Allosteric modulation of Protein Arginine Methyltransferase 5 (PRMT5)

Rachel L. Palte*†, Sebastian E. Schneider*†, Michael D. Altman†, Robert P. Hayes§, Shuhei Kawamura∫, Brian M. Lacey∅, My Sam Mansueto∅, Michael Reutershan∫, Phieng Siliphaivanh∫, Christopher Sondey∅, Haiyan Xu∅, Zangwei Xu∅, Yingchun Ye∫, Michelle R. Machacek∫

† Computational and Structural Chemistry, ∫ Discovery Chemistry, ∅ Quantitative Biosciences, Merck & Co., Inc., 33 Avenue Louis Pasteur, Boston, Massachusetts, 02115, United States § Computational and Structural Chemistry, West Point, Pennsylvania, 19486, United States

Table of contents

Expression and Purification of PRMT5/MEP50 Complex for Biochemical Assay

Avi-tagged PRMT5 (2-637) and His-tagged MEP50 (2-342) were individually cloned into pBAC1 vectors for co-expression in *sf21* cells. 10 L of 2.8 x 10^6 cells/mL were coinfected with P2 BIICs (Baculovirus Infected Insect Cells) at 0.2 MOI in a 20 L wave bag, rocking at 28 rpm for 72 hrs post infection. Cells were pelleted at 3,400 x g for 10 min and frozen at -80°C until date of purification. 1.5 L of Lysis buffer (50 mM HEPES pH 7.3, 300 mL NaCl, 1 mM DTT, 20 mM Imidazole, 5% Glycerol, 1 tablet/ 50 mL Protease Inhibitor Tablets w/o EDTA and 50 units/mL Benzonase) was added to cell pellet and homogenized with a dounce homogenizer. Lysate was further homogenized with a microfluidizer (2 cycles at 17 kPsi). The resulting lysate was spun for 120 mins at 45,000 g and clarified lysate was loaded onto a 5 mL HisTrap FF Crude column using an AKTA System. Protein was eluted over a gradient with 50 mM HEPES pH 7.3, 300 mM NaCl, 20-300 mM Imidazole, 5% Glycerol and 1 mM DTT. Fractions containing PRMT5/MEP50 proteins were pooled and biotinylated with BirA (8:1 protein to BirA molar ratio) during dialysis into SEC buffer (18 hrs at 4°C). Greater than 90% biotinylation was observed by streptavidin gel shift assay. The biotinylated complex was further purified over a Superdex 200 26/60 column in SEC buffer (50 mM HEPES pH 7.3, 200 mM NaCl, 10% Glycerol and 5 mM DTT). It should be noted that PRMT5 and MEP50 coelute as a complex throughout the purification and eluted as a mono-dispersed symmetrical peak on the Superdex 200 column. Final PRMT5-MEP50 complex was >70 % pure and was concentrated and flash frozen.

PRMT5-MEP50 Enzyme Methylation Assay

PRMT5-MEP50 biochemical assay is a direct measurement of the methylation activity of the enzyme complex on a short peptide substrate derived from the N-terminus of H4 histone. Methylation experiment is performed with recombinant PRMT5-MEP50 protein complex. The assessment of inhibitory effect of small molecules is measured by the effectiveness of the compounds to inhibit this reaction (EC_{50}). In this assay, the potency (EC_{50}) of each compound was determined from a twenty-point (1:2 serial dilution; top compound concentration of 100000 nM) titration curve using the following outlined procedure. To each well of a white ProxiPlus 384 well-plate, 100 nL of compound (1% DMSO in final assay volume of 10 μL) was dispensed, followed by the addition of 8 μL of 1x assay buffer (50 mM Bicine pH 8.0, 1 mM DTT, 0.004% Tween20, 0.01% BSA) containing 1.25 nM of Full-length (FL)-PRMT5-MEP50 enzyme complex (recombinant proteins from baculovirus-transfected Sf21 cells: FL-PRMT5; MW = 73837 kDa and FL-MEP50; MW = 38614) and 1 μL of 150 μM S-(5'-Adenosyl)-L-Methionine Chloride (SAM). Plates were sealed and placed in a 37 ^{o}C humidified chamber for a 60 minutes pre-incubation with compound. Subsequently, each reaction was initiated by the addition of 1 μL 1x assay buffer containing 750 nM biotinylated H4R3(Me1) peptide. The final reaction in each well of 10 µL consists of 1.0 nM PRMT5-MEP50, 75 nM biotinylated-peptide, and 15 μM SAM. Methylation reactions were allowed to proceed for 150 minutes in a sealed plate at 37 ^{o}C. Reactions were immediately quenched by the addition of 1 μL of 5% formic acid. Plates were then frozen and shipped to SAMDITM Tech Inc. to determine the percent conversion from H4R3(Me1) to H4R3(Me2). Dose-response curves were generated by plotting percent effect (% product conversion; Y-axis) vs. Log10 compound concentrations (X-axis). EC_{50} values were determined by non-linear regression according to model for sigmoidal (4 parameters) dose-response curves.

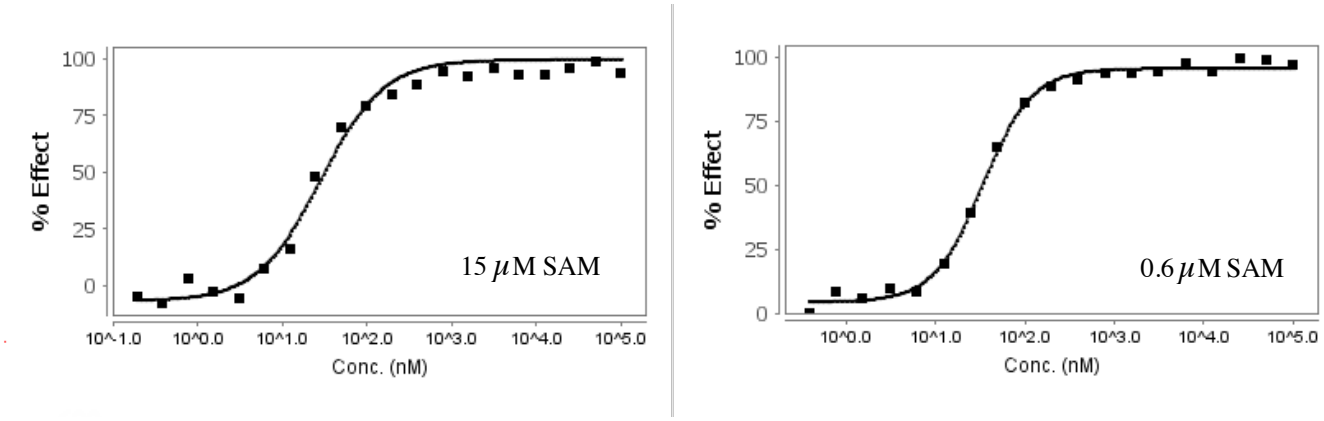


Fig S1. Percent inhibition calculated from biochemical PRMT5:MEP50 methylation assay with Compound 1a. Response curves from the biochemical enzyme methylation assay with Compound 1a with SAM at ~K_M and 25x K_M.

PRMT5 Cell Target Engagement (TE) Assay

The PRMT5 TE assay is a biomarker assay for identifying compounds that inhibit symmetric dimethylation of arginine (SDMA) of PRMT5 substrates. Specifically, symmetrically dimethylated nuclear proteins are detected using high content imaging technology. Detection of the expression of symmetrically dimethylated nuclear proteins is through a mixture of primary rabbit monoclonal antibodies to SDMA (CST 13222), which in turn recognized by an Alexafluor 488 dye-conjugated anti-rabbit IgG secondary antibody. The IN Cell Analyzer 2200 or Opera-Phenix measures nuclear Alexafluor 488 fluorescent dye intensity that is directly related to the level of expression of symmetrically dimethylated nuclear proteins at the single cell level. Nuclear AF488 dye intensities are compared to the mean value for DMSO treated cells (MIN) to report percent of inhibition for each compound-treated well.

In this assay, the cell potency (EC_{50}) of each compound was determined from a ten point (1:3 serial dilution; top compound concentration of 10000 nM) titration curve using the following outlined procedure. Each well of a BD falcon collagen coated black/clear bottom 384-well plate was seeded with 4000 MCF-7 cells in 30ul media and allowed to attach for 5 hours. Media is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, the following components were added to the base medium: 0.01 mg/ml human recombinant insulin; fetal bovine serum to a final concentration of 10%. Additional 30ul of media containing 2x compounds were added to each well. Cells were treated for 3 days in 37°C 5% CO_2 incubator. On day 3, cells were fixed with Cytofix, permeablized with 0.4% Triton-X-

100/Cytofix, and washed with D-PBS without Ca/Mg. Cells were blocked with Licor Odessey blocking reagent for 1hr at room temperature, followed by incubation with anti-SDMA (1:1000) antibody at 4°C overnight. 1° antibody was removed, followed by three washings with DPBS without Ca/Mg and 0.05% Tween20. Hoechst (5mg/ml), Cell Mask deep red stain (1:2000) and Alexa488-conjugated goat anti-rabbit IgG (2 μg/mL) was added for 1 hour at room temperature. A final washing step (three washes) was performed before sealing plate for imaging on In Cell Analyzer 2200 or Opera-Phenix. Images from analyzer were uploaded to Columbus (at WP or BOS) for image analysis. IC_{50} values were determined by 4 parameters robust fit of percent fluorescence units vs. (Log_{10}) compound concentrations.

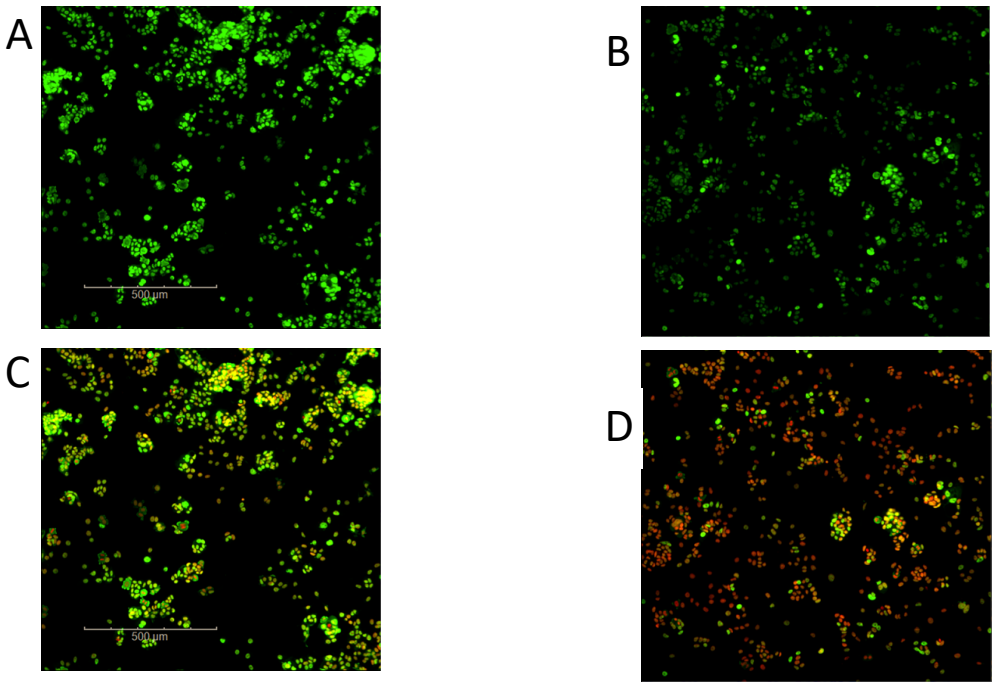
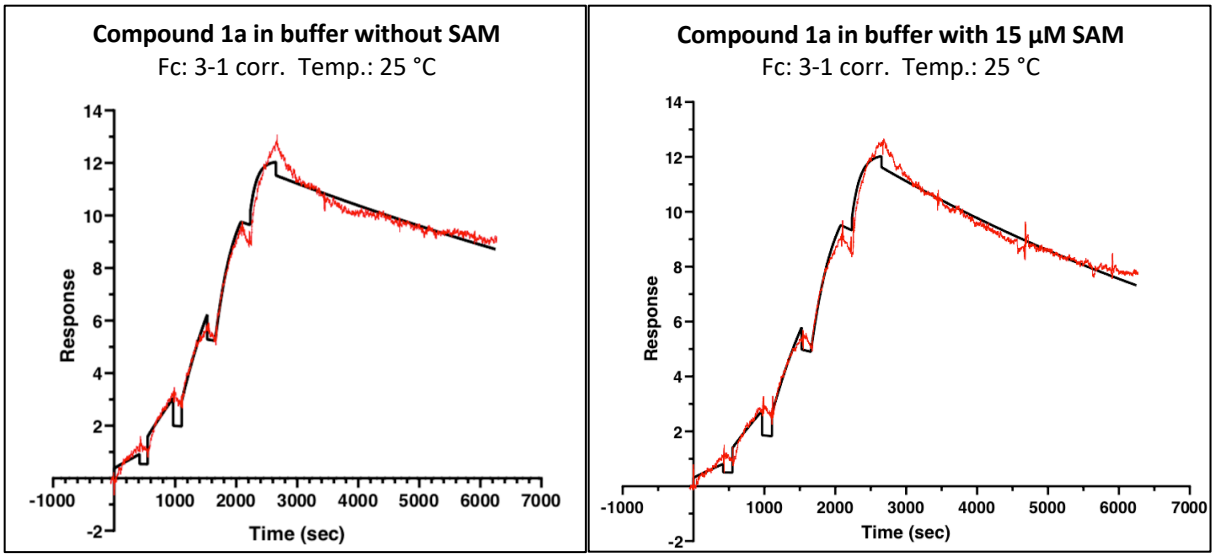


Figure S2. Expression of nuclear SDMA detected by high content imaging using SDMA antibody. MCF-7 cells were treated either with DMSO (A) or 10uM L-3934 (B). Secondary antibody was labeled with Alexa Fluor 488 dye (AF488) which is seen here as green fluorescence. These same cells were also stained with DAPI stain to show the presence of all nuclei. Images (C) and (D) are superimposed images the AF488-labeled cells (A) and (B), respectively, with the DAPI-stained cells, and the DAPI stain was given an artificial red color to more easily show the presence of all nuclei.

Surface Plasmon Resonance Binding Experiments

The Biacore T200 instrument was used to carry out surface plasmon resonance binding experiments. A biotinylated Human PRMT5:MEP50 complex was prepared in immobilization buffer (HBS-P+, GE Healthcare, supplemented with 5 mM dithiothreitol) and the ligand was flowed onto a Biacore Series S Sensor Chip SA, (following the manufacturer's general recommended conditions) to a final capture level of ~3500 RU onto channels 2-4. The sensor chip was then equilibrated in running buffer (immobilization buffer containing 3% v/v dimethyl sulfoxide) and compound binding experiments were carried out in this buffer. The compound interaction with PRMT5:MEP50 complex was tested on each channel (n = 3) using a 5-point, 3-fold dilution series (0.123-10uM) followed by a single dissociation step using single cycle kinetics mode (contact and dissociation times of 230 and 1800 sec, respectively). Resulting sensorgrams were solvent corrected and double reference subtracted (blank injection with reference flow cell). Conditions were repeated on a fresh Biacore Series S Sensor Chip SA in which 15 μM S-(5'-Adenosyl)-L-methionine (SAM) Chloride (American Radioligand Chemicals, Inc.) was included in the running buffer. The Biacore Evaluation software was used to fit the data to a 1:1 binding model.



Injection	Compound (nM)
Conc. 1	123.456
Conc. 2	370.370
Conc. 3	1111.111
Conc. 4	3333.333
Conc. 5	10000.000

compound	[SAM]	k_a (1/Ms)	k_d (1/s)	K_D (M)	K_D Fold change
1a	0 µM	930.5	7.8×10^{-5}	8.3×10^{-8}	1
1a	15 µM	860.4	12.9×10^{-5}	15.0×10^{-8}	1.8

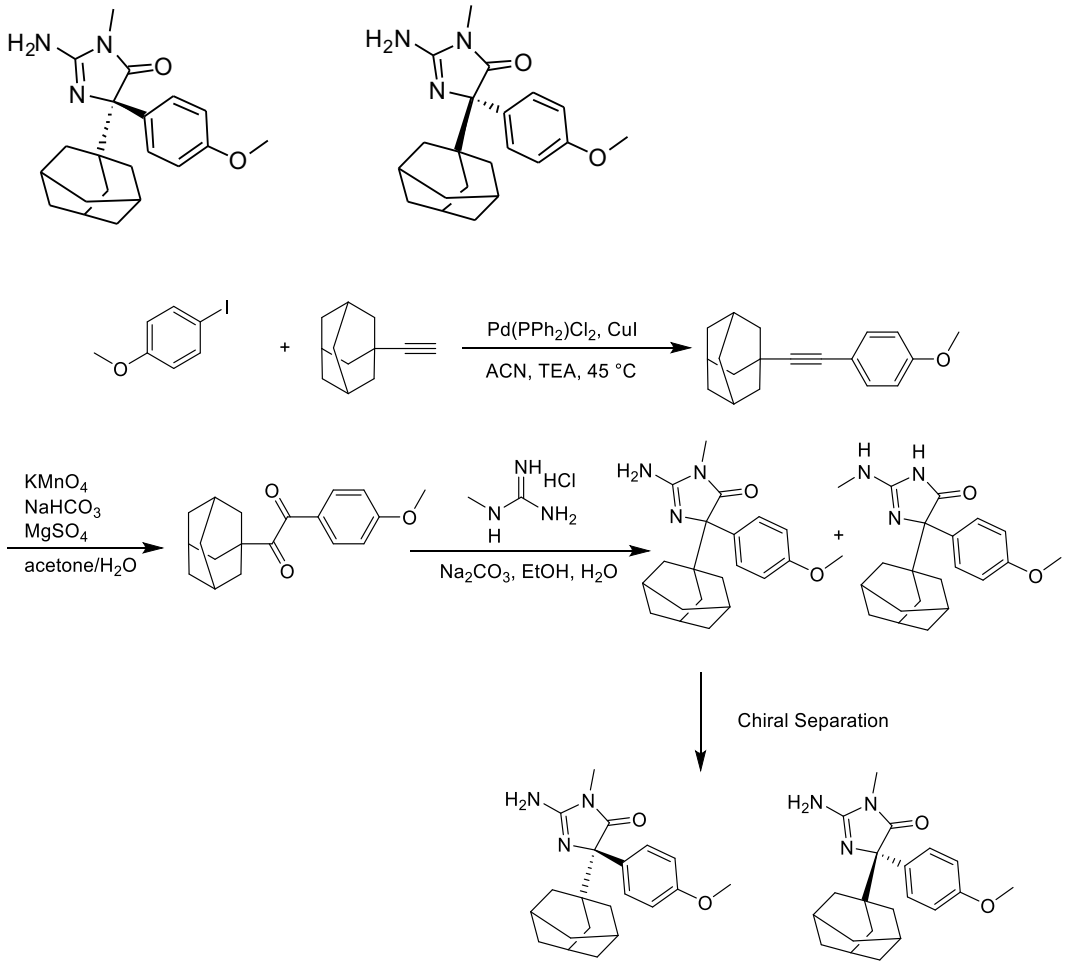
Figure S3. Single cycle kinetic SPR data for Compound 1a with and without SAM. Raw SPR data (red) is shown here with a 1:1 binding model overlaid (black). The concentration of compound 1a used at each injection are listed in the first table. The association rate constant (k_a), dissociation rate constant (k_d) and overall equilibrium dissociation constant (K_D) are shown in the table. The K_D values both in the presence and absence of SAM are within twofold, highlighting that the presence of SAM does not change the binding of Compound 1a. It should be noted that the 1:1 model does not optimally fit the experimental data and therefore these reported values are relative kinetic parameters.

Chemistry General Materials and Methods

All reagents and solvents were purchased from commercial sources and used as is without further purification. Reaction progress and synthetic intermediate analysis was assessed by LCMS (UV detection with ESI mass detection) when applicable using an Agilent or Shimadzu instrument with a MeCN/water gradient with either TFA or NH_4OH modifier. All reported yields are isolated yields. Silica gel and reverse-phase flash column chromatography were conducted with Teledyne ISCO CombiFlash or Biotage-Isolera One instruments and commercially available pre-packed columns. Reverse-phase preparative HPLC purification of final analogs was performed on a Gilson preparative HPLC instrument with UV detection using a MeCN/water gradient with either TFA or NH_4OH modifier. Chiral SFC separation was performed on Thar200 preparative SFC(SFC-10), Sepiatec Prep 100 or SFC80Q with solvent and modifier noted in the experimental. All reported compounds tested in the assays were ≥ 95% pure as determined by LCMS or HPLC analysis. ^1H NMR spectra were collected at room temperature. Chemical shifts are reported in ppm relative to the listed deuterated solvent, and multiplicities, coupling constants (where applicable), and signal integrations are listed parenthetically. Final compounds were isolated either as the TFA salts or the free base after purification. Safety statement: given the highly potent nature of the final compounds whose preparation is detailed in this section, and their demonstrated ability to inhibit PRMT5 *in vitro* and *in vivo*, proper procedures for the handling of these highly potent compounds should be followed at all times in accordance with institutional policies.

Synthetic procedures for representative compounds

Compound 1a: (5R)-5-(adamantan-1-yl)-2-amino-5-(4-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one and **Compound 1b: (5S)-5-(adamantan-1-yl)-2-amino-5-(4-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one**



Step 1: A mixture of 1-ethynyladamantane (250 mg, 1.56 mmol), 1-iodo-4-methoxybenzene (365 mg, 1.56 mmol), $Pd(PPh_3)_2Cl_2$ (44 mg, 0.06 mmol), copper(I) iodide (14.9 mg, 0.08 mmol) and Et_3N (3 ml, 21.5 mmol) in acetonitrile (3 ml) was degassed and backfilled with N_2 (three times). The mixture was stirred at 45 °C for 3 h. The mixture was cooled to room temperature and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (Pet. ether) to provide 1-((4-methoxyphenyl)ethynyl)adamantane (350 mg, 1.25 mmol, 80 % yield) as

colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.35 (m, 2H), 6.76-6.82 (m, 2H), 3.79 (s, 3H), 1.93-2.01 (m, 9H), 1.71 (br s, 6H).

Step 2: To a mixture 1-((4-methoxyphenyl)ethynyl)adamantane (2.2 g, 8.26 mmol) in acetone (30 mL) was added a solution of $NaHCO_3$ (0.41 g, 4.96 mmol) and $MgSO_4$ (1.49 g, 12.39 mmol) in H_2O (15 mL). $KMnO_4$ (3.92 g, 24.78 mmol) was then added in one portion. The resulting mixture was stirred at 30 °C for 16 h. Water (60 mL) was added and the mixture was extracted with EtOAc (30 mL × 3). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by flash silica gel chromatography (eluting with 8% ethyl acetate/petroleum ether) to give 1-(adamantan-1-yl)-2-(4-methoxyphenyl)ethane-1,2-dione (2.0 g, 73% yield) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75-7.79 (m, 2H), 6.96 (d, *J* = 8.77 Hz, 2H), 3.88 (s, 3H), 2.04 (s, 3H), 1.94-1.97 (m, 6H), 1.68-1.77 (m, 6H)

Step 3: A mixture of 1-(adamantan-1-yl)-2-(4-methoxyphenyl)ethane-1,2-dione (100 mg, 0.34 mmol) and 1-methylguanidine hydrochloride (184 mg, 1.68 mmol) in 1,4-Dioxane (1 mL) and ethanol (1mL) was stirred at 15 °C for 5 min, and then treated with an aqueous solution of sodium carbonate (178 mg, 1.68 mmol) in water (1.0 mL). The mixture was heated at 85° C with stirring for 3 h. The mixture was cooled to room temperature and concentrated *in vacuo*. The residue was purified by Prep-HPLC (YMC-Actus Pro C18 150*30 5u column, water (0.1%TFA)-MeCN (25%B- 55%B) to give 4-(adamantan-1-yl)-2-amino-4-(4-methoxyphenyl)-1-methyl-1H-imidazol-5(4H)-one (63.5 mg, 0.17 mmol, 51% yield) as white solid. (LCMS, ESI+) m/z Calcd for $[(C_{21}H_{27}N_3O_2)+H]^+$: 354, found, 354. ^{1}H NMR (400MHz, CD_3OD) δ = 7.50 (d, *J* = 8.8 Hz, 2H), 6.95 (d, *J* = 9.2 Hz, 2H), 3.80 (s, 3H), 3.17 (s, 3H), 1.99 (br s, 3H), 1.81 (br d, *J* = 11.8 Hz, 3H), 1.73 - 1.65 (m, 3H), 1.55 (br d, *J* = 11.8 Hz, 3H), 1.47 (br d, *J* = 11.8 Hz, 3H).

4-(adamantan-1-yl)-2-amino-4-(4-methoxyphenyl)-1-methyl-1H-imidazol-5(4H)-one was separated by chiral SFC (IC, 21x250 mm column, methanol + 0.25% dimethyl ethyl amine as modifier, 30% modifier in CO_2) to afford (5S)-5-(adamantan-1-yl)-2-amino-5-(4-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one (peak 1, Compound 1b) and (5R)-5-

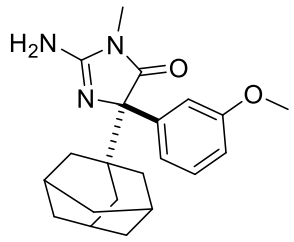
(adamantan-1-yl)-2-amino-5-(4-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one (peak 2, Compound 1a).

Compound 1a: $[\alpha]_D^{25}$ = +14.9° (methanol, c 1). (HRMS, ESI+) m/z Calcd. for $[(C_{21}H_{27}N_3O_2)+H]^+$: 354.2181, found 354.2182. ^{1}HNMR: (400 MHz, $CDCl_3$) δ: 7.59-7.64 (m, 2H), 6.82-6.87 (m, 2H), 5.31 (s, 1H), 3.80 (s, 3H), 3.01 (s, 3H), 1.95 (bs, 3H), 1.82 (bd, *J* = 11.6 Hz, 3H), 1.43-1.65 (m, 10H).

Compound 1b:

(LCMS, ESI+) m/z Calcd. for $[(C_{21}H_{27}N_3O_2)+H]^+$: 354, found 354. ^{1}HNMR: (400 MHz, $CDCl_3$) δ: 7.59-7.64 (m, 2H), 6.82-6.87 (m, 2H), 5.31 (s, 1H), 3.80 (s, 3H), 3.01 (s, 3H), 1.95 (s, 3H), 1.82 (bd, *J* = 10.8 Hz, 3H), 1.43-1.65 (m, 10H).

Compound 2: (5R)-5-(adamantan-1-yl)-2-amino-5-(3-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one

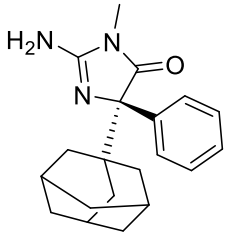


5-(adamantan-1-yl)-2-amino-5-(3-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one (58.9 mg, 0.162 mmol, 19% yield) was prepared in analogy to the procedures described for Compound 1a/1b, starting with 1-iodo-3-methoxybenzene and 1-ethynyladamantane. The racemic material was separated by chiral SFC (IC, 21x250 mm column, methanol + 0.25% dimethyl ethyl amine as modifier, 30% modifier in CO_2) to afford (5S)-5-(adamantan-1-yl)-2-amino-5-(3-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one (peak 1) and (5R)-5-(adamantan-1-yl)-2-amino-5-(3-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one (peak 2, Compound 2).

Compound 2: (HRMS, ESI+) m/z Calcd for $[(C_{21}H_{27}N_3O_2)+H]^+$, 354.2181, found, 354.2177. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.29 – 7.23 (m, 2H), 7.17 (t, *J* = 7.9 Hz, 1H), 6.79 (d, *J* = 8.1 Hz,

1H), 3.70 (s, 3H), 2.87 (s, 3H), 1.93 – 1.84 (m, 5H), 1.73 – 1.64 (m, 3H), 1.60 – 1.51 (m, 3H), 1.47 – 1.35 (m, 6H).

Compound 3: (5R)-5-(adamantan-1-yl)-2-amino-3-methyl-5-phenyl-3,5-dihydro-4H-imidazol-4-one



1-(adamantan-1-yl)-2-phenylethane-1,2-dione (706 mg, 2.2 mmol, 48 % yield) was prepared in analogy to Steps 1 and 2 of the procedures described for Compound 1a/1b, starting with iodobenzene and 1-ethynyladamantane.

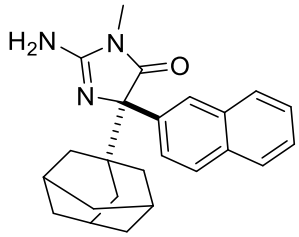
A mixture of 1-(adamantan-1-yl)-2-phenylethane-1,2-dione (200 mg, 0.745 mmol) and 1-methylguanidine hydrochloride (408 mg, 3.73 mmol) in 1,4-dioxane (2 ml) and ethanol (2 mL) was stirred at 28 °C for 5 min. Then, a solution of Na_2CO_3 (395 mg, 3.73 mmol) in water (2 mL) was added. The reaction was stirred at 85 °C for 5 h. The resulting mixture was cooled and then concentrated under vacuum. The resulting residue was purified by prep-HPLC (Xtimate C18 150*25mm*5um column, water (10mM NH_4HCO_3) : MeCN, 52%B – 82%B) to give 4-(adamantan-1-yl)-2-amino-1-methyl-4-phenyl-1H-imidazol-5(4H)-one (73.3 mg, 0.23 mmol, 30% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (d, *J* = 7.34 Hz, 2H), 7.17-7.31 (m, 3H), 6.39 (br s, 2H), 2.87 (s, 3H), 1.88 (br s, 3H), 1.70 (br d, *J* = 11.74 Hz, 3H), 1.56 (br d, *J* = 11.74 Hz, 3H), 1.42 (br d, *J* = 11.25 Hz, 6H). (LCMS ESI+) m/z Calcd. for $[(C_{20}H_{25}N_3O)+H]^+$, 324, found, 324.

Chiral SFC separation of 4-(adamantan-1-yl)-2-amino-1-methyl-4-phenyl-1H-imidazol-5(4H)-one (IC, 21x250 mm column, methanol +0.1% NH_3H_2O as modifier, 40% modifier in CO_2) afforded (5R)-5-(adamantan-1-yl)-2-amino-3-methyl-5-phenyl-3,5-dihydro-4H-imidazol-4-one

(peak 1, Compound 3) and (5S)-5-(adamantan-1-yl)-2-amino-3-methyl-5-phenyl-3,5-dihydro-4H-imidazol-4-one (peak 2).

Compound 3: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67 (br d, *J* = 7.45 Hz, 2H), 7.15-7.29 (m, 3H), 6.38 (br s, 2H), 2.85 (s, 3H), 1.85 (br s, 3H), 1.67 (br d, *J* = 11.40 Hz, 3H), 1.53 (br d, *J* = 11.84 Hz, 3H), 1.39 (br d, *J* = 11.40 Hz, 6H). (HRMS, ESI+) m/z Calcd for. for $[(C_{20}H_{25}N_3O)+H]^+$, 324.2076, found, 324.2081.

Compound 4: (5R)-5-(adamantan-1-yl)-2-amino-3-methyl-5-(naphthalen-2-yl)-3,5-dihydro-4H-imidazol-4-one



1-(adamantan-1-yl)-2-(naphthalen-2-yl)ethane-1,2-dione (73 mg, 0.19 mmol, 26 % yield) was prepared in analogy to Steps 1 and 2 of the procedures described for Compound 1a/1b, starting with 2-iodonaphthalene and 1-ethynyladamantane.

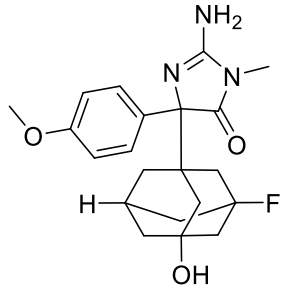
A mixture of 1-(adamantan-1-yl)-2-(naphthalen-2-yl)ethane-1,2-dione (240 mg, 0.754 mmol) in dioxane (2.2 mL) and EtOH (2.2 mL) was stirred at 25 °C for 15 min. 1-Methylguanidine hydrochloride (413 mg, 3.77 mmol) was added and stirred at 25 °C for 15 min. A solution of Na_2CO_3 (399.4 mg, 3.77 mmol) in water (2.2 mL) was added and the mixture was stirred at 85 °C for 12 h. The solution was cooled and concentrated under vacuum. The residue was purified by Prep-HPLC (Xtimate C18 150*25mm*5um column, water (10mM NH_4HCO_3) : MeCN, 60%B-90%B) to give 4-(adamantan-1-yl)-2-amino-1-methyl-4-(naphthalen-2-yl)-1H-imidazol-5(4H)-one (73 mg, 0.19 mmol, 26 % yield). ^{1}H NMR (400 MHz, *d6*-acetone) δ 8.28 (s, 1H), 7.99 (dd, *J* = 1.75, 8.77 Hz, 1H), 7.82-7.89 (m, 2H), 7.78 (d, *J* = 8.77 Hz, 1H), 7.43-7.50 (m, 2H), 3.02 (s, 3H), 1.83-1.94 (m, 6H), 1.55-1.65 (m, 6H), 1.46-1.53 (m, 3H). *Note:* Presumably, due to moisture

in *d6*-acetone, resonances for the two amino protons were not observed. (LCMS ESI+) m/z Calcd for $[(C_{24}H_{27}N_3O)+H]^+$, 374, found, 374.

Chiral SFC separation of 4-(adamantan-1-yl)-2-amino-1-methyl-4-(naphthalen-2-yl)-1H-imidazol-5(4H)-one ($CCOF_4$, 21 x 250, methanol 0.25% DMEA as modifier, 20% modifier in CO_2) afforded (5R)-5-(adamantan-1-yl)-2-amino-3-methyl-5-(naphthalen-2-yl)-3,5-dihydro-4H-imidazol-4-one (peak 1, Compound 4) and (5S)-5-(adamantan-1-yl)-2-amino-3-methyl-5-(naphthalen-2-yl)-3,5-dihydro-4H-imidazol-4-one (peak 2).

Compound 4: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.89 (d, J = 8.6 Hz, 1H), 7.87 – 7.82 (m, 2H), 7.79 (d, J = 8.7 Hz, 1H), 7.50 – 7.43 (m, 2H), 2.90 (s, 3H), 1.93 – 1.83 (m, 5H), 1.80 – 1.71 (m, 3H), 1.59 – 1.52 (m, 3H), 1.52 – 1.45 (m, 3H), 1.44 – 1.38 (m, 3H). (HRMS, ESI+) m/z Calcd. for $[(C_{24}H_{27}N_3O)+H]^+$, 374.2232, found, 374.2232.

Compound 5: 2-amino-5-(3-fluoro-5-hydroxyadamantan-1-yl)-5-(4-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one



Step 1:

A mixture of 3-ethynyl-5-fluoroadamantan-1-ol (310 mg, 1.596 mmol), 1-iodo-4-methoxybenzene (374 mg, 1.596 mmol), $Pd(PPh_3)_2Cl_2$ (44.8 mg, 0.064 mmol), copper(I) iodide (15.20 mg, 0.080 mmol) and triethyl amine (3 ml, 21.5 mmol) in acetonitrile (3 ml) was degassed and backfilled with N_2 (three times). The mixture was stirred at 45 °C for 3 hours and then concentrated under vacuum. The resulting residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 5/1 to 2/1) to give 3-fluoro-5-((4-methoxyphenyl)ethynyl)adamantan-1-ol (437 mg, 1.31 mmol, 82 % yield) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.34

(m, 2H), 6.81 (d, *J* = 8.77 Hz, 2H), 3.80 (s, 3H), 2.42 (td, *J* =2.96, 5.48 Hz, 1H), 2.03 (br s, 2H), 1.86-1.94 (m, 4H), 1.81 (br s, 2H), 1.75 (br s, 2H), 1.65 (br s, 2H).

Step 2:

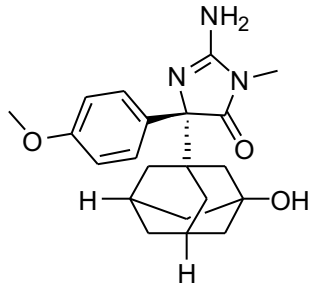
To a solution of 3-fluoro-5-((4-methoxyphenyl)ethynyl)adamantan-1-ol (437 mg, 1.46 mmol) in acetone (15 ml) was added a solution of $MgSO_4$ (263 mg, 2.18 mmol) and sodium bicarbonate (73.3 mg, 0.87 mmol) in water (8 ml), followed by the addition of $KMnO_4$ (690 mg, 4.36 mmol) in one portion. The reaction was stirred for 12 h at 30 °C. Water (50 mL) was added and the reaction mixture was extracted with EtOAc (20 mL x 3). The combined organic extracts were dried over $MgSO_4$, filtered, and the filtrate was concentrated under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 2/1) to give 1-(3-fluoro-5-hydroxyadamantan-1-yl)-2-(4-methoxyphenyl)ethane-1,2-dione (390 mg, 1.12 mmol, 77 % yield) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75-7.80 (m, 2H), 6.97 (d, *J* = 8.77 Hz, 2H), 3.89 (s, 3H), 2.47-2.54 (m, 1H), 2.03-2.06 (m, 2H), 1.88-1.95 (m, 4H), 1.77-1.85 (m, 4H), 1.67 (br d, *J* = 3.95 Hz, 2H).

Step 3:

A mixture of 1-(3-fluoro-5-hydroxyadamantan-1-yl)-2-(4-methoxyphenyl)ethane-1,2-dione (390 mg, 1.17 mmol) and 1-methylguanidine hydrochloride (643 mg, 5.87 mmol) in 1,4-dioxane (2.5 ml) and ethanol (2.5 mL) was stirred at 25 °C for 5 min. Then, a solution of Na_2CO_3 (622 mg, 5.87 mmol) in water (2.5 mL) was added. The reaction was stirred at 85 °C for 5 h. The mixture was then cooled and concentrated under vacuum. The residue was purified by prep-HPLC to give 2-amino-4-(3-fluoro-5-hydroxyadamantan-1-yl)-4-(4-methoxyphenyl)-1-methyl-1H-imidazol-5(4H)-one (133 mg, 0.34 mmol, 29 % yield) as a white solid.

Compound 5: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (d, *J* = 8.80 Hz, 2H), 6.86 (d, *J* = 8.80 Hz, 2H), 6.53 (br s, 2H), 4.75 (s, 1H), 3.73 (s, 3H), 2.89 (s, 3H), 2.22 (br s, 1H), 1.56-1.75 (m, 3H), 1.37-1.55 (m, 6H), 1.12-1.31 (m, 3H). (HRMS, ESI+) m/z Calcd. for $[(C_{21}H_{26}FN_3O_3)+H]^+$, 388.2036, found, 388.2037.

Compound 6: (5R)-2-amino-5-(3-hydroxyadamantan-1-yl)-5-(4-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one



Step 1:

A mixture of 3-ethynyladamantan-1-ol (160 mg, 0.908 mmol), 1-iodo-4-methoxybenzene (212 mg, 0.908 mmol), $Pd(PPh_3)_2Cl_2$ (25.5 mg, 0.036 mmol), copper(I) iodide (8.6 mg, 0.05 mmol) and triethyl amine (3 ml, 21.52 mmol) in acetonitrile (3 ml) was degassed and backfilled with N_2 (three times), the mixture was stirred at 45 °C) for 3 h. The mixture was then cooled to room temperature and concentrated under vacuum. The resulting residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 5/1 to 2/1) to give 3-((4-methoxyphenyl)ethynyl)adamantan-1-ol (210 mg, 0.71 mmol, 78 % yield) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.33 (m, 2H), 6.77-6.82 (m, 2H), 3.79 (s, 3H), 2.22 (br s, 2H), 1.89 (s, 2H), 1.84 (d, *J* = 1.76 Hz, 4H), 1.70 (d, *J* = 2.65 Hz, 4H), 1.62 (s, 2H).

Step 2:

To a solution of 3-((4-methoxyphenyl)ethynyl)adamantan-1-ol (210 mg, 0.74 mmol) in acetone (15 ml) was added a solution of $MgSO_4$ (134 mg, 1.12 mmol) and sodium bicarbonate (38 mg, 0.45 mmol) in water (8 ml), followed by the addition of $KMnO_4$ (353 mg, 2.23 mmol) in one portion. The reaction was stirred for 12 h at 30 °C. Water (50 mL) was added and the reaction mixture was extracted with ethyl acetate (20 mL x 3). The combined organic extracts were dried over $MgSO_4$, filtered, and the filtrate was concentrated under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 1/1) to give 1-(3-hydroxyadamantan-1-yl)-2-(4-methoxyphenyl)ethane-1,2-dione (150 mg, 0.43 mmol, 58 % yield)

as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74-7.79 (m, 2H), 6.96 (d, *J* = 9.21 Hz, 2H), 3.88 (s, 3H), 2.29 (br s, 2H), 1.80-1.91 (m, 6H), 1.64-1.75 (m, 6H)

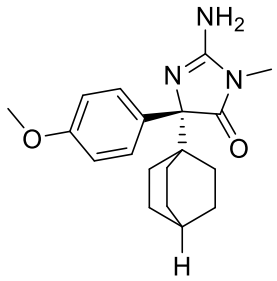
Step 3:

A mixture of 1-(3-hydroxyadamantan-1-yl)-2-(4-methoxyphenyl)ethane-1,2-dione (150 mg, 0.48 mmol) and 1-methylguanidine hydrochloride (261 mg, 2.39 mmol) in 1,4-dioxane (2 ml) and ethanol (2 mL) was stirred at 30 °C for 5 min. Then, a solution of Na_2CO_3 (253 mg, 2.386 mmol) in water (2 mL) was added. The reaction was stirred at 85 °C for 2 h. The mixture was then cooled to room temperature and concentrated *in vacuo*. The resulting residue was purified by prep-HPLC (Xtimate C18 150*25mm*5um column, water(10mM NH_4HCO_3):MeCN, 30%B- 50%B) to give 2-amino-4-(3-hydroxyadamantan-1-yl)-4-(4-methoxyphenyl)-1-methyl-1H-imidazol-5(4H)-one (85 mg, 0.23 mmol, 48 % yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 (d, *J*=8.77 Hz, 2H), 6.81 (d, *J*=8.77 Hz, 2H), 6.37 (br s, 1H), 4.31 (s, 1H), 3.70 (s, 3H), 2.85 (s, 3H), 2.00 (br s, 2H), 1.47-1.58 (m, 3H), 1.37-1.45 (m, 2H), 1.20-1.37 (m, 7H). (LCMS, ESI+) m/z Calcd. for $[(C_{21}H_{27}N_3O_3)+H]^+$, 370, found, 370.

Chiral SFC separation of 2-amino-4-(3-hydroxyadamantan-1-yl)-4-(4-methoxyphenyl)-1-methyl-1H-imidazol-5(4H)-one (IG, 21 x 250, 2-propanol with 0.25% DMEA as modifier, 45% modifier in CO_2) afforded (5R)-2-amino-5-(3-hydroxyadamantan-1-yl)-5-(4-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one (peak 2, Compound 6) and (5S)-2-amino-5-(3-hydroxyadamantan-1-yl)-5-(4-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one (peak 1).

Compound 6: ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.59 (d, *J* = 8.9 Hz, 2H), 6.84 (d, *J* = 8.9 Hz, 2H), 4.29 (s, 1H), 3.73 (s, 3H), 2.87 (s, 3H), 1.91 (s, 2H), 1.60 – 1.50 (m, 3H), 1.46 – 1.40 (m, 2H), 1.39 – 1.21 (m, 8H), 1.04 (d, *J* = 6.1 Hz, 1H). (HRMS, ESI+) m/z Calcd. for $[(C_{21}H_{27}N_3O_3)+H]^+$, 370.2130, found, 370.2130.

Compound 7: (R)-2-amino-5-(bicyclo[2.2.2]octan-1-yl)-5-(4-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one



Step 1:

A mixture of 1-ethynylbicyclo[2.2.2]octane (70 mg, 0.52 mmol), 1-iodo-4-methoxybenzene (122 mg, 0.52 mmol), $Pd(PPh_3)_2Cl_2$ (14.6 mg, 0.02 mmol), copper(I) iodide (5.0 mg, 0.026 mmol) and triethyl amine (3 ml, 21.5 mmol) in acetonitrile (3 ml) was degassed and backfilled with N_2 (three times). The mixture was stirred at 45 °C for 3 h, cooled to room temperature, and concentrated under vacuum. The resulting residue was purified by column chromatography on silica gel (Pet. ether/ethyl acetate = 10/1) to give 1-((4-methoxyphenyl)ethynyl)bicyclo[2.2.2]octane (50 mg, 0.20 mmol, 38 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 8.77 Hz, 2H), 6.78 (d, *J* = 8.77 Hz, 2H), 3.79 (s, 3H), 1.78 (br dd, *J* = 4.17, 10.74 Hz, 6H), 1.58-1.62 (m, 7H).

Step 2:

To a solution of 1-((4-methoxyphenyl)ethynyl)bicyclo[2.2.2]octane (50 mg, 0.21 mmol) in acetone (20 ml) was added a solution of $MgSO_4$ (37.6 mg, 0.31 mmol) and sodium bicarbonate (10.5 mg, 0.13 mmol) in water (10 ml), followed by the addition of $KMnO_4$ (99 mg, 0.624 mmol) in one portion. The reaction was stirred for 12 h at 30 °C. Water (50 mL) was added and the reaction mixture was extracted with ethyl acetate (20 mL x 3). The combined organic extracts were dried over $MgSO_4$, filtered, and the filtrate was concentrated under vacuum. The residue was purified by column chromatography on silica gel (Pet. ether/ethyl acetate = 15/1 to 5/1, v/v) to give 1-(bicyclo[2.2.2]octan-1-yl)-2-(4-methoxyphenyl)ethane-1,2-dione (40 mg, 0.14 mmol, 67 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74-7.78 (m, 2H), 6.95 (d, *J* = 9.21 Hz, 2H), 3.88 (s, 3H), 1.80-1.86 (m, 6H), 1.66 (dd, *J* = 3.07, 5.70 Hz, 1H), 1.60 (dt, *J* = 2.85, 7.78 Hz, 6H).

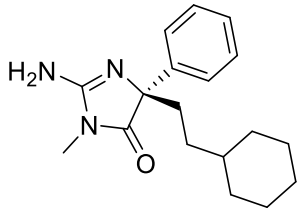
Step 3:

A mixture of 1-(bicyclo[2.2.2]octan-1-yl)-2-(4-methoxyphenyl)ethane-1,2-dione (40 mg, 0.15 mmol) and 1-methylguanidine hydrochloride (80 mg, 0.73 mmol) in 1,4-dioxane (2 ml) and ethanol (2 mL) was stirred at 30 °C for 5 min. Then, a solution of Na_2CO_3 (78 mg, 0.73 mmol) in water (2 mL) was added. The reaction was stirred at 85 °C for 5 h. The mixture was cooled to room temperature and concentrated under vacuum. The resulting residue was purified by pre-HPLC (Xtimate C18 150*25mm*5um column, water(10mM NH_4HCO_3):MeCN, 50%B – 80%B) to give 2-amino-4-(bicyclo[2.2.2]octan-1-yl)-4-(4-methoxyphenyl)-1-methyl-1H-imidazol-5(4H)-one (31 mg, 0.09 mmol, 62 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (br d, *J* = 8.33 Hz, 2H), 6.79 (br d, *J* = 8.33 Hz, 2H), 6.31 (br s, 2H), 3.69 (s, 3H), 2.84 (s, 3H), 1.52 (br d, *J* = 10.52 Hz, 3H), 1.18-1.44 (m, 10H). (LCMS, ESI+) m/z Calcd. for $[(C_{19}H_{25}N_3O_2)+H]^+$, 328, found 328.

Chiral SFC separation of 2-amino-4-(bicyclo[2.2.2]octan-1-yl)-4-(4-methoxyphenyl)-1-methyl-1H-imidazol-5(4H)-one ($CCOF_4$, 21 x 250, methanol 0.25% DMEA as modifier, 20% modifier in CO_2) afforded (R)-2-amino-5-(bicyclo[2.2.2]octan-1-yl)-5-(4-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one (peak 1, Compound 7) and (S)-2-amino-5-(bicyclo[2.2.2]octan-1-yl)-5-(4-methoxyphenyl)-3-methyl-3,5-dihydro-4H-imidazol-4-one (peak 2).

Compound 7: ^{1}H NMR (499 MHz, DMSO-d_6) δ 7.56 (d, *J* = 8.6 Hz, 2H), 6.81 (d, *J* = 8.6 Hz, 2H), 3.71 (s, 3H), 2.85 (s, 3H), 1.89 (s, 3H), 1.61 – 1.50 (m, 3H), 1.44 – 1.34 (m, 6H), 1.30 – 1.22 (m, 3H). (HRMS, ESI+) m/z Calcd. for $[(C_{19}H_{25}N_3O_2)+H]^+$, 328.2025, found, 328.2026.

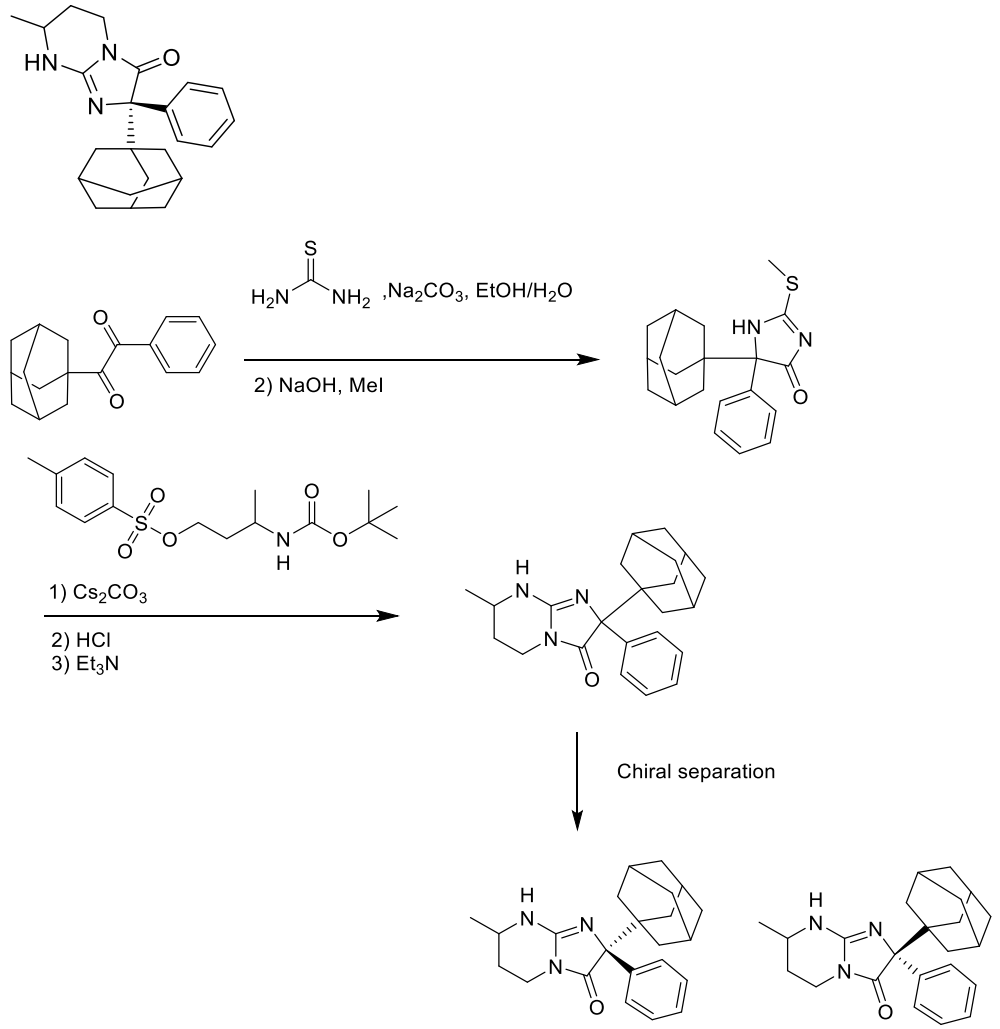
Compound 8: (R)-2-amino-5-(2-cyclohexylethyl)-3-methyl-5-phenyl-3,5-dihydro-4H-imidazol-4-one



5-(2-cyclohexylethyl)-2-imino-3-methyl-5-phenylimidazolidin-4-one was prepared in analogy to the procedures described in Method C in patent WO2005058311(A1) to provide 5-(3-bromophenyl)-5-(2-cyclohexylethyl)-2-imino-3-methylimidazolidin-4-one (200 mg, 0.529 mmol) which was then reduced using Pd/C (10%, 20 mg) catalyst in MeOH (38 mL) under H_2 atmosphere overnight. The resulting mixture was filtered through celite, concentrated *in vacuo,* and treated with HCl/ether to provide racemic 2-amino-5-(2-cyclohexylethyl)-3-methyl-5-phenyl-3,5-dihydro-4H-imidazol-4-one as a HCl salt. The resulting racemic material was purified by chiral SFC (AD-H column, IPA/CO_2 with 0.25% DMEA modifier) to provide (R)-2-amino-5-(2-cyclohexylethyl)-3-methyl-5-phenyl-3,5-dihydro-4H-imidazol-4-one (peak 1, Compound 8) and (S)-2-amino-5-(2-cyclohexylethyl)-3-methyl-5-phenyl-3,5-dihydro-4H-imidazol-4-one (peak 2).

Compound 8: ^{1}H NMR (600 MHz, DMSO-d6) δ 7.55-7.52 (m, 2H), 7.32-7.28 (m, 2H), 7.24-7.20 (m, 1H), 6.61-6.28 (m, 2H), 2.89 (s, 3H), 1.89-1.76 (m, 2H), 1.63-1.55 (m, 4H), 1.19-1.04 (m, 5H), 1.02-0.93 (m, 2H), 0.81-0.73 (m, 2H). (HRMS, ESI+) m/z Calcd. for $[(C_{18}H_{25}N_3O)+H]^+$ 300.2076, found, 300.2082.

Compound 9: (2R)-2-(adamantan-1-yl)-7-methyl-2-phenyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrimidin-3(2H)-one



Step 1:

To a solution of 1-(adamantan-1-yl)-2-phenylethane-1,2-dione (690 mg, 2.31 mmol) in dioxane (6 mL) and EtOH (6 mL) was added thiourea (1.3 g, 11.56 mmol), the mixture was stirred at 28 °C for 15 min. Then a solution of Na_2CO_3 (1.23 g, 11.56 mmol) in water (6 mL) was added and the mixture was stirred at 85 °C for 12 h. The resulting solution was cooled to room temperature and concentrated under vacuum. Water (15 mL) was added, and the mixture stirred at 28 °C for 30 min and filtered. The filter cake solid was dried *in vacuo* to afford 5-(adamantan-1-yl)-5-phenyl-2-

thioxoimidazolidin-4-one (810 mg, quantitative yield) as a white solid, which was used directly in next step without further purification.

Step 2:

To a mixture of 5-(adamantan-1-yl)-5-phenyl-2-thioxoimidazolidin-4-one (800 mg, 2.45 mmol) and NaOH (98 mg, 2.45 mmol) in THF (5 ml), MeOH (5mL) and water (5.0 ml) was added iodomethane (1.17 g, 8.24 mmol). The resulting mixture was stirred at 25 °C for 12 h and then concentrated under reduced pressure. Water (15 mL) was added and the resulting mixture was stirred at 25 °C for 30 min and filtered. The filter cake solid was collected and dried *in vacuo* to afford 4-(adamantan-1-yl)-2-(methylthio)-4-phenyl-1H-imidazol-5(4H)-one (700 mg, 1.95 mmol, 80 % yield) as a white solid, which was used directly in next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.19 (br s, 1H), 7.66 (br s, 2H), 7.14-7.43 (m, 3H), 2.53 (s, 3H), 1.88 (br s, 3H), 1.30-1.79 (m, 12H)

Step 3:

A solution of 4-(adamantan-1-yl)-2-(methylthio)-4-phenyl-1H-imidazol-5(4H)-one (200 mg, 0.59 mmol), 3-((tert-butoxycarbonyl)amino)butyl 4-methylbenzenesulfonate (303 mg, 0.88 mmol) and Cs_2CO_3 (574 mg, 1.76 mmol) in DMF (4 ml) was stirred at 80 °C for 12 h. The mixture was then diluted with water (60 mL) and extracted with ethyl acetate (30 mL x 5). The combined organic layers were washed with brine (30 mL), dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by flash silica gel chromatography (ISCO®; 4 g SepaFlash® Silica Flash Column, eluent of [0~8]% ethyl acetate/petroleum ether gradient @ 45 mL/min) to give tert-butyl (4-(4-(adamantan-1-yl)-2-(methylthio)-5-oxo-4-phenyl-4,5-dihydro-1H-imidazol-1-yl)butan-2-yl)carbamate (200 mg, 0.32 mmol, 55 % yield) as a colorless oil. (LCMS, ESI+) m/z Calcd. for $[(C_{29}H_{41}N_3O_3S)+H]^+$ 512, found, 512.

Step 4:

A mixture of tert-butyl (4-(4-(adamantan-1-yl)-2-(methylthio)-5-oxo-4-phenyl-4,5-dihydro-1H-imidazol-1-yl)butan-2-yl)carbamate (200 mg, 0.39 mmol) in HCl/dioxane (4 M) (10 ml) was stirred at 20 °C for 2 h. The mixture was concentrated to give crude 4-(adamantan-1-yl)-1-(3-aminobutyl)-2-(methylthio)-4-phenyl-1H-imidazol-5(4H)-one (170 mg, 0.41 mmol) as a yellow

oil, which was used in next step directly. (LCMS, ESI+) m/z Calcd. for $[(C_{24}H_{33}N_3OS)+H]^+$ 412, found, 412.

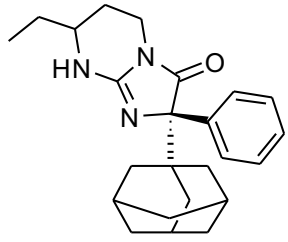
Step 5:

A mixture of 4-(adamantan-1-yl)-1-(3-aminobutyl)-2-(methylthio)-4-phenyl-1H-imidazol-5(4H)-one (170 mg, 0.413 mmol) and triethyl amine (0.576 ml, 4.13 mmol) in MeOH (10 ml) was stirred at 85 °C for 12 h. The mixture was concentrated and purified by Prep-HPLC (Waters XSELECT C18 150*30mm*5um column, water(0.1%TFA):MeCN 30%B-60%B) to give 2-(adamantan-1-yl)-7-methyl-2-phenyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrimidin-3(2H)-one (50 mg, 0.14 mmol, 33 % yield) as a white solid. (LCMS, ESI+) m/z Calcd. for $[(C_{23}H_{29}N_3O)+H]^+$ 364, found, 364.

2-(adamantan-1-yl)-7-methyl-2-phenyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrimidin-3(2H)-one (50 mg, 0.138 mmol) was further purified by SFC (DAICEL CHIRALPAK IC(250mm*30mm,10um) column, IPA, 0.1% NH_3H_2O as modifier, 45% modified in CO_2) to give (2R)-2-(adamantan-1-yl)-7-methyl-2-phenyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrimidin-3(2H)-one (peak 1, Compound 9) and (2S)-2-(adamantan-1-yl)-7-methyl-2-phenyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrimidin-3(2H)-one (peak 2).

Compound 9: ^{1}H NMR (400 MHz, METHANOL-d_4) δ 7.69 (br d, *J* = 7.02 Hz, 2H), 7.18-7.31 (m, 3H), 3.65 (td, *J* = 4.66, 12.61 Hz, 1H), 3.47-3.56 (m, 1H), 1.95-2.05 (m, 1H), 1.91 (br s, 3H), 1.82 (br d, *J* = 11.84 Hz, 3H), 1.61-1.69 (m, 3H), 1.46-1.57 (m, 7H), 1.24-1.37 (m, 1H), 1.22 (d, *J* = 6.14 Hz, 3H). (HRMS, ESI+) m/z Calcd. for $[(C_{23}H_{29}N_3O)+H]^+$: 364.2389, found 364.2397.

Compound 10: (2R)-2-(adamantan-1-yl)-7-ethyl-2-phenyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrimidin-3(2H)-one



Step 1:

A solution of 4-(adamantan-1-yl)-2-(methylthio)-4-phenyl-1H-imidazol-5(4H)-one (200 mg, 0.59 mmol), 3-((tert-butoxycarbonyl)amino)pentyl 4-methylbenzenesulfonate (252 mg, 0.71 mmol) and Cs_2CO_3 (574 mg, 1.76 mmol) in DMF (3 ml) was stirred at 80 °C for 12 h. The mixture was diluted with water (60 mL) and extracted with EtOAc (30 mL x 5). The combined organic layers were washed with brine (30 mL), dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated under vacuum to give tert-butyl (1-(4-(adamantan-1-yl)-2-(methylthio)-5-oxo-4-phenyl-4,5-dihydro-1H-imidazol-1-yl)pentan-3-yl)carbamate (300 mg, 0.57 mmol), which was used in next step directly. (LCMS, ESI+) m/z Calcd. for $[(C_{30}H_{43}N_3O_3S)+H]^+$:526, found 526.

Step 2:

A solution of tert-butyl (1-(4-(adamantan-1-yl)-2-(methylthio)-5-oxo-4-phenyl-4,5-dihydro-1H-imidazol-1-yl)pentan-3-yl)carbamate (300 mg, 0.57 mmol) in HCl/EtOAc (4M) (5 ml) was stirred at 15 °C for 1.5 hours. The mixture was concentrated to give 4-(adamantan-1-yl)-1-(3-aminopentyl)-2-(methylthio)-4-phenyl-1H-imidazol-5(4H)-one (250 mg, 0.587 mmol), which was used in next step directly. (LCMS, ESI+) m/z Calcd. for $[(C_{25}H_{35}N_3OS)+H]^+$: 426, found 426.

Step 3:

A mixture of 4-(adamantan-1-yl)-1-(3-aminopentyl)-2-(methylthio)-4-phenyl-1H-imidazol-5(4H)-one (300 mg, 0.71 mmol) and TEA (0.98 ml, 7.1 mmol) in MeOH (10 ml) was stirred at 85 °C for 12 h. The mixture was cooled to room temperature and concentrated under vacuum. The resulting residue was purified by Prep-HPLC (Waters XSELECT C18 150*30mm*5um column, water(0.1%TFA):MeCN 30%B-60%B) to give 2-(adamantan-1-yl)-7-ethyl-2-phenyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrimidin-3(2H)-one (100 mg, 0.26 mmol, 37 % yield) as a white solid. (LCMS, ESI+) m/z Calcd. for $[(C_{24}H_{31}N_3O)+H]^+$: 378, found 378.

Chiral SFC separation of 2-(adamantan-1-yl)-7-ethyl-2-phenyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrimidin-3(2H)-one (DAICEL CHIRALCEL OD-H (250mm*30mm, 5 um) column, EtOH, 0.1% NH_3H_2O as modifier, 40% modifier in CO_2) afforded (2R)-2-(adamantan-1-yl)-7-ethyl-2-phenyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrimidin-3(2H)-one (peak 1, Compound 10) and (2S)-2-(adamantan-1-yl)-7-ethyl-2-phenyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrimidin-3(2H)-one (peak 2).

Compound 10: ^{1}H NMR (400 MHz, DMSO-d6) δ 7.65-7.67 (m, 2H), 7.21-7.38 (m, 3H), 3.51-3.54 (m, 1H), 3.2 (br, 2H), 1.87 (br, 4H), 1.68-1.71 (m, 4H), 1.43-1.56 (m, 4H), 1.33-1.43 (m, 8H), 0.85 (t, *J* = 7.2Hz, 3H). (HRMS, ESI+) m/z Calcd. for $[(C_{24}H_{31}N_3O)+H]^+$: 378.2545, found 378.2550.

Cloning, Expression, and Purification of Protein for Crystallography

Human PRMT5 (amino acids 2-637) with an N-terminal Flag tag and MEP50 (amino acids 2-342) with an N-terminal 8x-histidine tag was cloned into a pFastBac vector and used to transform High Five™ (BTI-TN-5B1-4) competent cells (ThermoFisher). Cells were grown in Express Five SFM media (Gibco) to a density of $1.5x10^6$ at 27℃ for 48hrs with an infected ratio ofPRMT5/MEP50: 1:200/1:50 (v/v). Harvested cells were sonicated by first resuspending the pellet in buffer A (50 mM Tris-HCl, 500 mM NaCl, 100 mM Glu, 100 mM Arg, 1 mM TCEP, pH 8.0) with a ratio of 6 mL per gram of pellet, followed by sonication at 200W for 3 s on and 3 s off for 20 minutes. Lysate was centrifuged at 13,500rpm for 30 min at 4°C and supernatant was collected. Clarified lysate in buffer B (50 mM Tris-HCl, 500 mM NaCl, 100 mM Glu, 100 mM Arg, 1 mM TCEP, pH 8.0) was IMAC-purified using pre-equilibrated Ni-NTA resin (Qiagen) and washed with buffers C and D until no protein was detected in flow through (Buffer C: 50 mM Tris-HCl, 250 mM NaCl, 1 mM TCEP, pH 8.0, 20 mM imidazole; Buffer D: Buffer C with 50 mM imidazole).PRMT5:MEP50 protein was eluted with buffer E (Buffer C with 250 mM imidazole). Eluate was pooled and dialyzed against Buffer F (20 mM Tris, 150 mM NaCl, 5% (v/v) glycerol, pH 8.0) at 4°C overnight. Protein was further purified via Flag column chromatography using a pre-equilibrated (Buffer F) ANTI-FLAG M2 affinity gel column (Sigma-Aldrich). Column was eluted with buffer G (200 μg/ml peptide, 20 mM Tris, 150 mM NaCl, 5% glycerol, pH 7.8) until no protein was detected in flow-through, followed by a column wash in buffer H (0.1 M glycine-HCl, pH 3.5). The FLAG column eluate was concentrated and centrifuged at 13,500 rpm for 10 min at 4°C. The supernatant was then loaded onto a Superdex 200 column (GE Healthcare), which was preequilibrated with buffer I (10 mM Hepes, 150mM NaCl, 1 mM TCEP, 10%(v/v) glycerol, pH 8.0) and run at a flow rate of 1.0 mL/min. Final purified protein was concentrated to approximately 15 mg/mL, centrifuged at 13,500 rpm for 10 min at 4°C, and the supernatant was aliquoted, flash frozen, and stored at -80°C until utilized in crystallography studies.

Crystallization and Structural Analysis

X-ray diffraction-quality crystals of the PRMT5:MEP50 protein complex were obtained by hanging drop vapor diffusion at 18°C by mixing a 1:1 ratio of the protein solution (15 mg/mL preincubated with 2 mM SAM and 2 mM compound at 4°C for 3hr, centrifuged for 30 min at 13,000 rpm) and a precipitant solution containing 0.1 M sodium citrate pH 6.0, 0.2 M sodium acetate, 10-12% PEG 4000, and streak seeding all drops. Crystals were seen after approximately 24 hours.

These crystals diffracted to nominal resolutions of 2.6-2.7 Å and belonged to the space group *I*222 with one PRMT5:MEP50 dimer in the asymmetric unit and the following approximate unit cell dimensions: a = 102 Å, b = 139 Å, and c = 178 Å. Crystals were transferred to cryoprotectant containing 0.1 M sodium citrate pH 6.0, 0.2 M sodium acetate, 10-12% PEG 4000, and 20% ethylene glycol prior to being harvested and plunged into LN2 prior to synchrotron data collection. X-ray data sets were collected at Shanghai Synchrotron Radiation Facility Beamline BL19U1 (**Compound 1a**) and Canadian Macromolecular Crystallography Facility Beamline CMCF-ID (08ID-1) at the Canadian Light Source (**Compound 8**), using Pilatus 6M detectors (Dectris). Data were processed using HKL3000[1], refined using Refmac5[2,3] and autoBUSTER[4], with manual compound building using Coot[5], compound geometrical restraints prepared using grade[6], and figures prepared using PyMOL[7]. Relevant X-ray data collection and refinement statistics are listed in Table S1.

Part of the research described in this paper was performed at the Canadian Light Source, a national research facility of the University of Saskatchewan, which is supported by the Canada Foundation for Innovation (CFI), the Natural Sciences and Engineering Research Council (NSERC), the National Research Council (NRC), the Canadian Institutes of Health Research (CIHR), the Government of Saskatchewan, and the University of Saskatchewan.

Table S1. Crystal Data Collection and Refinement Statistics Table

PDB code	6UXX	6UXY
Compound name	**Compound 1a**	**Compound 8**
Data collection		
Space group	I222	I222
Cell dimensions a, b, c (Å)	103.1, 138.8, 178.6	101.8, 139.2, 179.4
Resolution (Å)	50.00-2.69 (2.79-2.69)*	60.61-2.35 (2.41-2.35)
Rmerge	0.07 (0.65)	0.09 (1.51)
I/σI	23.9 (2.2)	15.5 (1.9)
Completeness (%)	99.0 (94.4)	99.9 (99.8)
Redundancy	6.4 (5.6)	13.5 (13.4)
Refinement		
Resolution (Å)	36.5-2.7	46.5-2.6
No. reflections	35526	41318
Rwork / Rfree	0.22/0.27	0.21/0.26
No. atoms		
Protein	7272	7312
Ligand	26	22
Solvent	57	241
B-factors		
Protein (Å2)	91.6	83.0
Ligands (Å2)	64.4	56.0
Solvent (Å2)	71.1	71.5
R.m.s. deviations		
Bond lengths (Å)	0.010	0.010
Bond angles (°)	1.18	0.88

*Values in parentheses are for highest-resolution shell

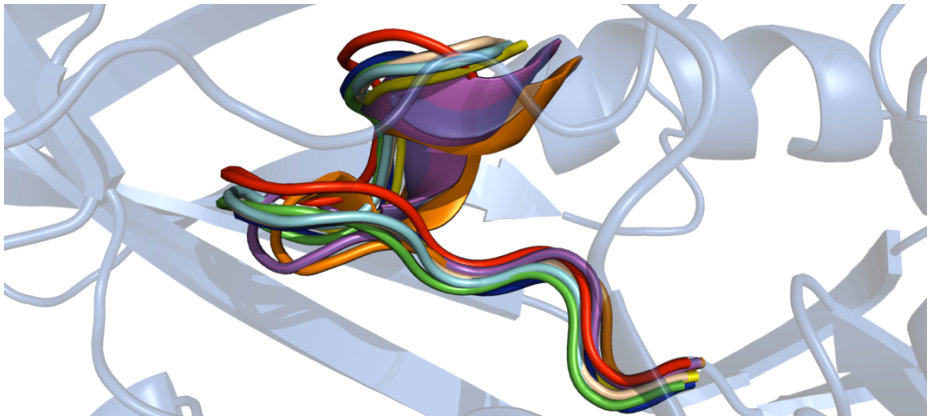


Figure S4. A cartoon overlay of PRMT1 (3q7e, red), PRMT2 (5jmq, green), PRMT3 (4hsg, blue), PRMT4 (5ih3, yellow), PRMT5 (6ckc, magenta), PRMT6 (5egs, cyan), PRMT7 (5eku, orange), PRMT8 (5dst, wheat) highlight the conserved structure of the 12-residue loop in the orthosteric conformation.

```
PRMT5       SELLGSFADNEL 12
PRMT7       SEIFGTMMLGES 12
PRMT4       SEPMGYMLFNER 12
PRMT2       SEWMGTCLLFEF 12
PRMT1       SEWMGYCLFYES 12
PRMT8       SEWMGYCLFYES 12
PRMT3       SEWMGYFLLFES 12
PRMT6       SEWMGYGLLHES 12
            ** :*      *
```

Figure S5. Alignment of the loop residues for each PRMT with a known public structure, listed in order of highest sequence identity to PRMT5.

Table S2. Cα RMSD values for the 12 residue loop

	PRMT1	PRMT2	PRMT3	PRMT4	PRMT5	PRMT6	PRMT7	PRMT8
PRMT1	0.00	1.33	1.27	1.49	1.98	1.25	2.67	1.22
PRMT2	1.33	0.00	1.22	0.95	1.60	0.87	2.31	0.53
PRMT3	1.27	1.22	0.00	1.20	1.57	1.56	2.11	1.34
PRMT4	1.49	0.95	1.20	0.00	1.03	1.12	1.70	0.77
PRMT5	1.98	1.60	1.57	1.03	0.00	1.70	1.04	1.42
PRMT6	1.25	0.87	1.56	1.12	1.70	0.00	2.37	0.57
PRMT7	2.67	2.31	2.11	1.70	1.04	2.37	0.00	2.14
PRMT8	1.22	0.53	1.34	0.77	1.42	0.57	2.14	0.00

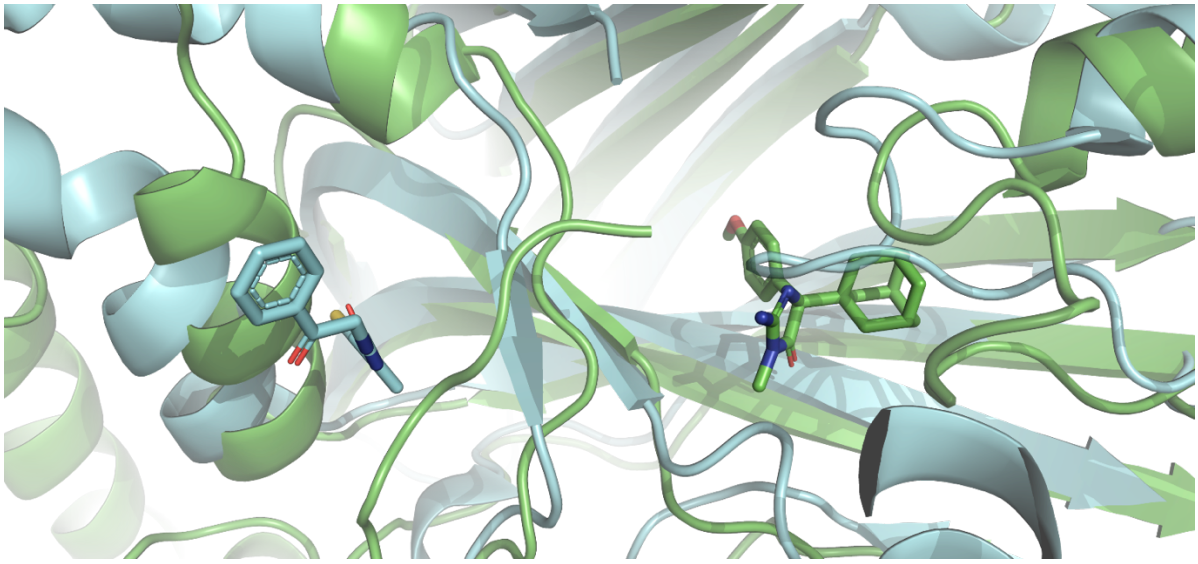


Figure S6. Overlay of PRMT3 and PRMT5 crystal structures. The PRMT3 allosteric inhibitor (PDB 4shg) is shown in cyan, the allosteric PRMT5 inhibitor is shown in green (PDB 6uxx). The allosteric inhibitors for these two PRMT subtypes bind to different pockets.

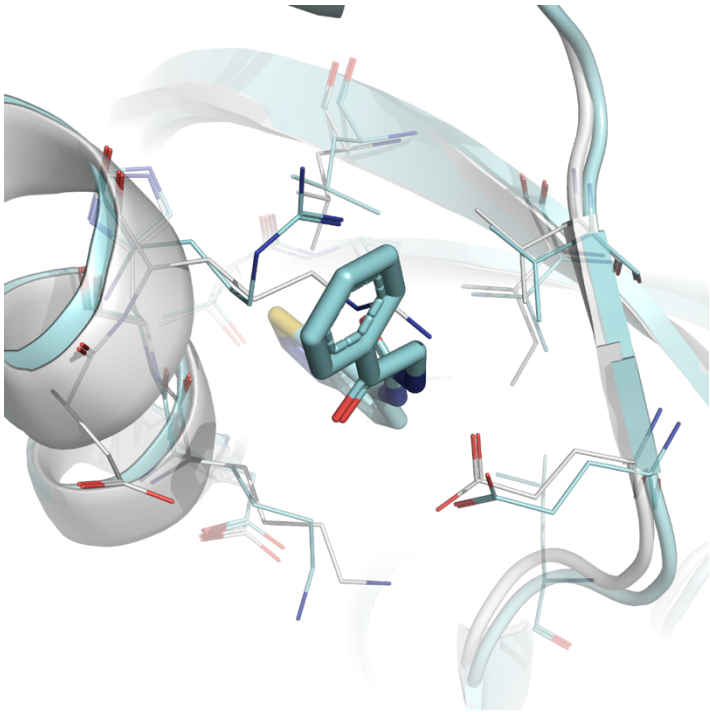
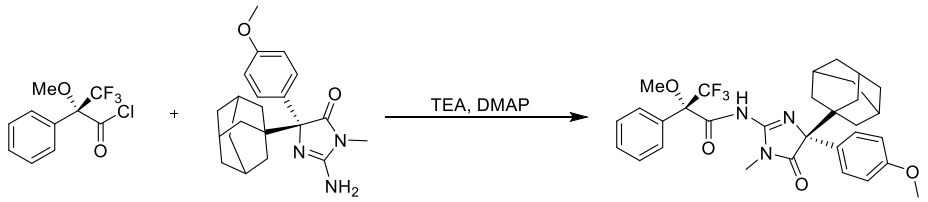
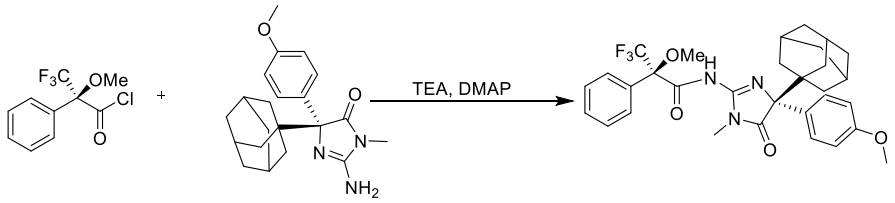


Figure S7. Binding site of the allosteric PRMT3 compound. Overlay of two PRMT3 crystal structures, bound with an allosteric inhibitor (cyan, PDB 4shg) and without (gray, PDB 1f3l). Binding of the allosteric inhibitor results in adjustment of the sidechains in this pocket but does not result in major backbone movement.

Synthetic Procedures and Characterization Data for Mosher Amides of Compound 1a



(2S)-N-((4R)-4-(adamantan-1-yl)-4-(4-methoxyphenyl)-1-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide: TEA (13 μl, 0.09 mmol), DMAP (1.2 mg, 10 μmol), and (R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride 8.6 μl, 0.05 mmol) were added to a solution of **Compound 1a** (10.8 mg, 0.03 mmol) in $CDCl_3$ (611 μl). The reaction mixture was stirred at room temperature for 6 hours and then left in the refrigerator overnight. The resulting solution was used for NMR characterization directly. (LCMS, ESI+) m/z Calcd. for $[(C_{31}H_{34}F_3N_3O_4)+H]^+$, 570, found, 570.



(2R)-N-((4R)-4-(adamantan-1-yl)-4-(4-methoxyphenyl)-1-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide: TEA (12 μl, 0.08 mmol), DMAP (1.6 mg, 0.01 mmol) and (S)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride 7.8 μl, 0.04 mmol) were added to a solution of **Compound 1a** (9.8 mg, 0.03 mmol) in $CDCl_3$ (277 μl). The reaction mixture was stirred at room temperature for 2 hours and then used for NMR characterization directly. (LCMS, ESI+) m/z Calcd. for $[(C_{31}H_{34}F_3N_3O_4)+H]^+$, 570, found, 570.

^{1}H, ^{13}C, PS-HSQC, HMBC, and COSY were performed at 25 °C at 500 MHz on the crude reaction mixtures in $CDCl_3$ and were utilized to fully assign both ^{1}H and ^{13}C shifts of (2S)-N-((4R)-4-(adamantan-1-yl)-4-(4-methoxyphenyl)-1-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide and (2R)-N-((4R)-4-(adamantan-1-yl)-4-(4-methoxyphenyl)-1-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide. PS-HSQC singlets were used to assign the absolute position of ^{1}H shifts.[15] All NMR spectra were referenced to TMS at 0.0 ppm.

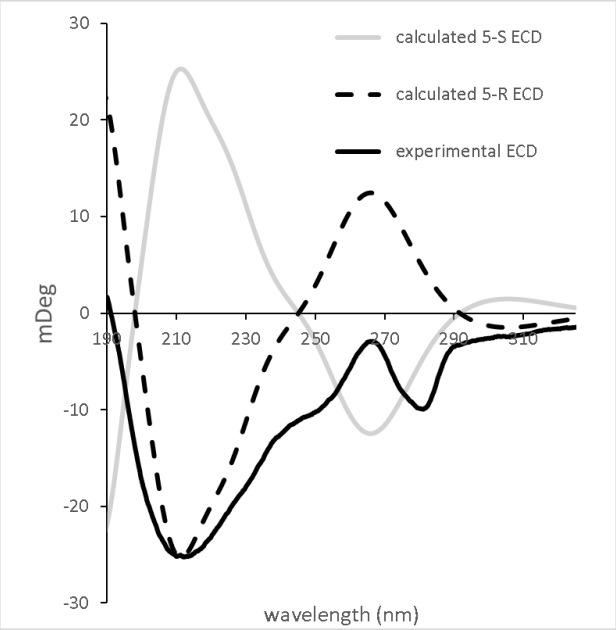
(2R)-N-((4R)-4-(adamantan-1-yl)-4-(4-methoxyphenyl)-1-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide. ^{13}C NMR (126 MHz, Chloroform-*d*) δ 179.5, 172.4, 160.3, 158.5, 133.2, 127.9, 127.5, 127.1, 126.6, 126.6, 123.6 (dd, *J* = 288.6, 79.7 Hz), 123.2, 112.2, 84.9 (q, *J* = 84.9 Hz), 72.0, 54.3, 39.5, 35.2, 34.8, 27.1, 24.4. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.97 (s, 1H), 7.55-7.52 (m, overlapped, 2H), 7.41 (d, *J* = 8.9 Hz, 2H), 7.31 – 7.27 (m, overlapped, 3H), 6.84 (d, *J* = 8.9 Hz, 2H), 3.75 (s, 3H), 3.56 (br s, 3H), 2.93 (s, 3H), 1.93 (p, *J* = 3.0 Hz, 3H), 1.77 – 1.69 (m, 3H), 1.57 (d, *J* = 11.9 Hz, 3H), 1.47 (d, *J* = 11.9 Hz, 3H), 1.39 – 1.31 (m, 3H).

(2S)-N-((4R)-4-(adamantan-1-yl)-4-(4-methoxyphenyl)-1-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide. ^{13}C NMR (126 MHz, Chloroform-*d*) δ 179.5, 172.4, 160.2, 158.5, 133.3, 127.9, 127.5, 127.1, 126.7, 123.6 (dd, *J* = 288.6, 80.8 Hz), 123.2, 112.2, 84.9 (d, *J* = 26.0), 71.9, 54.3, 54.3, 39.6, 35.2, 34.8, 27.1, 24.4. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.97 (s, 1H), 7.54-7.51 (m, overlapped, 2H), 7.42 (d, *J* = 9.0 Hz, 2H), 7.32 – 7.28 (m, overlapped, 3H), 6.83 (d, *J* = 8.9 Hz, 2H), 3.74 (s, 3H), 3.53 (br s, 3H), 2.92 (s, 3H), 1.93 (p, *J* = 3.3 Hz, 3H), 1.70 (dd, *J* = 12.0, 2.6 Hz, 3H), 1.58 (d, *J* = 12.5 Hz, 3H), 1.47 (d, *J* = 12.4 Hz, 3H), 1.34 (dd, *J* = 11.8, 2.2 Hz, 3H).

Table S3. Corrected Mean Average Error for DFT chemical shifts calculated at the IEFPCM-mPW1PW91/6-311+G(2d,p)// M062X/6-311+G(2d,p) level for ^{13}C and and WP04/aug-cc-pVDZ//B3LYP/6-31+G(d,p) level for ^{1}H for both (2S)-N-((4R)-4-(adamantan-1-yl)-4-(4-methoxyphenyl)-1-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide and (2R)-N-((4R)-4-(adamantan-1-yl)-4-(4-methoxyphenyl)-1-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide indicating position 4 of the Mosher's amides have an *R* absolute configuration, corresponding to a 5-*R* configuration in **Compound 1a**.

	***S* Mosher's amide 1a**		***R* Mosher's amide 1a**	
	4-*R*	**4-*S***	**4-*R***	**4-*S***
^{1}H - CMAE:	0.12	0.13	0.08	0.22
^{13}C - CMAE:	3.49	3.56	3.52	3.57

Figure S8. Experimental (black solid) and sTDDFT calculated ECD spectra for 5-*R* (black dashed) and 5-*S* (grey solid) indicating a 5-*R* configuration in **1a**.



ECD experimental details:

ECD spectra of **1a** were acquired on a Jasco J-1500 CD polararimeter at 25°C in acetonitrile at 0.025c from 190 to 500 nm scanned at 100 nm/min with a 1nm bandwidth with two acquisitions averaged and baseline corrected against an acetonitrile blank.

ECD data for **1a**: (0.025c, acetonitrile) 212.1 (-25.4), 238.8 (-12.9), 265.9 (-2.9), 281.0 (-9.9)

Computational details:

Conformers of **1a**, (2S)-N-((4R)-4-(adamantan-1-yl)-4-(4-methoxyphenyl)-1-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide, and (2R)-N-((4R)-4-(adamantan-1-yl)-4-(4-methoxyphenyl)-1-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide utilized for DFT and xtb4stda calculations were generated utilizing ForceGen 4.4 pquant conformational sampling.[8] Gaussian '16 was utilized for all DFT calculations.[9] Quantum mechanical DFT GIAO NMR chemical shift calculations were employed at the IEFPCM-mPW1PW91/6-311+G(2d,p)//M062X/6-311+G(2d,p) level for ^{13}C and

WP04/aug-cc-pVDZ//B3LYP/6-31+G(d,p) level for ^{1}H shifts.[10-11] Chemical shifts were Boltzmann averaged based on Gibb's Free Energies from frequence calculations at the ωB97xD/aug-cc-PVTZ level. The amide NH was excluded from CMAE ^{1}H comparisons of the Mosher's amides.

GFN2-xTB was utilized for geometry optimizations and Hessian calculations with GBSA solvation in acetonitrile for downstream use in computation of ECD spectra of **1a**.[12] sTDDFT at the GBSA-ωB97xD level was used to generate ECD spectra.[13] Computed spectra were generated in SpecDis 1.71 and compared to experimental ECD spectra of **1a**.[14]

References:

1. Otwinowski, Z. and Minor, W. (**1997**) Processing of X-ray diffraction data collected in oscillation mode*Methods Enzymol*. 276, 307– 326
2. "Refinement of Macromolecular Structures by the Maximum-Likelihood method" G.N. Murshudov, A.A.Vagin and E.J.Dodson, (1997) in Acta Cryst. **D53**, 240-255.
3. M. D. Winn et al. *Acta*. *Cryst*. **D67**, 235-242 (2011) "Overview of the CCP4 suite and current developments"
4. Bricogne G., Blanc E., Brandl M., Flensburg C., Keller P., Paciorek W., Roversi P, Sharff A., Smart O.S., Vonrhein C., Womack T.O. (2017). BUSTER. Cambridge, United Kingdom: Global Phasing Ltd.
5. Emsley P., Lohkamp B., Scott W. G. & Cowtan K. (2010) Features and Development of Coot. Acta Cryst. D66, 486-501.
6. Smart O. S., Womack T. O., Sharff A., Flensburg C., Keller P., Paciorek W., Vonrhein C. & Bricogne G. (2011) grade. Cambridge, United Kingdom, Global Phasing Ltd.
7. The PyMOL Molecular Graphics System, Schrödinger, LLC
8. Jain, A. N.; Cleves, A. E.; Gao, Q.; Wang, X.; Liu, Y.; Sherer, E. C.; Reibarkh, M. Y., Complex macrocycle exploration: parallel, heuristic, and constraint-based conformer generation using ForceGen. *Journal of Computer-Aided Molecular Design* **2019,** *33*, 531-558.
9. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.;

Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. *Gaussian 16 Rev. B.01*: Wallingford, CT, 2016.

10. Lodewyk, M. W.; Siebert, M. R.; Tantillo, D. J., Computational Prediction of 1H and 13C Chemical Shifts: A Useful Tool for Natural Product, Mechanistic, and Synthetic Organic Chemistry. *Chemical Reviews* **2012,** *112*, 1839-1862.
11. Wiitala, K. W.; Hoye, T. R.; Cramer, C. J., Hybrid Density Functional Methods Empirically Optimized for the Computation of 13C and 1H Chemical Shifts in Chloroform Solution. *Journal of Chemical Theory and Computation* **2006,** *2*, 1085-1092.
12. Bannwarth, C.; Ehlert, S.; Grimme, S., GFN2-xTB—An Accurate and Broadly Parametrized Self-Consistent Tight-Binding Quantum Chemical Method with Multipole Electrostatics and Density-Dependent Dispersion Contributions. *Journal of Chemical Theory and Computation* **2019,** *15*, 1652-1671.
13. Grimme, S.; Bannwarth, C., Ultra-fast computation of electronic spectra for large systems by tight-binding based simplified Tamm-Dancoff approximation (sTDA-xTB). *The Journal of Chemical Physics* **2016,** *145*, 054103.
14. Bruhn, T.; Schaumlöffel, A.; Hemberger, Y.; Bringmann, G., SpecDis: quantifying the comparison of calculated and experimental electronic circular dichroism spectra. *Chirality* **2013,** *25*, 243-9.
15. Castañar, L.; Sistaré, E.; Virgili, A.; Williamson, R. T.; Parella, T., Suppression of phase and amplitude J(HH) modulations in HSQC experiments. *Magnetic Resonance in Chemistry* **2015,** *53*, 115-119.