow. Dashed lines depict hydrogen bonds.)

Detailed Method of Mass-spectroscopic Analysis of Human Menin Protein Incubated with Menin Inhibitors

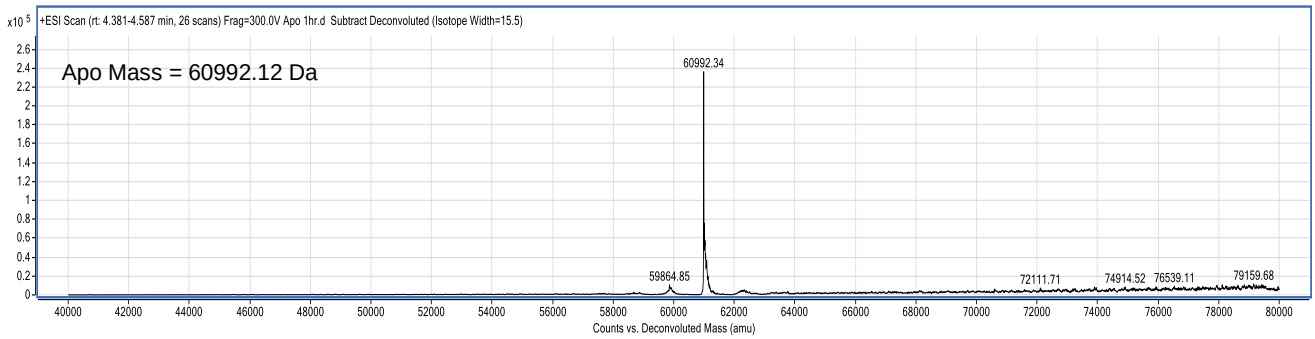
Samples of menin (25 mg/mL in 25 mM T ris 8.0, 150 mM NaCl and 5mM DTT) were incubated with compounds **8, 9** or **10** in a protein to compound molar ratio of 1: 1.2 for 1 h or overnight at 4 °C. Following incubation, the sample was diluted to 1 mg/mL with water. 0.1 mL of each sample was applied to a reverse phase HPLC column (Phenomenex Aeris widepore C4 column 3.6 µM, 50 × 2.10 mm) at a flow rate of 0.5 mL/min in H_2O with 0.2% (v/v) formic acid. Protein was eluted using a gradient of 5-100% acetonitrile with 0.2% (v/v) formic acid over 4 minutes. LC-MS experiment (Agilent Q-TOF 6545) was carried out under the following conditions: fragmentor voltage, 300 V; skimmer voltage, 75 V; nozzle voltage, 100 V; sheath gas temperature, 350 °C; drying gas temperature, 325 °C. MassHunter Qualitative Analysis Software (Agilent) was used to analyze the data. Intact protein masses were obtained using the maximum entropy deconvolution algorithm.

Table S1. Mass-spectrometry of Menin Incubated with or without Menin Inhibitors.

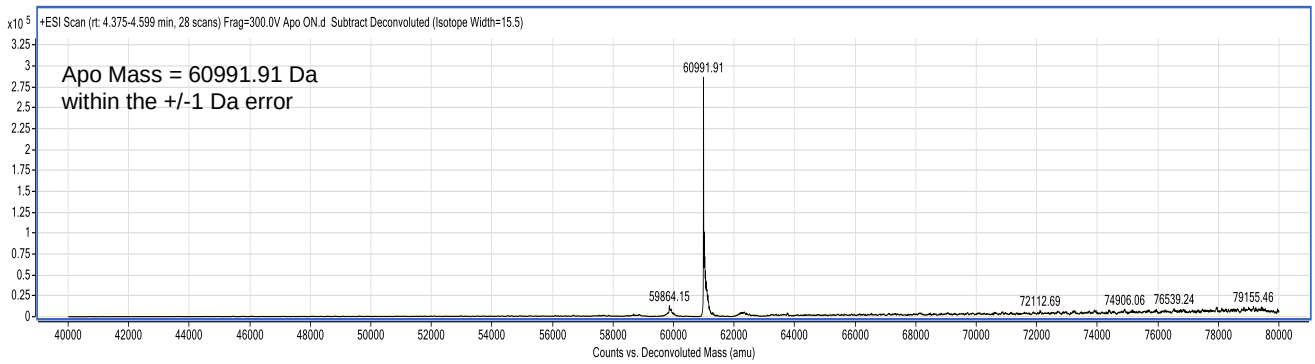
ID	Calculated exact mass	Incubation time	Analyzed exact mass	Difference in mass	Notes
Apo Menin	60992.12	1 h	60992.34		
		overnight	60991.91		
8	677.3	1 h	61669.64	677.3	Only about 50% incorporation after 1 h
		overnight	61669.42	677.51	100% incorporation of compound overnight
9	679.32	1 h	60991.76		No binding
		overnight	60991.56		No binding
10	734.36	1 h	61726.83	734.49	more than 95% incorporation of compound after 1 h
		overnight	61726.58	734.67	100% incorporation of compound after overnight

Mass-spectra

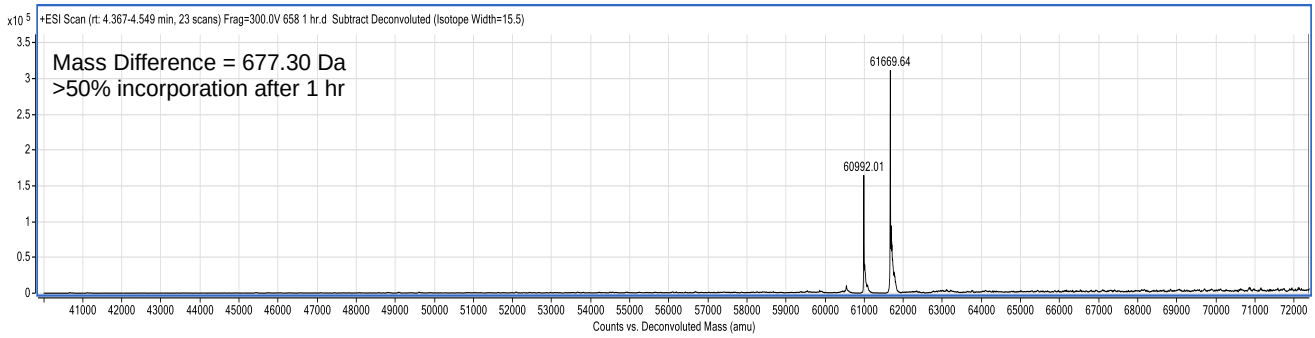
Mass of Apo Menin after 1 h:



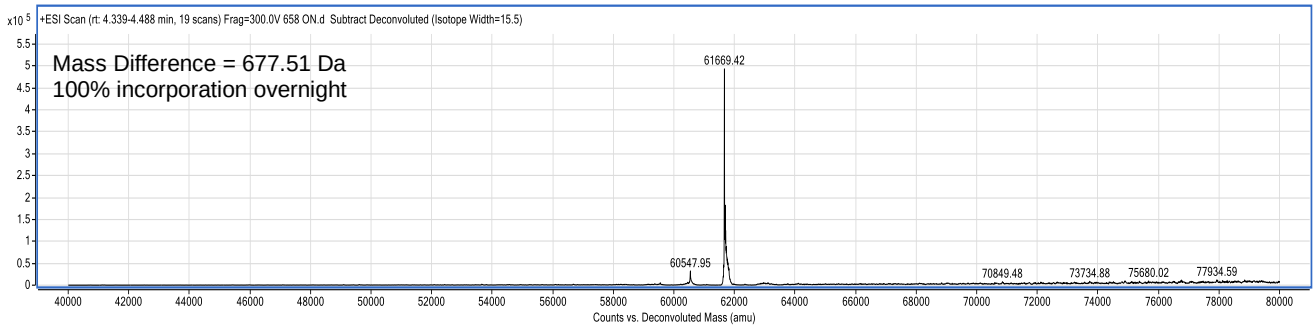
Mass of Apo Menin overnight:



Mass of Menin with compound **8** after 1h:



Mass of Menin with compound **8** overnight:



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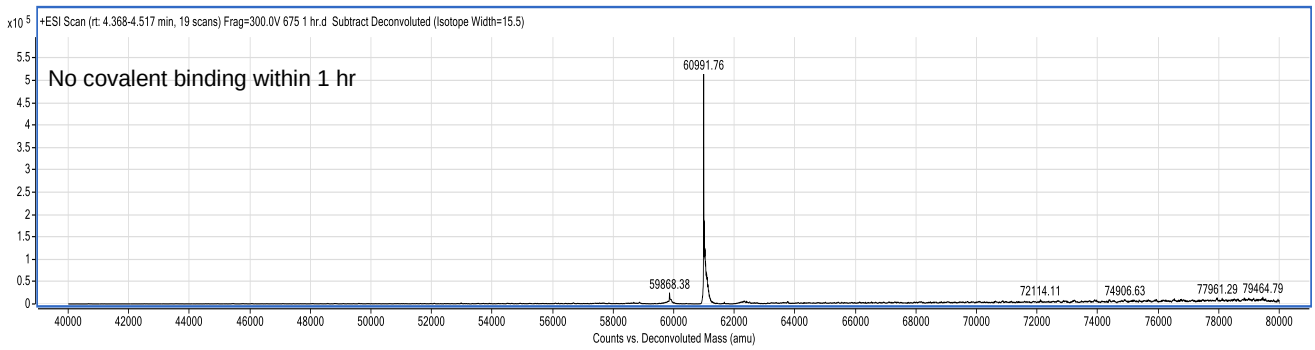
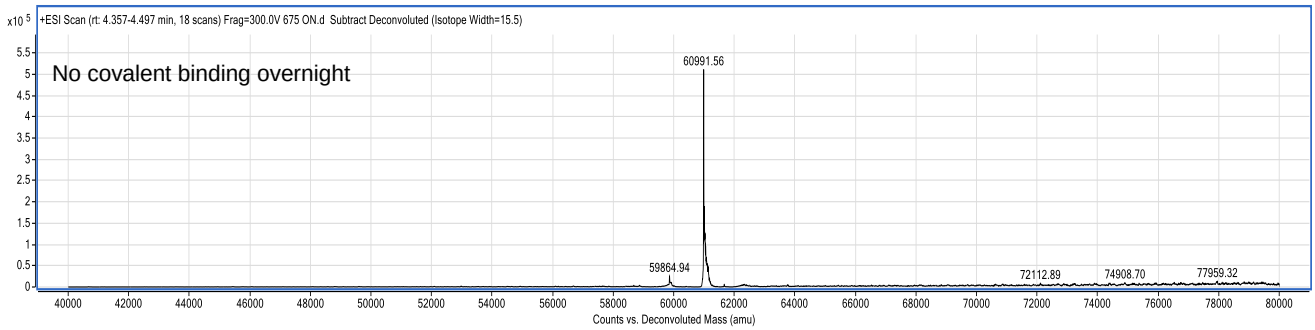
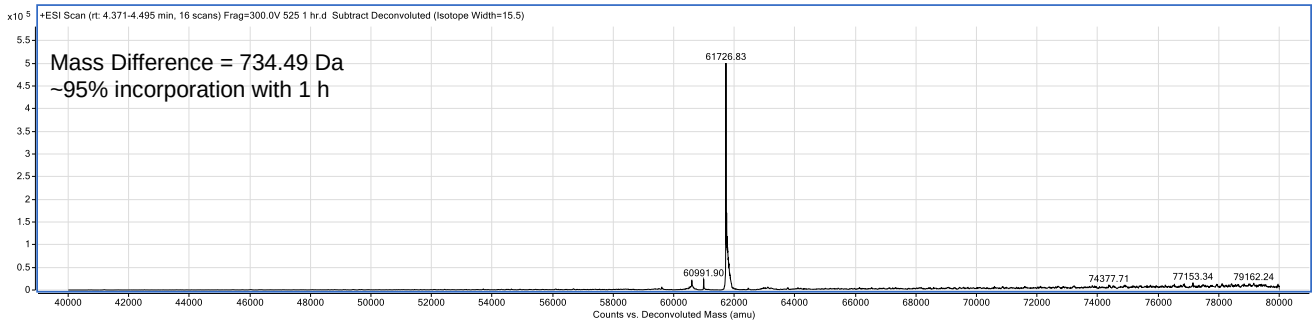
Mass of Menin with compound **9** after 1h:



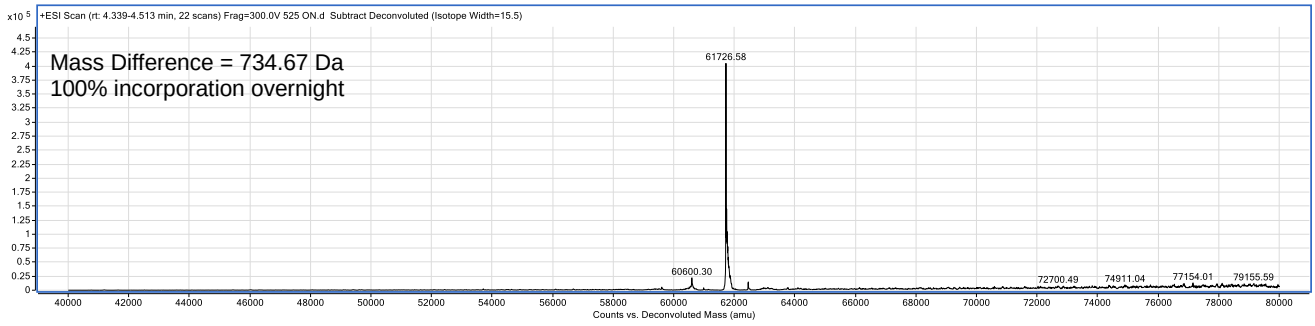
Mass of Menin compound **9** overnight:



Mass of Menin with compound **10** after 1 h:



Mass of Menin with compound **10** overnight:



Association/dissociation Kinetic Assay Using Octet Red

Biotinylation:
Purified recombinant menin protein was biotinylated using the Thermo EZ-Link long-chain biotinylation reagent. Menin protein and biotinylation reagent were mixed with 1:1 molar ratio in PBS at 4 °C. This reaction mixture was incubated at 4 °C for 2 h. Reaction mixture was then dialyzed using Fishersci 10K MWCO dialysis cassettes to remove unreacted biotinylation reagent.
Bio-Layer Interferometry (BLI) binding assay:
Bio-Layer Interferometry (BLI) binding assays were performed in 96-well microplates at room temperature with continuous 1000 rpm shaking using the Octet Red 96 system (Fortebio, Menlo Park, CA, USA). PBS with 0.1% BSA, 0.01% Tween-20 and 2% DMSO was used as the assay buffer. Biotinylated menin protein was tethered on Super Streptavidin (SSA) biosensors (ForteBio) by dipping sensors into 200 µL per well 10 µg/mL protein solutions. Average saturation response level of 8-10 nm was achieved in 20 minutes. The measurement processes were all under computer control. Program procedures were established as follows: For the initial step, biosensors were washed in assay buffer for 60 sec to form a baseline; the biosensors labeled with biotin-Menin were exposed to 100 nM compounds for association, and were monitored for 1200 sec; and then, the biosensors were moved back into assay buffer to disassociate for another 1800 sec. Data were fit globally and generated automatically by Octet User software (version 9.0; Fortebio).

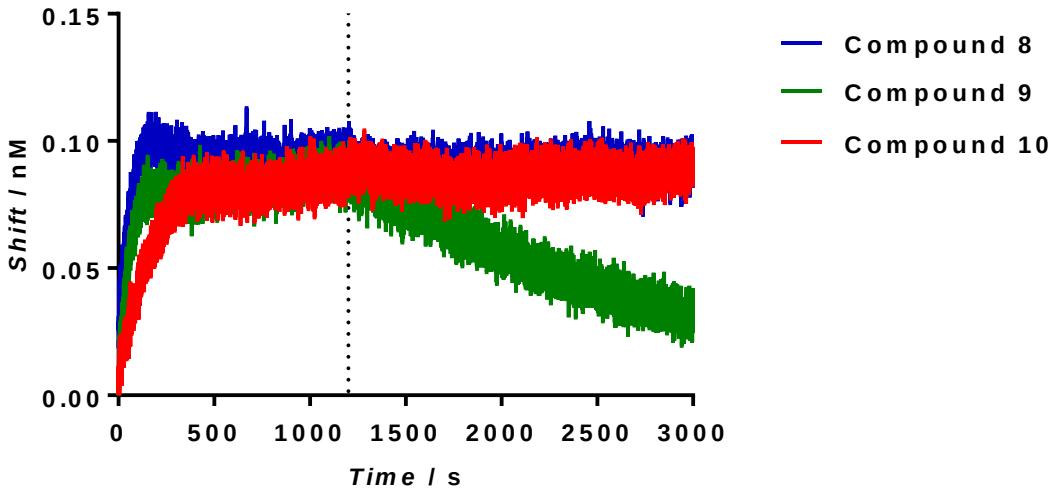


Figure S2. Association/dissociation kinetics of compounds for menin determined by Octet. Association time was 1200 s and dissociation time was 1800 s. Compounds **8** and **10** do not show obvious dissociation, while compound **9** shows dissociation.

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Detailed Method for X-ray Crystallography Determination of Compound 10 Bound to Menin

Prior to crystallization, Menin (25 mg/mL in 25 mM Tris 8.0, 150 mM NaCl and 5mM DTT) was incubated with compound **10** in a protein to compound ratio of 1:1.2 at 4 °C for 24 hr. Crystals grew in drops containing 1 µL of complex and 1 µL of well solution (1.96 M NaCl, 89 mM Bis-Tris pH 6.8, 0.178 M $MgCl_2$ and 10.7 mM Pr Acetate). Crystals were cryoprotected by progressively soaking crystals in well solution with increasingly higher amounts of sodium formate (1 M - 5 M in 1M steps). Diffraction data were collected on a Mar300 detector at the Advanced Photon Source LS-CAT 21-ID-G beamline at Argonne National Laboratory with a wavelength of 0.9786 Å and processed with HKL2000[5]. The structure of menin bound to compound **10** was solved by molecular replacement (Molrep[6]) using the apo menin structure (PDB ID 3U84) as the search model. The menin-**10** structure went through iterative rounds of electron density fitting and structural refinement using Coot[7] and Buster[8], respectively. The coordinates and restraint files for the ligand were created from smiles in Grade4 with the mogul+qm option. The initial Fo-Fc electron density map showed the presence of compound **10** in the active site covalently bound to C329 (Figure S3). The following regions were disordered in the structure: 71-73, 386-401, 528-547 and 582-610. Data collection and structural refinement statistics are shown in Table S2.

Table S2. Crystallography Data Collection and Refinement Statistics.

Data Collection	**Menin-10**
PDB ID	**6B41**
Space Group	$I4_1$
Unit Cell (Å)	a = b =153.920 c = 81.618 $\alpha = \beta = \gamma = 90°$
Wavelength (Å)	0.9786
Resolution (Å)[1]	2.61 (2.61—2.66)
Rmerge[2]	0.102 (0.665)
$\langle I/\sigma I \rangle$[3]	10 (2)
Completeness (%)[4]	99.8 (96.6)
Redundancy	12.1 (11.0)
Refinement	
Resolution (Å)	2.61
R-Factor[5]	0.208
Rfree[6]	0.251
Protein atoms	3714
Ligands	1
Water Molecules	141
Unique Reflections	25715
R.m.s.d.[7]	
Bonds	0.01
Angles	1.14
MolProbity Score[8]	1.59
Clash Score[8]	2.12
RSCC[9]	0.94
RSR[9]	0.21

[1]Statistics for highest resolution bin of reflections in parentheses.
[2]$R_{merge} = \Sigma_h \Sigma_j | I_{hj} - \langle I_h \rangle | / \Sigma_h \Sigma_j I_{hj}$, where I_{hj} is the intensity of observation j of reflection h and $\langle I_h \rangle$ is the mean intensity for multiply recorded reflections.
[3]Intensity signal-to-noise ratio.
[4]Completeness of the unique diffraction data.
[5]R-factor = $\Sigma_h | IF_oI - IF_cI | / \Sigma_h |F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes for reflection h.
[6]R_{free} is calculated against a 5% random sampling of the reflections that were removed before structure refinement.
[7]Root mean square deviation of bond lengths and bond angles.
[8]Chen et al. (2010) MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallographica D66:12-21.
[9]wwPDB Validation Server.
[9]wwPDB Validation Server.

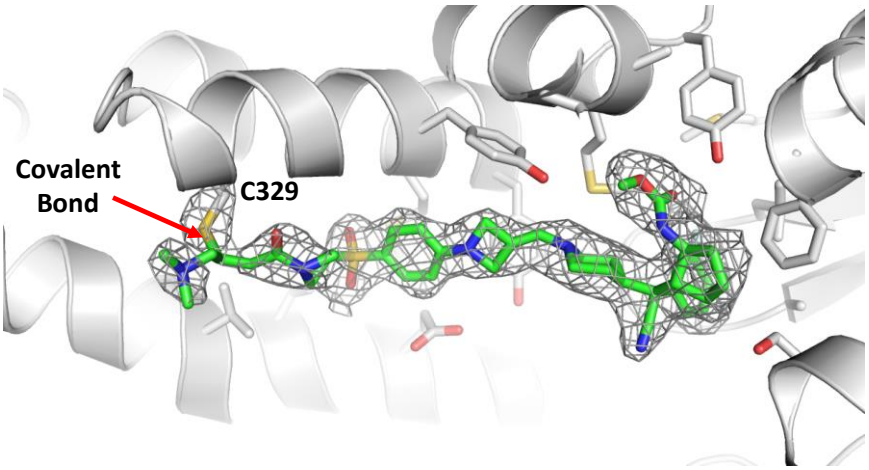


Figure S3. Fo-Fc electron density map (dark gray grid contoured at 2.5 σ) created by omitting compound **10** and the side chain of C329, shows the presence of a covalent bond between the Sγ of C329 (yellow) and the second carbon of compound **10** ethyl group (green).

Cellular Thermal Shift Assay

Cellular thermal shift assays were employed to assess target engagement of compound **10** in live cells. MV4;11 or MOLM-13 cells were plated in 6-well plates at a density of 5 × 10^6 cells per well and treated for 1 h with compound **10** at concentrations ranging from 0.4 to 300 nM. Cells were collected by centrifugation and washed with PBS. Cell pellets were resuspended in 100 µL of PBS containing Halt protease inhibitors, transferred to PCR tubes, and heated to 45 °C for 3 minutes. Cell lysis was achieved by two freeze-thaw cycles in liquid nitrogen. Lysates were clarified by centrifugation at 15,000 rpm. Supernatants were transferred to clean tubes, mixed with loading buffer, heated, and proteins were separated by SDS-PAGE. Membranes were blotted with anti-menin (CST, catalog # 6891) or anti-WDR5 antibodies (Santa Cruz biotechnology, sc-393080).

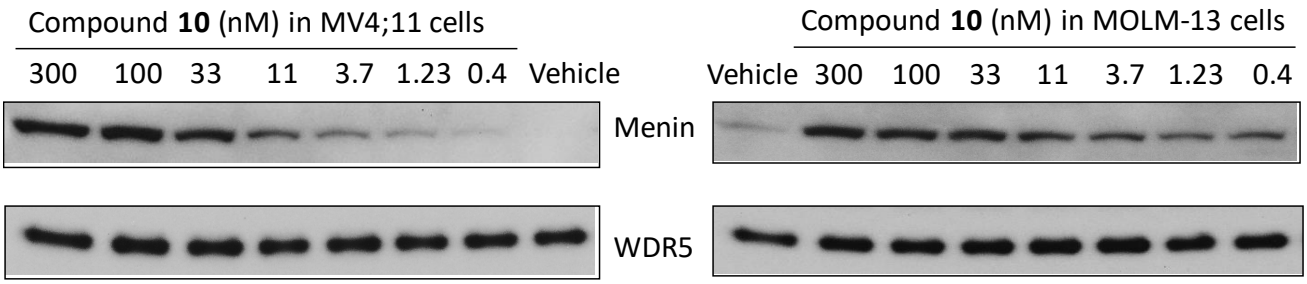


Figure S4. Cellular thermal shift assay (CETSA) to assess the stabilization of cellular menin protein by compound **10** in MV4;11 and MOLM-13 cells. Cells were treated with compound **10** for 1 h and then were heated at 45 °C for 3 min, lysed, and the proteins separated by SDS-PAGE. Membranes were blotted with anti-menin or anti-WDR5 antibody.

Real-Time PCR

Cells were treated with menin inhibitors at indicated concentrations, or with vehicle, giving a final concentration of 0.1% DMSO in all the samples. Total RNA was isolated from cells treated with various doses of the inhibitors at indicated treatment time, using the RNEASY kit (QIAGEN) according to the manufacturer's protocol[9]. The cDNA was generated using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Real-time PCR amplifications of *HOXA9*, *HOXA11, MEIS1*, and *GAPDH* genes were carried out with primers specific for each gene, using TaqMan gene expression assays (Applied Biosystems). Relative quantification of each gene transcript was calculated by a comparative cycle threshold (Ct) method. The results were presented as relative expression to vehicle treatment after normalizing to an internal loading control *GAPDH*.

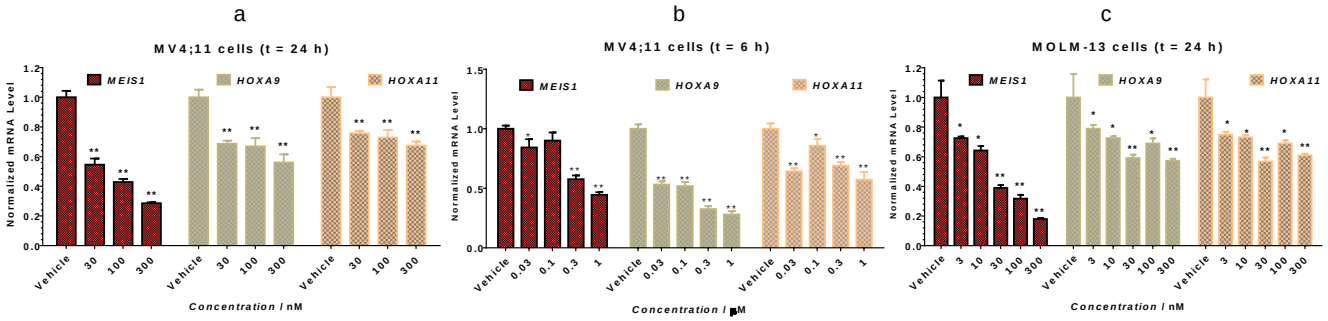


Figure S5. a), b) Suppression of *MEIS1* and *HOXA* gene expression by compound **10** in MV4;11cell lines. Cells were treated with different concentrations of compound **10** for 24 h or 6 h. c) Suppression of *MEIS1* and *HOXA* gene expression by compound **10** in MOLM13 cell lines. Cells were treated with different concentrations of compound **10** for 24 h. mRNA levels of *MEIS1*, *HOXA9* and *HOXA11* were determined by RT-PCR. * ($p < 0.05$), ** ($p < 0.01$).

In vivo Pharmacodynamic Assay

Female SCID mice were injected subcutaneously in the right flank with 5 million MV4;11 cells in a 5 mg/ml solution of Matrigel. When tumors reached 100-200 mm^3, mice were injected with a single intravenous dose of either vehicle or compound **10** at 50 mg/kg. Tumors were harvested at 24 h or 48 h after the drug administration (3 per time point), immediately frozen in liquid nitrogen, ground into fine powder, placed on dry ice and stored at -80°C for analysis.

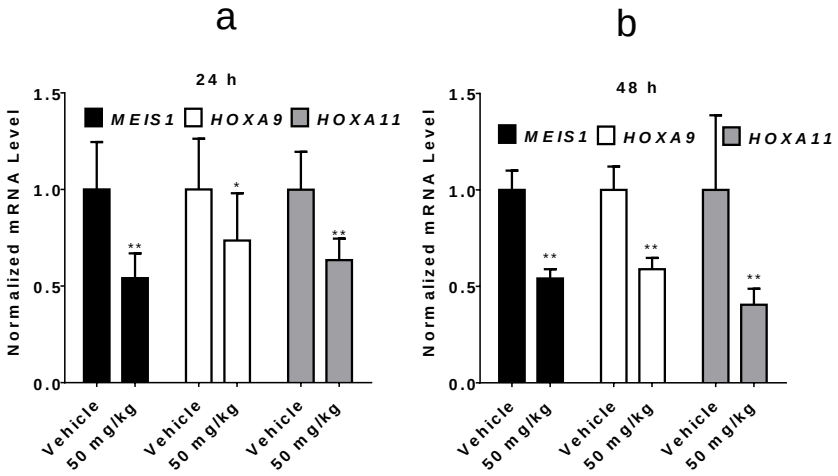


Figure S6. a), b) Pharmacodynamic effect of compound **10** on expression of *MEIS1* and *HOX* genes in the MV4;11 xenograft tumor tissues. Mice bearing MV4;11 tumors were injected intravenously with a single dose of either vehicle or compound **10** at 50 mg/kg. Tumors were harvested 24 h or 48 h after the injection with 3 tumors evaluated at each time point, and immediately frozen in liquid nitrogen, ground into fine powder, placed on dry ice and stored at -80 °C prior to RT-PCR analysis for expression levels of *MEIS1*, *HOXA9* and *HOXA11*. * ($p < 0.05$); ** ($p < 0.01$).

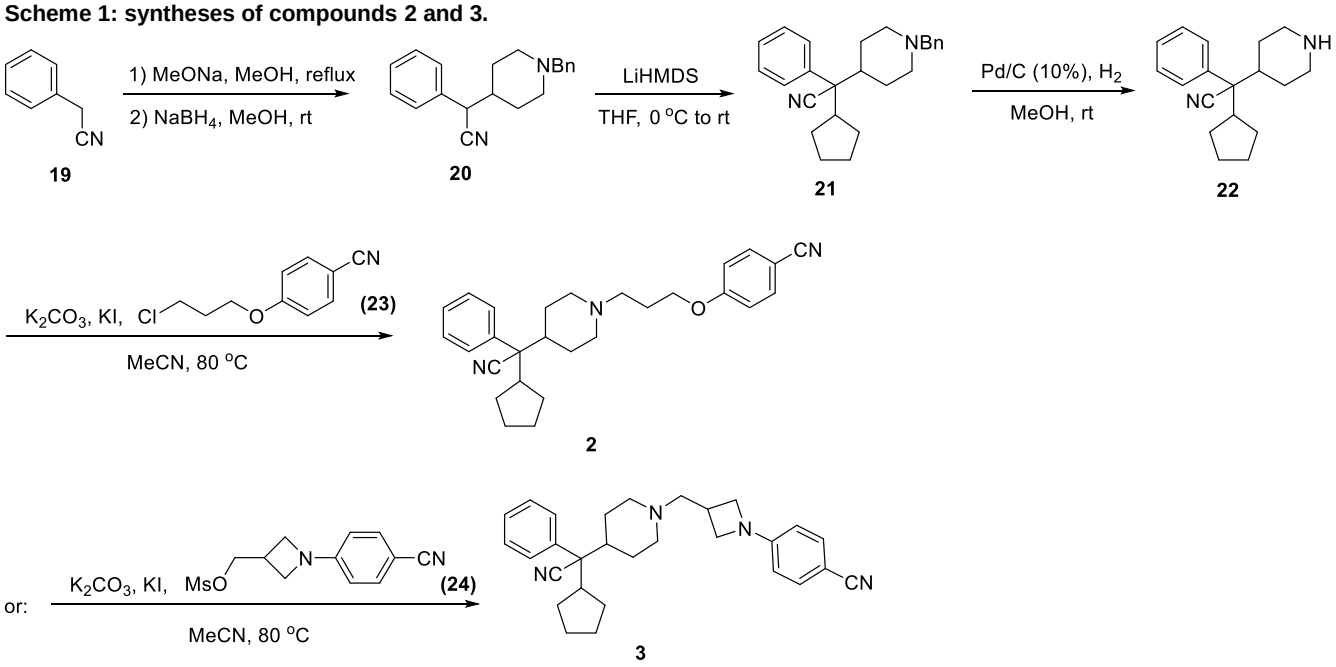
SUPPORTING INFORMATION

General Chemistry Experiment and Information.

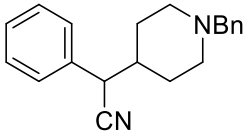
Unless otherwise noted, commercial solvents and reagents were used without further purification with the following exception: THF was freshly distilled from sodium wire. Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker Advance 400 MHz spectrometer. ^{1}H NMR spectra were reported in parts per million (ppm) downfield from tetramethylsilane (TMS). All ^{13}C NMR spectra were reported in ppm and obtained with ^{1}H decoupling. In reported spectral data, the format (δ) chemical shift (multiplicity, *J* values in Hz, integration) was used with the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. MS analyses were carried out with a Waters UPLC−mass spectrometer. The final compounds were all purified by C18 reverse phase preparative HPLC column with solvent A (0.1% TFA in H_2O) and solvent B (0.1% TFA in MeCN) as eluents. The purity of all the final compounds was confirmed to be >95% by UPLC analysis (10% to 100% MeCN in H_2O containing 0.1% CF_3COOH in 10 min). The optical rotation was measured on Autopol® III automatic polarimetor (Rudolph Research Analytical). The specific optical rotation was calculated by application of the following formula: $[\alpha]_D^T = \alpha / (l \times c)$, where α is the observed angular rotation, l is the length of the cell in decimeters (l = 1 dm), c is the concentration in grams per milliliter (g/mL).

Chemistry Experimental Procedures.

Scheme 1: syntheses of compounds 2 and 3.



Method A: synthesis of 2-(1-benzylpiperidin-4-yl)-2-phenylacetonitrile (20)[10]



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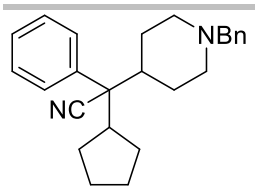
To a solution of 2-phenylacetonitrile (**19**) (10 g, 85.4 mmol) and 1-benzylpiperidin-4-one (16g, 85.4 mmol) in MeOH (200mL) was added MeONa (25% Wt. in MeOH, 23.4 mL, 102.4 mmol) under nitrogen, and the mixture was stirred under reflux overnight. Then, the reaction mixture was cooled to room temperature and concentrated to remove the solvent MeOH. The resulting mixture was dissolve in dichloromethane and poured into brine, and extracted with dichloromethane twice. The combined organic solution was dried over Na_2SO_4, filtered and the solvent was evaporated in vacuum. The crude intermediate was dissolved in MeOH (200 mL) and $NaBH_4$ (6.5g, 170.7 mmol) was added into the solution. The mixture was stirred at room temperature for 2 days. Then, the reaction mixture was concentrated, dissolved in dichloromethane and brine, and extracted with dichloromethane. Extract was dried over Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by flash chromatography to obtain the title compound as a yellow solid (15g, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.25 (m, 3H), 7.24-7.20 (m, 5H), 7.20-7.17 (m, 2H), 3.52 (d, *J* = 7.6 Hz, 1H), 3.43 (s, 2H), 2.90-2.81 (m, 2H), 1.92-1.79 (m, 3H), 1.74-1.64 (m, 1H), 1.52-1.34 (m, 3H); ESI-MS calculated for $C_{20}H_{22}N_2$ $[M + H]^+$ = 291.18, found: 291.19.

2-(1-Benzylpiperidin-4-yl)-2-cyclopentyl-2-phenylacetonitrile (21)



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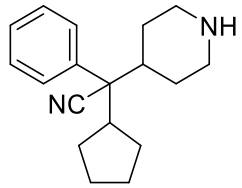
To a solution of 2-(1-benzylpiperidin-4-yl)-2-phenylacetonitrile (**20**) (2g, 6.9 mmol) in dry THF (50 mL) was added dropwise LiHMDS (1M in THF, 13.8 mL, 13.8 mmol) at 0 °C under nitrogen. After stirring for 30 min, bromocyclopentane (0.89 mL, 8.3 mmol) was added dropwise and the reaction mixture was allowed to slowly warm to room temperature. After stirring overnight, the reaction was quenched with saturated NH_4Cl, and concentrated to remove THF. The resulting mixture was extracted with dichloromethane twice, dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by flash chromatography to obtain the title compound as a yellow solid (2 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.24 (m, 4H), 7.23-7.15 (m, 6H), 3.39 (s, 2H), 2.88-2.83 (m, 2H), 2.71-2.62 (m, 1H), 1.96-1.78 (m, 5H), 1.70-1.64 (m, 1H), 1.58-1.49 (m, 4H), 1.46-1.41 (m, 2H), 1.26-1.11 (m, 3H).; ESI-MS calculated for $C_{25}H_{30}N_2$ $[M + H]^+$ = 359.24, found: 359.32.

Method B: synthesis of 2-cyclopentyl-2-phenyl-2-(piperidin-4-yl)acetonitrile (22)



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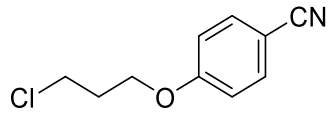
To a solution of the intermediate **21** (100 mg, 0.28 mmol) in methanol (5 mL) was added 10% Pd/C (30 mg). The mixture was stirred for 3 h at room temperature under hydrogen atmosphere (normal pressure). After the Pd/C catalyst was filtered off, the solvent was removed by rotary evaporation to give the title compound (74 mg, 99%). The product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.26 (m, 4H), 7.24-7.20 (m, 1H), 3.06-3.02 (m, 2H), 2.72-2.63 (m, 1H), 2.60-2.46 (m, 2H), 2.00-1.84 (m, 3H), 1.71-1.40 (m, 7H), 1.23-0.94 (m, 3H). ESI-MS calculated for $C_{18}H_{24}N_2$ $[M + H]^+$ = 269.19, found: 269.25.

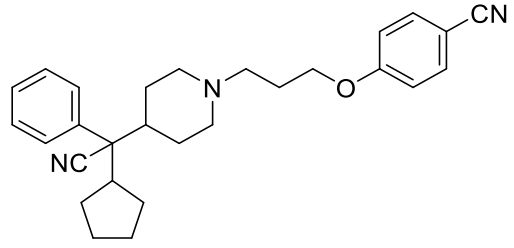
4-(3-Chloropropoxy)benzonitrile (23)[11]



23

To a solution of 4-hydroxybenzonitrile (1.5 g, 12.6 mmol) and 1-bromo-3-chloropropane (2.2 g, 13.9 mmol) in acetonitrile was added K_2CO_3 (2.6 g, 18.9 mmol). The mixture was stirred at 80 °C. Then, the reaction mixture was cooled to room temperature and concentrated to remove the solvent acetonitrile. The resulting mixture was dissolve in dichloromethane and poured into brine, and extracted with dichloromethane twice. The combined organic solution was dried over Na_2SO_4, filtered and the solvent was evaporated in vacuum. The residue was purified by flash chromatography to obtain the title compound as a white solid (1.5 g, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.9 Hz, 2H), 6.89 (d, *J* = 8.9 Hz, 2H), 4.10 (t, *J* = 5.8 Hz, 2H), 3.68 (t, *J* = 6.2 Hz, 2H), 2.23-2.17 (m, 2H); ESI-MS calculated for $C_{10}H_{10}ClNO$ $[M + H]^+$ = 196.05, found: 196.18.

Method C: synthesis of 4-(3-(4-(cyano(cyclopentyl)(phenyl)methyl)piperidin-1-yl)propoxy)benzonitrile (2)

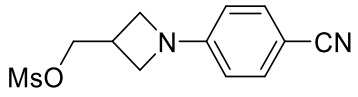


2

To a solution of the intermediate **22** (19 mg, 0.072 mmol) in acetonitrile (2 mL) was added 4-(3-chloropropoxy)benzonitrile (**23**) (16.8 mg, 0.086 mmol), K_2CO_3 (20 mg, 0.11 mmol) and KI (1 mg, 0.007 mmol). The mixture was stirred at 80 °C overnight. Then, the mixture was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, and the solvent was evaporated under vacuum. The residue was purified by reverse phase preparative HPLC to give the title compound as a salt of trifluoroacetic acid (15 mg, 40%). ^{1}H NMR (400 MHz, MeOD) δ 7.65 (d, *J* = 9.0 Hz, 2H), 7.50-7.42 (m, 4H), 7.39-7.35 (m, 1H), 7.04 (d, *J* = 9.0 Hz, 2H), 4.13 (t, *J* = 5.8 Hz, 2H), 3.67-3.59 (m, 2H), 3.27-3.22 (m, 2H), 3.11-2.98 (m, 2H), 2.97-2.89 (m, 1H), 2.44-2.37 (m, 1H), 2.32-2.27 (m, 1H), 2.22-2.15 (m, 2H), 2.08-2.01 (m, 2H), 1.78-1.66 (m, 2H), 1.65-1.55 (m, 4H), 1.53-1.38 (m, 2H), 1.29-1.16 (m, 1H); ^{13}C NMR (100 MHz, MeOD) δ 163.3, 136.0,

135.2, 129.8, 129.4, 129.2, 122.2, 119.9, 116.5, 105.3, 66.3, 57.3, 55.5, 53.6, 44.7, 41.5, 30.6, 30.0, 27.9, 26.5, 25.9, 25.8, 25.0; ESI-MS calculated for $C_{28}H_{33}N_3O$ $[M + H]^+$ = 428.26, found: 428.41; t_R (UPLC) = 4.55 min; Purity > 99%.

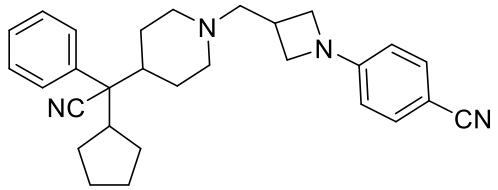
(1-(4-Cyanophenyl)azetidin-3-yl)methyl methanesulfonate (24)



24

To a solution of 4-fluorobenzonitrile (2.6 g, 21.2 mmol) and azetidin-3-ylmethanol hydrochloride (3.4 g, 27.6 mmol) in DMSO was added K_2CO_3 (8.8 g, 63.7 mmol). The mixture was stirred at 80 °C overnight. Then, the reaction mixture was cooled to room temperature, pour into ice, and extracted with ethyl acetate twice. The combined organic solution was dried over Na_2SO_4, filtered and the solvent was evaporated in vacuum. The residue was dissolved in dichloromethane (100 mL) and Et_3N (8.9 mL, 63.6 mmol) and methanesulfonyl chloride (2.5 mL, 31.8 mmol) were added dropwise successively at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for 5 h. Then, the reaction mixture was quenched with saturated $NaHCO_3$, and washed with brine, dried over Na_2SO_4, and the solvent was evaporated under vacuum. The residue was purified by flash chromatography to obtain the title compound as a white solid (2.5 g, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.8 Hz, 2H), 6.31 (d, *J* = 8.8 Hz, 2H), 4.38 (d, *J* = 6.7 Hz, 2H), 4.02 (t, *J* = 8.2 Hz, 2H), 3.74-3.70 (m, 2H), 3.16-3.08 (m, 1H), 2.99 (s, 3H); ESI-MS calculated for $C_{12}H_{14}N_2O_3S$ $[M + H]^+$ = 267.07, found: 267.11.

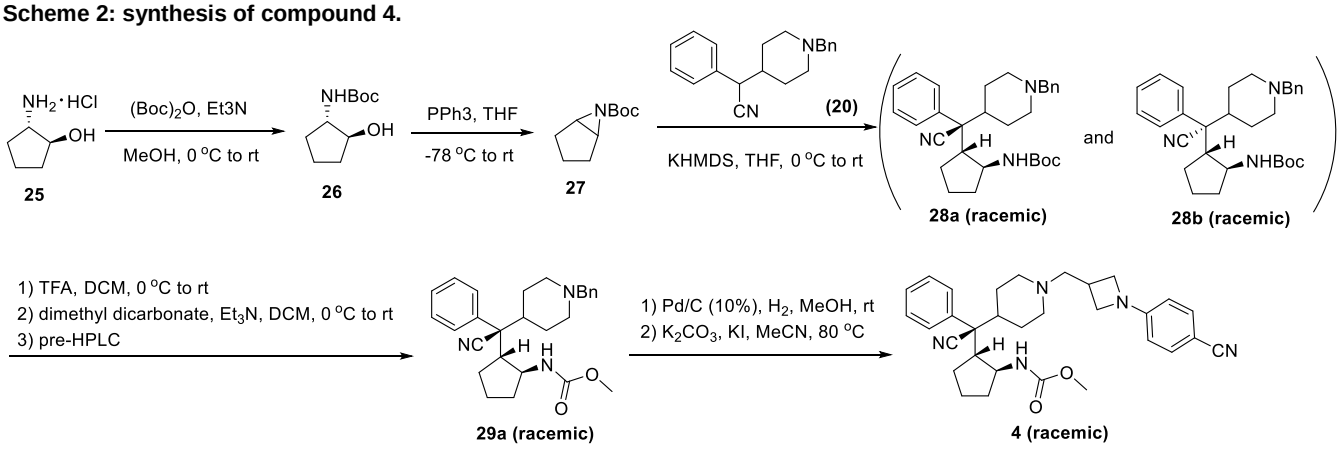
4-(3-((4-(Cyano(cyclopentyl)(phenyl)methyl)piperidin-1-yl)methyl)azetidin-1-yl)benzonitrile (3)



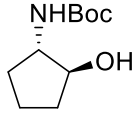
3

Method C using the intermediate **22** and **24**. ^{1}H NMR (400 MHz, MeOD) δ 7.50-7.42 (m, 6H), 7.40-7.36 (m, 1H), 6.46 (d, *J* = 8.8 Hz, 2H), 4.16-4.12 (m, 2H), 3.72-3.69 (m, 2H), 3.54 (t, *J* = 14.1 Hz, 2H), 3.41 (d, *J* = 7.2 Hz, 2H), 3.24-3.16 (m, 1H), 3.12-2.98 (m, 2H), 2.97-2.89 (m, 1H), 2.42-2.36 (m, 1H), 2.28 (d, *J* = 14.3 Hz, 1H), 2.08-2.00 (m, 2H), 1.76-1.67 (m 2H), 1.65-1.55 (m, 4H), 1.53-1.38 (m, 2H), 1.29-1.16 (m, 1H); ^{13}C NMR (100 MHz, MeOD) δ 154.8, 136.0, 134.4, 129.8, 129.5, 129.2, 122.2, 121.2, 112.0, 99.6, 60.9, 57.4, 56.2, 53.6, 44.7, 41.4, 30.6, 30.0, 27.8, 26.7, 26.5, 25.9, 25.6; ESI-MS calculated for $C_{29}H_{34}N_4$ $[M + H]^+$ = 439.23, found: 439.42; t_R (UPLC) = 4.53 min; Purity > 99%.

Scheme 2: synthesis of compound 4.



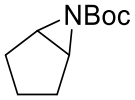
***tert*-Butyl ((1*S*,2*S*)-2-hydroxycyclopentyl)carbamate (26)**



26

To a solution of (1*S*,2*S*)-2-Aminocyclopentanol hydrochloride (**25**) (4.6 g, 33.7 mmol) and Et_3N (9.4 mL, 67.4 mmol) in MeOH (50 mL) was added Boc_2O (8.1 g, 37 mmol) at 0°C. The reaction was allowed to warm to room temperature and after overnight the reaction was concentrated and the crude was purified by column chromatography to give **26** as a white solid (6.5 g, 96%).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (s, 1H), 4.00-3.96 (m, 2H), 3.66-3.59 (m, 1H), 2.12-2.06 (m, 1H), 2.05-1.98 (m, 1H), 1.81-1.74 (m, 1H), 1.70-1.63 (m, 2H), 1.45 (s, 9H), 1.38-1.31 (m, 1H).

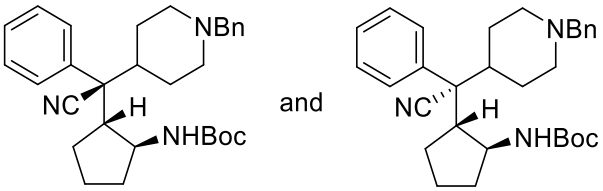
***tert*-Butyl 6-azabicyclo[3.1.0]hexane-6-carboxylate (27)[12]**



27

To a solution of PPh_3 (10.2 g, 38.8 mmol) in dry THF (100 mL) was added diisopropyl azodicarboxylate (7.84 g, 38.8 mmol) at -78°C. After stirring for 1h at -78°C, a solution of the intermediate **26** (6.5 g, 32.3 mmol) in dry THF (60 mL) was added dropwise. After overnight at room temperature, the reaction mixture was concentrated and then diluted with ethyl ether. The white precipitate was filtered off and the solvent was concentrated. The residue was purified by column chromatography to give the title compound **27** as oil (5.4 g, 91 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.9 (s, 2H), 2.11-2.05 (m, 2H), 1.66-1.56 (m, 3H), 1.46 (s, 9H), 1.30-1.13 (m, 1H).

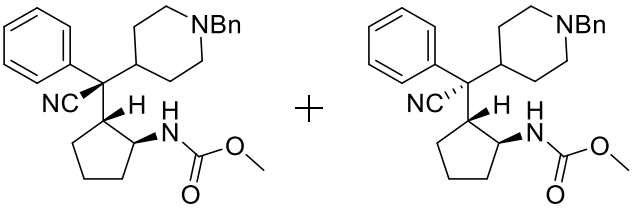
Mixture of *rac*-*tert*-butyl ((1*S*,2*R*)-2-((*S*)-(1-benzylpiperidin-4-yl)(cyano)(phenyl)methyl)cyclopentyl)carbamate (28a) and *rac*-*tert*-butyl ((1*S*,2*R*)-2-((*R*)-(1-benzylpiperidin-4-yl)(cyano)(phenyl)methyl)cyclopentyl)carbamate (28b)



28a (racemic) **28b (racemic)**

Compound **20** (1 g, 3.44 mmol), 18-Crown-6 (2.73 g, 10.33 mmol), and compound **27** (1.89 g, 10.33 mmol) were added to a dry round-bottom flask. Then, the flask was covered with a kimwipe and dried in a desiccator under vacuum for 1-2 days. After the drying step, the flask was removed from the desiccator and quickly capped with a septum. The system was vacuumed and protected under nitrogen atmosphere. The contents in the flask were then dissolved completely with 30 mL of freshly distilled THF. The solution was then briefly vacuumed then put under nitrogen atmosphere (This purging was repeated two more times). The reaction was cooled to -78 °C, KHMDS (0.5 M in toluene, 20.66 mL, 10.33 mmol) was added dropwise and then the reaction was allowed to warm to room temperature and stirred overnight. After overnight, the reaction was quenched with saturated NH_4Cl, extracted with ethyl acetate three times. The combined organic solvent was dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatograph to give a 3:2 diastereomer mixture of **28a**/**28b** as a white solid (1.1g, 67%). ESI-MS calculated for $C_{30}H_{39}N_3O_2$ $[M + H]^+$ = 474.30, found: 474.50.

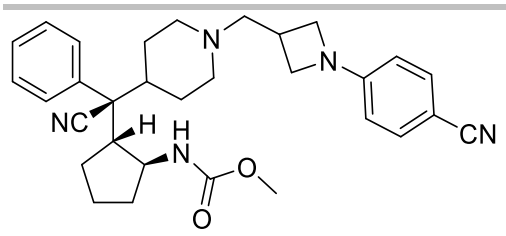
***rac*-Methyl ((1*S*,2*R*)-2-((*S*)-(1-benzylpiperidin-4-yl)(cyano)(phenyl)methyl)cyclopentyl)carbamate (29a)** and ***rac*-methyl ((1*S*,2*R*)-2-((*R*)-(1-benzylpiperidin-4-yl)(cyano)(phenyl)methyl)cyclopentyl)carbamate (29b)**



29a (racemic) **29b (racemic)**

To a solution of mixture **28a**/**28b** (30 mg, 0.063 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (5 mL) at 0 °C. After stirring at room temperature for 1h, the reaction mixture was concentrated under vacuum, then basified with saturated $NaHCO_3$, and extracted with dichloromethane three times. The combined organic solvent was dried over Na_2SO_4, filtered and concentrated under vacuum. The resulting residue was redissolved in dry dichloromethane (5 mL), and Et_3N (0.018 mL, 0.129 mmol), dimethyl dicarbonate (10 mg, 0.077 mmol) were added at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was quenched with H_2O, and extracted with dichloromethane three times. The combined organic solvent was dried over Na_2SO_4, filtered and concentrated under vacuum. Then, the mixture **29a**/**29b** were separated by reverse phase preparative HPLC to give the title compounds **29a** (13 mg, 44%), and **29b** (8 mg, 27%) as salts of trifluoroacetic acid. Date for **29a**: ^{1}H NMR (400 MHz, MeOD) δ 7.51-7.34 (m, 10H), 4.24 (s, 2H), 3.91-3.86 (m, 1H), 3.53-3.46 (m, 2H), 3.43 (s, 3H), 3.06-2.96 (m,, 2H), 2.86-2.80 (m, 1H), 2.47 (t, *J* = 12.1 Hz, 1H), 2.26 (d, *J* = 14.4 Hz, 1H), 2.13-2.07 (m, 1H), 1.90 (d, *J* = 14.4 Hz, 1H), 1.79-1.72 (m, 1H), 1.70-1.60 (m, 2H), 1.58-1.40 (m, 4H); ESI-MS calculated for $C_{27}H_{33}N_3O_2$ $[M + H]^+$ = 432.26, found: 432.53; t_R (UPLC) = 3.31 min. Date for **29b**: ^{1}H NMR (400 MHz, MeOD) δ 7.48-7.41 (m, 9H), 7.39-7.35 (m, 1H), 4.24 (s, 2H), 4.12-4.07 (m, 1H), 3.66 (s, 3H), 3.47 (t, *J* = 12.8 Hz, 2H), 3.11-3.04 (m, 1H), 2.93-2.87 (m, 1H), 2.81 (t, *J* = 11.3 Hz, 1H), 2.55 (t, *J* = 12.1 Hz, 1H), 2.26 (d, *J* = 14.2 Hz, 1H), 2.00 (d, *J* = 14.4 Hz, 1H), 1.95-1.89 (m, 1H), 1.83-1.75 (m, 1H), 1.67-1.55 (m, 3H), 1.44-1.34 (m, 2H), 1.30-1.21 (m, 1H); ESI-MS calculated for $C_{27}H_{33}N_3O_2$ $[M + H]^+$ = 432.26, found: 432.51; t_R (UPLC) = 3.82 min.

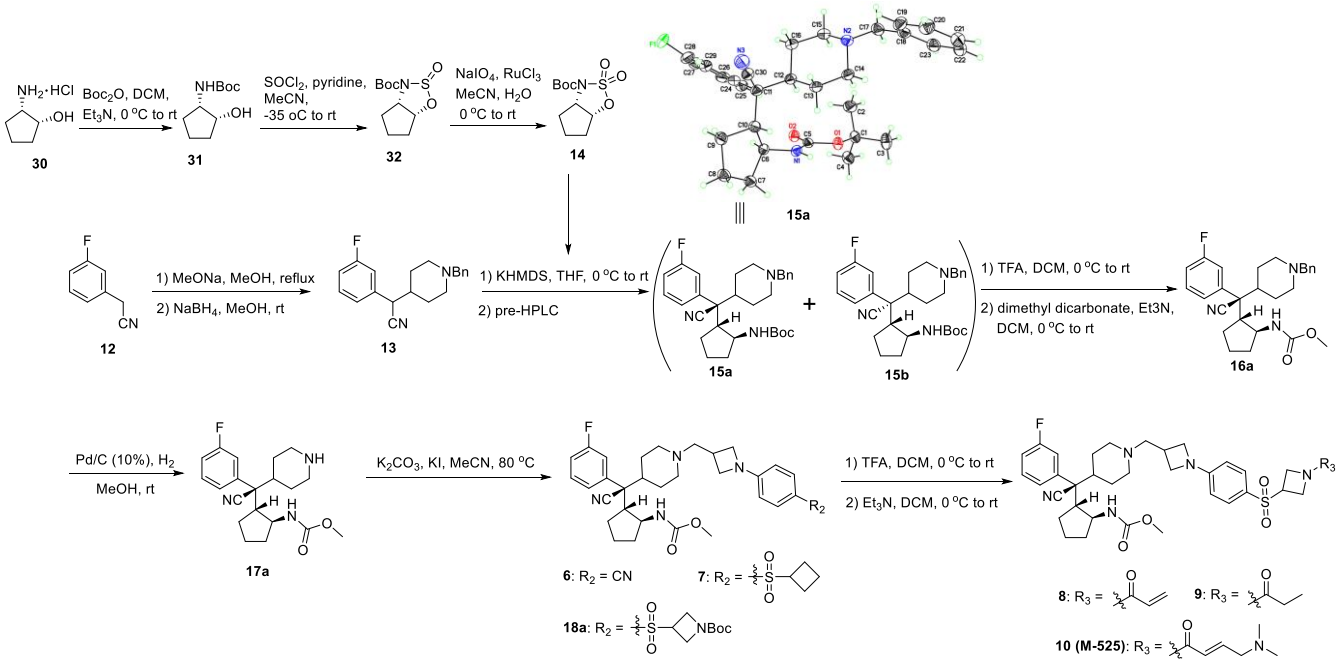
***rac*-Methyl ((1*S*,2*R*)-2-((*S*)-cyano(1-((1-(4-cyanophenyl)azetidin-3-yl)methyl)piperidin-4-yl)(phenyl)methyl)cyclopentyl)carbamate (4)**



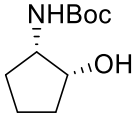
4 (racemic)

Compound **4** was synthesized using the method described for compound **2** from the intermediates **29a** and **24**. ^{1}H NMR (400 MHz, MeOD) δ 7.52 (d, *J* = 7.2 Hz, 2H), 7.47 (d, *J* = 8.8 Hz, 2H), 7.45-7.36 (m, 3H), 6.47 (*d*, *J* = 8.8 Hz, 2H), 4.17-4.12 (m, 2H), 3.93-3.88 (m, 1H), 3.73-3.70 (m, 2H), 3.60-3.51 (m, 2H), 3.44 (s, 3H), 3.42 (s, 2H), 3.23-3.17 (m, 1H), 3.07-2.99 (m, 2H), 2.89-2.83 (m, 1H), 2.50 (t, *J* = 10.2 Hz, 1H), 2.28 (d, *J* = 14.6 Hz, 1H), 2.16-2.10 (m, 1H), 1.94 (d, *J* = 14.3 Hz, 1H), 1.79-1.44 (m, 7H); ^{13}C NMR (100 MHz, MeOD) δ 158.2, 154.8, 134.4, 134.3, 129.7, 129.6, 122.0, 121.2, 112.0, 99.7, 61.0, 56.8, 56.2, 55.5, 53.6, 52.3, 40.7, 35.2, 30.1, 27.7, 26.8, 26.1, 23.9; ESI-MS calculated for $C_{31}H_{37}N_5O_2$ $[M + H]^+$ = 512.29, found: 512.42. t_R (UPLC) = 3.65 min; Purity > 97%.

Scheme 3: syntheses of compounds 6, 7, 8, 9 and 10 (The absolute stereochemistry of **15a** was determined by X-ray crystallography, fluorine in green, oxygens in red, nitrogens in blue, carbons in gray, and hydrogens in light green, CDCC: 1581872)



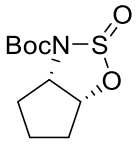
***tert*-Butyl ((1*S*,2*R*)-2-hydroxycyclopentyl)carbamate (31)**



31

To a solution of (1*R*,2*S*)-2-aminocyclopentanol hydrochloride (**30**) (11 g, 79.9 mmol) and Boc_2O (20.9 g, 95.9 mmol) in dichloromethane (200 mL) was added dropwise Et_3N (20.9 mL, 119.9 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature. After stirring overnight, the reaction mixture was washed with saturated brine and the water phase was extracted with dichloromethane twice. The combined organic solvent was dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash column chromatography to give the intermediate **31** as oil (15.5 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.85 (s, 1H), 4.16 (s, 1H), 3.80 (s, 1H), 2.02-1.95 (m, 1H), 1.93-1.87 (m, 1H), 1.86-1.77 (m, 2H), 1.70-1.65 (m, 1H), 1.59-1.51 (m, 2H), 1.45 (s, 9H).

***tert*-Butyl (3a*S*,6a*R*)-tetrahydrocyclopenta[*d*][1,2,3]oxathiazole-3(3a*H*)-carboxylate 2-oxide (32)**

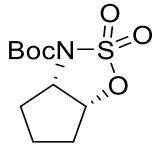


32

SUPPORTING INFORMATION

To a solution of thionyl chloride (7 mL, 96.3 mmol) in dry acetonitrile (150 mL) was added a solution of the intermediate **31** (15.5g, 77.0 mmol) in acetonitrile (150mL) at -35ºC. Then, pyridine (18.7 mL, 231 mmol) was added dropwise and the reaction mixture was allowed to slowly warm to room temperature. After stirring overnight, the reaction mixture was concentrated, and water and ethyl acetate were added. The organic layer was separated and the aqueous layer was extracted three times with ethyl acetate. The combined organic solvent was dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography to produce the intermediate **32** as oil (18.8 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (t, *J* = 4.6 Hz, 1H), 4.46 (s, 1H), 2.14-2.09 (m, 1H), 1.90-1.68 (m, 5H), 1.52 (s, 9H).

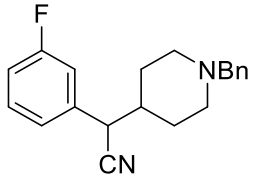
***tert*-Butyl (3a*S*,6a*R*)-tetrahydrocyclopenta[*d*][1,2,3]oxathiazole-3(3a*H*)-carboxylate 2,2-dioxide (14)[13]**



14

To a solution of the intermediate **32** (18.8 g, 76 mmol) in acetonitrile (100 mL) and H_2O (100 mL) was added $NaIO_4$ (24.4 g, 114 mmol) in portions, followed by addition of $RuCl_3.3H_2O$ (315 mg, 1.5 mmol) at 0 °C. The reaction was stirred at room temperature for 2 hours. Then, the aqueous layer was extracted with diethyl ether three time. The combined organic solvent was dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography to produce the title compound **14** as a white solid (19 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.18-5.15 (m, 1H), 4.56-4.53 (m, 1H), 2.23-2.18 (m, 1H), 2.06-1.95 (m, 3H), 1.87-1.77 (m, 2H), 1.55 (s, 9H). ESI-MS calculated for $C_{10}H_{17}NO_5S$ [M + Na]$^+$ = 286.07, found: 286.10.

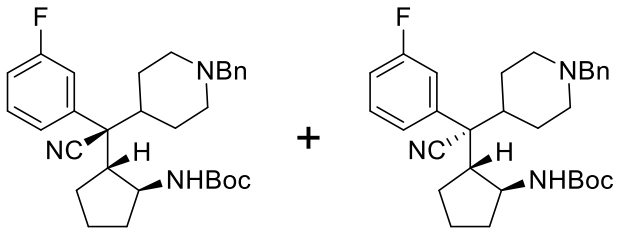
2-(1-Benzylpiperidin-4-yl)-2-(3-fluorophenyl)acetonitrile (13)



13

Method A using 2-(3-fluorophenyl)acetonitrile (**12**) and 1-benzylpiperidin-4-one. ^{1}H NMR (400 MHz, MeOD) δ 7.44-7.38 (m, 1H), 7.32-7.28 (m, 4H), 7.27-7.22 (m, 1H), 7.18-7.16 (m, 1H), 7.13-7.05 (m, 2H), 3.98 (d, *J* = 7.1 Hz, 1H), 3.48 (s, 2H), 2.96-2.87 (m, 2H), 2.00-1.92 (m, 2H), 1.87-1.80 (m, 1H), 1.79-1.72 (m, 1H), 1.59-1.52 (m, 1H), 1.50-1.39 (m, 2H); ESI-MS calculated for $C_{20}H_{21}FN_2$ [M + H]$^+$ = 309.17, found: 309.16.

***tert*-Butyl ((1*S*,2*R*)-2-((*S*)-(1-benzylpiperidin-4-yl)(cyano)(3-fluorophenyl)methyl)cyclopentyl)carbamate (15a) and *tert*-butyl ((1*S*,2*R*)-2-((*R*)-(1-benzylpiperidin-4-yl)(cyano)(3-fluorophenyl)methyl)cyclopentyl)carbamate (15b)**

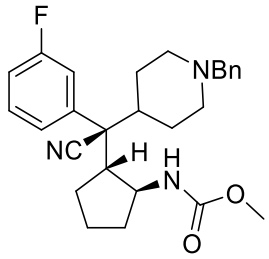


15a **15b**

Compound **13** (2.18 g, 7.07 mmol), 18-Crown-6 (5.61 g, 21.21 mmol), and compound **14** (5.58 g, 21.21 mmol) were added to a dry round-bottom flask. Then, the flask was covered with a kimwipe and dried in a desiccator under vacuum for 1-2 days. After the drying step, the flask was removed from the desiccator and quickly capped with a septum. The system was vacuumed and protected under nitrogen atmosphere. The contents in the flask were then dissolved completely with 60 mL of freshly distilled THF. The solution was then briefly vacuumed then put under nitrogen atmosphere (This purging was repeated two more times). The reaction was cooled to 0 ºC, KHMDS (0.5M in toluene, 42.4 mL, 21.21 mmol) was added dropwise and then the reaction was allowed to warm to room temperature and stirred overnight. After overnight, a solution of concentrated H_2SO_4 (0.6 mL, 11.31 mmol) in H_2O (10 mL) was added (Note: PH of solution should be < 7) and the solution was vigorously stirred overnight. Then, the reaction mixture was slowly quenched and basified with saturated $NaHCO_3$, extracted with ethyl acetate three times. The combined organic solvent was dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatograph to give the mixture of diastereomers in a ratio of 3:2 as a yellow solid (2.5 g, 73%). Then, the diastereomers were separated by reverse phase preparative HPLC to give the enantiopure title compounds **15a** (1.2 g, 36%) and **15b** (0.8 g, 24%) as salts of trifluoroacetic acid, respectively. (Note: In large-scale synthesis, the enantiopure compound **15a** can be isolated by recrystallization in a solution of hexane and dichloromethane with a ratio of 4:1). Date for **15a**: ^{1}H NMR (400 MHz, MeOD) δ 7.44-7.39 (m, 1H), 7.35 (d, *J* = 7.9 Hz, 1H), 7.31-7.22 (m, 6H), 7.11-7.06 (m, 1H), 3.82-3.77 (m, 1H), 3.46 (s, 2H), 2.91 (t, *J* = 12.5 Hz, 2H), 2.81-2.76 (m, 1H), 2.07-1.93 (m, 5H), 1.80-1.72 (m, 1H), 1.62-1.46 (m, 5H), 1.33 (s, 9H), 1.27-1.17 (m, 2H); ESI-MS calculated for $C_{30}H_{38}FN_3O_2$ [M + H]$^+$ = 492.29, found: 492.36. $[\alpha]_D^{20}$ = + 23.1, (c 1.17×10^{-3} g/mL, MeOH); t_R (UPLC) = 4.46 min. Date for **15b:** ^{1}H NMR (400 MHz, MeOD) δ 7.50-7.43 (m, 6H), 7.27 (d, *J* = 7.3 Hz, 1H), 7.20 (d, *J* = 9.9 Hz, 1H), 7.14 (t, *J* = 8.3 Hz, 1H), 4.24 (s, 2H), 4.02-3.98 (m, 1H), 3.54-3.45 (m, 2H), 3.08 (t, *J* = 11.4 Hz, 2H), 2.88-2.83 (m, 2H), 2.59 (t, *J* = 11.8 Hz, 1H), 2.25 (d, *J* = 14.0 Hz, 1H), 1.99-1.87 (m, 2H), 1.79-1.74 (m, 1H), 1.67-1.57 (m, 3H), 1.46 (s, 9H), 1.43-1.37 (m, 2H), 1.33-1.18

(m, 1H); ESI-MS calculated for $C_{30}H_{38}FN_3O_2$ $[M + H]^+$ = 492.29, found: 492.36. $[\alpha]_D^{20}$ = + 9.4, (c 1.07 ×10^{-3} g/mL, MeOH); t_R (UPLC) = 4.63 min.

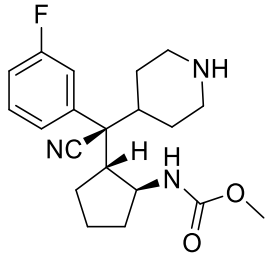
Methyl ((1*S*,2*R*)-2-((*S*)-(1-benzylpiperidin-4-yl)(cyano)(3-fluorophenyl)methyl)cyclopentyl)carbamate (16a)



16a

To a solution of compound **15a** as a salt of trifluoroacetic acid (1.2 g, 2.04 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (10 mL) at 0 °C. After stirring at room temperature for 3 h, the reaction mixture was concentrated under vacuum, then basified with saturated $NaHCO_3$, and extracted with dichloromethane three times. The combined organic solvent was dried over Na_2SO_4, filtered and concentrated under vacuum. The resulting residue was redissolved in dry dichloromethane (50 mL), and Et_3N (0.43 mL, 3.1 mmol), dimethyl dicarbonate (329 mg, 2.45 mmol) were added at 0 °C. After stirring at room temperature for 2h, the reaction mixture was quenched with H_2O, and extracted with dichloromethane three times. The combined organic solvent was dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by flash column chromatograph to give the title compound **16a** as a white solid (0.82 g, 89 %). ^{1}H NMR (400 MHz, MeOD) δ 7.43-7.37 (m, 1H), 7.33-7.30 (m, 1H), 7.29-7.22 (m, 6H), 7.11-7.06 (m, 1H), 3.88-3.83 (m, 1H), 3.46 (s, 2H), 3.43 (m, 3H), 2.91 (t, *J* = 11.0 Hz, 2H), 2.84-2.78 (m, 1H), 2.09-1.94 (m, 5H), 1.82-1.77 (m, 1H), 1.66-1.56 (m, 3H), 1.55-1.48 (m, 2H), 1.39-1.29 (m, 1H), 1.26-1.16 (m, 1H); ESI-MS calculated for $C_{27}H_{32}FN_3O_2$ $[M + H]^+$ = 450.25, found: 450.40.

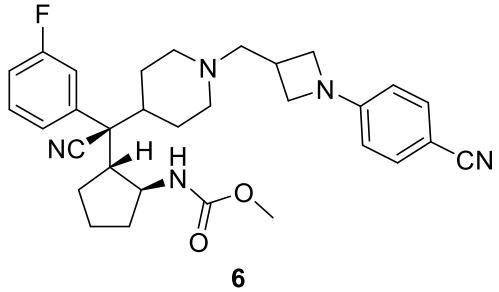
Methyl ((1*S*,2*R*)-2-((*S*)-(1-benzylpiperidin-4-yl)(cyano)(3-fluorophenyl)methyl)cyclopentyl)carbamate (17a)



17a

Method B using the intermediate **16a**. ^{1}H NMR (400 MHz, MeOD) δ 7.48-7.42 (m, 1H), 7.36 (d, *J* = 7.8 Hz, 1H), 7.27 (d, *J* = 10.5 Hz, 1H), 7.16-7.11 (m, 1H), 3.91-3.85 (m, 1H), 3.44 (s, 3H), 3.41-3.37 (m, 2H), 3.00 (t, *J* = 12.4 Hz, 2H), 2.87-2.81 (m, 1H), 2.46 (t, *J* = 12.1 Hz, 1H), 2.22 (d, *J* = 13.9 Hz, 1H), 2.15-2.12 (m, 1H), 1.90 (d, *J* = 13.8 Hz, 1H), 1.84-1.77 (m, 1H), 1.73-1.44 (m, 5H), 1.39-1.29 (m, 1H); ESI-MS calculated for $C_{20}H_{26}FN_3O_2$ $[M + H]^+$ = 360.20, found: 360.25.

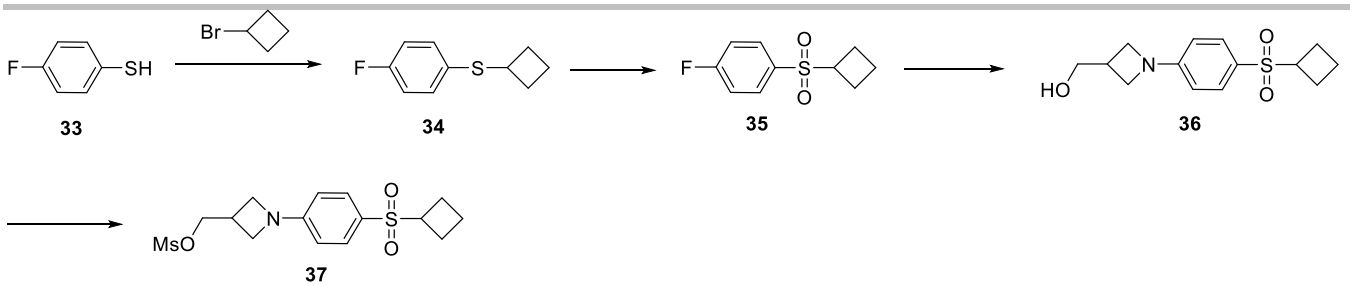
Methyl ((1*S*,2*R*)-2-((*S*)-cyano(1-((1-(4-cyanophenyl)azetidin-3-yl)methyl)piperidin-4-yl)(3-fluorophenyl)methyl)cyclopentyl)carbamate (6)



6

Method C using the intermediate **17a** and **24**. ^{1}H NMR (400 MHz, MeOD) δ 7.50-7.43 (m, 3H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.27 (d, *J* = 10.4 Hz, 1H), 7.17-7.13 (m, 1H), 6.47 (d, *J* = 8.8 Hz, 2H), 4.17-4.13 (m, 2H), 3.90-3.86 (m, 1H), 3.74-3.70 (m, 2H), 3.57 (t, *J* = 13.6 Hz, 2H), 3.44 (s, 3H), 3.43-3.36 (m, 2H), 3.25-3.17 (m, 1H), 3.08-3.00 (m, 2H), 2.87-2.81 (m, 1H), 2.47 (t, *J* = 11.8 Hz, 1H), 2.27 (d, *J* = 14.1 Hz, 1H), 2.16-2.13 (m, 1H), 1.97 (d, *J* = 14.6 Hz, 1H), 1.84-1.78 (m, 1H), 1.74-1.51 (m, 5H), 1.48-1.39 (m, 1H); ^{13}C NMR (100 MHz, MeOD) δ 165.2, 162.8, 158.1, 154.8, 137.2, 137.1, 134.4, 131.5, 131.4, 125.6, 121.6, 121.2, 116.5, 116.3, 112.0, 99.8, 61.0, 56.8, 56.1, 55.7, 53.6, 52.3, 40.9, 35.0, 30.0, 27.6, 26.7, 25.8, 23.8; ESI-MS calculated for $C_{31}H_{36}FN_5O_2$ $[M + H]^+$ = 530.29, found: 530.32; t_R (UPLC) = 3.75 min; Purity > 99%. $[\alpha]_D^{20}$ = +1.5, (c 1.83 ×10^{-3} g/mL, MeOH).

Method D: synthesis of (1-(4-(cyclobutylsulfonyl)phenyl)azetidin-3-yl)methyl methanesulfonate (37)

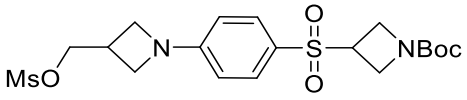


33 **34** **35** **36** **37**

To a solution of 4-fluorobenzenethiol (**33**) (1 g, 7.8 mmol) in DMSO (10 mL) was added sodium tert-butoxide (1.12 g, 11.7 mmol) at room temperature. After stirring for 30 min at room temperature, bromocyclobutane (1.16 g, 8.6 mmol) was added. Then, the reaction mixture was heated to 80 °C and stirred overnight. The reaction mixture was cooled to room temperature and poured into water and dichloromethane. The layers were separated, and the aqueous layer was extracted with dichloromethane three times. The combined organic layers were washed with aqueous NaOH (1M, 3 x 20 mL) and brine, dried over Na_2SO_4, filtered and concentrated under vacuum to give crude sulfide intermediate **34**. This intermediate was redissolved in dichloromethane (50 mL) and 3-Chloroperbenzoic acid (77% Wt., 3.49 g, 15.6 mmol) was added at 0 °C. Then the reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction was quench with a solution of $Na_2S_2O_3$ in water and washed with saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography to give the intermediate **35** as a yellow solid (1.2 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91-7.87 (m, 2H), 7.26-7.21(m, 2H), 3.83-3.75 (m, 1H), 2.61-2.51 (m, 2H), 2.25-2.15 (m, 2H), 2.05-1.94 (m, 2H).

To a solution of **35** (452 mg, 2.11 mmol) and azetidin-3-ylmethanol hydrochloride (287 mg, 2.32 mmol) in DMSO (5 mL) was added K_2CO_3 (875 mg, 6.33 mmol). The reaction mixture was heated to 80 °C and stirred overnight. The reaction mixture was cooled to room temperature and poured into water and dichloromethane. The layers were separated, and the aqueous layer was extracted with dichloromethane three times. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum to give crude intermediate **36**. This intermediate was redissolved in dichloromethane (20 mL), then Et_3N (0.59 mL, 2.53 mmol) and methanesulfonyl chloride (0.20 mL, 2.53 mmol) were added at 0 °C. After stirring for 2h at room temperature, the reaction was quench with saturated $NaHCO_3$ and extracted with dichloromethane three time. The combined organic layers was dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography to give the title compound **37** as a yellow solid (455 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 8.8 Hz, 2H), 6.42 (d, *J* = 8.8 Hz, 2H), 4.46 (d, *J* = 6.7 Hz, 2H), 4.10 (t, *J* = 8.1 Hz, 2H), 3.82-3.79 (m, 2H), 3.76-3.70 (m, 1H), 3.23-3.15 (m, 1H), 3.06 (s, 3H), 2.56-2.46 (m, 2H), 2.20-2.12 (m, 2H), 1.98-1.89 (m, 2H); ESI-MS calculated for $C_{15}H_{21}NO_5S_2$ $[M + H]^+$ = 360.09, found: 360.10.

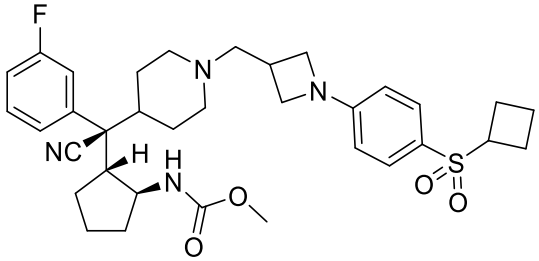
***tert*-Butyl 3-((4-(3-(((methylsulfonyl)oxy)methyl)azetidin-1-yl)phenyl)sulfonyl)azetidine-1-carboxylate (38)**



38

Method D using 4-fluorobenzenethiol and tert-butyl 3-bromoazetidine-1-carboxylate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.9 Hz, 2H), 6.43 (d, *J* = 8.8 Hz, 2H), 4.45 (d, *J* = 6.6 Hz, 2H), 4.23-4.19 (m, 2H), 4.12 (t, *J* = 8.2 Hz, 2H), 4.03 (t, *J* = 8.6 Hz, 2H), 3.92-3.87 (m, 1H), 3.85-3.81 (m, 2H), 3.25-3.16 (m, 1H), 3.06 (s, 3H), 1.42 (s, 9H); ESI-MS calculated for $C_{19}H_{28}N_2O_7S_2$ $[M + Na]^+$ = 483.12, found: 483.22.

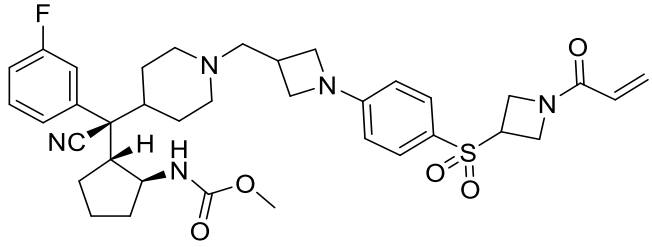
Methyl ((1*S*,2*R*)-2-((*S*)-cyano(1-((1-(4-(cyclobutylsulfonyl)phenyl)azetidin-3-yl)methyl)piperidin-4-yl)(3-fluorophenyl)methyl)cyclopentyl)carbamate (7)



7

Method C using intermediate **17a** and compound **37**. ^{1}H NMR (400 MHz, MeOD) δ 7.62 (d, *J* = 8.8 Hz, 2H), 7.48-7.42 (m, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.26 (d, *J* = 10.4 Hz, 1H), 7.17-7.12 (m, 1H), 6.51 (d, *J* = 8.8 Hz, 2H), 4.18-4.13 (m, 2H), 3.91-3.82 (m, 2H), 3.75-3.71 (m, 2H), 3.57 (t, *J* = 13.1 Hz, 2H), 3.43 (s, 3H), 3.42-3.35 (m, 2H), 3.24-3.18 (m, 1H), 3.08-3.03 (m, 2H), 2.87-2.82 (m, 1H), 2.49-2.35 (m, 3H), 2.26 (d, *J* = 14.5 Hz, 1H), 2.19-2.10 (m, 3H), 2.05-1.88 (m, 3H), 1.86-1.78 (m, 1H), 1.74-1.51 (m, 5H), 1.47-1.38 (m, 1H); ^{13}C NMR (100 MHz, MeOD) δ 165.2, 162.8, 158.0, 155.7, 137.1, 131.5, 131.4, 130.9, 125.5, 121.6, 116.5, 116.3, 111.5, 61.0, 58.4, 56.8, 56.2, 55.8, 53.6, 52.3, 41.0, 35.0, 30.0, 27.6, 26.8, 25.8, 23.8, 17.4; ESI-MS calculated for $C_{34}H_{43}FN_4O_4S$ $[M + H]^+$ = 623.30, found: 623.38; t_R (UPLC) = 3.92 min; Purity > 99%. $[\alpha]_D^{20}$ = -34.8, (c 2.57 ×10^{-3} g/mL, MeOH).

Methyl ((1*S*,2*R*)-2-((*S*)-(1-((1-(4-((1-acryloylazetidin-3-yl)sulfonyl)phenyl)azetidin-3-yl)methyl)piperidin-4-yl)(cyano)(3-fluorophenyl)methyl)cyclopentyl)carbamate (8)

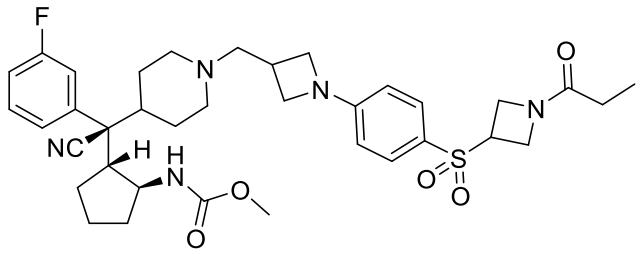


8

To a solution of the intermediate **17a** (45 mg, 0.125 mmol) in acetonitrile (2 mL) was added compound **38** (58 mg, 0.125 mmol), K_2CO_3 (35 mg, 0.25 mmol) and KI (2 mg, 0.013 mmol). The mixture was stirred at 80 °C overnight. Then, the mixture was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, and the solvent was evaporated under vacuum. The residue was purified by reverse phase preparative HPLC to give the salt of **18a**. Compound **18a** was dissolved in dichloromethane (5 mL) and trifluoroacetic acid (5 mL) was added at 0 °C. After stirring for 15 min at room temperature, the reaction mixture was concentrated under vacuum, basified with saturated $NaHCO_3$, extracted with dichloromethane three times. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum. The resulting residue was redissolved in dry dichloromethane (2 mL). Then, DIPEA (0.043 mL, 0.247 mmol) and acryloyl chloride (0.020 mL, 0.247 mmol) were added at 0 °C. After stirring for 2 h at room temperature, the reaction mixture was concentrated under vacuum. The residue was purified by reverse phase preparative HPLC to give the title compound as a salt of trifluoroacetic acid (40 mg, 48%). ^{1}H NMR (400 MHz, MeOD) δ 7.71 (d, *J* = 8.9 Hz, 2H), 7.48-7.43 (m, 1H), 7.35 (d, *J* = 7.8 Hz, 1H), 7.26 (d, *J* = 10.4 Hz, 1H), 7.17-7.13 (m, 1H), 6.85 (d, *J* = 9.0 Hz, 1H), 6.54 (d, *J* = 8.9 Hz, 2H), 6.33-6.20 (m, 2H), 5.75 (dd, *J* = 2.7, 9.4 Hz, 1H), 4.51-4.44 (m, 2H), 4.26-4.16 (m, 5H), 3.92-3.88 (m, 1H), 3.78-3.74 (m, 2H), 3.57 (t, *J* = 13.2 Hz, 2H), 3.44 (s, 3H), 3.45-3.36 (m, 2H), 3.25-3.19 (m, 1H), 3.08-3.01 (m, 2H), 2.87-2.81 (m, 1H), 2.47 (t, *J* = 11.9 Hz, 1H), 2.27 (d, *J* = 13.9 Hz, 1H), 2.16-2.13 (m, 1H), 1.97 (d, *J* = 14.0 Hz, 1H), 1.84-1.78 (m, 1H), 1.74-1.53 (m, 5H), 1.48-1.39 (m, 1H); ^{13}C NMR (100 MHz, MeOD) δ 167.6, 165.2, 162.8, 162.3, 161.9, 158.1, 156.0, 137.2, 137.1, 131.5, 131.4, 128.8, 126.6, 125.6, 124.0, 121.6, 116.5, 116.3, 111.6, 60.9, 56.8, 56.1, 55.8, 53.6, 52.3, 52.0, 50.0, 35.0, 30.0, 27.6, 26.7, 25.8, 23.8; ESI-MS calculated for $C_{36}H_{44}FN_5O_5S$ $[M + H]^+$ = 678.30, found: 678.40; t_R (UPLC) = 3.43 min; Purity > 99%. $[\alpha]_D^{20}$ = - 2.2, (c 1.07 ×10^{-3} g/mL, MeOH).

Methyl ((1*S*,2*R*)-2-((*S*)-cyano(3-fluorophenyl)(1-((1-(4-((1-propionylazetidin-3-yl)sulfonyl)phenyl)azetidin-3-yl)methyl)piperidin-4-yl)methyl)cyclopentyl)carbamate

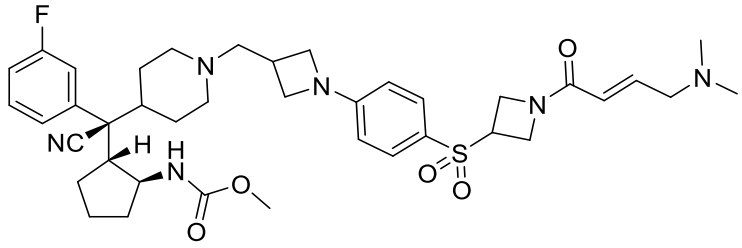
(9)



9

Compound **9** was synthesized using the method described for compound **8** from the intermediate **17a** and propionic anhydride.^{1}H NMR (400 MHz, MeOD) δ 7.71 (d, *J* = 8.8 Hz, 2H), 7.48-7.43 (m, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.27 (d, *J* = 10.4 Hz, 1H), 7.17-7.13 (m, 1H), 6.54 (d, *J* = 8.9 Hz, 2H), 4.41-4.34 (m, 2H), 4.21-4.15 (m, 3H), 4.09-4.07 (m, 2H), 3.91-3.86 (m, 1H), 3.78-3.74 (m, 2H), 3.57 (t, *J* = 14.4 Hz, 2H), 3.44 (s, 3H), 3.45-3.36 (m, 2H), 3.25-3.19 (m, 1H), 3.08-3.00 (m, 2H), 2.87-2.81 (m, 1H), 2.47 (t, *J* = 12.1 Hz, 1H), 2.27 (d, *J* = 13.5 Hz, 1H), 2.16-2.09 (m, 3H), 1.97 (d, *J* = 13.7 Hz, 1H), 1.84-1.78 (m, 1H), 1.74-1.53 (m, 5H), 1.48-1.39 (m, 1H), 1.05 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, MeOD) δ 176.4, 165.2, 162.8, 158.1, 156.0, 137.2, 137.1, 131.6, 131.5, 131.4, 125.6, 124.1, 121.6, 116.5, 116.3, 111.6, 60.9, 56.8, 56.1, 55.7, 53.6, 52.3, 52.2, 51.7, 50.0, 35.0, 30.0, 27.6, 26.7, 25.9, 25.2, 23.8, 9.0; ESI-MS calculated for $C_{36}H_{46}FN_5O5S$ $[M + H]^+$ = 680.32, found: 680.43; t_R (UPLC) = 3.40 min; Purity > 99%. $[\alpha]_D^{20}$ = -0.6, (c 1.7 ×10^{-3} g/mL, MeOH).

Methyl ((1*S*,2*R*)-2-((*S*)-cyano(1-((1-(4-((1-(*E*)-4-(dimethylamino)but-2-enoyl)azetidin-3-yl)sulfonyl)phenyl)azetidin-3-yl)methyl)piperidin-4-yl)(3-fluorophenyl)methyl)cyclopentyl)carbamate (10)

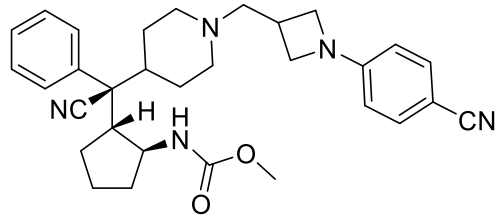


10

Compound **10** was synthesized using the method described for compound **8** from the intermediate **17a** and (2*E*)-4-(dimethylamino)but-2-enoyl chloride hydrochloride. ^{1}H NMR (400 MHz, MeOD) δ 7.70 (d, *J* = 8.9 Hz, 2H), 7.48-7.42 (m, 1H), 7.34 (d, *J* = 7.8 Hz, 1H), 7.26 (d, *J* = 10.5 Hz, 1H), 7.16-7.12 (m, 1H), 6.77-6.70 (m, 1H), 6.54 (d, *J* = 8.9 Hz, 2H), 6.47 (d, *J* = 15.3 Hz, 1H), 4.52 (d, *J* = 6.3 Hz, 2H), 4.29-4.16 (m, 5H), 3.95-3.93 (m, 2H), 3.90-3.85 (m, 1H), 3.78-3.74 (m, 2H), 3.57 (t, *J* = 13.0 Hz, 2H), 3.45-3.35 (m, 2H), 3.43 (s, 3H),

3.27-3.22 (m, 1H), 3.07-3.01 (m, 2H), 2.90 (s, 6H), 2.87-2.82 (m, 1H), 2.46 (t, *J* = 11.7 Hz, 1H), 2.30 (d, *J* = 14.5 Hz, 1H), 2.16-2.13 (m, 1H), 1.97 (d, *J* = 15.2 Hz, 1H), 1.85-1.78 (m, 1H), 1.74-1.53 (m, 5H), 1.48-1.38 (m, 1H); ^{13}C NMR (100 MHz, MeOD) δ 165.8, 165.2, 162.7, 158.0, 137.1, 133.0, 131.6, 131.5, 131.4, 131.3, 128.4, 125.6, 124.1, 121.6, 116.5, 116.2, 111.6, 60.9, 58.7, 56.8, 56.1, 55.8, 53.6, 52.3, 52.2, 51.8, 50.2, 43.3, 41.0, 35.0, 30.0, 27.6, 26.7, 25.8, 23.8; ESI-MS calculated for $C_{31}H_{37}N_5O_2$ [M + H]$^+$ = 735.36, found: 735.40. t_R (UPLC) = 2.91 min; Purity = 98%. $[\alpha]_D^{20}$ = -1.4, (c 2.47 ×10^{-3} g/mL, MeOH).

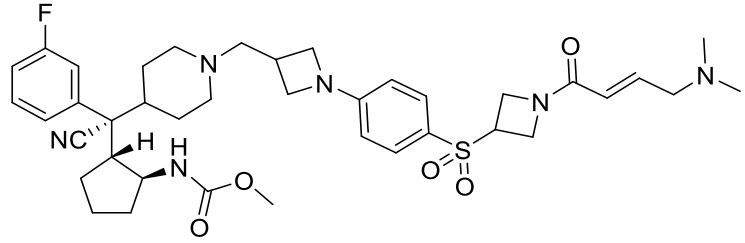
Methyl ((1*S*,2*R*)-2-((*S*)-cyano(1-((1-(4-cyanophenyl)azetidin-3-yl)methyl)piperidin-4-yl)(phenyl)methyl)cyclopentyl)carbamate (5)



5

Compound **5** was synthesized using the method described for compound **6** from the intermediate **20** and **14**. ^{1}H NMR (400 MHz, MeOD) δ 7.52 (d, *J* = 7.2 Hz, 2H), 7.47 (d, *J* = 8.8 Hz, 2H), 7.45-7.36 (m, 3H), 6.47 (*d*, *J* = 8.8 Hz, 2H), 4.17-4.12 (m, 2H), 3.93-3.88 (m, 1H), 3.73-3.70 (m, 2H), 3.60-3.52 (m, 2H), 3.44 (s, 3H), 3.42 (s, 2H), 3.23-3.17 (m, 1H), 3.07-2.99 (m, 2H), 2.89-2.83 (m, 1H), 2.50 (t, *J* = 12.4 Hz, 1H), 2.28 (d, *J* = 13.9 Hz, 1H), 2.16-2.10 (m, 1H), 1.94 (d, *J* = 13.5 Hz, 1H), 1.79-1.44 (m, 7H); ^{13}C NMR (100 MHz, MeOD) δ 158.1, 154.8, 134.4, 134.3, 129.7, 129.6, 122.0, 121.2, 112.0, 99.7, 61.0, 56.8, 56.1, 55.6, 53.6, 52.3, 40.7, 35.2, 30.1, 27.6, 26.7, 26.0, 23.9; ESI-MS calculated for $C_{31}H_{37}N_5O_2$ [M + H]$^+$ = 512.29, found: 512.39. t_R (UPLC) = 3.67 min; Purity > 99%. $[\alpha]_D^{20}$ = +10.3, (c 1.6 ×10^{-3} g/mL, MeOH).

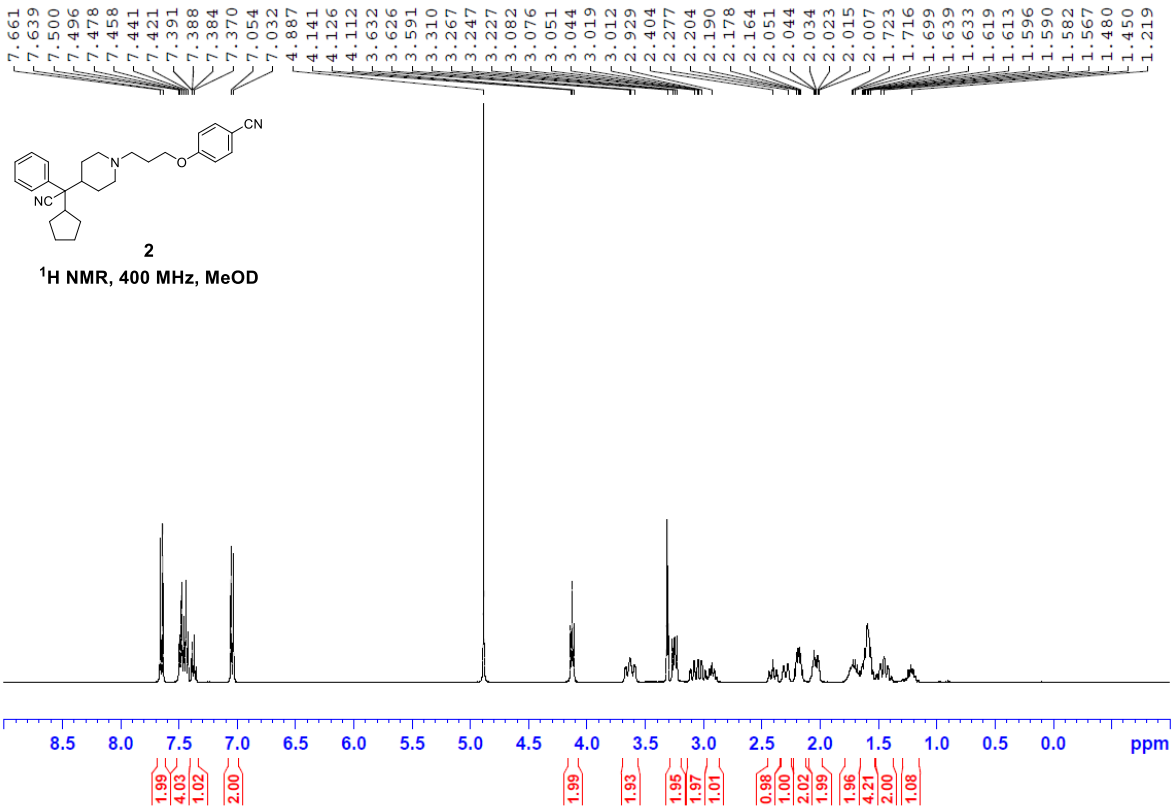
Methyl ((1*S*,2*R*)-2-((*R*)-cyano(1-((1-(4-((1-((*E*)-4-(dimethylamino)but-2-enoyl)azetidin-3-yl)sulfonyl)phenyl)azetidin-3-yl)methyl)piperidin-4-yl)(3-fluorophenyl)methyl)cyclopentyl)carbamate (11)

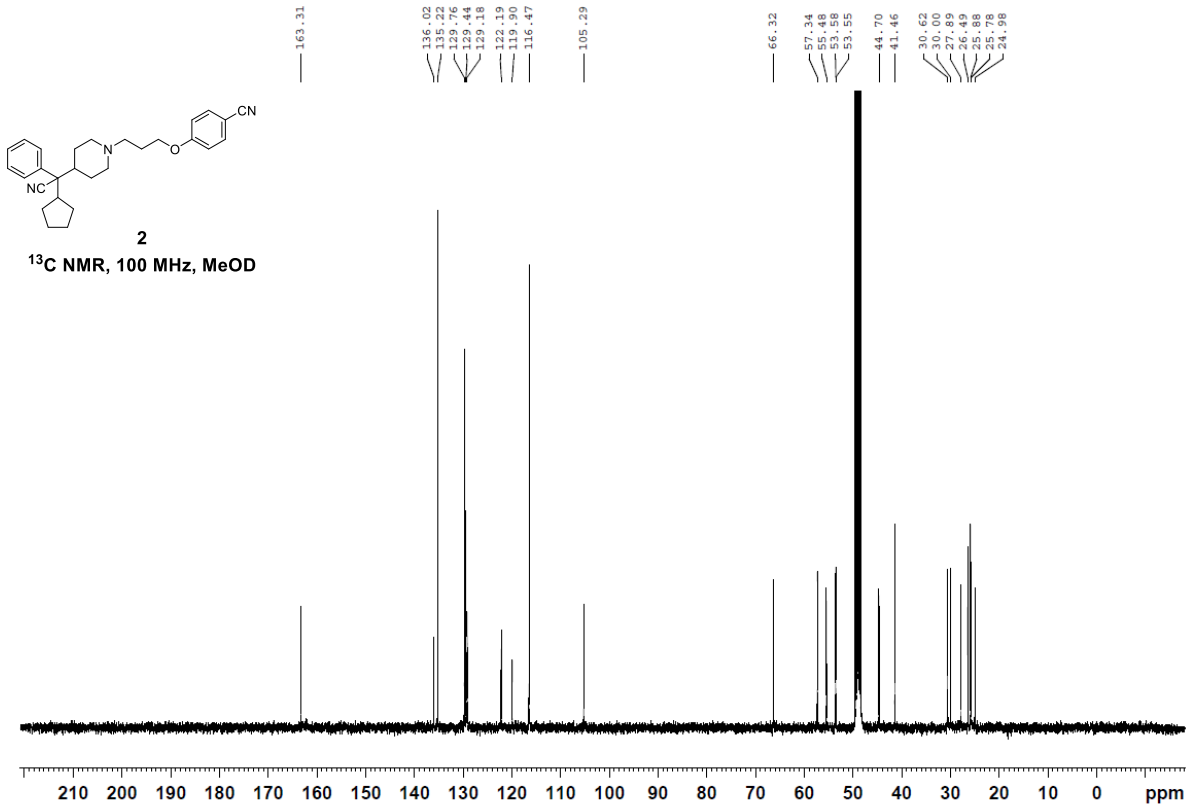


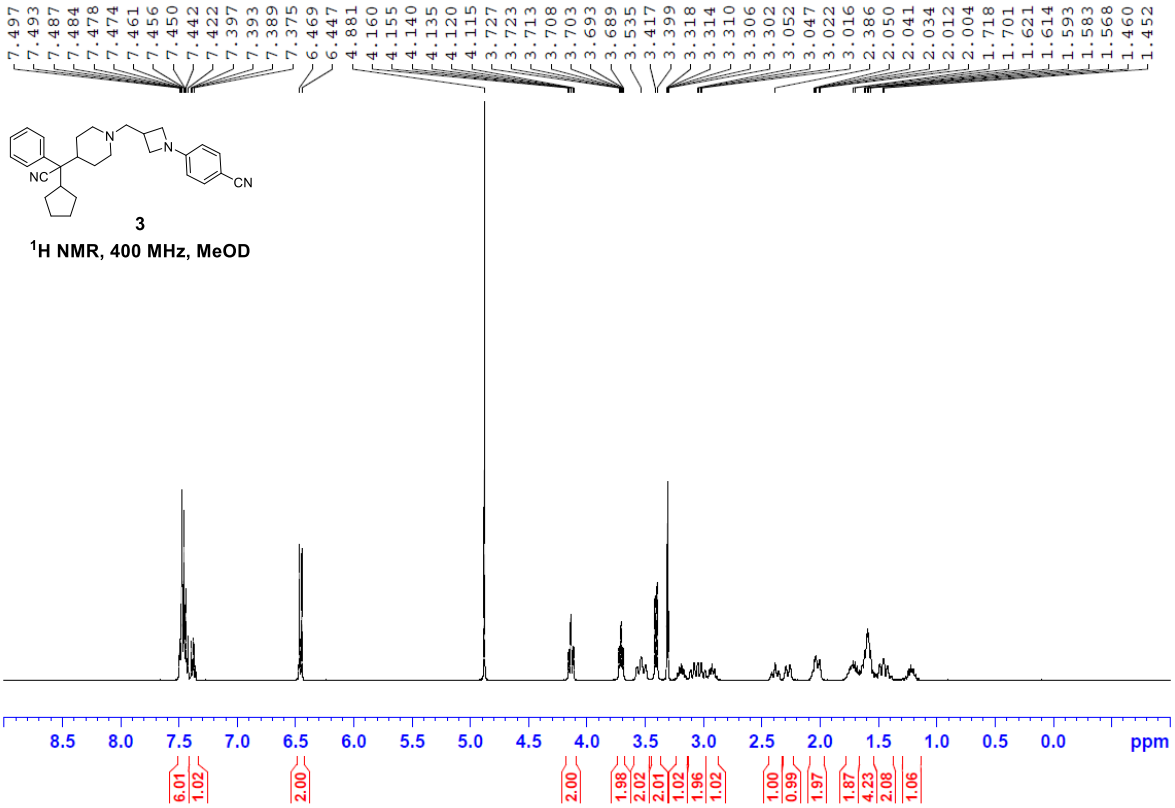
11

Compound **11** was synthesized using the method described for compound **8** from the intermediate **15b** and (2*E*)-4-(dimethylamino)but-2-enoyl chloride hydrochloride. ^{1}H NMR (400 MHz, MeOD) δ 7.70 (d, *J* = 8.8 Hz, 2H), 7.52-7.47 (m, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.25-7.15 (m, 2H), 6.77-6.70 (m, 1H), 6.64 (d, *J* = 8.9 Hz, 2H), 6.48 (d, *J* = 15.3 Hz, 1H), 4.54-4.52 (m, 2H), 4.29-4.14 (m, 5H), 4.12-4.06 (m, 1H), 3.95-3.93 (m, 2H), 3.79-3.74 (m, 2H), 3.69 (s, 3H), 3.58 (t, *J* = 13.6 Hz, 2H), 3.45 (d, *J* = 7.1 Hz, 2H), 3.26-3.19 (m, 1H), 3.14-3.08 (m, 1H), 2.90 (s, 6H), 2.88-2.84 (m, 2H), 2.63 (t, *J* = 12.0 Hz, 1H), 2.30 (d, *J* = 14. 1Hz, 1H), 2.03-1.96 (m, 2H), 1.87-1.79 (m, 1H), 1.68-1.59 (m, 3H), 1.51-1.42 (m, 2H), 1.32-1.23 (m, 1H); ^{13}C NMR (100 MHz, MeOD) δ 165.8, 165.4, 162.9, 158.7, 156.0, 137.9, 137.8, 133.0, 131.7, 131.6, 131.3, 128.4, 125.4, 124.0, 121.9, 116.6, 116.4, 111.6, 61.0, 58.7, 56.7, 56.1, 56.0, 55.8, 53.7, 52.7, 52.2, 51.9, 50.2, 50.0, 43.3, 40.4, 35.6, 30.6, 27.6, 26.7, 26.0, 24.3; ESI-MS calculated for $C_{31}H_{37}N_5O_2$ [M + H]$^+$ = 735.36, found: 735.45. t_R (UPLC) = 3.32 min; Purity > 99%. $[\alpha]_D^{20}$ = + 5.1, (c 2.4 ×10^{-3} g/mL, MeOH).

NMR Spectra

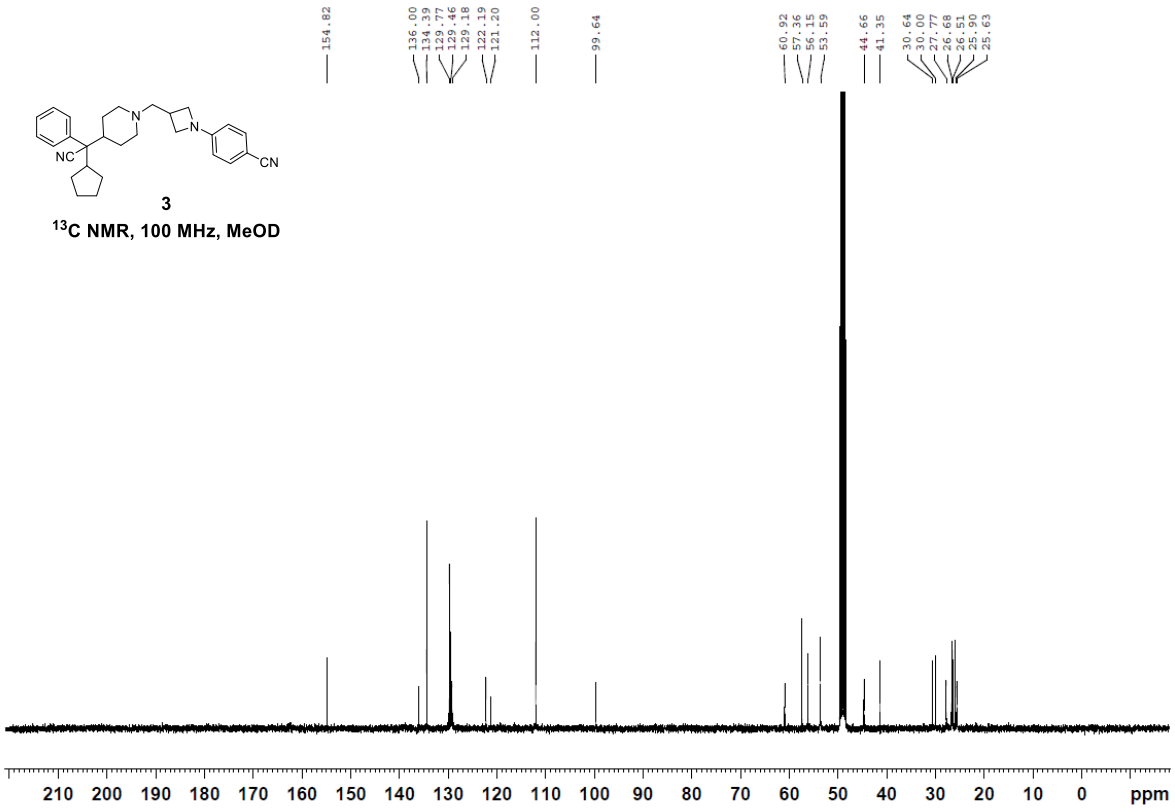

7.661
7.639
7.500
7.496
7.478
7.458
7.441
7.421
7.391
7.388
7.384
7.370
7.054
7.032
4.887
4.141
4.126
4.112
3.632
3.626
3.591
3.310
3.267
3.247
3.227
3.082
3.076
3.051
3.044
3.019
3.012
2.929
2.404
2.277
2.204
2.190
2.178
2.164
2.051
2.044
2.034
2.023
2.015
2.007
1.723
1.716
1.699
1.639
1.633
1.619
1.613
1.596
1.590
1.582
1.567
1.480
1.450
1.219
CN
NC
N
O
2
1H NMR, 400 MHz, MeOD
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
1.99
4.03
1.02
2.00
1.99
1.93
1.95
1.97
1.01
0.98
1.00
2.02
1.99
1.96
4.21
2.00
1.08


163.31
136.02
135.22
129.76
129.44
129.18
122.19
119.90
116.47
105.29
66.32
57.34
55.48
53.58
53.55
44.70
41.46
30.62
30.00
27.89
26.49
25.88
25.78
24.98
CN
NC
N
O
2
13C NMR, 100 MHz, MeOD
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm



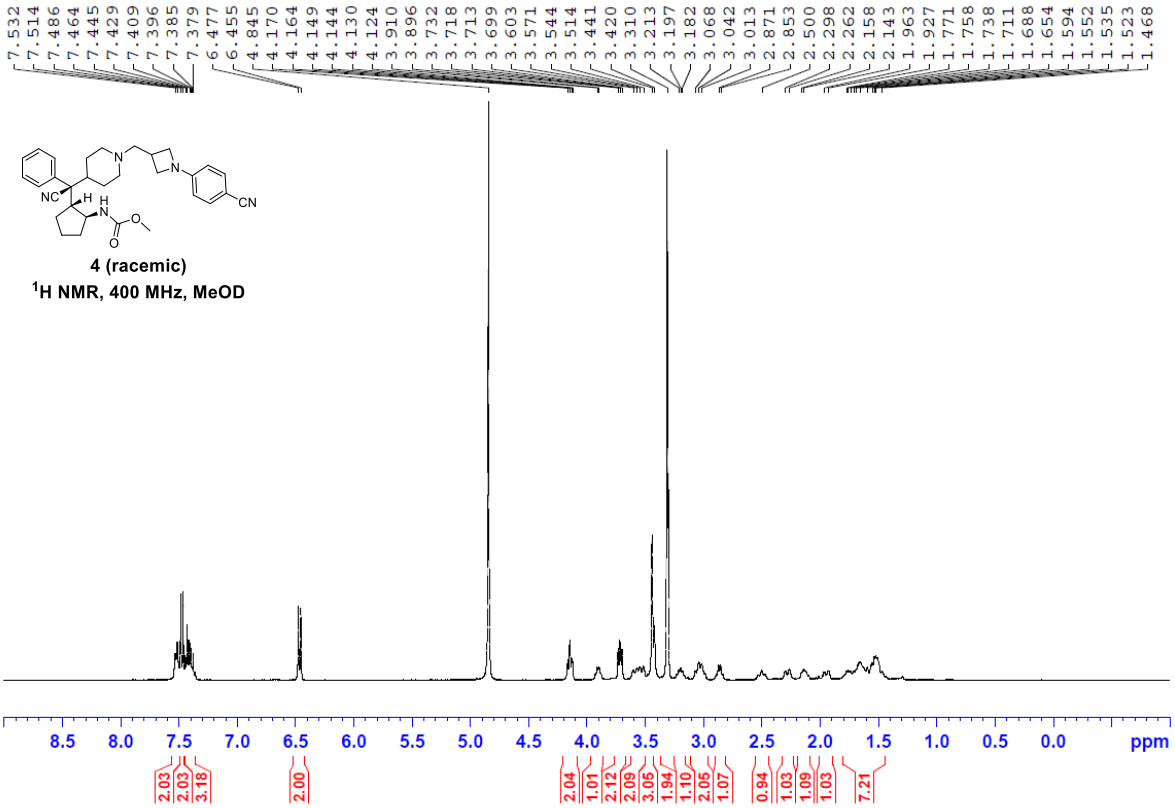
3

^{1}H NMR, 400 MHz, MeOD



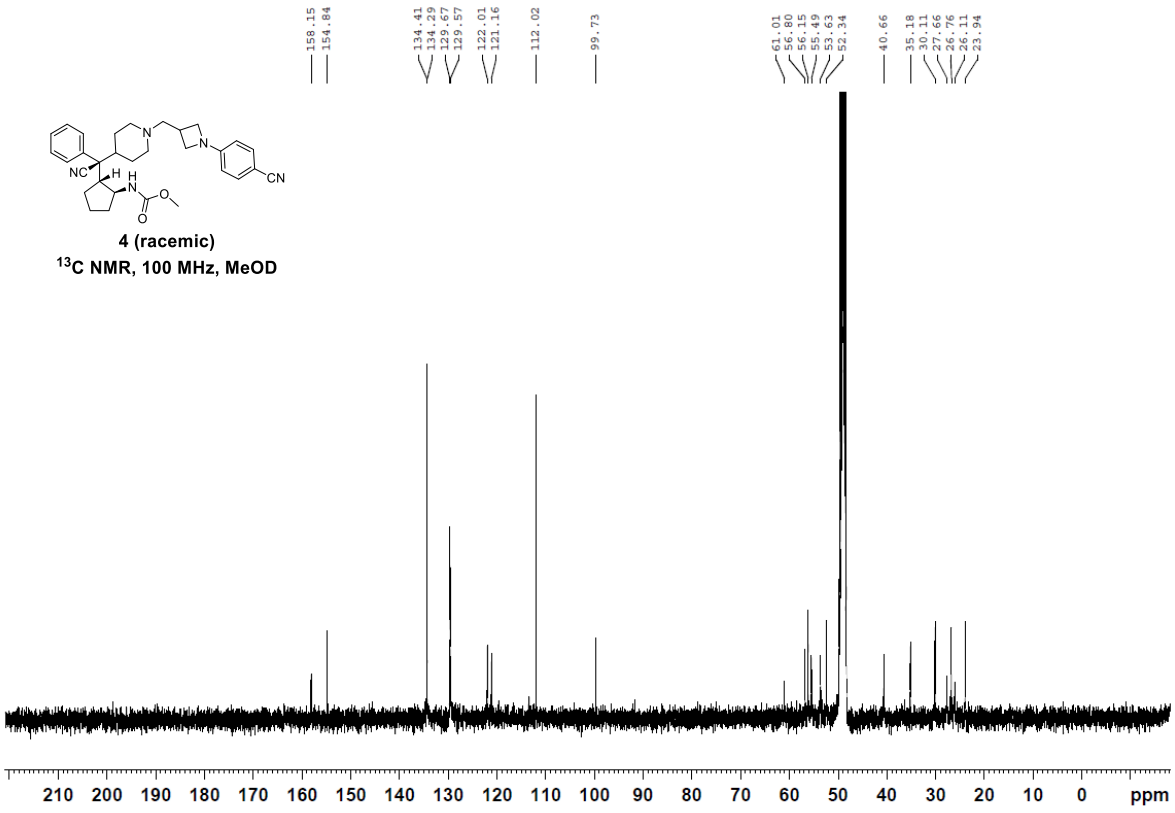
3

^{13}C NMR, 100 MHz, MeOD



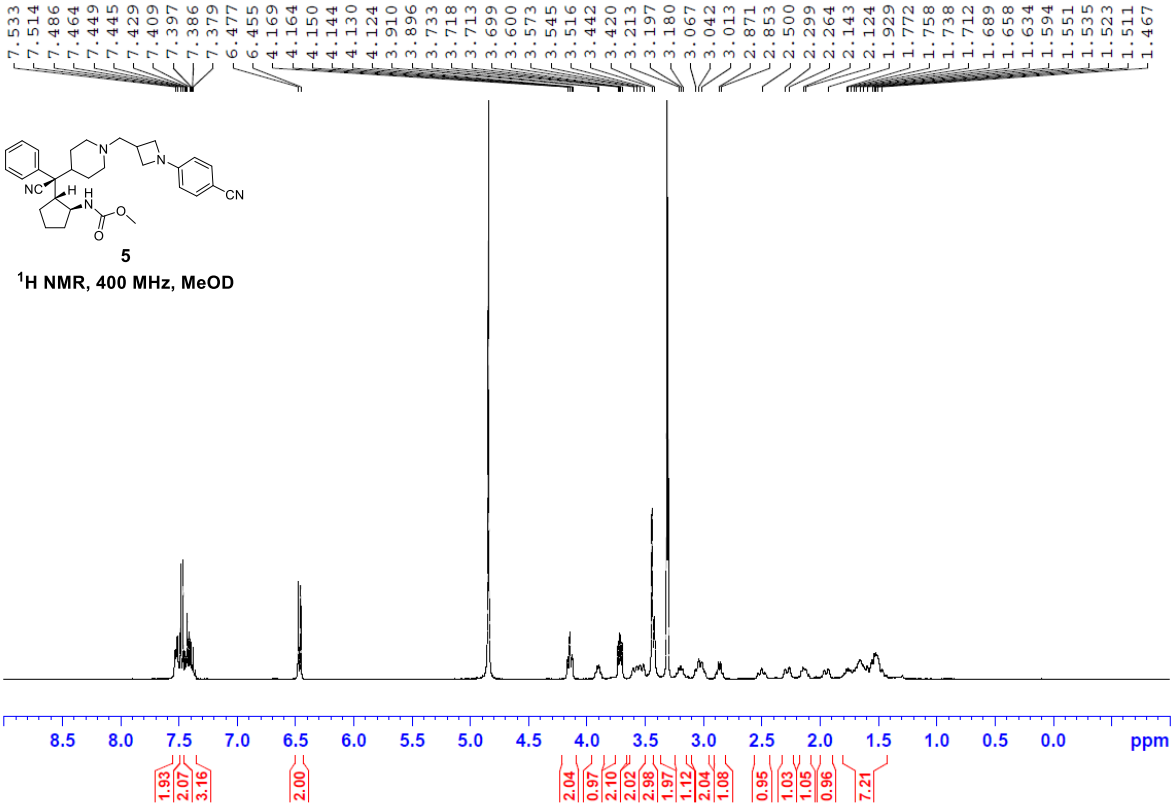
4 (racemic)

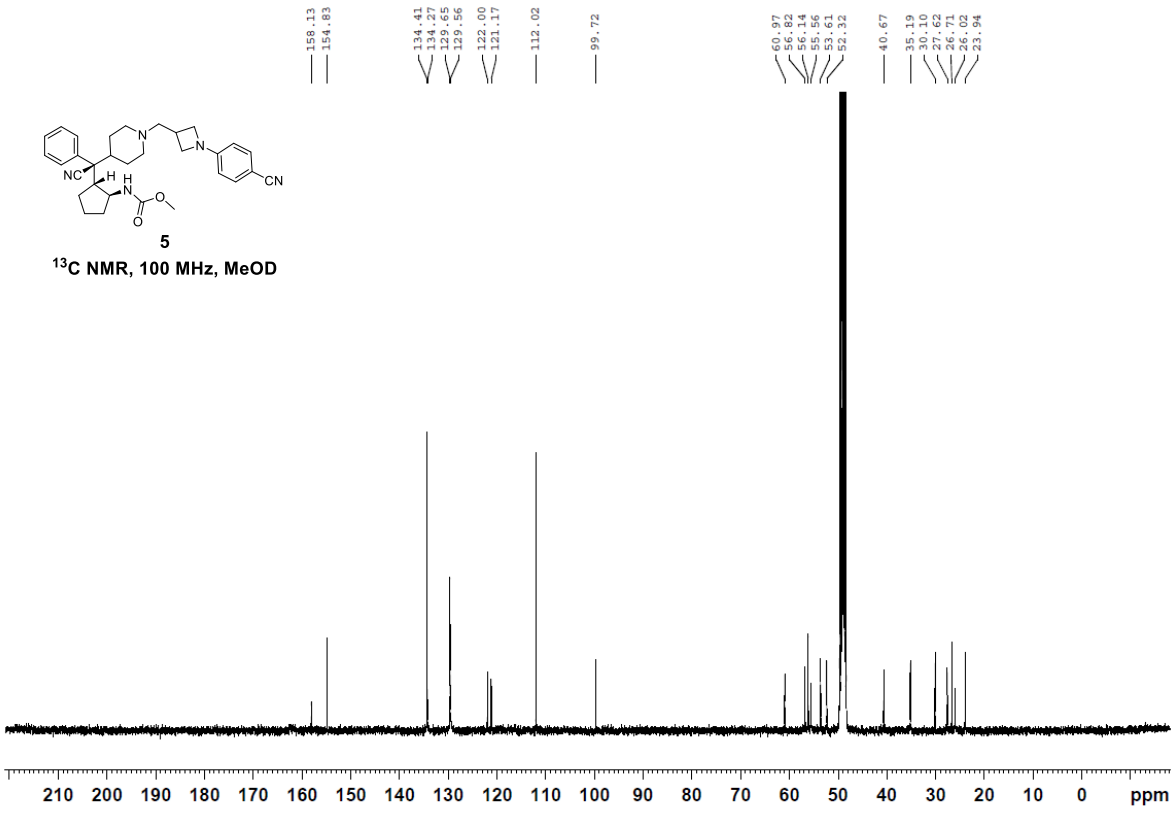
^{1}H NMR, 400 MHz, MeOD

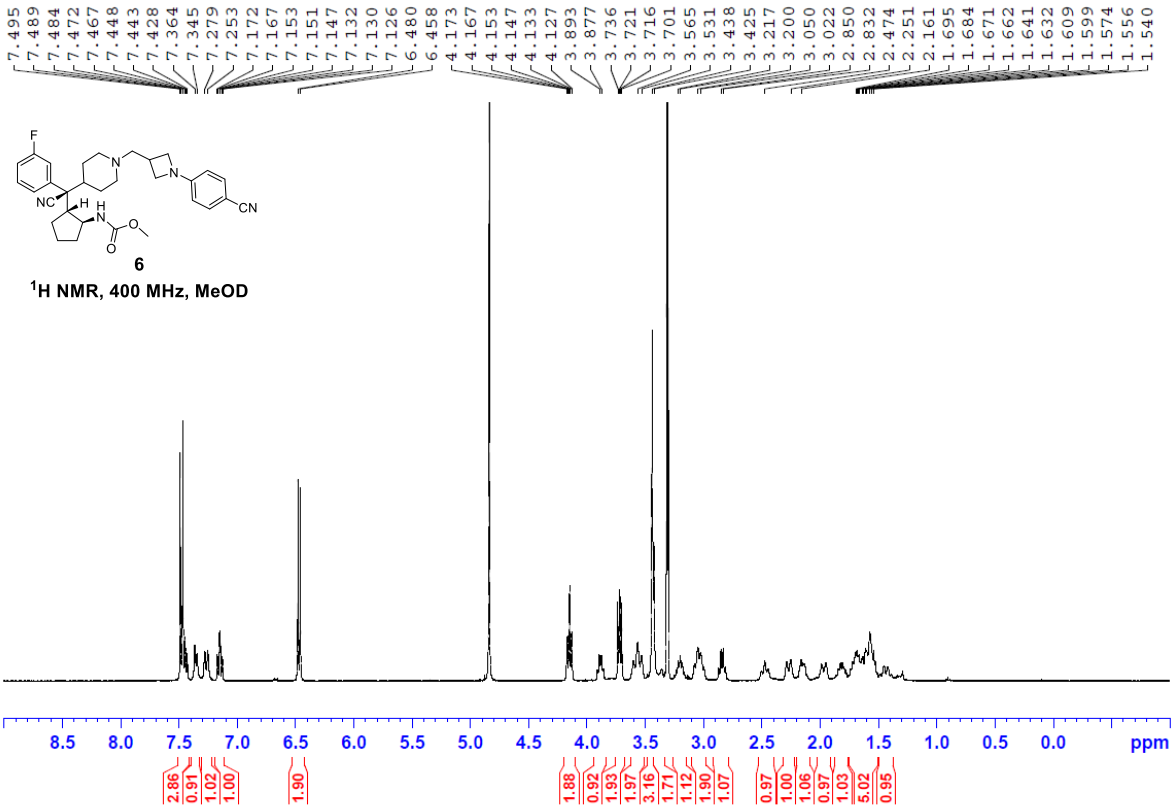


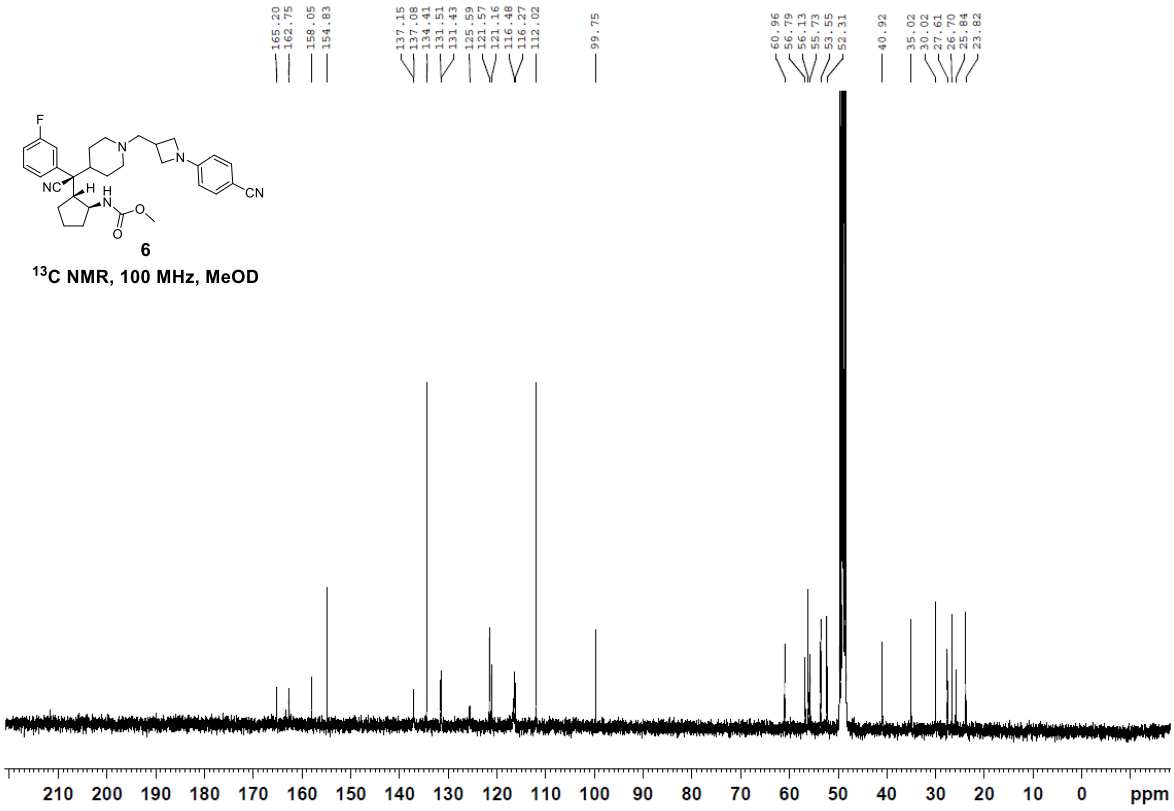
4 (racemic)

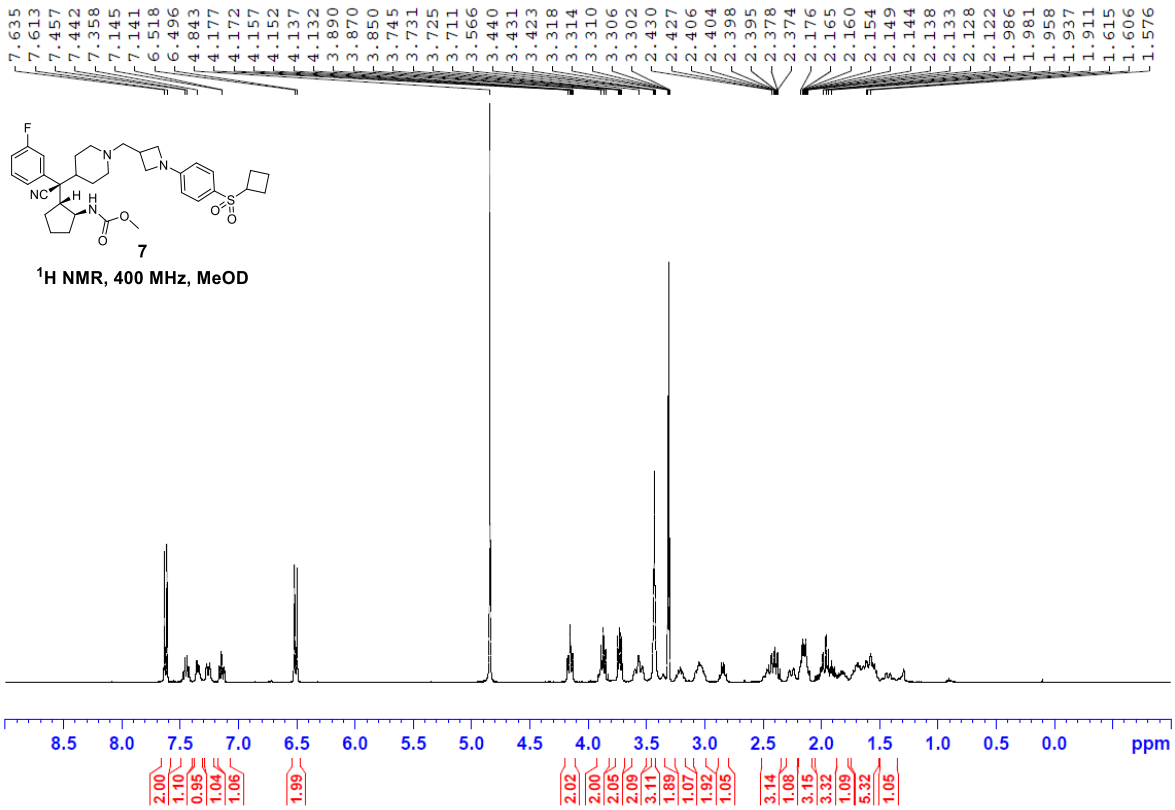
^{13}C NMR, 100 MHz, MeOD

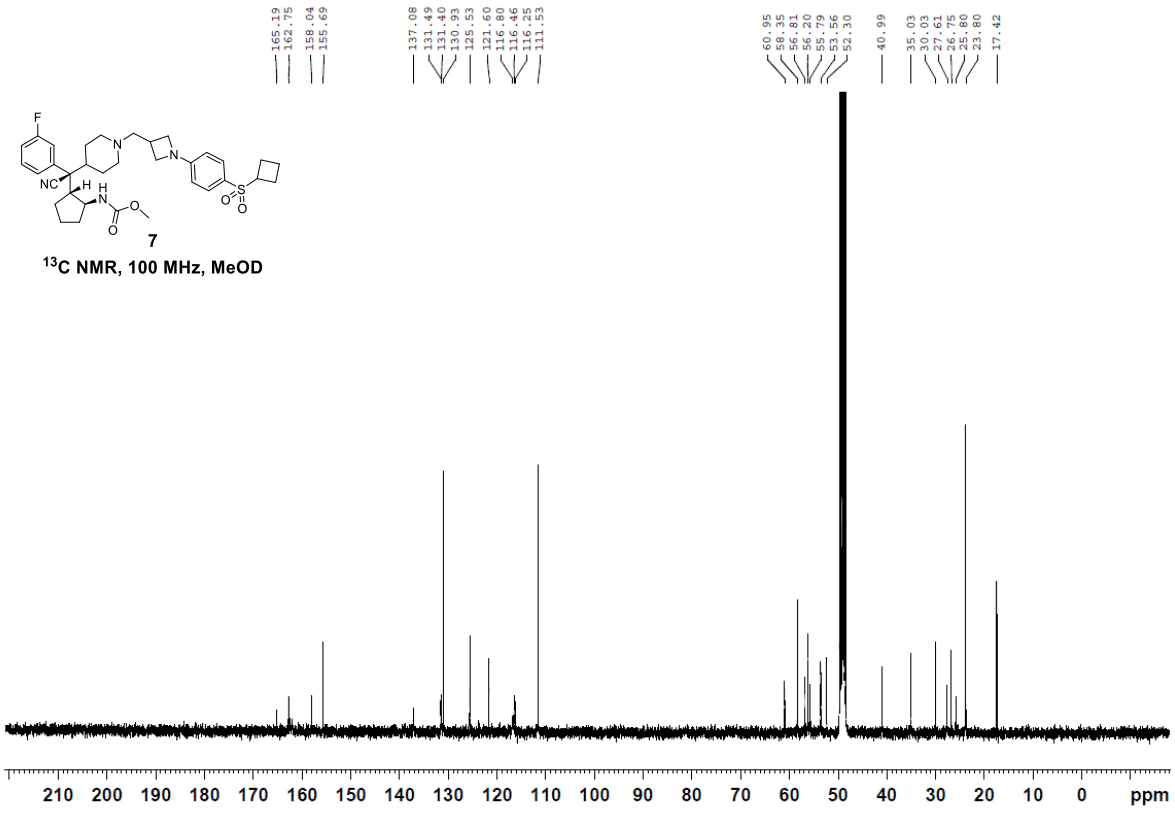

7.533
7.514
7.486
7.464
7.449
7.445
7.429
7.409
7.397
7.386
7.379
6.477
6.455
4.169
4.164
4.150
4.144
4.130
4.124
3.910
3.896
3.733
3.718
3.713
3.699
3.600
3.573
3.545
3.516
3.442
3.420
3.213
3.197
3.180
3.067
3.042
3.013
2.871
2.853
2.500
2.299
2.264
2.143
2.124
1.929
1.772
1.758
1.738
1.712
1.689
1.658
1.634
1.594
1.551
1.535
1.523
1.511
1.467
NC
H
N
O
N
N
CN
5
1H NMR, 400 MHz, MeOD
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
1.93
2.07
3.16
2.00
2.04
0.97
2.10
2.02
2.98
1.97
1.12
2.04
1.08
0.95
1.03
1.05
0.96
7.21

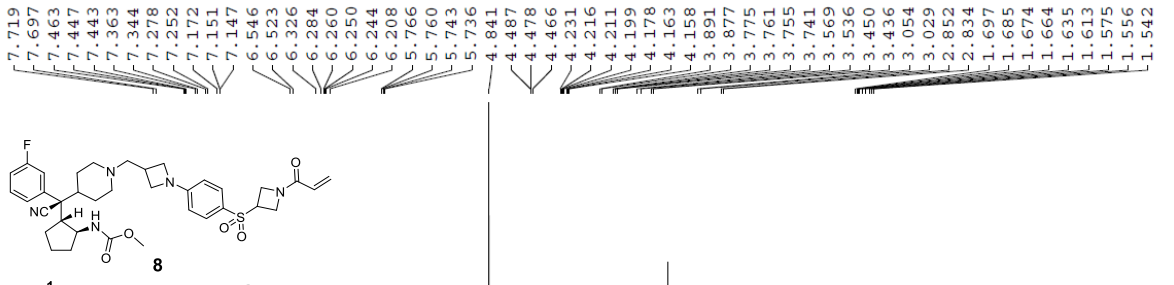

158.13
154.83
134.41
134.27
129.65
129.56
122.00
121.17
112.02
99.72
60.97
56.82
56.14
55.56
53.61
52.32
40.67
35.19
30.10
27.62
26.71
26.02
23.94
NC
H
N
O
N
N
CN
5
13C NMR, 100 MHz, MeOD
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


7.495
7.489
7.484
7.472
7.467
7.448
7.443
7.428
7.364
7.345
7.279
7.253
7.172
7.167
7.153
7.151
7.147
7.132
7.130
7.126
6.480
6.458
4.173
4.167
4.153
4.147
4.133
4.127
3.893
3.877
3.736
3.721
3.716
3.701
3.565
3.531
3.438
3.425
3.217
3.200
3.050
3.022
2.850
2.832
2.474
2.251
2.161
1.695
1.684
1.671
1.662
1.641
1.632
1.609
1.599
1.574
1.556
1.540
F
NC
H
N
O
N
N
CN
6
1H NMR, 400 MHz, MeOD
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
2.86
0.91
1.02
1.00
1.90
1.88
0.92
1.93
1.97
3.16
1.71
1.12
1.90
1.07
0.97
1.00
1.06
0.97
1.03
5.02
0.95


165.20
162.75
158.05
154.83
137.15
137.08
134.41
131.51
131.43
125.59
121.57
121.16
116.48
116.27
112.02
99.75
60.96
56.79
56.13
55.73
53.55
52.31
40.92
35.02
30.02
27.61
26.70
25.84
23.82
F
NC
H
N
O
N
N
CN
6
13C NMR, 100 MHz, MeOD
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

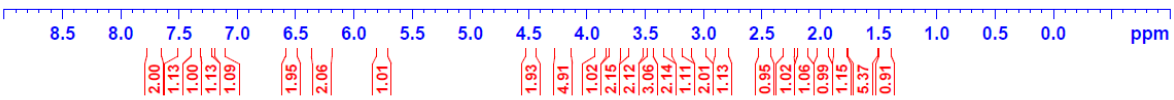

7.635
7.613
7.457
7.442
7.358
7.145
7.141
6.518
6.496
4.843
4.177
4.172
4.157
4.152
4.137
4.132
3.890
3.870
3.850
3.745
3.731
3.725
3.711
3.566
3.440
3.431
3.423
3.318
3.314
3.310
3.306
3.302
2.430
2.427
2.406
2.404
2.398
2.395
2.378
2.374
2.176
2.165
2.160
2.154
2.149
2.144
2.138
2.133
2.128
2.122
1.986
1.981
1.958
1.937
1.911
1.615
1.606
1.576
F
NC
H
N
O
S
7
1H NMR, 400 MHz, MeOD
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
2.00 1.10 0.95 1.04 1.06 1.99 2.02 2.00 2.05 2.09 3.11 1.89 1.07 1.92 1.05 3.14 1.08 3.15 3.32 1.09 5.32 1.05

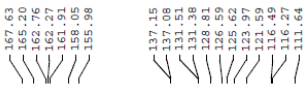

165.19
162.75
158.04
155.69
137.08
131.49
131.40
130.93
125.53
121.60
116.80
116.46
116.25
111.53
60.95
58.35
56.31
56.00
55.79
53.56
52.30
40.99
35.03
30.03
27.61
26.75
25.80
23.80
17.42
F
NC
H
N
O
S
7
13C NMR, 100 MHz, MeOD
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

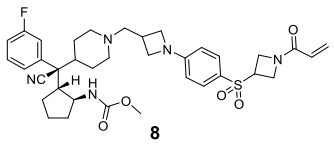


8

^{1}H NMR, 400 MHz, MeOD

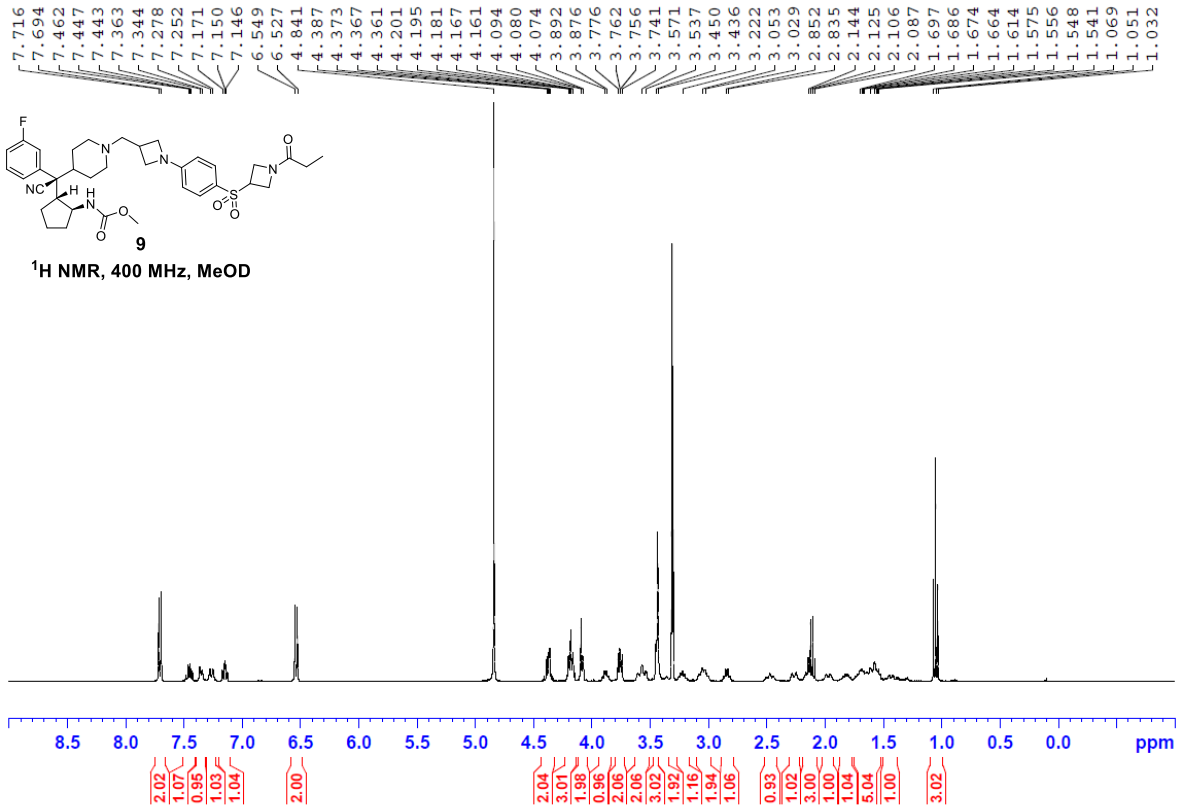




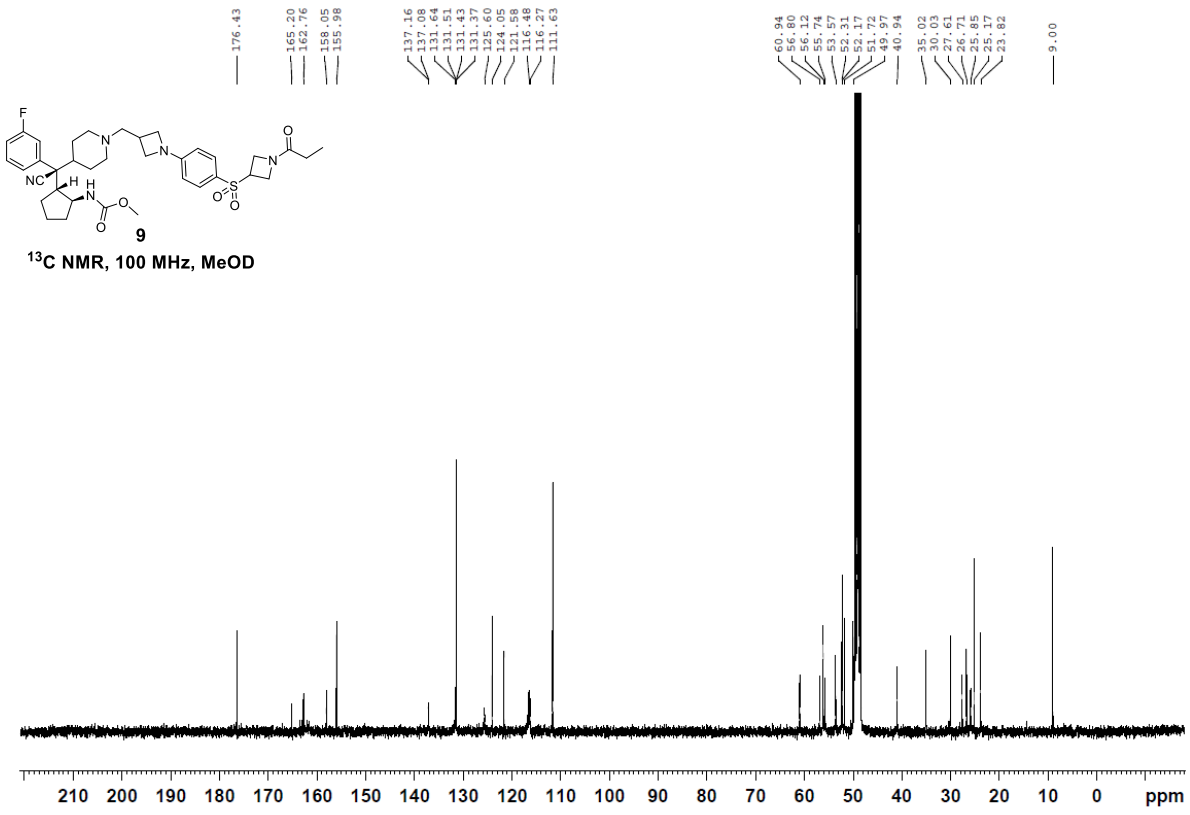


8

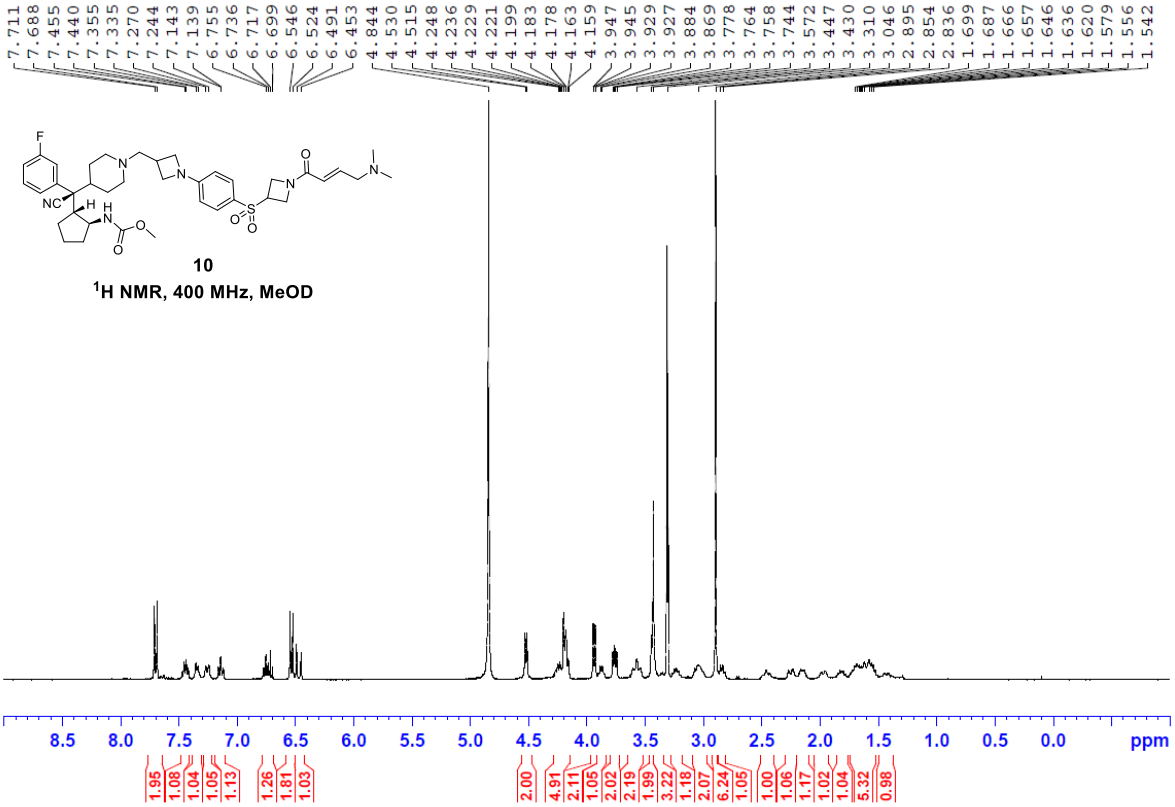
^{13}C NMR, 100 MHz, MeOD



^{1}H NMR, 400 MHz, MeOD



^{13}C NMR, 100 MHz, MeOD


7.711
7.688
7.455
7.440
7.355
7.335
7.270
7.244
7.143
7.139
6.755
6.736
6.717
6.699
6.546
6.524
6.491
6.453
4.844
4.530
4.515
4.248
4.236
4.229
4.221
4.199
4.183
4.178
4.163
4.159
3.947
3.945
3.929
3.927
3.884
3.869
3.778
3.764
3.758
3.744
3.572
3.447
3.430
3.310
3.046
2.895
2.854
2.836
1.699
1.687
1.666
1.657
1.646
1.636
1.620
1.579
1.556
1.542
F
NC
H
N
O
N
N
O
S
O
O
N
O
N
10
1H NMR, 400 MHz, MeOD
8.5
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7.5
7.0
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6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
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0.0
ppm
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1.08
1.04
1.05
1.13
1.26
1.81
1.03
2.00
4.91
2.11
1.05
2.02
2.19
1.99
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6.24
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1.00
1.06
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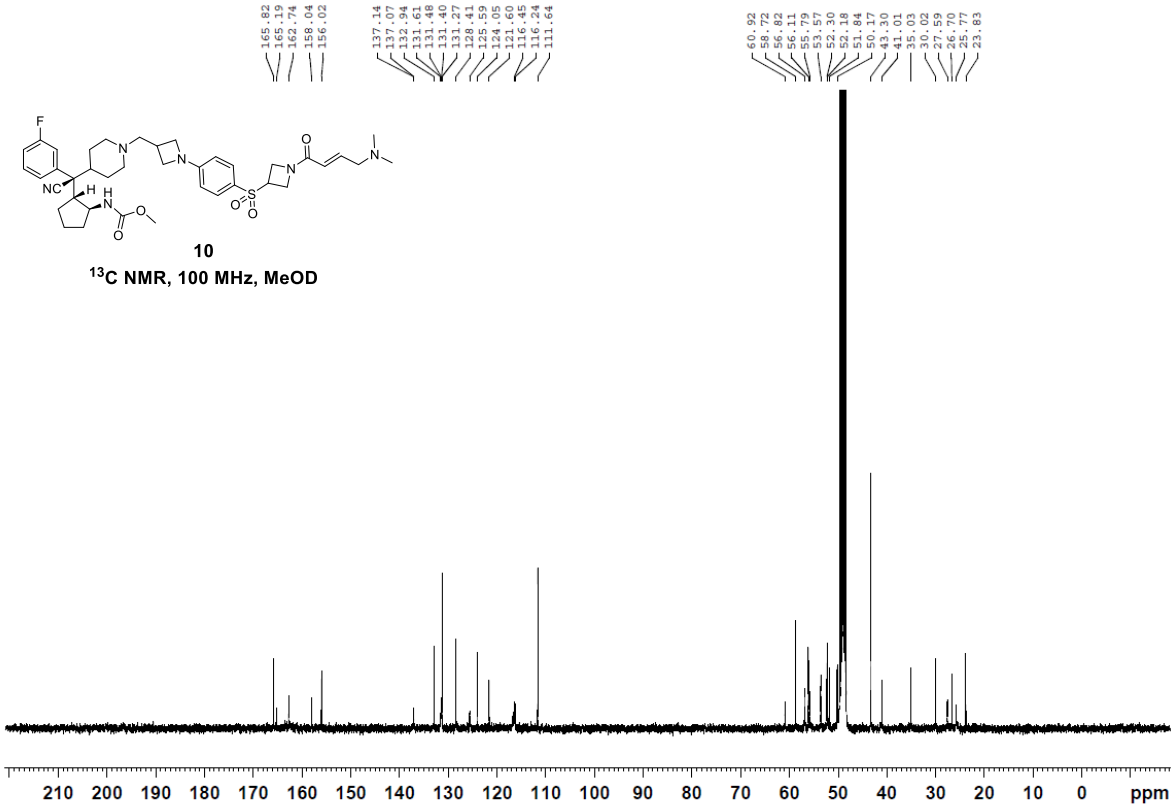
1.04
5.32
0.98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165.19
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156.02
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132.94
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128.41
125.59
124.05
121.60
116.45
116.24
111.64
60.92
58.72
56.82
56.11
55.79
53.57
52.30
52.18
51.84
50.17
43.30
41.01
35.03
30.02
27.59
26.70
25.77
23.83
F
NC
H
N
O
N
N
O
S
O
O
N
O
N
10
13C NMR, 100 MHz, MeOD
210
200
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120
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100
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60
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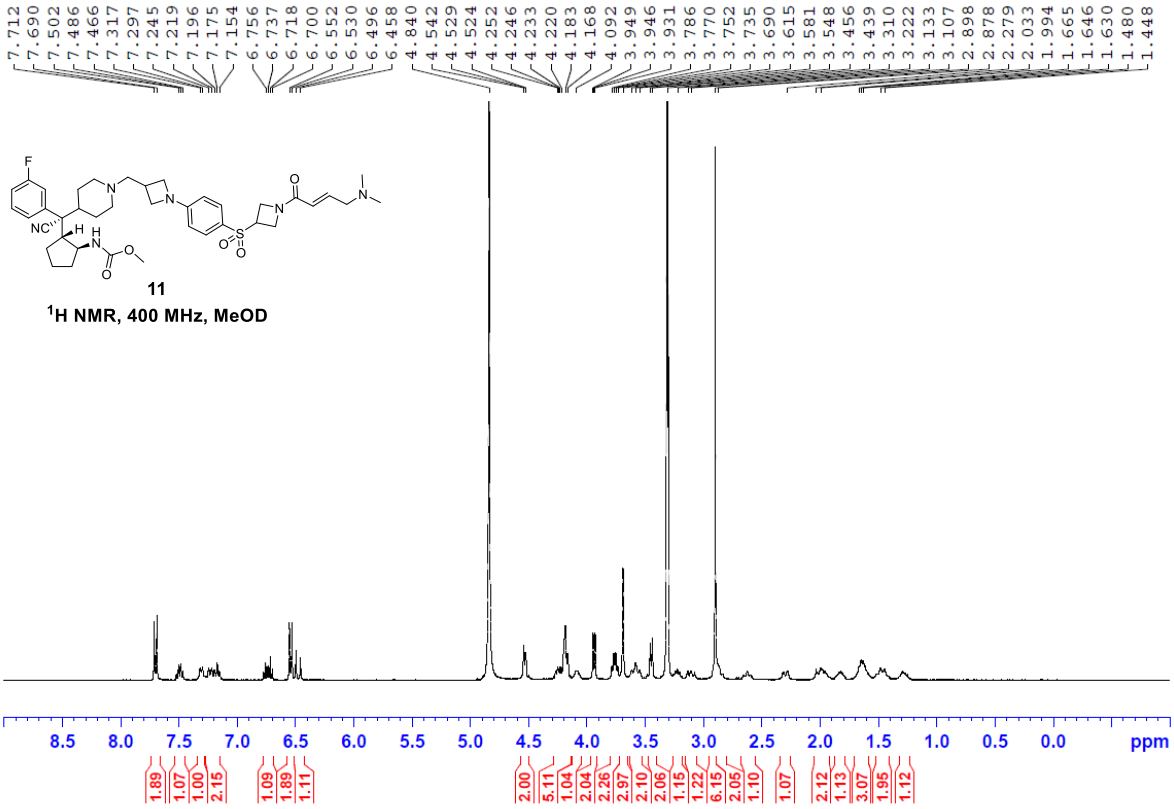
ppm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7.502
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6.756
6.737
6.718
6.700
6.552
6.530
6.496
6.458
4.840
4.542
4.529
4.524
4.252
4.246
4.233
4.220
4.183
4.168
4.092
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3.946
3.931
3.786
3.770
3.752
3.735
3.690
3.615
3.581
3.548
3.456
3.439
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3.222
3.133
3.107
2.898
2.878
2.279
2.033
1.994
1.665
1.646
1.630
1.480
1.448
F
NC
H
N
O
O
O
S
O
N
N
N
O
11
1H NMR, 400 MHz, MeOD
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7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
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1.07
1.00
2.15
1.09
1.89
1.11
2.00
5.11
1.04
2.04
2.26
2.97
2.10
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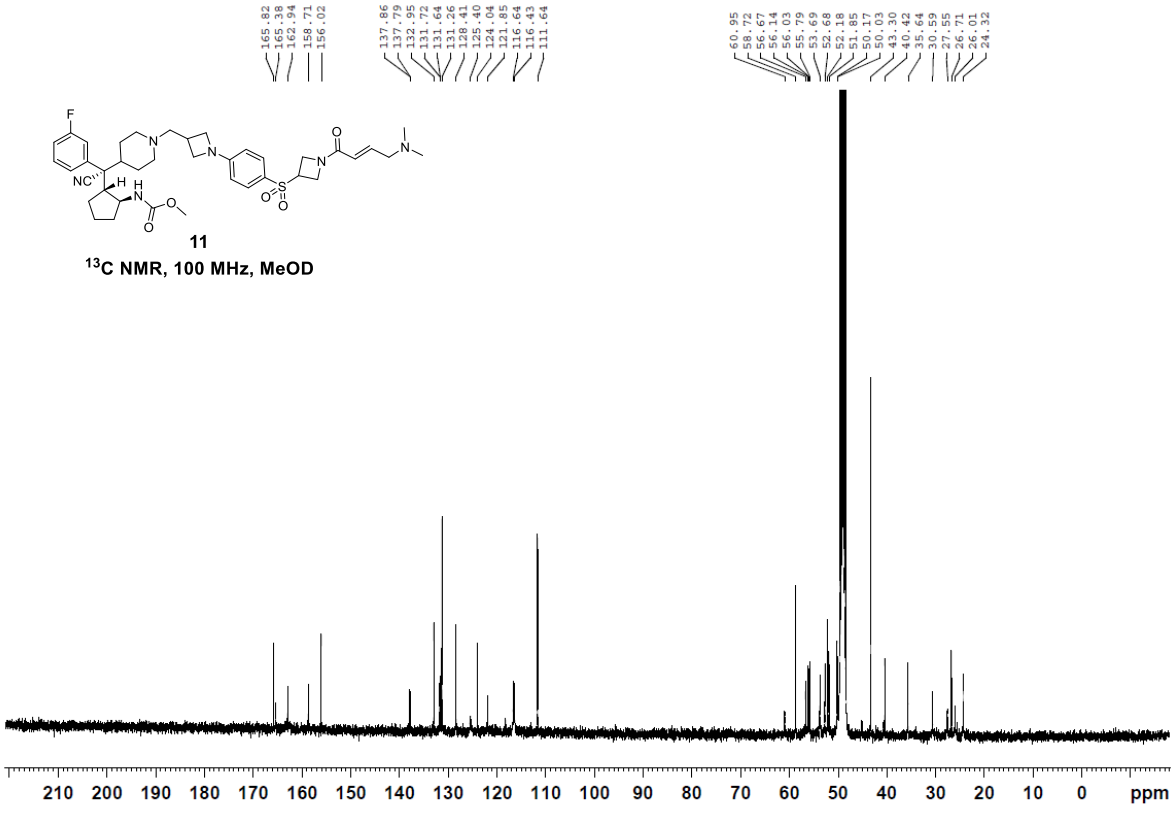
1.07
2.12
1.13
3.07
1.95
1.12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156.02
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125.40
124.04
121.85
116.64
116.43
111.64
60.95
58.72
56.67
56.14
56.03
55.79
53.69
52.68
52.18
51.85
50.17
50.03
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24.32
F
NC
H
N
O
O
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S
O
N
N
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11
13C NMR, 100 MHz, MeOD
210
200
190
180
170
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100
90
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50
40
30
20
10
0
ppm

Single Crystal Structure of Compound 15a

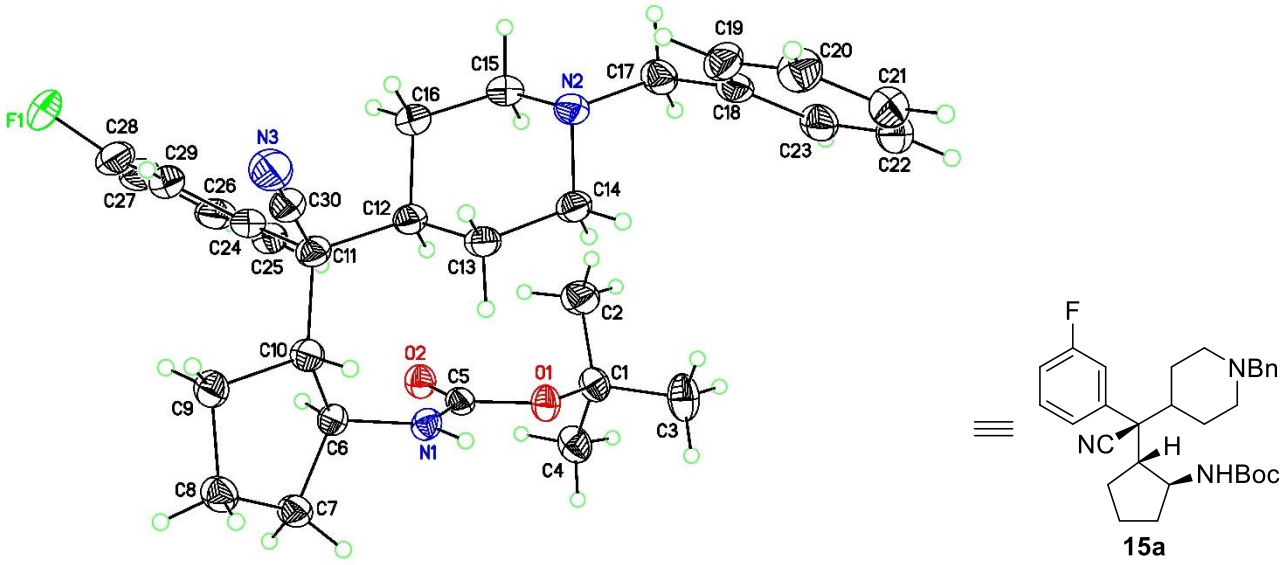

Figure S7. Single Crystal structure of compound **15a** (Fluorine in green, oxygens in red, nitrogens in blue, carbons in gray, and hydrogens in light green, CDCC: 1581872).

Colorless needles of **15a** were grown from a methanol solution of the compound at 22 °C. A crystal of dimensions 0.26 × 0.04 × 0.04 mm was mounted on a Rigaku AFC10K Saturn 944+ CCD-based X-ray diffractometer equipped with a low temperature device and Micromax-007HF Cu-target micro-focus rotating anode (λ = 1.54187 Å) operated at 1.2 kW power (40 kV, 30 mA). The X-ray intensities were measured at 85(1) K with the detector placed at a distance 42.00 mm from the crystal. A total of 2028 images were collected with an oscillation width of 1.0° in ω. The exposure times were 1 sec. for the low angle images, 3 sec. for high angle. Rigaku d*trek images were exported to CrysAlisPro for processing and corrected for absorption. The integration of the data yielded a total of 41135 reflections to a maximum 2θ value of 139.82° of which 4942 were independent and 4792 were greater than 2σ(I). The final cell constants (Table S3) were based on the xyz centroids of 18755 reflections above 10σ(I). Analysis of the data showed negligible decay during data collection. The structure was solved and refined with the Bruker SHELXTL (version 2016/6) software package, using the space group P2(1)2(1)2(1) with Z = 4 for the formula $C_{30}H_{38}N_3O_2F$. All non-hydrogen atoms were refined anisotropically with the hydrogen atoms placed in a combination of idealized and refined positions. The o-fluorine atom of the terminal aryl group is disordered (50/50) over two sites related by 180 rotation of the ligand around the C11-C24 bond. Full matrix least-squares refinement based on F^2 converged at R1 = 0.0371 and wR2 = 0.0900 [based on I > 2sigma(I)], R1 = 0.0389 and wR2 = 0.0944 for all data. Additional details are presented in Table S3 and are given as Supporting Information in a CIF file.

Table S3. Sample and crystal data for **15a.**

Identification code	**15a**	
Empirical formula	$C_{30}H_{38}FN_3O_2$	
Formula weight	491.63 g/mol	
Temperature	85(2) K	
Wavelength	1.54184 Å	
Crystal system, space group	Orthorhombic, P2(1)2(1)2(1)	
Unit cell dimensions	a = 9.76750(10) Å	α = 90°
	b = 14.6292(2) Å	β = 90°
	c = 18.6690(3) Å	γ = 90°
Volume	2667.63(6) $Å^3$	
Z, Density (calculated)	4, 1.224 g/cm^3	
Absorption coefficient	0.652 mm^{-1}	
F(000)	1056	
Crystal size	0.260 × 0.040 × 0.040 mm	
Thera range for data collection	3.839° to 69.909°	
Limiting indices	-11<=h<=11, -17<=k<=17, -21<=l<=19	
Reflections collected / unique	41135 / 4942 [R(int) = 0.0581]	
Completeness to theta = 67.684	99.6 %	

Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.00000 and 0.82570
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4942 / 0 / 343
Goodness-of-fit on F^2	1.043
Final R indices [I>2sigma(I)]	R1 = 0.0371, wR2 = 0.0899
R indices (all data)	R1 = 0.0389, wR2 = 0.0943
Extinction coefficient	0.0023(3)
Largest diff. peak and hole	0.195 and -0.183 $eÅ^{-3}$

G.M. Sheldrick (2015) "Crystal structure refinement with SHELXL", Acta Cryst., C71, 3-8 (Open Access).
CrystalClear Expert 2.0 r16, Rigaku Americas and Rigaku Corporation (2014), Rigaku Americas, 9009, TX, USA 77381-5209, Rigaku Tokyo, 196-8666, Japan.
CrysAlisPro 1.171.38.41 (Rigaku Oxford Diffraction, 2015).

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