A chemical probe for the methyl transferase PRMT5 with a novel binding mode

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Supporting Information

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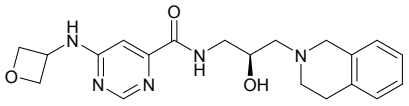
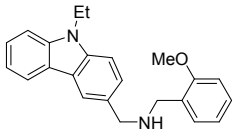
S1 Published Inhibitors

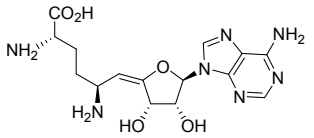
Few recent achievements to identify PRMT5 small molecule inhibitors are noteworthy (Figure S1). The most important is the discovery of the peptide substrate competitive (and SAM cooperative) inhibitor **EPZ015666**.[1] Other recent report of PRMT5 inhibitors include compounds **HLCL-61**, and **BLL-1**[2]. Interestingly, the SAM analog **A9145C** displays sub-micromolar PRMT5 inhibitory activity and its binding mode was confirmed by X-ray crystallography in the presence of a H4 peptide[3]. Another adenosine analog with micromolar inhibitory PRMT5 activity is **DS-437**[4]. Further, the metabolite methyl thioadenosine (**MTA**) that accumulates in MTAP deleted cells is in itself a weak PRMT5 inhibitor[5]. In addition, the potent DOT1L lysine methyl transferase inhibitor **EPZ-5676** has been reported to display weak PRMT5 inhibition, as a side activity[6].Finally, most recently **LLY-283** was reported as a chemical probe for PRMT5 [7] as well as the structurally related and highly potent molecule **PF-06829927** [8].



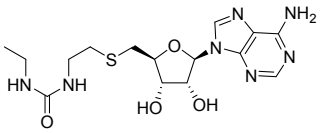
EPZ-015666 nM enzyme activity[1]



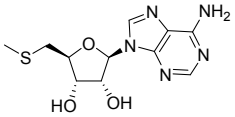
HLCL-61 μM activity[2]



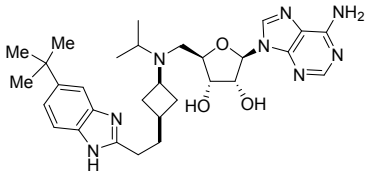
A9145C nM enzyme activity[3]



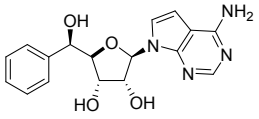
DS-437 μM enzyme activity[4]



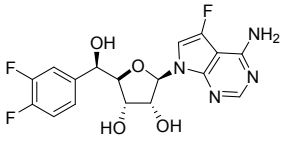
MTA μM enzyme activity[5]



EPZ-5676 μM PRMT5 activity[6]



LLY-283 PRMT5 probe compound[7]



PF-06829927 nM PRMT5 activity[8]

Figure S1. Published small molecules with PRMT5 inhibitory activity.

S2 Chemical Synthesis

S2.1 Instrumentation

Normal phase column chromatography purifications were conducted on silica gel 60 (40-63 μm; VWR). TLC was carried out on plastic TLC sheets, precoated with silica gel 60F254 (Merck); the spots were visualized under UV light (λ=254 nm) and/or $KMnO_4$ (aq.) was used as revealing system.

Preparative reverse phase high performance liquid chromatography (Prep. HPLC) was performed on a Gilson HPLC system accommodated with Gilson 322 pumps using the conditions as indicated in the Section S2.2.

Supercritical fluid chromatography (SFC) purifications were performed on a Acquity UPC2 system from Waters equipped with a UV, QDA and ELSD detector. The SFC measurement was performed using an Analytical system composed by a binary pump for delivering carbon dioxide (CO_2) and modifier, an autosampler, a column oven, a diode array detector equipped with a high-pressure flow cell standing up to 400 bars, and configured with a Mass Spectrometer (MS) Data acquisition was performed with MassLynx software from Waters.

Melting points were acquired on a Buchi Melting Point B-540.apparatus.

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 250 console, operating at 250 MHz for ^1H NMR and 63 MHz for ^{13}C NMR, a Bruker Avance III 300 operating at 300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR; a Bruker Avance II 500 console, operating at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR. Alternatively, ^1H and ^{13}C NMR spectra were recorded at 600 MHz for ^1H NMR and 151 MHz for ^{13}C NMR on a Bruker Avance III-HD console. The deuterated solvent is mentioned in the analysis section and tetramethylsilane was used as an internal standard. In solvents without TMS, the solvent peak was chosen as a reference value. Chemical shifts (δ) are given in parts per million (ppm), coupling constants (J) are given in Hertz (Hz). The following abbreviations are used in the description of spectra: singlet (s), doublet (d), triplet (t), quadruplet (q), quintet (qn), multiplet (m), doublet of doublets (dd),

High resolution mass spectrometry was performed on a Waters Acquity IClass UPLC -DAD and Xevo G2-S QTOF instrument. The samples were run on a Waters BEH C18 (1.7 μm, 2.1 x 50 mm) column using reverse phase chromatography with a gradient from 95% A to 40% A at 1.2 min, then to 5% A at 1.8 min and held for 0.2min (A: 95% 6.5 mM CH_3COONH_4 / 5% CH_3CN, B: CH_3CN). Flow=1 mL/min at 50ºC. Optical rotations were measured on a Jasco P-

2000 Polarimeter using a Sodium lamp at 589 nm and a cell of 3.5mm dia * 100mm path * 1mm ID * 50 mm and controlled by Spectra Manger-2 software.

Analytical Liquid Chromatography / Mass Spectrometry (LC/MS) instrumentation and methods are listed in Table S2, and referenced in the Section S2.2

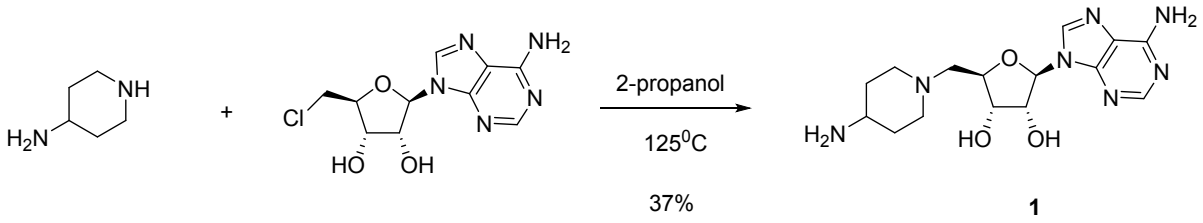
Table S2: Analytical LC/MS Instrumentation and Methods

Method code	Instrument	column	mobile phase	gradient	Flow ---------- Col T
Method A	Waters: Acquity® UPLC® - DAD and SQD	Waters :BEH C18 (1.8µm, 2.1*100mm)	A: 10mM CH_3COONH_4 in 95% H2O + 5% CH_3CN B: CH3CN	From 100% A to 90% A in 2.00min, to 0% A in 1.0min, to 5% A in 0.5min	0.6 ------- 55
Method B	Waters: Acquity® UPLC® - DAD and SQD	Waters :BEH C18 (1.8µm, 2.1*100mm)	A: 10mM CH_3COONH_4 in 95% H2O + 5% CH_3CN B: CH3CN	From 100% A to 90% A in 2.00min, to 0% A in 1.0min, to 5% A in 0.5min	0.6 ------- 55
Method C	Waters: Acquity® UPLC® - DAD and SQD	Waters :BEH C18 (1.8µm, 2.1*100mm)	A: 10mM CH_3COONH_4 in 95% H2O + 5% CH_3CN B: CH3CN	From 100% A to 5% A in 2.10min, to 0% A in 0.90min, to 5% A in 0.5min	0.7 ------- 55
Method D	Waters: Acquity® UPLC® - DAD and SQD	Waters :BEH C18 (1.8µm, 2.1*100mm)	A: 0.1% NH_4HCO3 in 95% H2O + 5% CH_3CN B: CH3CN	From 100% A to 90% A in 2.00min, to 0% A in 1.0min, to 5% A in 0.5min	0.6 ------- 55
Method E	Waters: Acquity® UPLC® - DAD and SQD	Waters :BEH C18 (1.8µm, 2.1*100mm)	A: 10mM CH_3COONH_4 in 95% H2O + 5% CH_3CN B: CH3CN	From 100% A to 5% A in 2.10min, to 0% A in 0.90min, to 5% A in 0.5min	0.7 ------- 55

Method F	Waters: Acquity® UPLC® -DAD and SQD	Waters : HSS T3 (1.8µm, 2.1*100mm)	A: 10mM CH_3COONH_4 in 95% H2O + 5% CH_3CN B: CH3CN	From 100% A to 5% A in 2.10min, to 0% A in 0.90min, to 5% A in 0.5min	0.7 ------- 55
Method G	Waters: Acquity® UPLC® - DAD and SQD	Waters : HSS T3 (1.8µm, 2.1*100mm)	A: 10mM CH_3COONH_4 in 95% H2O + 5% CH_3CN B: CH3CN	From 100% A to 5% A in 2.1min, to 0% A in 0.9min, to 5% A in 0.5min	0.7 ------- 55
Method H	Waters: Acquity® UPLC® - DAD and SQD	BEH C18 (1.7 µm, 2.1 x 50 mm; Waters Acquity)	A: 10mM CH_3COONH_4 in 95% H2O + 5% CH_3CN B: CH3CN	From 95% A to 5% A in 1.3min, held for 0.7 min	0.8 ------- 55
Method J	Waters: Acquity® UPLC® - DAD and SQD	Waters :BEH C18 (1.8µm, 2.1*100mm)	A: 10mM CH_3COONH_4 in 95% H2O + 5% CH_3CN B: CH3CN	From 100% A to 5% A in 2.1min, to 0% A in 0.9 min, to 5% A in 0.5min	0.7 ------- 55
Method K	Agilent 1100 HPLC DAD LC/MS G1956A	YMC-pack ODS-AQ C18 (50 x 4.6 mm, 3 µm)	A: 0.1% HCOOH in H2O B: CH3CN	From 95% A to 5% A in 4.8 min, held for 1.0 min, to 95% A in 0.2 min.	2.6 ------- 35

S2.2 Synthetic methods

Preparation of Compound **1**



1

A suspension of 5'-chloro-5'-deoxyadenosine (CAS 892-48-8) (2.9 g, 10 mmol) and 4-amino piperidine (CAS 13035-19-3) (5.0 g, 50 mmol) in 2-propanol (11 mL) was stirred at 125°C for 2.5 h in a pressure tube. The mixture was cooled to room temperature, followed by the addition of a mixture of 2-propanol and dichloro methane (25%, 100 mL) and solid sodium bicarbonate. The mixture was filtered and the filtrate concentrated *in vacuo*. The residue was

purified by prep HPLC (stationary phase: RP Vydac Denali C18 - 10µm, 200g, 5cm; mobile phase: 0.25% NH_4HCO_3 solution in water, CH_3CN). The combined fractions containing product were concentrated *in vacuo*, re-dissolved in MeOH - CH_3CN and again concentrated *in vacuo*. The residue was triturated from di*iso*propyl ether to afford pure compound **8** as a white solid (1.3 g, 37%).

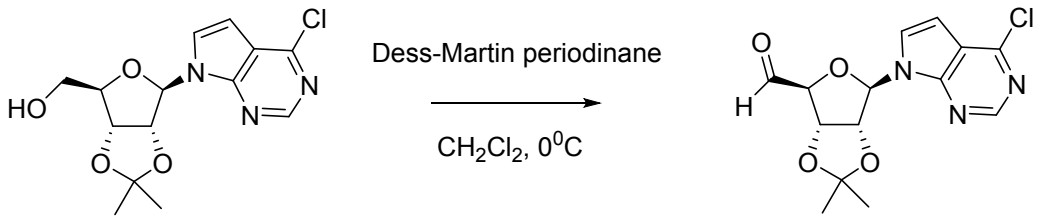
^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.16 - 1.25 (m, 1 H) 1.61 (br d, *J*=12.4 Hz, 2 H) 1.94 - 2.04 (m, 2 H) 2.43 - 2.50 (m, 2 H) 2.64 (dd, *J*=13.5, 4.3 Hz, 1 H) 2.78 (br dd, *J*=16.6, 11.7 Hz, 2 H) 3.97 (dt, *J*=6.7, 4.7 Hz, 1 H) 4.11 (t, *J*=5.1 Hz, 1 H) 4.58 (t, *J*=5.0 Hz, 1 H) 5.21 (br s, 1 H) 5.49 (br s, 1 H) 5.85 (d, *J*=5.0 Hz, 1 H) 7.30 (br s, 2 H) 8.15 (s, 1 H) 8.36 (s, 1 H).

^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 35.46 (s, 1 C) 35.49 (s, 1 C) 48.19 (s, 1 C) 52.80 (s, 1 C) 52.99 (s, 1 C) 60.21 (s, 1 C) 71.76 (s, 1 C) 72.77 (s, 1 C) 82.21 (s, 1 C) 87.61 (s, 1 C) 119.11 (s, 1 C) 139.74 (s, 1 C) 149.35 (s, 1 C) 152.64 (s, 1 C) 156.07 (s, 1 C)

LC/MS (Method H): m/z 350.3 $[M+H]^+$, retention time 0.21 min. Purity 100%

HRMS $[M+H]^+$ calcd. for $C_{15}H_{24}N_7O_3$ 350.1941; found 350.1941

Preparation of common intermediate **A**



common intermediate **A**

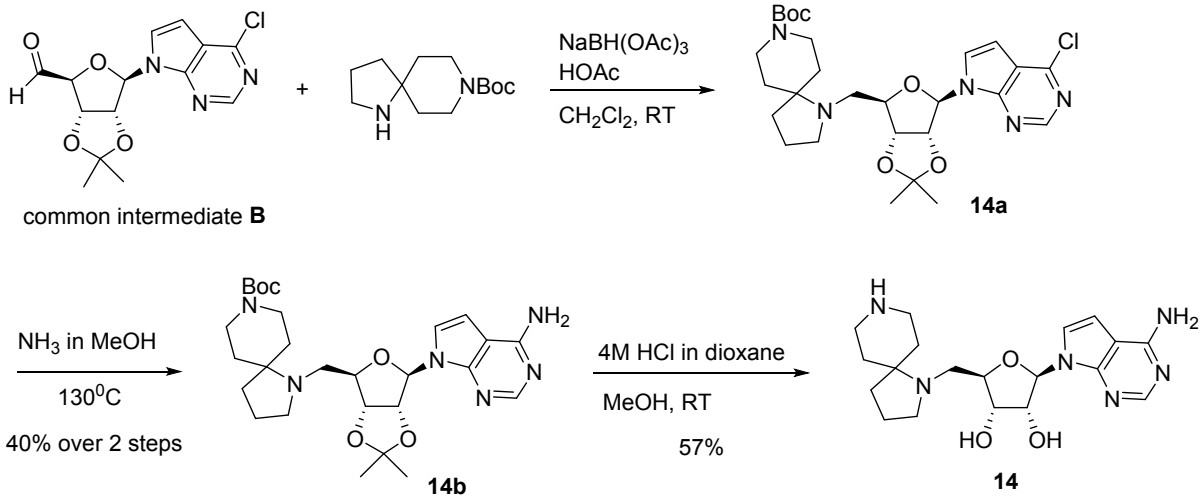
A solution of 4-chloro-7-[2,3-O-(1-methylethylidene)-β-D-ribofuranosyl]- 7H-pyrrolo[2,3-d]pyrimidine (CAS 158078-04-7) (47.11 g, 134.5 mmol) in anhydrous dichloro methane (200 mL) was added dropwise to a suspension of Dess-Martin periodinane (CAS 87413-09-0) (97.0 g, 229 mmol) in anhydrous dichloro methane (600 mL) at 0°C under nitrogen atmosphere. The reaction mixture was allowed to warm up to room temperature and was stirred for 2 hours. The reaction was quenched by the addition of a solution of 50 gram sodium thiosulfate dissolved in 300 mL of a saturated aqueous $NaHCO_3$ solution. The resulting mixture was stirred vigorously at room temperature for 30 minutes. The organic layer was separated, and dried with $MgSO_4$.The resulting solution was used without further purification in the next reactions.

For analytical purposes a small sample was concentrated *in vacuo* to afford the aldehyde **A** as a colorless oil.

^{1}H NMR (360 MHz, DMSO-*d*6) δ ppm 1.36 (br s, 3 H) 1.54 (br s, 3 H) 4.75 (d, J=1.8 Hz, 1 H) 5.37 (d, J=6.2 Hz, 1 H) 5.47 (dd, J=6.2, 1.8 Hz, 1 H) 6.53 (s, 1 H) 6.74 (d, J=3.7 Hz, 1 H) 7.93 (d, J=3.7 Hz, 1 H) 8.55 (s, 1 H) 9.25 (s, 1 H)

LC/MS (Method F): m/z 324.2 $[M+H]^+$, retention time 1.42 min. Purity 75%

Preparation of Compound **14**



Step 1: reductive amination

Compound 14a: Sodium triacetoxyborohydride (45.1 g, 212 mmol) was added to a stirred solution of 1,8-diazaspiro[4.5]decane-8-carboxylic acid tert butyl ester (CAS 937729-06-1) (21 g, 87 mmol) and acetic acid (4.6 mL, 80 mmol) in dichloromethane (1500 mL). Then a solution of the freshly prepared aldehyde common intermediate **A**, containing 106.3 mmol in dichloro methane (700 mL), was added dropwise (slow addition) to the reaction mixture at room temperature. The reaction mixture was stirred at room temperature for 18 hours. The reaction mixture was filtered over a pad of celite. The pad was washed three times with dichloromethane. The filtrate was partially concentrated and washed two times with a saturated aqueous $NaHCO_3$ solution, washed with brine, and dried with $MgSO_4$, to afford 58.2 g of crude product. The crude product was used as such in the subsequent step.

LC/MS (Method J): m/z 548.3 $[M+H]^+$, retention time 2.57 min. Purity 53%

Step 2: ammonia substitution

Compound 14b: A solution of the above product **14a** (58.2 g, 56.3 mmol) in a 7M ammonia solution in methanol (500 mL) was stirred and heated at 130°C for 4 hours in a stainless steel autoclave. The solvents were evaporated. The residue was dissolved in dichloromethane,

washed two times with a saturated aqueous $NaHCO_3$ solution, washed with brine, and dried with $MgSO_4$, to afford 51.7g of crude product. A purification was performed via Prep HPLC (stationary phase: RP SunFire Prep C18 OBD-10µm, 30x150mm, mobile phase: 0.25% NH_4HCO_3 solution in water, MeOH) to afford 23.6 g of almost pure product. A second purification was performed by silica gel column chromatography (eluens 100% dichloromethane – 5% methanol in dichloro methane) to afford 22.7 g (40%) as an oil.

LC/MS (Method J): m/z 529.4 $[M+H]^+$, retention time 1.97 min. Purity 97%

Step 3: deprotection

Compound 14: HCl (4M in dioxane; 104 mL, 416 mmol) was added to a stirred solution of the above product **14b** (22.7 g, 41.6 mmol) in methanol (1800 mL) at room temperature. The reaction mixture was stirred at room temperature for 18 hours. The reaction mixture was poured out into a beaker with 4L di*iso*propyl ether. The resulting suspension was stirred for 10 minutes at room temperature. The precipitate was filtered off and washed with di*iso*propyl ether. The residue was dissolved in methanol and slightly alkalized with a 7N solution of ammonia in methanol. Then the solvents were evaporated yielding 16.5 g of crude product. A purification was performed via prep HPLC (stationary phase: Uptisphere C18 ODB - 10µm, 200g, 5cm, Mobile phase: 0.25% NH_4HCO_3 solution in water, CH3CN) yielding 9.2 g (57%) pure product as a white solid. To obtain an analytical pure sample, a second purification using 100 mg by prep SFC (Stationary phase: Chiralpak Diacel AD 20 x 250 mm, Mobile phase: CO_2, EtOH + 0.4 $iPrNH_2$) afforded 83 mg pure product as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.09 - 1.18 (m, 2 H) 1.39 (td, *J*=12.5, 4.3 Hz, 1 H) 1.49 (td, *J*=12.6, 4.5 Hz, 1 H) 1.54 - 1.73 (m, 4 H) 2.41 - 2.49 (m, 2 H) 2.49 - 2.57 (m, 1 H) 2.61 - 2.71 (m, 1 H) 2.75 - 2.93 (m, 4 H) 3.83 - 3.89 (m, 1 H) 4.03 (t, *J*=4.5 Hz, 1 H) 4.36 (t, *J*=5.3 Hz, 1 H) 5.06 (br s, 1 H) 5.27 (br s, 1 H) 6.03 (d, *J*=5.7 Hz, 1 H) 6.60 (d, *J*=3.7 Hz, 1 H) 6.98 (s, 2 H) 7.32 (d, *J*=3.7 Hz, 1 H) 8.06 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 20.68 (s, 1 C) 31.83 (s, 1 C) 33.33 (s, 1 C) 33.48 (s, 1 C) 44.11 (s, 1 C) 44.32 (s, 1 C) 50.19 (s, 1 C) 51.22 (s, 1 C) 61.80 (s, 1 C) 71.56 (s, 1 C) 73.15 (s, 1 C) 83.14 (s, 1 C) 86.59 (s, 1 C) 99.80 (s, 1 C) 102.74 (s, 1 C) 121.47 (s, 1 C) 150.37 (s, 1 C) 151.74 (s, 1 C) 157.42 (s, 1 C)

LC/MS (Method E): m/z 389.3 $[M+H]^+$, retention time 0.81 min. Purity 100%

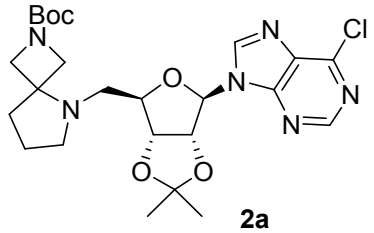
HRMS $[M+H]^+$ calcd. for $C_{19}H_{29}N_6O_3$ 389.2301; found 389.2303

Preparation of Compound **2**

The same sequence was followed as described for **Compound 14.**

Step 1: reductive amination

Compound **2a**



2a

was obtained as a colorless oil in 34% yield (281 mg) using t-butyl 2,5-diazaspiro[3.4]octane-2-carboxylate, CAS Number: 1086398-02-8, and (3aS,4S,6R,6aR)-6-(6-chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carbaldehyde, CAS 52719-20-7, following the general procedure to obtain **14a** after purification by SiO_2 chromatography (eluent heptane / ethyl acetate).

^{1}H NMR (300 MHz, CDCl3) δ 8.79 (s, 1H), 8.23 (s, 1H), 6.13 (s, 1H), 5.45 (d, J = 5.4 Hz, 1H), 5.04 (s, 1H), 4.40 (s, 1H), 3.99 (d, J = 9.0 Hz, 1H), 3.82 – 3.66 (m, 2H), 3.60 (d, J = 8.8 Hz, 1H), 3.03 (d, J = 5.9 Hz, 1H), 2.82 (d, J = 7.4 Hz, 2H), 2.69 (s, 1H), 2.00 (d, J = 7.1 Hz, 2H), 1.75 (d, J = 7.2 Hz, 2H), 1.63 (s, 3H), 1.45 (s, 9H), 1.40 (s,3H).

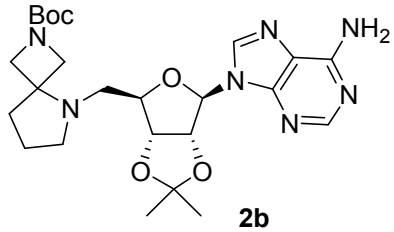
LC/MS (method K: m/z 522 $[M+H]^+$, retention time 1.07 min. Purity 96%

M.p.: 100°C

$[\alpha]_D^{23}$ -8.3° (c = 0.13 MeOH)

Step 2: ammonia substitution

Compound **2b**



2b

was obtained after a reaction time of 2 h at 100^0C, following the procedure described for **14b**. Purification by SiO_2 chromatography (eluent dichloro methane / methanol 9:1) afforded **2b** as a colorless oil in 66% yield.

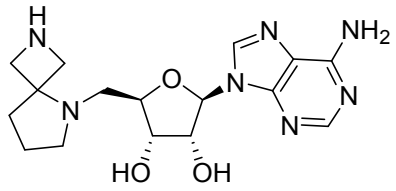
^{1}H NMR (300 MHz, CDCl3) δ 8.36 (s, 1H), 7.89 (s, 1H), 6.06 (s, 1H), 5.69 (s, 2H), 5.48 (d, *J* = 6.4 Hz, 1H), 5.05 (d, *J* = 6.0 Hz, 1H), 4.40 (s, 1H), 4.04 (d, *J* = 8.9 Hz, 1H), 3.75 (d, *J* = 9.0 Hz, 2H), 3.58 (d, *J* = 9.2 Hz, 1H), 3.10 (s, 1H), 2.92 (d, *J* = 21.6 Hz, 2H), 2.65 (s, 1H), 2.02 (t, *J* = 7.3 Hz, 2H), 1.82 – 1.70 (m, 2H), 1.62 (s, 3H), 1.45 (s, 9H), 1.40 (s, 3H).

LC/MS (method K: m/z 502 $[M+H]^+$, retention time 1.981 min. Purity 100%

$[\alpha]_D^{23}$ -17.57° (c = 0.13 MeOH).

Step 3: deprotection

Compound 2



2

The procedure to obtain compound **14** was followed. Upon completion, the solvent was evaporated under reduced pressure. The crude material was then dissolved in methanol/water (2 mL/mmol, 1:1). To the solution, basic Amberlyst resin (619 mg, Amberlyst A26, CAS: 39339-85-0) was added and gently shaked for 10 min until the pH increased to between 8-9. The resin was filtered off and washed with MeOH. The solution was then evaporated to afford compound **2** in 74% yield as the free base.

An analytically pure sample was obtained by Prep SFC (Stationary phase: Chiralpak Diacel AD 20 x 250 mm, Mobile phase: CO_2, MeOH + 0.4% (v/v) $iPrNH_2$). The solvents of the pure fractions were evaporated under reduced pressure. The residue was co-evaporated with methanol.

Yield: 25 mg as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.55 - 1.65 (m, 2 H) 1.89 - 2.00 (m, 2 H) 2.58 - 2.75 (m, 2 H) 2.94 (dd, *J*=13.4, 6.1 Hz, 1 H) 3.13 (dd, *J*=13.4, 4.9 Hz, 1 H) 3.16 - 3.26 (m, 2 H) 3.58 - 3.68 (m, 2 H) 3.99 (dt, *J*=5.7, 4.9 Hz, 1 H) 4.19 (t, *J*=5.1 Hz, 1 H) 4.65 (t, *J*=5.1 Hz, 1 H) 5.89 (d, *J*=1.0 Hz, 1 H) 6.88 (br s, 2 H) 8.16 (s, 1 H) 8.24 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 20.39 (s, 1 C) 37.30 (s, 1 C) 50.98 (s, 1 C) 52.01 (s, 1 C) 53.92 (s, 1 C) 54.13 (s, 1 C) 65.71 (s, 1 C) 71.42 (s, 1 C) 72.79 (s, 1 C) 83.57 (s, 1 C) 87.30 (s, 1 C) 119.05 (s, 1 C) 139.68 (s, 1 C) 149.41 (s, 1 C) 152.61 (s, 1 C) 156.00 (s, 1 C)

LC/MS (method A): m/z 362.3 $[M+H]^+$, retention time 1.23 min. Purity 98%

HRMS $[M+H]^+$ calcd. for $C_{16}H_{24}N_7O_3$ 362.1941; found 362.1938

$[\alpha]_D^{23}$ +3.85° (c = 0.13 MeOH)

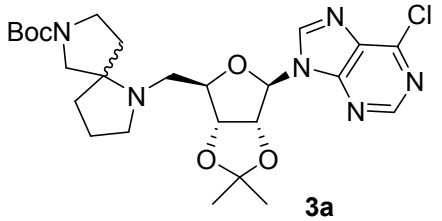
M.p.: 133 °C

Preparation of Compounds **3a** and **3b**

The same sequence was followed as described for **Compound 14.**

Step 1: Reductive amination

Compound 3a (mixture of diastereomers).



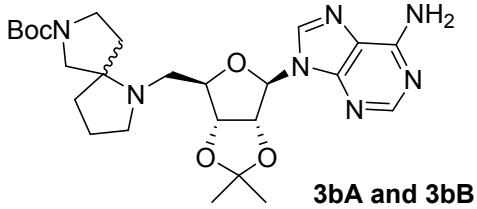
3a

was obtained as a colorless oil (mixture of diastereoisomers) in 31% yield (265 mg) using t-butyl 1,7-diazaspiro[4.4]nonane-7-carboxylate, CAS: 646055-63-2 and (3aS,4S,6R,6aR)-6-(6-chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carbaldehyde, CAS 52719-20-7, following the general procedure to obtain **14a** after purification by SiO_2 chromatography (eluent heptane / ethyl acetate).

HRMS $[M+H]^+$ calcd. for $C_{25}H_{36}ClN_6O_5$ 535.2436; found 535.4933

Step 2: ammonia substitution

Compound 3bA and 3bB (mixture of diastereomers)



3bA and 3bB

Compounds **3b** (mixture of diastereoisomers) were obtained after a reaction time of 2 h at 100^0C in 59% yield, following the procedure described for **14b**. Both diastereoisomers were separated by RP-HPLC into diastereoisomer-A and diastereoisomer B.

RP-HPLC conditions: Aqueous phase (A): H_2O (containing 0.1% HCOOH). Organic Phase (B): MeOH/MeCN 1:1. From A:B 95:5 to 63:37, flow: 40 mL/min. on a C-18, Phenomenex Gemini column, 5µ, 100x30 mm.

Diastereoisomer-3bA (method K) retention time = 1.878 min

^{1}H NMR (300 MHz, DMSO) δ 8.32 (s, 1H), 8.14 (s, 1H), 7.32 (s, 2H), 6.15 (s, 1H), 5.75 (s, 1H), 5.46 (d, *J* = 6.2 Hz, 1H), 4.96 (s, 1H), 4.17 (s, 1H), 3.12 (d, *J* = 8.8 Hz, 1H), 2.98 (d, *J* = 10.6 Hz, 1H), 2.91 – 2.75 (m, 4H), 1.95 (d, *J* = 16.2 Hz, 1H), 1.67 (s, br., 3H), 1.53 (s, 3H), 1.34 (s, 9H)., 1.32 (s, 3H).

HRMS $[M+H]^+$ calcd. for $C_{25}H_{38}N_7O_5$ 516.2934; found 516.5257

$[\alpha]_D^{23}$ +11.33° (c = 1.00 MeOH)

M.p.:110°C

Diastereoisomer-3bB (method K) retention time 1.983 min.

^{1}H NMR (300 MHz, DMSO) δ 8.32 (s, 1H), 8.13 (d, *J* = 9.5 Hz, 1H), 7.34 (s, 2H), 6.19 (s, 1H), 5.75 (s, 1H), 5.47 (d, *J* = 9.6 Hz, 1H), 4.97 (d, *J* = 9.4 Hz, 1H), 4.20 (s, 1H), 3.15 – 2.96 (m, 2H), 2.85 (d, *J* = 21.9 Hz, 2H), 1.69 (s, br., 3H), 1.52 (s, 3H), 1.42 (s, 9H), 1.32 (s, 3H).

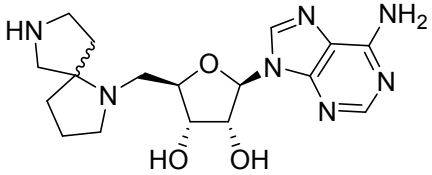
HRMS $[M+H]^+$ calcd. for $C_{25}H_{38}N_7O_5$ 516.2934; found 516.5067

$[\alpha]_D^{23}$ -94.37° (c = 1.00 MeOH)

M.p.: 126°C.

Step 3: deprotection

Compound 3a and 3b



3a & **3b**

stereochemistry in spiro cyclic diamine not assigned

The procedure to obtain compound **14** was followed starting from diastereomer **3bA**. Upon completion, the solvent was evaporated under reduced pressure. The crude material was then dissolved in methanol/water (2 mL/mmol, 1:1). To the solution, basic Amberlyst resin (619 mg, Amberlyst A26, CAS: 39339-85-0) was added and gently shaked for 10 min until the pH increased to between 8-9. The resin was filtered off and washed with MeOH. The solution was then evaporated to afford compound **3a** in 40% yield as the free base, retention time 0.265 min. An analytically pure sample was obtained by Prep SFC (Stationary phase: Chiralpak Diacel AD 20 x 250 mm, Mobile phase: CO_2, MeOH + 0.4% (v/v) $iPrNH_2$). The solvents of the pure fraction were evaporated under reduced pressure. The residue was co-evaporated with methanol.

Yield compound **3a**: 5 mg as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.39 - 1.49 (m, 1 H) 1.56 - 1.82 (m, 5 H) 2.52 - 2.99 (m, 8 H) 3.90 - 3.98 (m, 1 H) 4.16 (t, *J*=5.0 Hz, 1 H) 4.64 (t, *J*=5.2 Hz, 1 H) 5.87 (d, *J*=5.1 Hz, 1 H) 6.93 (br s, 2 H) 8.15 (s, 1 H) 8.26 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 21.21 (s, 1 C) 32.52 (s, 1 C) 38.77 (s, 1 C) 45.95 (s, 1 C) 51.29 (s, 1 C) 52.12 (s, 1 C) 52.99 (s, 1 C) 71.91 (s, 1 C) 72.03 (s, 1 C) 73.22 (s, 1 C) 83.94 (s, 1 C) 87.73 (s, 1 C) 119.57 (s, 1 C) 140.24 (s, 1 C) 149.92 (s, 1 C) 153.06 (s, 1 C) 156.51 (s, 1 C).

LC/MS (method B): m/z 376.3 $[M+H]^+$, retention time 1.39 min. Purity 100%

HRMS $[M+H]^+$ calcd. for $C_{17}H_{26}N_7O_3$ 376.2097; found 376.2097

$[\alpha]_D^{23}$ -86.0° (c = 1.00 MeOH)

M.p.: 97.8°C

Compound **3b** was obtained as a colorless solid in 55% yield following the procedure as described for compound **3a**

Compound **3b**: An analytically pure sample was obtained by Prep SFC (Stationary phase: Chiralpak Diacel AD 20 x 250 mm, Mobile phase: CO_2, MeOH + 0.4% (v/v) $iPrNH_2$). The solvents of the pure fraction were evaporated under reduced pressure. The residue was co-evaporated with methanol.

Yield: 13 mg as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.29 - 1.41 (m, 1 H) 1.60 - 1.76 (m, 5 H) 2.50 - 2.66 (m, 3 H) 2.72 - 2.94 (m, 5 H) 3.91 - 3.97 (m, 1 H) 4.15 (t, *J*=4.8 Hz, 1 H) 4.66 (t, *J*=5.2 Hz, 1 H) 5.87 (d, *J*=5.1 Hz, 1 H) 6.93 (br s, 2 H) 8.14 (s, 1 H) 8.26 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 21.28 (s, 1 C) 31.90 (s, 1 C) 38.99 (s, 1 C) 46.46 (s, 1 C) 51.55 (s, 1 C) 53.07 (s, 1 C) 53.76 (s, 1 C) 72.00 (s, 1 C) 72.28 (s, 1 C) 73.10 (s, 1 C) 84.26 (s, 1 C) 87.66 (s, 1 C) 119.59 (s, 1 C) 140.28 (s, 1 C) 149.95 (s, 1 C) 153.02 (s, 1 C) 156.51 (s, 1 C).

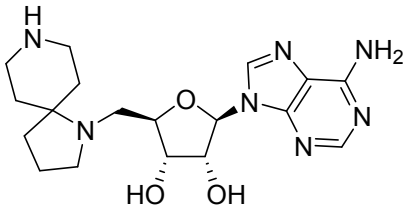
LC/MS (method B): m/z 376.2 $[M+H]^+$, retention time 1.49 min. Purity 100%

HRMS $[M+H]^+$ calcd. for $C_{17}H_{26}N_7O_3$ 376.2097; found 376.2096

$[\alpha]_D^{23}$ -76° (c = 1.00 MeOH)

M.p.: 208°C

Preparation of Compound **4**

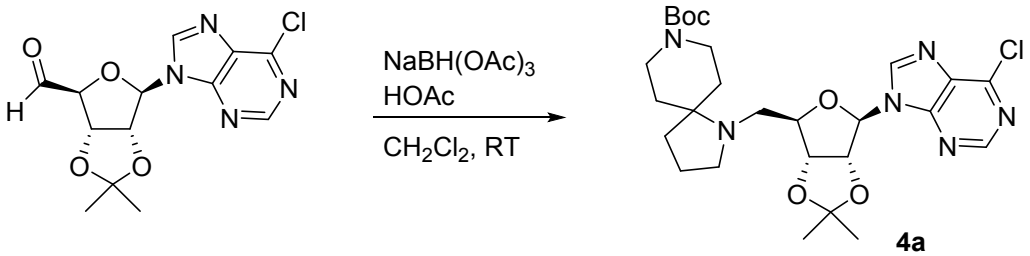


4

The same sequence was followed as described for **Compound 14.**

Step 1: reductive amination

Compound **4a**



was obtained as a colorless powder in 24% yield (203 mg) using tert-butyl 1,8-diazaspiro [4.5] decane-8-carboxylate, CAS: 937729-06-1 and (3aS,4S,6R,6aR)-6-(6-chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carbaldehyde, CAS 52719-20-7, following the general procedure to obtain **14a** after purification by SiO_2 chromatography (eluent heptane / ethyl acetate).

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.77 (s, 1H), 8.26 (s, 1H), 6.12 (s, 1H), 5.42 (d, J = 5.9 Hz, 1H), 5.07 – 4.82 (m, 1H), 4.36 (s, 1H), 4.11 (s, 2H), 3.09–2.42 (m, 6H), 1.75 (s, 4H), 1.62 (s, 3H), 1.54 (d, J = 4.6 Hz, 1H), 1.46 (s, 9H), 1.39 (s, 3H), 1.25 -1.31 (m, 3H).

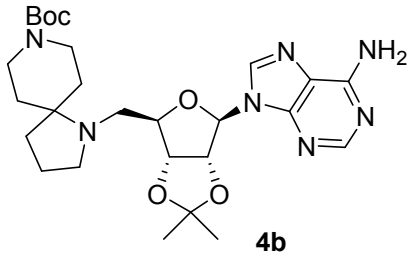
HRMS $[M+H]^+$ calcd. for $C_{26}H_{38}ClN_6O_5$ 549.2592; found 549.4882

M.p.: 100°C

$[\alpha]_D^{23}$ -58.86° (c = 1.00 MeOH)

Step 2: ammonia substitution

Compound **4b**



4b

Compound **4b** was obtained after a reaction time of 2 h at 100^0C, following the procedure described for **14b**. Purification by SiO_2 chromatography (eluent dichloro methane / methanol 9:1) afforded **4b** as a colorless solid in 59% yield.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.34 (s, 1H), 7.91 (s, 1H), 6.05 (s, 1H), 5.76 (s, 2H), 5.47 (s, 1H), 4.97 (s, 1H), 4.33-4.11 (m, br., 3H), 2.95-2.67 (m, br., 4H), 1.76 (s ,br. 4H), 1.61 (s, 3H), 1.46 (s, 9H), 1.39 (s, 3H), 1.25-1.21 (m, br., 4H) 0.82 (s, br., 2H).

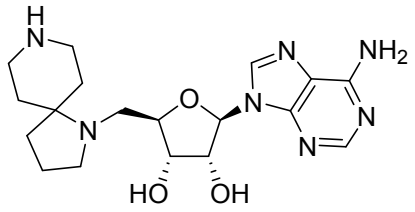
HRMS $[M+H]^+$ calcd. for $C_{26}H_{40}N_7O_5$ 530.3091; found 530.5586

$[\alpha]_D^{23}$ -42.17° (c = 1.00 MeOH).

M.p.:121°C

Step 3: deprotection

Compound **4**



4

The procedure to obtain compound **14** was followed. Upon completion, the solvent was evaporated under reduced pressure. The crude material was then dissolved in methanol/water (2 mL/mmol, 1:1). To the solution, basic Amberlyst resin (619 mg, Amberlyst A26, CAS: 39339-85-0) was added and gently shaked for 10 min until the pH increased to between 8-9. The resin was filtered off and washed with MeOH. The solution was then evaporated to afford compound **4** in 89% yield as the free base.

An analytically pure sample was obtained by Prep SFC (Stationary phase: Chiralpak Diacel AD 20 x 250 mm, Mobile phase: CO_2, EtOH + 0.4% (v/v) $iPrNH_2$). The solvents of the pure fraction were evaporated under reduced pressure. The residue was co-evaporated with methanol.

Yield: 10 mg as a colorless oil.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.15 (br d, *J*=12.5 Hz, 2 H) 1.38 - 1.72 (m, 6 H) 2.41 - 2.47 (m, 1 H) 2.51 - 2.55 (m, 1 H) 2.58 - 2.73 (m, 2 H) 2.77 - 2.90 (m, 4 H) 3.91 - 3.98 (m, 1 H) 4.16 (t, *J*=4.8 Hz, 1 H) 4.67 (t, *J*=5.3 Hz, 1 H) 5.87 (d, *J*=5.1 Hz, 1 H) 6.92 (br s, 2 H) 8.16 (s, 1 H) 8.26 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 21.35 (s, 1 C) 32.88 (s, 1 C) 33.93 (s, 1 C) 34.13 (s, 1 C) 44.57 (s, 1 C) 44.71 (s, 1 C) 50.44 (s, 1 C) 51.82 (s, 1 C) 62.23 (s, 1 C) 72.21 (s, 1 C) 73.49 (s, 1 C) 84.82 (s, 1 C) 88.48 (s, 1 C) 119.99 (s, 1 C) 140.20 (s, 1 C) 150.17 (s, 1 C) 153.01 (s, 1 C) 156.60 (s, 1 C).

LC/MS (method B): m/z 390.3 $[M+H]^+$, retention time 1.55 min. Purity 100%

HRMS $[M+H]^+$ calcd. for $C_{18}H_{28}N_7O_3$ 390.2254; found 390.2251

$[\alpha]_D^{23}$ -27.3° (c = 1.00 MeOH)

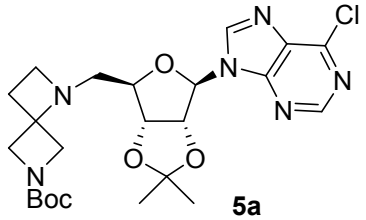
M.p.: 131 °C

Preparation of Compound **5**

The same sequence was followed as described for **Compound 14.**

Step 1: reductive amination

Compound 5a.

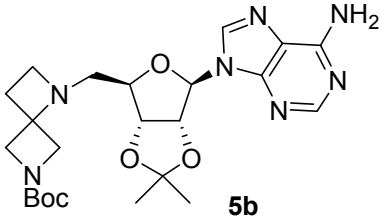


5a

was obtained as a colorless oil in 15% yield (115 mg) using t-butyl 1,6-diazaspiro[3.3]heptane-6-carboxylate (oxalate salt), CAS Number: 1272412-72-2, and (3aS,4S,6R,6aR)-6-(6-chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carbaldehyde, CAS 52719-20-7, following the general procedure to obtain **14a** after purification by SiO_2 chromatography (eluent heptane / ethyl acetate).

LC/MS (method K): m/z 507 $[M+H]^+$, retention time 1.23 min. Purity 75%

Step 2: ammonia substitution



5b

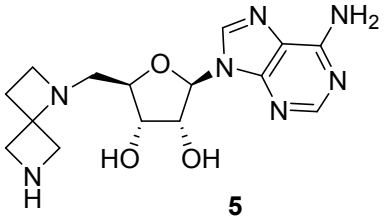
Compound **5b** was obtained after a reaction time of 2 h at 100^0C, following the procedure described for **14b**. Purification by SiO_2 chromatography (eluent dichloro methane / methanol 9:1) afforded **5b** as a colorless oil in 51% yield.

^{1}H NMR (300 MHz, DMSO) δ 8.34 (s, 1H), 8.14 (s, 1H), 7.32 (s, 2H), 6.12 (s, 1H), 5.75 (s, 3H), 5.45 (d, *J* = 6.2 Hz, 1H), 4.97 (d, *J* = 6.1 Hz, 1H), 4.07 (s, 1H), 3.96 (d, *J* = 9.4 Hz, 2H), 3.79 – 3.69 (m, 2H), 3.02 (t, *J* = 6.4 Hz, 1H), 2.94 (t, *J* = 6.8 Hz, 1H), 2.81 (dd, *J* = 12.4, 7.6 Hz, 1H), 2.65 (dd, *J* = 12.4, 6.3 Hz, 1H), 2.18 (d, *J* = 6.0 Hz, 2H), 1.52 (s, 3H), 1.35 (s, 9H), 1.32 (s, 2H).

LC/MS (method K): m/z 488 $[M+H]^+$, retention time 1.851 min. Purity 98%

Step 3: deprotection

Compound 5



5

The procedure to obtain compound **14** was followed. Upon completion, the solvent was evaporated under reduced pressure. The crude material was then dissolved in methanol/water (2 mL/mmol, 1:1). To the solution, basic Amberlyst resin (619 mg, Amberlyst A26, CAS: 39339-85-0) was added and gently shaked for 10 min until the pH increased to between 8-9. The resin was filtered off and washed with MeOH. The solution was then evaporated to afford compound **5** in 69% yield as the free base.

An analytically pure sample was obtained by Prep HPLC (Stationary phase: RP XBridge Prep C18 OBD-10µm,30x150mm, Mobile phase: 0.25% NH_4HCO_3 solution in water, MeOH). The solvents of the purified fraction were evaporated. The residue was dissolved in

water with some acetonitrile. After removal of the solvents by lyophilization the product was obtained as a white solid.

Yield: 2 mg as a colorless oil.

^{1}H NMR (600 MHz, DMSO-d6) δ ppm 2.12 - 2.18 (m, 2 H) 2.76 - 2.80 (m, 1 H) 2.88 (br dd, J=13.0, 5.1 Hz, 1 H) 2.99 - 3.06 (m, 2 H) 3.25 - 3.36 (m, 2 H) 3.67 - 3.76 (m, 2 H) 3.89 - 3.93 (m, 1 H) 4.15 (t, J=1.0 Hz, 1 H) 4.65 (t, J=5.3 Hz, 1 H) 5.86 (d, J=5.1 Hz, 1 H) 6.93 (br s, 2 H) 8.15 (s, 1 H) 8.27 (s, 1 H).

^{13}C NMR (151 MHz, DMSO-d6) δ ppm 31.17 (s, 1 C) 40.92 (s, 1 C) 51.09 - 51.33 (m, 1 C) 54.04 - 54.28 (m, 1 C) 56.75 (s, 1 C) 69.66 (s, 1 C) 72.17 (s, 1 C) 73.49 (s, 1 C) 83.81 (s, 1 C) 88.18 (s, 1 C) 119.84 (s, 1 C) 140.12 (s, 1 C) 150.00 - 150.29 (m, 1 C) 153.04 (s, 1 C) 156.59 (s, 1 C).

LC/MS (method C): m/z 348.3 $[M+H]^+$, retention time 0.77 min. Purity 91%

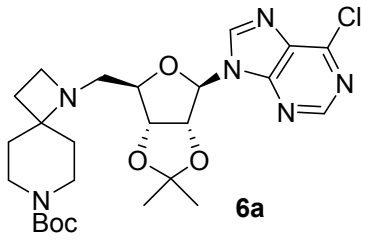
HRMS $[M+H]^+$ calcd. for $C_{15}H_{22}N_7O_3$ 348.1784; found 348.1781

Preparation of Compound **6**

The same sequence was followed as described for **Compound 14.**

Step 1: reductive amination

Compound **6a**



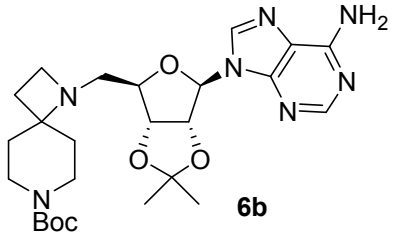
6a

was obtained as a colorless oil in 36% yield (307 mg) using t-butyl 1,7-diazaspiro[3.5]nonane-7-carboxylate, CAS Number: 1180112-41-7, and (3aS,4S,6R,6aR)-6-(6-chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carbaldehyde, CAS 52719-20-7, following the general procedure to obtain **14a** after purification by SiO_2 chromatography (eluent heptane / ethyl acetate).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.43 (s, 1H), 6.13 (s, 1H), 5.35 (d, J = 5.9 Hz, 1H), 4.94 – 4.79 (m, 1H), 4.23 (s, 1H), 4.11 (dd, J = 20.9, 13.7 Hz, 2H), 3.30 – 3.14 (m, 2H), 2.77 – 2.56 (m, 4H), 2.04 (s, 1H), 1.95 (t, J = 6.9 Hz, 2H), 1.73 (s, 2H), 1.62 (s, 3H), 1.44 (s, 9H), 1.39 (s, 3H), 1.26 (t, J = 7.1 Hz, 1H).

LC/MS (method K): m/z 536 $[M+H]^+$, retention time 2.150 min. Purity 98%

Step 2: ammonia substitution



6b

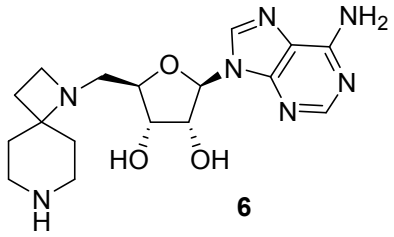
Compound **6b** was obtained after a reaction time of 2 h at 100^0C, following the procedure described for **14b**. Purification by SiO_2 chromatography (eluent dichloro methane / methanol 9:1) afforded **6b** as a colorless oil in 57% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.99 (s, 1H), 6.04 (s, 1H), 5.72 (s, 2H), 5.42 (d, *J* = 6.1 Hz, 1H), 4.95 – 4.85 (m, 1H), 4.18 (s, 1H), 4.01 (s, 2H), 3.20 (dd, *J* = 17.8, 7.1 Hz, 2H), 2.75 – 2.57 (m, 4H), 1.93 (t, *J* = 6.7 Hz, 3H), 1.70 (s, br., 3H), 1.59 (s, 3H), 1.44 (s, 9H), 1.38 (s, 3H).

LC/MS (method K): m/z 516 $[M+H]^+$, retention time 1.802 min. Purity 95%

Step 3: deprotection

Compound **6**



6

The procedure to obtain compound **14** was followed. Upon completion, the solvent was evaporated under reduced pressure. The crude material was then dissolved in methanol/water (2 mL/mmol, 1:1). To the solution, basic Amberlyst resin (619 mg, Amberlyst A26, CAS: 39339-85-0) was added and gently shaked for 10 min until the pH increased to between 8-9. The resin was filtered off and washed with MeOH. The solution was then evaporated to afford compound **6** in 66% yield as the free base.

An analytically pure sample was obtained by Prep HPLC (Stationary phase: RP XBridge Prep C18 OBD-10µm,50x250mm, Mobile phase: 0.25% NH4HCO3 solution in water,

CH3CN). The solvents of the purified fraction were evaporated. The residue was dissolved in water with some acetonitrile. After removal of the solvents by lyophilization the product was obtained as a white solid.

Yield: 27 mg as a white solid.

^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 1.40 - 1.54 (m, 2 H) 1.59 - 1.68 (m, 2 H) 1.84 (t, *J*=7.0 Hz, 2 H) 2.37 - 2.47 (m, 2 H) 2.64 - 2.81 (m, 2 H) 2.82 - 2.92 (m, 2 H) 3.08 - 3.20 (m, 2 H) 3.85 (q, *J*=5.4 Hz, 1 H) 4.14 (m, *J*=4.9, 4.9, 4.9 Hz, 1 H) 4.64 (t, *J*=5.3 Hz, 1 H) 5.88 (d, *J*=5.2 Hz, 1 H) 6.93 (br s, 2 H) 8.18 (s, 1 H) 8.33 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-*d*6) δ ppm 27.61 (s, 1 C) 33.60 (s, 1 C) 33.93 (s, 1 C) 41.59 (s, 2 C) 48.83 (s, 1 C) 50.40 (s, 1 C) 64.15 (s, 1 C) 70.23 (s, 1 C) 71.71 (s, 1 C) 82.31 (s, 1 C) 86.27 (s, 1 C) 117.92 (s, 1 C) 138.22 (s, 1 C) 148.23 (s, 1 C) 151.12 (s, 1 C) 154.64 (s, 1 C).

LC/MS (method D): m/z 376.3 $[M+H]^+$, retention time 2.16 min. Purity 100%

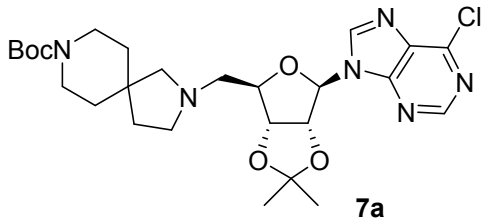
HRMS $[M+H]^+$ calcd. for $C_{17}H_{26}N_7O_3$ 376.2097; found 376.2097

Preparation of Compound **7**

The same sequence was followed as described for **Compound 14.**

Step 1: reductive amination

Compound **7a**



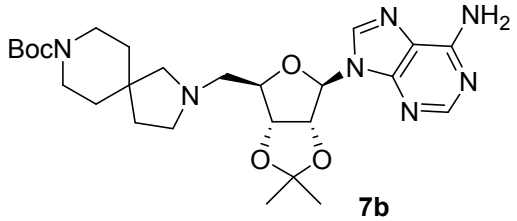
7a

was obtained as a colorless oil in 28% yield (240 mg) using t-butyl 2,8-diazaspiro[4.5]decane-8-carboxylate, CAS Number: 236406-39-6, and (3aS,4S,6R,6aR)-6-(6-chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carbaldehyde, CAS 52719-20-7, following the general procedure to obtain **14a** after purification by SiO_2 chromatography (eluent heptane / ethyl acetate).

^{1}H NMR (300 MHz, DMSO) δ 8.92 (s, 1H), 8.84 (s, 1H), 6.27 (s, 1H), 5.51 (d, *J* = 6.2 Hz, 1H), 4.93 (s, 1H), 4.33 (s, 1H), 4.02 (d, *J* = 11.5 Hz, 1H), 3.24 (d, *J* = 5.2 Hz, 3H), 2.31 (s, 2H), 1.53 (s, 3H), 1.38 (s, 9H), 1.30 (s, 3H).

LC/MS (method K): m/z 550 $[M+H]^+$, retention time 0.986 min. Purity 93%

Step 2: ammonia substitution



7b

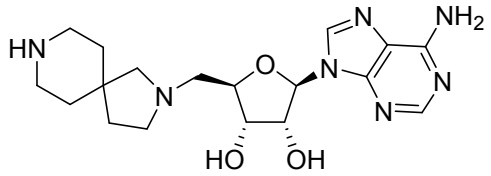
Compound **7b** was obtained after a reaction time of 2 h at 100^0C, following the procedure described for **14b**. Purification by SiO_2 chromatography (eluent dichloro methane / methanol 9:1) afforded **7b** as a colorless oil in 56% yield.

^{1}H NMR (300 MHz, DMSO-d6) δ 8.35 (s, 1H), 8.17 (s, 1H), 7.33 (s, 2H), 6.12 (s, 1H), 5.76 (s, 2H), 5.47 (d, J = 6.1 Hz, 1H), 4.22 (s, 1H), 4.95 (s, br., 1H), 4.21 (s, br., 1H), 3.29 – 3.17 (m, 5H), 2.62 (s, br., 1H), 2.32 (s, 2H), 1.53 (s, 4H), 1.39 (s, 13H), 1.33 (s, 3H)

LC/MS (method K): m/z 530 $[M+H]^+$, retention time 2.023 min. Purity 94%

Step 3: deprotection

Compound **7**



7

The procedure to obtain compound **14** was followed. Upon completion, the solvent was evaporated under reduced pressure. The crude material was then dissolved in methanol/water (2 mL/mmol, 1:1). To the solution, basic Amberlyst resin (619 mg, Amberlyst A26, CAS: 39339-85-0) was added and gently shaked for 10 min until the pH increased to between 8-9. The resin was filtered off and washed with MeOH. The solution was then evaporated to afford compound **7** in 78% yield as the free base.

An analytically pure sample was obtained by Prep SFC (Stationary phase: Chiralpak Diacel AD 20 x 250 mm, Mobile phase: Acetonitrile, EtOH + 0.4% (v/v) $iPrNH_2$). The solvents of the pure fraction were evaporated under reduced pressure. The residue was co-evaporated with methanol.

Yield: 12 mg as a white solid.

^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 1.38 - 1.55 (m, 6 H) 2.40 (br s, 2 H) 2.52 - 2.81 (m, 8 H) 3.92 - 4.00 (m, 1 H) 4.17 (t, *J*=5.2 Hz, 1 H) 4.61 (t, *J*=5.1 Hz, 1 H) 5.86 (d, *J*=4.8 Hz, 1 H) 6.92 (br s, 2 H) 8.14 (s, 1 H) 8.22 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-*d*6) δ ppm 34.31 (s, 1 C) 35.90 - 36.36 (m, 2 C) 36.91 - 37.26 (m, 1 C) 41.86 (s, 2 C) 53.40 (s, 1 C) 57.62 (s, 1 C) 65.50 - 66.14 (m, 1 C) 71.81 (s, 1 C) 73.04 (s, 1 C) 83.26 (s, 1 C) 88.26 (s, 1 C) 119.26 - 119.71 (m, 1 C) 139.44 - 139.93 (m, 1 C) 149.64 (s, 1 C) 152.52 (s, 1 C) 156.11 (s, 1 C)

LC/MS (method B): m/z 390.2 $[M+H]^+$, retention time 1.22 min. Purity 100%

HRMS $[M+H]^+$ calcd. for $C_{18}H_{28}N_7O_3$ 390.2254; found 390.2249

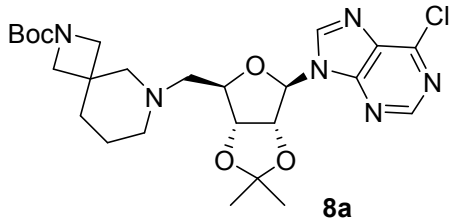
M.p.: 176.5°C

Preparation of Compound **8**

The same sequence was followed as described for **Compound 14.**

Step 1: reductive amination

Compound **8a**



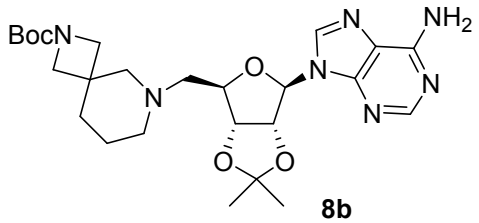
8a

was obtained as a colorless oil in 12% yield (100 mg) using t-butyl 2,6-diazaspiro[3.5]nonane-2-carboxylate, CAS Number: 1086394-57-1, and (3aS,4S,6R,6aR)-6-(6-chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carbaldehyde, CAS 52719-20-7, following the general procedure to obtain **14a** after purification by SiO_2 chromatography (eluent heptane / ethyl acetate).

^{1}H NMR (300 MHz, CDCl3) δ 8.78 (s, 1H), 8.38 (s, 1H), 6.14 (s, 1H), 5.43 (d, *J* = 5.5 Hz, 1H), 4.91 (s, 1H), 4.47 (s, 1H), 3.58 (t, *J* = 8.3 Hz, 4H), 2.71 – 2.22 (m,6H), 1.79 (s, 1H), 1.63 (s, 5H), 1.44 (s, 9H), 1.40 (s, 4H).

LC/MS (method K): m/z 536 $[M+H]^+$, retention time 2.232 min. Purity 98%

Step 2: ammonia substitution



8b

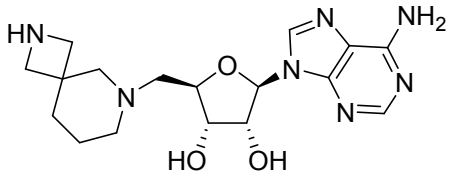
Compound **8b** was obtained after a reaction time of 2 h at 100^0C, following the procedure described for **14b**. Purification by SiO_2 chromatography (eluent dichloro methane / methanol 9:1) afforded **8b** as a colorless oil in 65% yield.

^{1}H NMR (300 MHz, DMSO) δ 8.35 (s, 1H), 7.99 (s, 1H), 7.26 (s, 2H), 6.11 (s, 1H), 5.75 (s, 1H), 5.55 (s, 2H), 5.40 (s, 1H), 4.99 (s, 1H), 3.66-3.50 (m, 4H), 2.60-2.40 (m, 6H), 1.52 (s, br., 2H), 1.43 (s, 3H), 1.37 (s, 9H), 1.32 (s,3H).

LC/MS (method K): m/z 516 $[M+H]^+$, retention time 0.83 min. Purity 99%

Step 3: deprotection

Compound **8**



8

The procedure to obtain compound **14** was followed. Upon completion, the solvent was evaporated under reduced pressure. The crude material was then dissolved in methanol/water (2 mL/mmol, 1:1). To the solution, basic Amberlyst resin (619 mg, Amberlyst A26, CAS: 39339-85-0) was added and gently shaked for 10 min until the pH increased to between 8-9. The resin was filtered off and washed with MeOH. The solution was then evaporated to afford compound **8** in 74% yield as the free base.

An analytically pure sample was obtained by Prep SFC (Stationary phase: Chiralpak Diacel AD 20 x 250 mm, Mobile phase: Acetonitrile, EtOH + 0.4% (v/v) $iPrNH_2$). The solvents of the pure fraction were evaporated under reduced pressure. The residue was co-evaporated with methanol.

Yield: 14 mg as a colorless oil.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.36 - 1.54 (m, 4 H) 2.32 (t, *J*=5.3 Hz, 2 H) 2.42 - 2.48 (m, 1 H) 2.51 - 2.58 (m, 2 H) 2.69 (dd, *J*=13.8, 4.5 Hz, 1 H) 3.11 - 3.24 (m, 4 H) 4.00 -

4.07 (m, 1 H) 4.17 (t, *J*=5.1 Hz, 1 H) 4.60 (t, *J*=5.1 Hz, 1 H) 5.88 (d, *J*=4.9 Hz, 1 H) 6.91 (br s, 2 H) 8.15 (s, 1 H) 8.22 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 22.08 (s, 1 C) 33.89 (s, 1 C) 38.68 (s, 1 C) 53.87 (s, 1 C) 55.25 (s, 1 C) 55.31 (s, 1 C) 60.23 (s, 1 C) 62.75 (s, 1 C) 71.63 (s, 1 C) 72.84 (s, 1 C) 82.21 (s, 1 C) 87.61 (s, 1 C) 119.08 (s, 1 C) 139.66 (s, 1 C) 149.33 (s, 1 C) 152.60 (s, 1 C) 156.01 (s, 1 C).

LC/MS (method B): m/z 376.3 $[M+H]^+$, retention time 1.70 min. Purity 100%

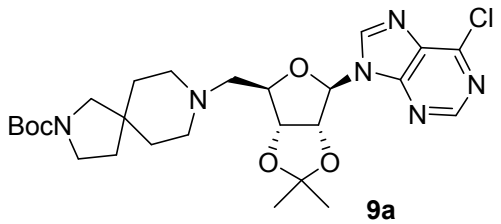
HRMS $[M+H]^+$ calcd. for $C_{17}H_{26}N_7O_3$ 376.2097; found 376.2101

Preparation of Compound **9**

The same sequence was followed as described for **Compound 14.**

Step 1: reductive amination

Compound **9a**



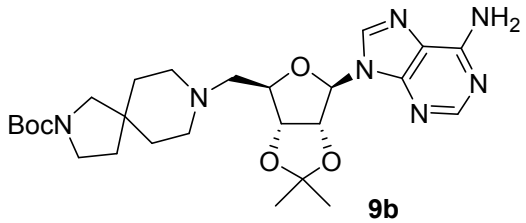
9a

was obtained as a colorless oil in 19% yield (159 mg) using t-butyl 2,8-diazaspiro[4.5]decane-2-carboxylate, CAS Number: 336191-17-4, and (3aS,4S,6R,6aR)-6-(6-chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carbaldehyde, CAS 52719-20-7, following the general procedure to obtain **14a** after purification by SiO_2 chromatography (eluent heptane / ethyl acetate).

^{1}H NMR (300 MHz, DMSO) δ 8.92 (s, 1H), 8.84 (s, 1H), 6.27 (s, 1H), 5.50 (d, *J* = 5.9 Hz, 1H), 4.91 (d, *J* = 6.2 Hz, 1H), 4.38 (s, 1H), 2.36 (dd, *J* = 25.5, 18.1 Hz, 4H), 2.15 – 1.84 (m, 2H), 1.66 (t, *J* = 18.8 Hz, 3H), 1.42 (s, 4H), 1.46 – 1.23 (m, 18H).

LC/MS (method K): m/z 550 $[M+H]^+$, retention time 2.352 min. Purity 89%

Step 2: ammonia substitution



9b

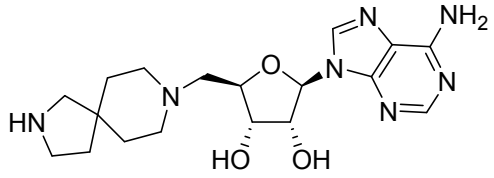
Compound **9b** was obtained after a reaction time of 2 h at 100^0C, following the procedure described for **14b**. Purification by SiO_2 chromatography (eluent dichloro methane / methanol 9:1) afforded **9b** as a colorless oil in 56% yield.

^{1}H NMR (300 MHz, DMSO) δ 8.35 (s, 1H), 8.16 (s, 1H), 7.32 (s, 2H), 6.11 (s, 1H), 5.75 (s, 1H), 5.47 (d, *J* = 6.0 Hz, 1H), 4.97 – 4.87 (m, 1H), 4.26 (s, 1H), 4.10 (q, *J* = 5.1 Hz, 3H), 3.17 (d, *J* = 5.2 Hz, 6H), 3.01 (s, 1H), 2.36 (dd, *J* = 18.6, 12.9 Hz, 3H), 1.62 (d, *J* = 6.2 Hz, 1H), 1.52 (s, 1H), 1.43 (s, 3H), 1.37 (s, 9H), 1.32 (s,3H).

LC/MS (method K): m/z 530 $[M+H]^+$, retention time 2.023 min. Purity 100%

Step 3: deprotection

Preparation of compound **9**



9

The procedure to obtain compound **14** was followed. Upon completion, the solvent was evaporated under reduced pressure. The crude material was then dissolved in methanol/water (2 mL/mmol, 1:1). To the solution, basic Amberlyst resin (619 mg, Amberlyst A26, CAS: 39339-85-0) was added and gently shaked for 10 min until the pH increased to between 8-9. The resin was filtered off and washed with MeOH. The solution was then evaporated to afford compound **9** in 53% yield as the free base.

An analytically pure sample was obtained by Prep SFC (Stationary phase: Chiralpak Diacel AD 20 x 250 mm, Mobile phase: Acetonitrile, EtOH + 0.4% (v/v) $iPrNH_2$). The solvents of the pure fraction were evaporated under reduced pressure. The residue was co-evaporated with methanol.

Yield: 4 mg as a colorless oil.

^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 1.42 - 1.55 (m, 5 H) 1.61 (t, *J*=7.3 Hz, 2 H) 2.32 - 2.47 (m, 5 H) 2.56 (br dd, *J*=13.6, 6.6 Hz, 1 H) 2.68 (br dd, *J*=13.5, 4.3 Hz, 1 H) 2.99 - 3.06

(m, 2 H) 3.98 - 4.03 (m, 1 H) 4.15 (t, *J*=5.3 Hz, 1 H) 4.60 (t, *J*=5.0 Hz, 1 H) 5.86 (d, *J*=4.6 Hz, 1 H) 6.93 (br s, 2 H) 8.15 (s, 1 H) 8.24 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-*d*6) δ ppm 38.29 (s, 1 C) 40.03 (s, 2 C) 47.50 (s, 1 C) 53.89 (s, 2 C) 57.96 (s, 1 C) 62.51 (s, 1 C) 74.03 (s, 1 C) 75.13 (s, 1 C) 84.62 (s, 1 C) 90.26 (s, 1 C) 141.64 (s, 1 C) 151.64 (s, 1 C) 154.61 (s, 1 C) 158.12 (s, 1 C).

LC/MS (method A): m/z 390.3 $[M+H]^+$, retention time 1.05 min. Purity 94%

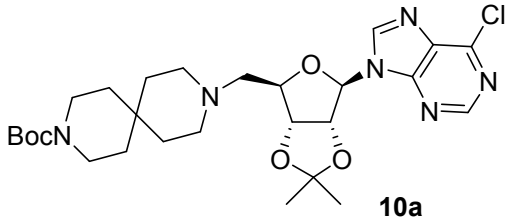
HRMS $[M+H]^+$ calcd. for $C_{18}H_{28}N_7O_3$ 390.2254; found 390.2247

Preparation of Compound **10**

The same sequence was followed as described for **Compound 14.**

Step 1: reductive amination

Compound **10a**



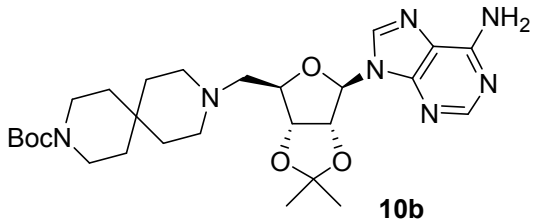
10a

was obtained as a colorless oil in 28% yield (251 mg) using t-butyl 3,9-diazaspiro[5.5]undecane-3-carboxylate, CAS Number: 87413-09-0, and (3aS,4S,6R,6aR)-6-(6-chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carbaldehyde, CAS 52719-20-7, following the general procedure to obtain **14a** after purification by SiO_2 chromatography (eluent heptane / ethyl acetate).

^{1}H NMR (300 MHz, DMSO) δ 8.92 (s, 1H), 8.83 (s, 1H), 6.26 (s, 1H), 5.50 (d, *J* = 6.2 Hz, 1H), 4.90 (d, *J* = 6.0 Hz, 1H), 4.37 (s, 1H), 4.03 (q, *J* = 7.1 Hz, 1H), 2.46 – 2.23 (m, 5H), 1.99 (t, *J* = 27.1 Hz, 2H), 1.53 (s, 3H), 1.38-1.37 (m, 13H), 1.34 – 1.11 (m, 9H).

LC/MS (method K): m/z 564 $[M+H]^+$, retention time 2.295 min. Purity 96%

Step 2: ammonia substitution



10b

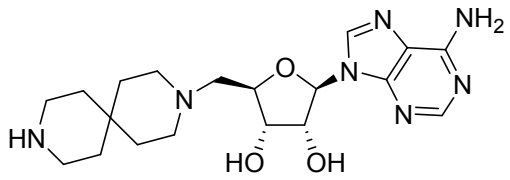
Compound **10b** was obtained after a reaction time of 2 h at 100^0C, following the procedure described for **14b**. Purification by SiO_2 chromatography (eluent dichloro methane / methanol 9:1) afforded **10b** as a colorless oil in 64% yield.

^{1}H NMR (300 MHz, DMSO) δ 8.35 (s, 1H), 8.16 (s, 1H), 7.32 (s, 2H), 6.11 (s, 1H), 5.75 (s, 1H), 5.46 (d, *J* = 6.3 Hz, 1H), 4.92 (s, 1H), 4.25 (s, 1H), 3.25 (s, br.,4H), 2-40-2.32 (s, br.., 4H), 1.52 (s, 3H), 1.37 (s, 9H), 1.32 (s, 3H), 1.29 – 1.20 (m, 9H).

LC/MS (method K)): m/z 544 $[M+H]^+$, retention time 1.977 min. Purity 99%

Step 3: deprotection

Preparation of compound **10**



10

The procedure to obtain compound **14** was followed. Upon completion, the solvent was evaporated under reduced pressure. The crude material was then dissolved in methanol/water (2 mL/mmol, 1:1). To the solution, basic Amberlyst resin (619 mg, Amberlyst A26, CAS: 39339-85-0) was added and gently shaked for 10 min until the pH increased to between 8-9. The resin was filtered off and washed with MeOH. The solution was then evaporated to afford compound **10** in 80% yield as the free base.

An analytically pure sample was obtained by Prep SFC (Stationary phase: Chiralpak Diacel AD 20 x 250 mm, Mobile phase: Acetonitrile, EtOH + 0.4% (v/v) $iPrNH_2$). The solvents of the pure fraction were evaporated under reduced pressure. The residue was co-evaporated with methanol.

Yield: 14 mg as a colorless oil.

^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 1.42 (br t, *J*=5.5 Hz, 8 H) 2.35 - 2.47 (m, 4 H) 2.55 (dd, *J*=13.4, 6.5 Hz, 1 H) 2.68 (dd, *J*=13.6, 4.3 Hz, 1 H) 2.79 (br s, 4 H) 3.97 - 4.03 (m, 1 H)

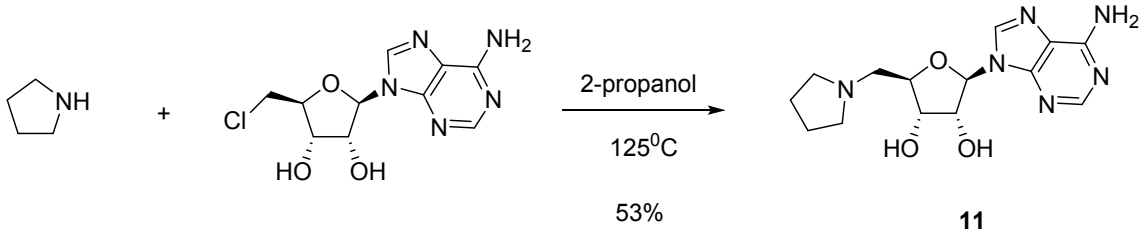
4.15 (t, *J*=5.3 Hz, 1 H) 4.57 - 4.62 (m, 1 H) 5.86 (d, *J*=4.5 Hz, 1 H) 6.90 (br s, 2 H) 8.15 (s, 1 H) 8.24 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-*d*6) δ ppm 29.32 (s, 1 C) 35.43 (s, 2 C) 36.10 (s, 2 C) 40.92 (s, 2 C) 49.70 (s, 2 C) 60.80 (s, 1 C) 72.48 (s, 1 C) 73.58 (s, 1 C) 83.13 (s, 1 C) 88.73 (s, 1 C) 120.02 (br s, 1 C) 140.15 (s, 1 C) 150.13 (s, 1 C) 153.02 (s, 1 C) 156.60 (s, 1 C).

LC/MS (method B): m/z 404.3 $[M+H]^+$, retention time 0.95 min. Purity 99%

HRMS $[M+H]^+$ calcd. for $C_{19}H_{30}N_7O_3$ 404.2410; found 404.2409

Preparation of Compound **11**



Compound **11** was prepared via the synthesis described for compound **1**, starting from 5'-chloro-5'-deoxyadenosine (400 mg, 1.4 mmol) (CAS 892-48-8) and pyrrolidine (2.0 mL) (CAS 123-75-1), reaction time overnight at 60^{0}C. HPLC purification conditions: Stationary phase: RP SunFire Prep C18 OBD-10µm,30x150mm, Mobile phase: 0.25% NH_4HCO_3 solution in water, MeOH. Yield 0.24 g, 53% as a white solid. To obtain an analytical pure sample, a second purification using 100 mg by prep SFC (Stationary phase: Chiralpak Diacel AD 20 x 250 mm, Mobile phase: CO_2, EtOH + 0.4 $iPrNH_2$) afforded 80 mg pure product as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.59 - 1.69 (m, 4 H) 2.41 - 2.48 (m, 4 H) 2.61 (dd, *J*=12.9, 6.9 Hz, 1 H) 2.79 (dd, *J*=12.9, 5.0 Hz, 1 H) 3.97 (dt, *J*=6.8, 4.7 Hz, 1 H) 4.13 (q, *J*=4.8 Hz, 1 H) 4.64 (q, *J*=5.4 Hz, 1 H) 5.18 (d, *J*=5.3 Hz, 1 H) 5.42 (d, *J*=5.9 Hz, 1 H) 5.86 (d, *J*=5.3 Hz, 1 H) 7.26 (s, 2 H) 8.15 (s, 1 H) 8.34 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 23.11 (s, 2 C) 54.20 (s, 1 C) 57.86 (s, 1 C) 71.75 (s, 1 C) 72.66 (s, 1 C) 83.08 (s, 1 C) 87.40 (s, 1 C) 119.11 (s, 1 C) 139.76 (s, 1 C) 149.43 (s, 1 C) 152.59 (s, 1 C) 156.02 (s, 1 C)

LC/MS (Method G): m/z 321.2 $[M+H]^+$, retention time 0.76 min. Purity 100%

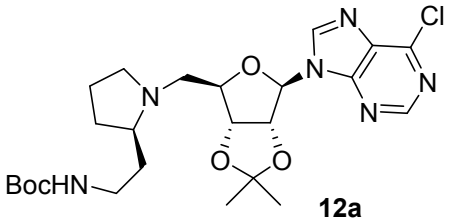
HRMS $[M+H]^+$ calcd. for $C_{14}H_{21}N_7O_3$ 321.1675; found 321.1674

Preparation of Compound **12**

The same sequence was followed as described for **Compound 14.**

Step 1: reductive amination

Compound **12a**



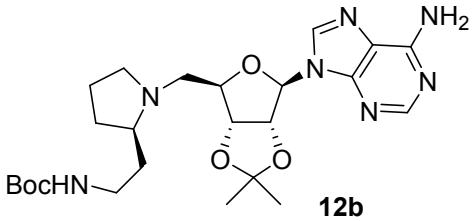
12a

was obtained as a colorless oil in 42% yield (345 mg) using t-butyl (*R*)-(2-(pyrrolidine-2-yl)ethyl)carbamate, CAS Number: 720000-05-0, and (3aS,4S,6R,6aR)-6-(6-chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carbaldehyde, CAS 52719-20-7, following the general procedure to obtain **14a** after purification by SiO_2 chromatography (eluent heptane / ethyl acetate).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.45 (s, 1H), 6.15 (s, 1H), 5.38 (d, *J* = 6.0 Hz, 1H), 4.88 (s, 1H), 4.78 (s, 1H), 4.47– 4.36 (m, 1H), 3.21 (s, 1H), 3.12 – 2.92 (m, 4H), 2.87 (s, 1H), 2.57 – 2.28 (m, 2H), 2.18 (d, *J* = 8.5 Hz, 1H), 1.85 (d, *J* = 8.2 Hz, 2H), 1.80 – 1.68 (m, 2H), 1.62 (s, 3H), 1.41 (s, 9H), 1.39(s, 3H).

LC/MS (method K): m/z 524 $[M+H]^+$, retention time 2.221 min. Purity 97%

Step 2: ammonia substitution



12b

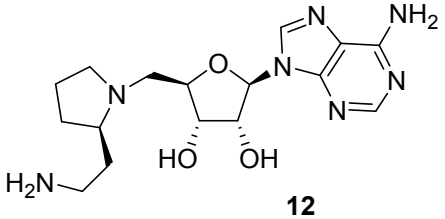
Compound **12b** was obtained after a reaction time of 2 h at 100^0C, following the procedure described for **14b**. Purification by SiO_2 chromatography (eluent dichloro methane / methanol 9:1) afforded **12b** as a colorless oil in 65% yield.

^{1}H NMR (300 MHz, CDCl3) δ 8.35 (s, 1H), 8.03 (s, 1H), 6.08 (s, 1H), 5.73 (s, 2H), 5.50 (s, 1H), 4.94 – 4.88 (m, 2H), 4.39(s, 1H), 3.24 (s, 1H), 3.01 (s, br., 3H), 2.40-2.10 (m, 5H), 1.84-1.82 (m,4H), 1.72 (s, 3H), 1.43 (s, 9H), 1.40 (s, 3H).

LC/MS (method K): m/z 504 $[M+H]^+$, retention time 0.905 min. Purity 100%

Step 3: deprotection

Preparation of compound **12**



12

The procedure to obtain compound **14** was followed. Upon completion, the solvent was evaporated under reduced pressure. The crude material was then dissolved in methanol/water (2 mL/mmol, 1:1). To the solution, basic Amberlyst resin (619 mg, Amberlyst A26, CAS: 39339-85-0) was added and gently shaked for 10 min until the pH increased to between 8-9. The resin was filtered off and washed with MeOH. The solution was then evaporated to afford compound **12** in 77% yield as the free base.

An analytically pure sample was obtained by Prep SFC (Stationary phase: Chiralpak Diacel AD 20 x 250 mm, Mobile phase: Acetonitrile, EtOH + 0.4% (v/v) $iPrNH_2$). The solvents of the pure fraction were evaporated under reduced pressure. The residue was co-evaporated with methanol.

Yield: 38 mg as a colorless oil.

^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 1.23 - 1.38 (m, 2 H) 1.55 - 1.68 (m, 3 H) 1.76 - 1.86 (m, 1 H) 2.12 - 2.21 (m, 1 H) 2.37 - 2.45 (m, 1 H) 2.50 - 2.66 (m, 2 H) 2.52 - 2.57 (m, 1 H) 2.92 - 2.98 (m, 1 H) 2.98 - 3.04 (m, 1 H) 3.98 (ddd, *J*=6.5, 5.1, 3.9 Hz, 1 H) 4.17 (t, *J*=5.3 Hz, 1 H) 4.61 (t, *J*=5.1 Hz, 1 H) 5.87 (d, *J*=4.5 Hz, 1 H) 6.89 (br s, 2 H) 8.15 (s, 1 H) 8.22 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-*d*6) δ ppm 22.09 (s, 1 C) 29.69 (s, 1 C) 37.59 (s, 1 C) 38.94 (s, 1 C) 54.49 (s, 1 C) 55.86 (s, 1 C) 62.46 (s, 1 C) 71.59 (s, 1 C) 72.90 (s, 1 C) 82.78 (s, 1 C) 87.45 (s, 1 C) 119.04 (s, 1 C) 139.63 (s, 1 C) 149.37 (s, 1 C) 152.60 (s, 1 C) 156.00 (s, 1 C)

LC/MS (method B): m/z 364.2 $[M+H]^+$, retention time 1.43 min. Purity 100%

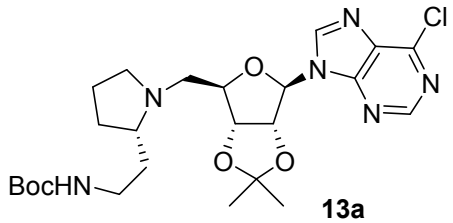
HRMS $[M+H]^+$ calcd. for $C_{16}H_{26}N_7O_3$ 364.2097; found 364.2100

Preparation of Compound **13**

The same sequence was followed as described for **Compound 14.**

Step 1: reductive amination

Compound **13a**



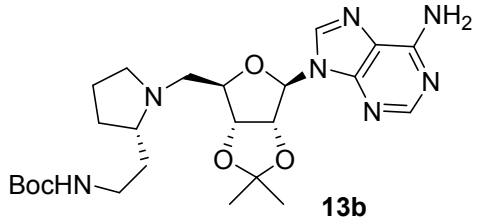
13a

was obtained as a colorless oil in 42% yield (345 mg) using t-butyl (*S*)-(2-(pyrrolidine-2-yl)ethyl)carbamate, CAS Number: 719999-55-0, and (3aS,4S,6R,6aR)-6-(6-chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carbaldehyde, CAS 52719-20-7, following the general procedure to obtain **14a** after purification by SiO_2 chromatography (eluent heptane / ethyl acetate).

^{1}H NMR (300 MHz, CDCl3) δ 8.79 (s, 1H), 8.32 (s, 1H), 6.16 (s, 1H), 5.48 (d, *J* = 5.3 Hz, 1H), 5.07 (s, 1H), 4.84 (s, 1H), 4.44 (s, 1H), 3.12 (ddd, *J* = 49.6, 29.9, 21.5 Hz, 4H), 2.38 (s, 2H), 2.10 (s, 1H), 1.84 (s, 2H), 1.73 – 1.61 (m, 4), 1.52 (s, 3H), 1.42 (s, 9H), 1.41 (s, 3H)

LC/MS (method VILLA): m/z 524 $[M+H]^+$, retention time 2.241 min. Purity 97%

Step 2: ammonia substitution



13b

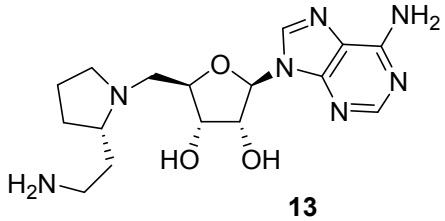
Compound **13b** was obtained after a reaction time of 2 h at 100^0C, following the procedure described for **14b**. Purification by SiO_2 chromatography (eluent dichloro methane / methanol 9:1) afforded **13b** as a colorless oil in 48% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.93 (s, 1H), 6.08 (s, 1H), 5.68 (s, 2H), 5.54 (d, *J* = 5.4 Hz, 1H), 5.14 – 5.04 (m, 1H), 4.91 (s, 1H), 4.42 (s, 1H), 3.16-3.01 (m, 4H), 2.37 (s, 2H), 2.10 (s, 1H), 1.84-1.71 (m, 6H), 1.61 (s,3H), 1.44 (s, 9H), 1.40 (s, 3H).

LC/MS (method K): m/z 504 $[M+H]^+$, retention time 1.197 min. Purity 99%

Step 3: deprotection

Preparation of compound **13**



13

The procedure to obtain compound **14** was followed. Upon completion, the solvent was evaporated under reduced pressure. The crude material was then dissolved in methanol/water (2 mL/mmol, 1:1). To the solution, basic Amberlyst resin (619 mg, Amberlyst A26, CAS: 39339-85-0) was added and gently shaked for 10 min until the pH increased to between 8-9. The resin was filtered off and washed with MeOH. The solution was then evaporated to afford compound **13** in 74% yield as the free base.

An analytically pure sample was obtained by Prep SFC (Stationary phase: Chiralpak Diacel AD 20 x 250 mm, Mobile phase: Acetonitrile, EtOH + 0.4% (v/v) $iPrNH_2$). The solvents of the pure fraction were evaporated under reduced pressure. The residue was co-evaporated with methanol.

Yield: 9 mg as a colorless oil.

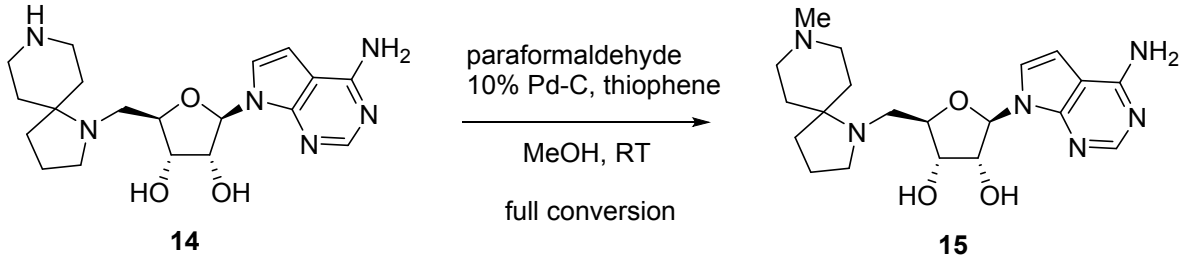
^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 1.24 - 1.42 (m, 2 H) 1.53 - 1.75 (m, 3 H) 1.76 - 1.89 (m, 1 H) 2.16 (q, *J*=8.5 Hz, 1 H) 2.34 - 2.45 (m, 2 H) 2.55 - 2.74 (m, 2 H) 3.03 - 3.13 (m, 2 H) 4.01 (td, *J*=6.3, 4.5 Hz, 1 H) 4.13 - 4.18 (m, 1 H) 4.71 (t, *J*=5.3 Hz, 1 H) 5.89 (d, *J*=5.3 Hz, 1 H) 6.91 (br s, 2 H) 8.17 (s, 1 H) 8.26 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-*d*6) δ ppm 22.15 (s, 1 C) 29.63 (s, 1 C) 37.28 - 37.50 (m, 1 C) 54.39 (s, 1 C) 56.38 (s, 1 C) 62.54 (s, 1 C) 71.83 (s, 1 C) 72.36 (s, 1 C) 83.75 (s, 1 C) 87.21 (s, 1 C) 119.16 (s, 1 C) 139.90 (s, 1 C) 149.47 (s, 1 C) 152.58 (s, 1 C) 156.01 (s, 1 C)

LC/MS (method B): m/z 364.3 $[M+H]^+$, retention time 1.61 min. Purity 99%

HRMS $[M+H]^+$ calcd. for $C_{16}H_{26}N_7O_3$ 364.2097; found 364.2095

Preparation of Compound **15**



14 **15**

Pd/C (10%) 250 mg, 0.235 mmol) was suspended in MeOH (200 mL) under nitrogen atmosphere. A 0.4% solution of thiophene in di*iso*propyl ether was added, followed by

compound **14** (2.5 g, 6.4mmol) and paraformaldehyde (0.58 g, 19.3 mmol). The reaction was stirred under hydrogen atmosphere until 6.4 mmol H_2.was absorbed. The catalyst was filtered off over a path of celite. The path was washed several times with methanol. The solvents of the filtrate were evaporated yielding 2.7 g (100%) compound **15**.

LC/MS (Method E): m/z 403.3 $[M+H]^+$, retention time 0.89 min. Purity 89%

To obtain an analytical pure sample, 100 mg was separated by prep SFC (Stationary phase: Chiralpak Diacel AD 20 x 250 mm, Mobile phase: CO_2, EtOH + 0.4% $iPrNH_2$) to afford 80 mg pure product **15** as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.10 - 1.24 (m, 2 H) 1.49 - 1.73 (m, 6 H) 1.84 - 1.96 (m, 2 H) 2.15 (s, 3 H) 2.60 - 2.68 (m, 1 H) 2.68 - 2.76 (m, 2 H) 2.76 - 2.85 (m, 2 H) 3.82 - 3.88 (m, 1 H) 4.02 (br q, *J*=4.4 Hz, 1 H) 4.37 (q, *J*=5.6 Hz, 1 H) 5.03 (br d, *J*=5.0 Hz, 1 H) 5.22 (d, *J*=6.2 Hz, 1 H) 6.04 (d, *J*=5.6 Hz, 1 H) 6.60 (d, *J*=3.6 Hz, 1 H) 6.98 (s, 2 H) 7.34 (d, *J*=3.7 Hz, 1 H) 8.06 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 20.63 (s, 1 C) 30.18 (s, 1 C) 32.03 (s, 1 C) 33.32 (s, 1 C) 45.89 (s, 1 C) 50.16 (s, 1 C) 51.37 (s, 1 C) 53.37 (s, 1 C) 53.58 (s, 1 C) 60.82 (s, 1 C) 71.55 (s, 1 C) 73.10 (s, 1 C) 83.02 (s, 1 C) 86.52 (s, 1 C) 99.78 (s, 1 C) 102.72 (s, 1 C) 121.53 (s, 1 C) 150.38 (s, 1 C) 151.72 (s, 1 C) 157.41 (s, 1 C)

LC/MS (Method E): m/z 403.3 $[M+H]^+$, retention time 0.92 min. Purity 99%

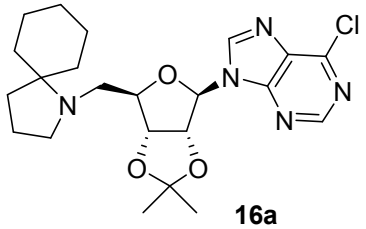
HRMS $[M+H]^+$ calcd. for $C_{20}H_{31}N_7O_3$ 432.2458; found 432.2456

Preparation of Compound **16**

The same sequence was followed as described for **Compound 40.**

Step 1: reductive amination

Compound **16a**



16a

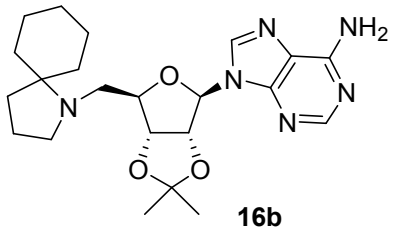
was obtained as a colorless oil in 25% yield (181 mg) using 1-azaspiro[4.5]decane hydrochloride, CAS Number: 176-80-7, and (3aS,4S,6R,6aR)-6-(6-chloro-9*H*-purin-9-yl)-

2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carbaldehyde, CAS 52719-20-7, following the general procedure to obtain **14a** after purification by SiO_2 chromatography (eluent heptane / ethyl acetate).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.28 (s, 1H), 6.13 (s, 1H), 5.43 (d, *J* = 5.5 Hz, 1H), 4.95 (s, 1H), 4.30 (d, *J* = 32.7 Hz, 1H), 3.05 – 2.42 (m, 4H), 1.65 (s, br., 5H), 1.59 (s, 3H), 1.37 (s, 3H), 1.31 – 1.14 (m, 5H), 0.94 – 0.74 (m, 4H)

LC/MS (method K): m/z 448 $[M+H]^+$, retention time 0.899 min. Purity 91%

Step 2: ammonia substitution



16b

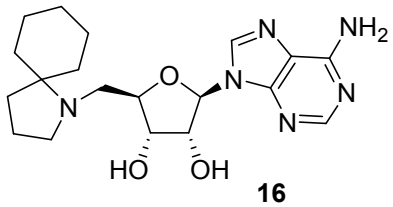
Compound **16b** was obtained after a reaction time of 2 h at 100^{0}C, following the procedure described for **14b**. Purification by SiO_2 chromatography (eluent dichloro methane / methanol 9:1) afforded **16b** as a colorless oil in 58% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.93 (s, 1H), 6.08 (s, 1H), 5.59 (s, br., 3H), 5.02 (s, br., 1H), 4.36 (s, 1H), 2.83-2.58 (m, 3H), 1.69-1.63 (m, 6H), 1.61 (s, 3H), 1.40 (s, 3H), 1.29-1.20 (m, 6H), 0.94-0.88 (m, 3H).

LC/MS (method K): m/z 429 $[M+H]^+$, retention time 0.709 min. Purity 96%

Step 3: deprotection

Preparation of compound **16**



16

The procedure to obtain compound **14** was followed. Upon completion, the solvent was evaporated under reduced pressure. The crude material was then dissolved in methanol/water (2 mL/mmol, 1:1). To the solution, basic Amberlyst resin (619 mg, Amberlyst A26, CAS: 39339-85-0) was added and gently shaked for 10 min until the pH increased to between 8-9.

The resin was filtered off and washed with MeOH. The solution was then evaporated to afford compound **16** in 55% yield as the free base.

An analytically pure sample was obtained by Prep SFC ((Stationary phase: RP XBridge Prep C18 OBD-10µm,50x250mm, Mobile phase: 0.25% NH_4HCO_3 solution in water, MeOH). The residue was dissolved in water with some acetonitrile. After removal of the solvents by lyophilization the product was obtained.

Yield: 13 mg as a white solid.

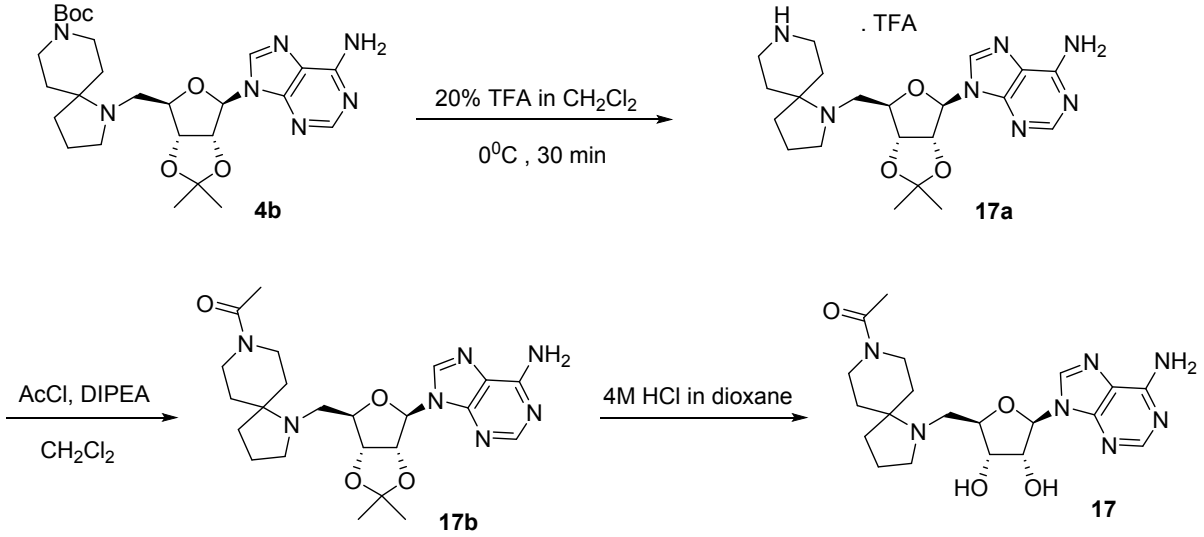
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.19 - 1.42 (m, 4 H) 1.22 - 1.28 (m, 3 H) 1.50 - 1.69 (m, 4 H) 1.61 - 1.66 (m, 3 H) 2.63 (dd, *J*=13.0, 5.7 Hz, 1 H) 2.67 - 2.74 (m, 1 H) 2.80 - 2.85 (m, 1 H) 2.88 (dd, *J*=13.4, 6.1 Hz, 1 H) 3.94 - 3.99 (m, 1 H) 4.18 (t, *J*=4.7 Hz, 1 H) 4.71 (t, *J*=4.1 Hz, 1 H) 5.89 (d, *J*=1.0 Hz, 1 H) 6.90 (br s, 2 H) 8.16 (s, 1 H) 8.26 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 20.87 (s, 1 C) 23.74 (s, 2 C) 23.93 (s, 1 C) 25.73 (s, 1 C) 32.77 (s, 1 C) 33.79 (s, 1 C) 50.00 (s, 1 C) 51.42 (s, 1 C) 62.87 (s, 1 C) 71.66 (s, 1 C) 72.82 (s, 1 C) 84.28 (s, 1 C) 87.81 (s, 1 C) 119.41 (s, 1 C) 139.76 (s, 1 C) 149.59 (s, 1 C) 152.44 (s, 1 C) 156.06 (s, 1 C).

LC/MS (method K): m/z 389 $[M+H]^+$, retention time 1.163 min. Purity 99%

HRMS $[M+H]^+$ calcd. for $C_{19}H_{29}N_7O_3$ 389.2301; found 389.2301

Preparation of Compound **17**



Step 1: Boc deprotection

Compound **17 TFA salt:**

Compound **4b** (227 mg, 0.43 mmol) was dissolved in 22 mL of 20% TFA/CH_2Cl_2 solution at 0°C. The ice/H_2O bath was removed, and the reaction mixture stirred at r.t. for 30 min. Upon completion of the reaction, the solvent was removed *in vacuo*, to afford compound **17a**, as a TFA salt, that was used as such in the next step.

LC/MS (method K): m/z 430 $[M+H]^+$, retention time 0.216 min. Purity 77%

Step 2: Acetylation

Compound 17b

Crude TFA salt **17a** was dissolved in 10 equiv. of DIPEA at -10°C. A solution of acetyl chloride (0.43 mmol) in CH_2Cl_2 (33 mL/mmol) was added dropwise. The reaction mixture was stirred at -10°C during 10 min. Volatiles were removed *in vacuo* to afford crude acetyl derivative **17b**. The product was purified by SiO_2 chromatography (eluent dichloro methane / methanol 9:1) to afford **17b** as a colorless oil (65% yield over 2 steps).

LC/MS (method K): m/z 430 $[(M-CH_3-CO)+H]^+$, retention time 0.634 min. Purity 70%

Step 3: deprotection

Preparation of Compound **17**

HCl (4N in dioxane, 10 eq.) was added to a stirred solution of **17b** in MeOH (43 mL/mmol) at r.t. The reaction mixture was stirred at r.t. for 18 hrs. Upon completion, the solvent was evaporated under reduced pressure. The crude material was then dissolved in MeOH/H_2O (2 mL/mmol, 1:1). To the solution, basic Amberlyst resin (619 mg, Amberlyst A26, CAS: 39339-85-0) was added and slowly shaked for 10 min until pH raised between 8-9. The resin was filtered off and washed with MeOH. The solution was then evaporated to afford **17** as the free base in 55% yield as a colorless oil. To obtain an analytical pure sample, the compound was purified by prep SFC (stationary phase: Chiralpak Diacel AD 20 x 250 mm, mobile phase: Acetonitrile, EtOH + 0.4% (v/v) $iPrNH_2$). The solvents of the pure fraction were evaporated under reduced pressure. The residue was co-evaporated with methanol.

Yield: 32 mg as a white solid

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.20 - 1.31 (m, 2 H) 1.43 (br d, *J*=12.6 Hz, 1 H) 1.52 (br d, *J*=12.6 Hz, 1 H) 1.63 - 1.79 (m, 4 H) 1.97 (s, 3 H) 2.53 - 2.90 (m, 6 H) 3.72 - 4.51 (m,

2 H) 3.90 - 3.99 (m, 1 H) 4.11 - 4.20 (m, 1 H) 4.70 (br s, 2 H) 5.00 (br s, 1 H) 5.86 (d, *J*=4.9 Hz, 1 H) 6.78 (br s, 2 H) 8.13 (s, 1 H) 8.21 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 20.54 (s, 1 C) 20.64 (s, 1 C) 31.06 (br s, 1 C) 32.25 (br s, 1 C) 32.95 (s, 1 C) 49.68 (s, 1 C) 51.13 (s, 1 C) 61.39 (s, 1 C) 71.42 (s, 1 C) 72.57 (s, 1 C) 83.94 (s, 1 C) 87.76 (s, 1 C) 119.26 (s, 1 C) 139.61 (s, 1 C) 149.37 (s, 1 C) 152.18 (s, 1 C) 155.84 (s, 1 C) 167.58 (s, 1 C)

LC/MS (method K): m/z 432 $[M+H]^+$, retention time 0.593 min. Purity 97%

HRMS $[M+H]^+$ calcd. for $C_{20}H_{30}N_7O_3$ 432.2359; found 432.2363

S3 Computational Chemistry

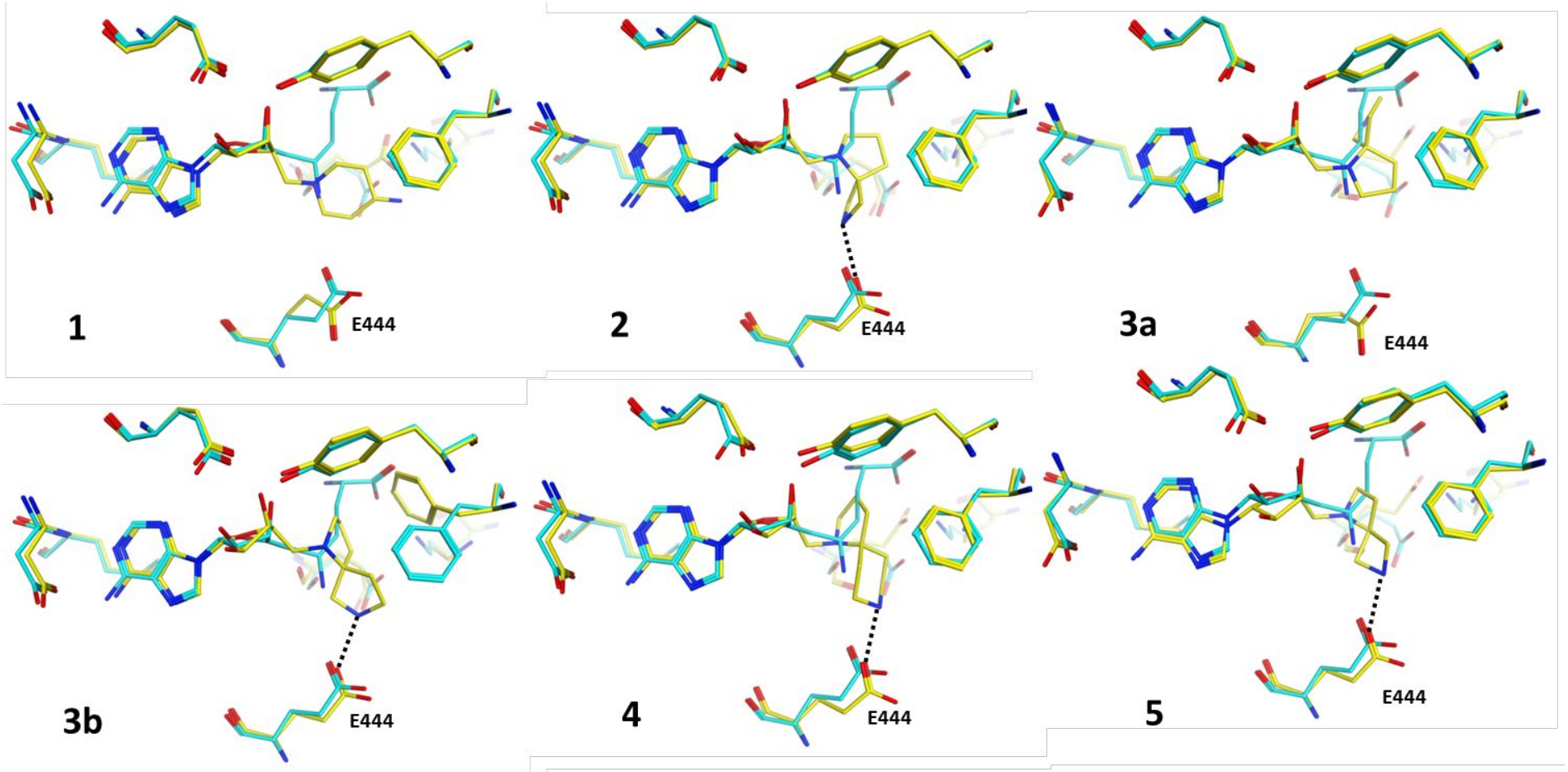
S3.1 Methods

Retrospective docking calculations presented here were performed on a selection of **13** compounds (**1-10**, **12** and**13**). For compound **3**, both the diastereomers were considered and for all compounds only the distal amines were considered as protonated. Induced Fit Docking (IFD) calculations and associated calculations were performed using Schrödinger Suite 2016-3[9]. Ligands and receptor (PDB ID: 4GQB without water molecules and histone peptide) were prepared with LigPrep and Protein Preparation Wizard, respectively as implemented in Maestro. IFD protocol was used with default settings (A9145C was selected as the ligand to define the docking box), except, the Glide redocking step was performed at XP precision level. Resulting poses were ranked with IFD score and visually evaluated superimposing the starting PRMT5:A9145C complex coordinates.

S3.2 Results

Figure S3 shows finally selected IFD poses for each ligand considered in this retrospective study. A general observation with this class of spirocylic SAM mimetics is that protein flexibility is essential in order to accommodate them in the SAM binding site, while maintaining shape and pharmacophore similarity with the adenosine-like substructure of SAM analogue A9145C. Our approach to selecting final docking poses for SAR analysis (Figure S3) was to use both the best IFD score as well as a general overlap (based on visual

inspection) with the adenosine substructure of the SAM analogue A9145C. This allowed us to not select top IFD ranking poses for SAR analysis, where the ligand was flipped, for instance. With this approach, a semi-quantitative model justifying the SAR emerged, where most of the active compounds formed a hydrogen-bond via their distal amines with Glu444 residue at the boundary of substrate site, while all the inactive and several very weak compounds did not form such a hydrogen-bond. This model was further substantiated with donor-less analogues **16** and **17**, where either the distal nitrogen was simply removed or acetylated, respectively, and these modifications resulted in nearly inactive compounds. Eventually, the Glu444 interaction model was further validated by a co-crystal structure of compound 14 (close analogue of compound 4) bound to PRMT5.


1
E444
2
E444
3a
E444
3b
E444
4
E444
5
E444
6
7
E444
8
E444

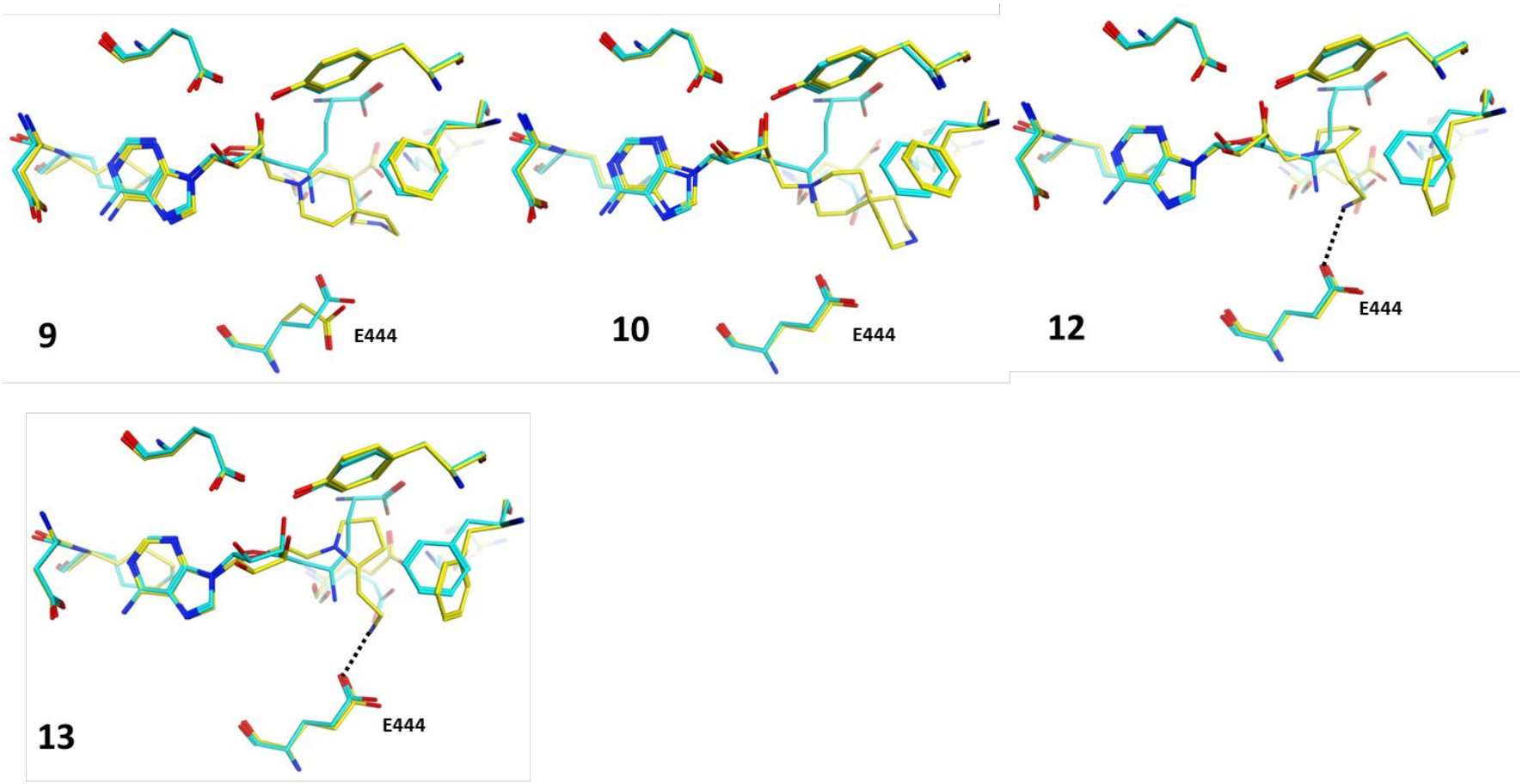


Figure S3. Induced fit docking models (yellow) superimposed with PRMT5:A9145C crystallographic coordinates (cyan). The numbers correspond to the selection of thirteen compounds (**3a** and **3b** refer to diasteromers). Hydrogen bond with E444 is depicted as dotted line

S4 Enzyme Assays

S4.1 Materials

PRMT5:MEP50 protein was purchased from Charles River Laboratories (Wilmington, Massachusetts). The protein complex was produced in Sf9 insect cells infected simultaneously with two baculoviruses. One virus expresses full length human PRMT5 with Flag-tag at N-terminus, the second virus expresses full length MEP50 with His6-TEV cleavage sequence at N-terminus. The protein was affinity purified using anti-Flag beads eluted with 3xFlag peptide, followed by His-select eluted with 0.5 M imidazole. Eluted protein was then dialyzed against TBS buffer, pH 8.0 containing 20% glycerol and 3 mM DTT.

Full length recombinant human histone H2A (Genebank Accession# NM_021052, Cat# HMT-11-146) expressed in *E. coli* was purchased from Reaction Biology Corporation (Malvern, Pennsylvania). SAM (Cat# 13956) and SAH (Cat# 13603) were purchased from Cayman Chemical Company (Ann Arbor, Michigan). Reagents used for making reaction buffer and stopping reaction were purchased. Trizma base (Cat# T1503), sodium chloride solution 5 M (Cat# 59222C), magnesium chloride hexahydrate (Cat # M0250) were purchased from Sigma-Aldrich Corporation (Ronkonkoma, NY). DTT solution 1 M (Cat# P2325) was purchased from ThermoFisher Scientific (Waltham, MA). Formic acid (Cat# 33015) was purchased from Riedel deHaen (Mexico City, Mexico).

S4.2 RapidFire mass spectrometry assay

Enzyme activity was measured by following the production of SAH generated by transferring a methyl group of SAM to histone H2A by PRMT5:MEP50. Assay was performed in a buffer of 20 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM $MgCl_2$ and 1 mM DTT. Reaction was stopped by 1% formic acid (final concentration). Reaction substrate SAM and product SAH were detected using a RapidFire 300 high-throughput solid-phase extraction chromatography system coupled to a Sciex 4000 QTrap triple quadrupole mass spectrometry (Agilent Technologies, Santa Clara, CA). Reaction mixtures were injected onto an Agilent Graphite Type D cartridge in 0.1% trifluoacetic acid, and eluted with 80% acetonitrile and 0.1% trifluoacetic acid. The multiple reaction monitoring (MRM) method was used for detecting SAM and SAH at Q1/Q3 of 399.2/250.1 and 385.2/136.1, respectively.

S4.3 Measurement of inhibition

Compound IC_{50} values were determined using a 11-point dosing series made for each compound by 2-fold serially diluted in DMSO, with point 12 being a DMSO control for an uninhibited enzymatic reaction. Compounds were first spotted to 384-well assay plates, and followed by addition of 30 µl of a mixture of 2 µM SAM and 0.6 µM H2A. The enzymatic reaction was initiated by addition of 30 µl of 20 nM PRMT5:MEP50. The final concentrations of the reaction mixture contained 1 µM SAM, 0.3 µM H2A and 10 nM PRMT5:MEP50. The reaction mixture was incubated at 30 ˚C for 60 min and then quenched by adding 10 µl formic acid solution (final concentration 1%). Inhibition of SAH formation

in the presence of compound was calculated as a percentage of the control relative to the uninhibited reaction as a function of inhibitor concentrations. Data were fit to eq 1:

$$y = \frac{100}{1 + \left(\frac{x}{IC_{50}}\right)^h} \quad (1)$$

Y is the percent of inhibition, X is log of compound concentration, IC_{50} is the inhibitor concentration at 50% inhibition, and h is the Hill slope.

For the compounds that reach the assay limit ($IC_{50} \leq 5$ nM), inhibition potency was measured in a mixture with a reduced enzyme concentration that is ≤ the value of 2x IC_{50} of the inhibitor.

S4.4 Inhibition mechanism studies

The initial velocity of the enzymatic reaction was determined by measuring product SAH formation. The inhibition of reaction was studied by varying the concentration of SAM while keeping the concentration of histone H2A at 0.3 μM) or by varying the concentration of substrate histone H2A at a fixed concentration of SAM at 1 μM. 5nM PRMT5:MEP50 was used in both cases and the reaction mixtures were incubated for 80 minutes at 30 °C. The initial velocities derived from the inhibition experiments were fitted to equation 2 by a nonlinear least squares approach.

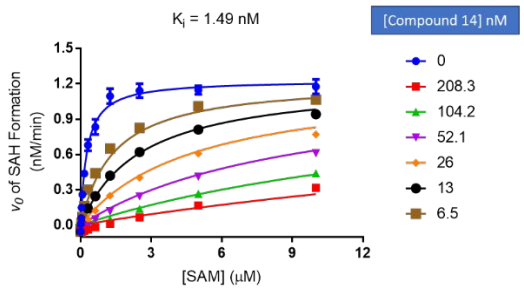
$$v = \frac{v_{max} * [S]}{[S]\left(1 + \frac{[I]}{\alpha K_i}\right) + K_M\,(1 + \frac{[I]}{K_i})} \quad (2)$$

v_{max} is the maximum velocity of the uninhibited reaction, and K_i represents the equilibrium dissociation constant for the enzyme-inhibitor (EI) complex and αKi represents the equilibrium constant for the substrate bound enzyme-inhibitor (ESI) complex. Constant α determines the inhibition mechanism. α = 1 suggests a non-competitive inhibition, while competitive and an uncompetitive inhibition mechanisms are reflected by the α value to be greater than 10 and to be smaller than 0.1 (but greater than 0), respectively.

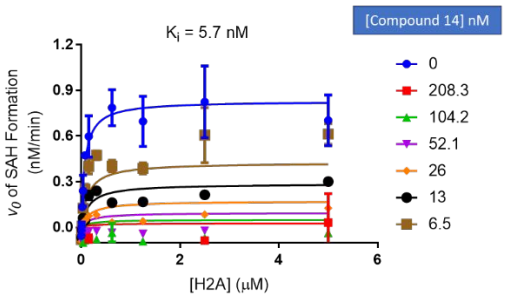
S4.4.1 Data Analysis.

Kinetic data were fitted to the appropriate equations by GraphPad Prism 7.0.

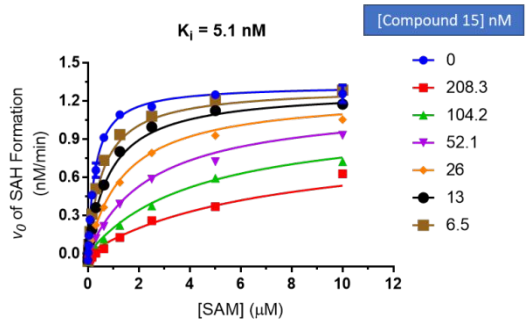
S4.4.2 Results



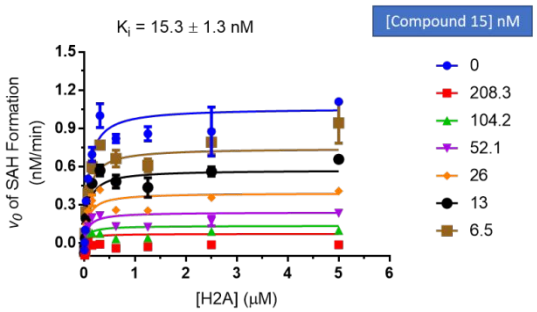
a) Compound 14 is competitive with SAM



b) Compound 14 is non-competitive with H2A



c) Compound 15 is competitive with SAM



d) Compound 15 is non-competitive with H2A

S5 Cellular Assays

A549 cells (ATCC CCL-185) were cultured in DMEM supplemented with 10% Foetal Calf Serum, 2 mM L-Glutamine, 1 mM Sodium Pyruvate and 50 µg/ml Gentamycine. They were harvested using 0.05% Trypsin-EDTA, loaded into black-wall clear-bottom 384-well plates and treated with compound in a dilution of 10^{-5} to 10^{-9} M or 10^{-6} to 10^{-10} M in 9 3.1x serial steps, resulting in a final concentration of 0.2% DMSO. Following 48 h of compound incubation, cells were fixed in 10% formalin for 15 min, permeabilized with ice-cold methanol for 20 min, washed with PBS, and probed overnight at 4ºC in blocking buffer (a 0.22 µm filtrated solution of PBS with 1% BSA and 0.5% Triton X-100) containing 1/4000 anti-SMD1/3 antibody SYM10 (Millipore [Billerica, MA] 07-412-100KL). Subsequently, the plates were washed 0.1% PBS/Triton X-100. SYM10 was detected by a green-fluorescent secondary antibody Alexa fluor 488 goat aRb (1/200, ThermoFisher Scientific A11034) and staining of cell structures was done with Hoechst (nucleus, blue) and Cellmask (cytoplasm, deep-red). Images were obtained with a confocal High Content Imaging device (Yokogawa CV7000) using a 3-wavelengths protocol (488, 540 and 633nm). Primary image analysis was

done by Columbus software using a dedicated script to localize and quantify the fluorescence intensity. 50% inhibitory concentration (IC_{50}) values were determined by use of a 2-parameter fitting strategy with fixed top and bottom.

Cell loss IC50s

The compounds (**2, 4, 5-7, 12, 13, 16, 17**) that were active in the biochemical assay were tested in a cell-based assay, measuring the cell count

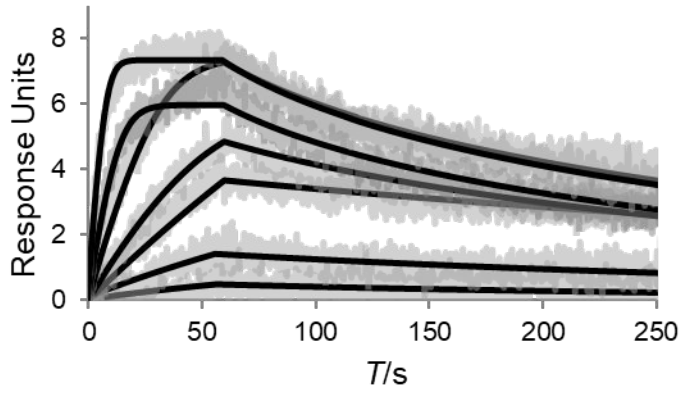
A549 cells were incubated with compounds in a dose range for 48h and subsequently fixed. Staining of cell structures was done with Hoechst (nucleus, blue) and Cellmask (cytoplasm, deep-red). Images were obtained with a confocal High Content Imaging device (Yokogawa CV7000) using a 2 wavelengths protocol (540 and 633nm). Primary image analysis was done by Columbus software using a dedicated script to localize and quantify the fluorescence intensity. The nuclear staining is used to monitor the toxicity of the compounds by normalizing the median nuclear fluorescence intensity of compound treated cells to that of 0.2% DMSO treated cells. log10(50% inhibitory concentration) (pIC50) values were determined by use of a 2-parameter fitting strategy with fixed top and bottom.

None of the compounds tested displayed cell loss up to the maximal concentration tested, 10 μM

S6 Surface plasmon resonance

Surface Plasmon Resonance. Surface plasmon resonance data were collected on a Biacore S51 or T200 system (GE Healthcare) at 25 °C. Streptavidin was immobilized on a CM5 sensor chip (GE Healthcare) or CMD500d chip (Xantec Bioanalytics) using standard amine-coupling chemistry at 25 °C with HBS-N (10 mM HEPES and 0.15 M NaCl, pH 7.4) as the running buffer. The carboxymethyldextran surface was activated with a 12 min injection of a 1:1 ratio of 0.4 M 1-ethyl-3-(3-(dimethylamino) propyl) carbodiimide hydrochloride (EDC)/0.1 M N-hydroxy succinimide (NHS) at a flow rate of 10 μL/min. For capture of streptavidin, protein was diluted to 0.1 mg/mL in 10 mM sodium acetate (pH 4.5) and captured by injecting 300 μL onto the activated chip surface. Residual activated groups were blocked with a 7 min injection of 1 M ethanolamine (pH 8.5). PRMT5/MEP50 (crystallographic construct), bearing an N-terminal His_6 and Avi-tag, was captured on the streptavidin surface by injection of purified protein diluted to 100 μg/mL in 50mM Tris pH 8.0, 150mM NaCl, 5mM MgCl2, 2mM DTT, 0.05% Tween 20. Typical surface densities obtained were 4000−6000 RU. SPR binding data were obtained using appropriate dilution series of each compound at a flow rates of 30−90 μL/min, with a capture time of 120 s and

dissociation times up to 900s. Running buffer for compound binding studies was 50mM Tris pH 8.0, 150mM NaCl, 5mM MgCl2, 2mM DTT, 0.05% Tween 20, 1% DMSO. Data were corrected for DMSO excluded volume effects. All data were double-referenced for blank injections and reference surface using standard processing procedures, and data processing and fitting were performed using Scrubber software, version 2.0c (BioLogic Software). Data were fitted using a simple 1:1 binding model.



k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	$t_{1/2}$ (sec)	K_D (nM)
8.6e5	0.00653	106s	7.6nM

Figure S6.SPR sensorgram (in grey are experimental and black are fitted curves) of **Compound 15** binding to PRMT5 in increasing concentrations (bottom to top).

S7 Crystallography

S7.1 Expression and Purification of PRMT5:MEP50 Complex

Full-length protein arginine methyltransferase 5 (PRMT5) (NP_006100) encompassing residues 1–637 with an amino terminal FLAG tag was cloned into pFastBac1 vector (Life Technologies). Full-length MEP50 (NP_077007) encompassing residues 2–342 was cloned into pFastBac1 vector (Life Technologies) introducing an N terminal, thrombin His tag. Standard baculovirus expression using the Bac-to-Bac system protocol (LifeTechnologies) was used to generate virus for each clone. Expressions of PRMT5 and MEP50 (at 1:1 ratio) in Sf21 cells were harvested by centrifugation and pellets were stored at −80 °C until purification. Purification was conducted at 4 °C. Frozen pellets were resuspended in 50 mM Hepes pH 7.5, 500 mM NaCl, 5% (vol/vol) glycerol, 10mM Imidazole, 0.5mM TCEP were broken using cell disrupter (Constant systems). The homogenate was clarified by centrifugation for 30 min at 18,000 rpm. The supernatant was loaded onto a 5ml His-select

column (Sigma) and washed with 50 mM Hepes pH 7.5, 500 mM NaCl, 5% (vol/vol) glycerol, 10mM Imidazole, 0.5mM TCEP. The PRMT5:MEP50 complex was eluted with buffer 50 mM Hepes pH 7.5, 500 mM NaCl, 5% (vol/vol) glycerol, 300mM Imidazole, 0.5mM TCEP and directly desalted back into buffer with 10mM Imidazole. Thrombin was added and incubated overnight at 4°C and then run through a secondary His-select column and flow through collected. This was concentrated and passed over a size-exclusion chromatography column (16/60 S200; GE LifeSciences) equilibrated in a buffer containing 20 mM Hepes, pH 7.6, 0.4 M NaCl, 5%(vol/vol) glycerol, 2 mM TCEP. Fractions containing PRMT5:MEP50 were concentrated to 10mg/mL for crystallization.

S7.2 Crystallization

The PRMT5:MEP50 complex was incubated with 1 mM **Compound 14** for 60 minutes and screened in crystallization. Crystallization conditions were optimized, and single crystals were grown at 4 °C by sitting-drop method by mixing 1 μL of protein complex at 10mg/mL, with 0.5 μL of reservoir solution containing 20–30%(wt/vol) PEG3350 and 150mM ammonium sulfate. Crystals grew to 50 × 50 × 200 μm within 2 days following seeding. Crystals were cryo-protected in reservoir solution supplemented with 20%(vol/vol) glycerol and flash-cooled in liquid nitrogen for data collection.

S7.3 Structure Determination and Refinement

X-ray diffraction data for the crystal was collected at Diamond Light Source on Beamline I04-1. Data was processed DIALS and AIMLESS. The structure solved by molecular replacement using PHASER with model 4GQB as a starting model. The solution shows the same octameric packing as the previously solved 4GQB. Iterative refinement model building using REFMAC and COOT showed clear unambiguous density for the ligand, which allowed for the stereochemistry of the ligand to be defined. The ligand dictionary was prepared using AceDRG and the structure further refined with the final model produced having an R factor of 21.2% and an R_{free} of 25.6%. All programs are part of the CCP4 suite. The refined structure was deposited in the Protein Data Bank with the accession code: 6RLL.

Table S7.1 Data collection and processing

	Compound 14
Synchrotron	Diamond
Beam line	I04-1
Wavelength (Å)	0.91739
Detector type	Pilatus 6M
Temperature (°K)	100
Oscillation range per frame (°)	0.1
Overall rotation (°)	180
Resolution range (Å)	89.65–2.22 (2.31)
Number of observations	394569 (31870)
Number of unique reflections	60395 (6070)
Multiplicity	6.5 (5.3)
CC(1/2)	0.99 (0.85)
Completeness (%) (overall and last) shell)	97.1 (87.9)
R_{merge} (%) (overall and last shell)	5.8 (94.8)
Mean I/sigma(I) (overall and last) shell)	13.5 (1.4)
Space group	I222
Unit cell parameters (Å), (°)	102.96 136.05 179.30 90.0 90.0 90.0

Table S7.2 Refinement statistics

	Compound 14
Refinement program	Refmac
Resolution range (Å)	89.44-2.22 (2.28)
Number of reflections	57140 (3626)
R_{work} %	21.25 (43.2)
R_{free} %	25.66 (45.7)
Protein residues	920
Ligand Atoms	28
Number of water atoms modeled	94
RMSD Bond lengths (Å)	0.012
RMSD Bond angles (°)	1.923
Ramachandran plot preferred (%)	94.78
Ramachandran plot allowed (%)	3.48
Ramachandran plot generously (%) (5) allowed (%)	1.74

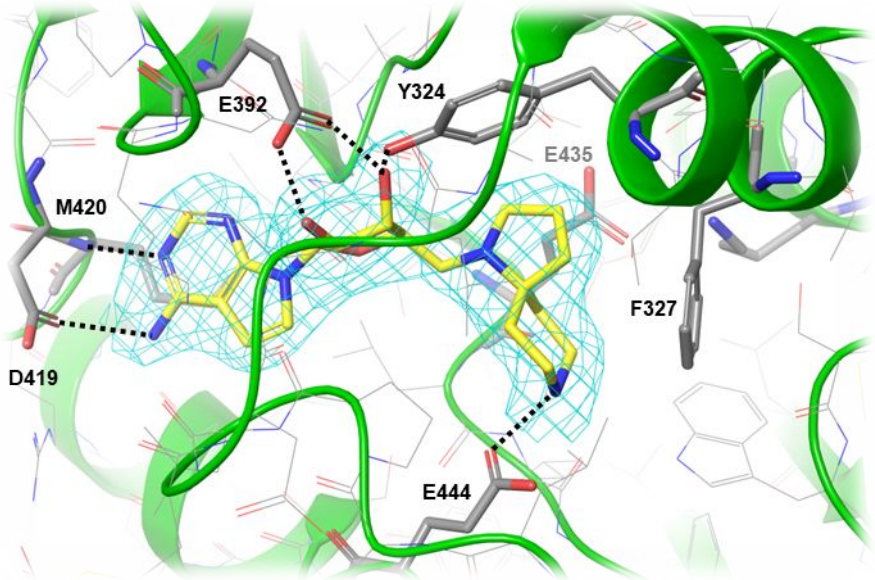


Figure S7.1. Interactions of **Compound 14** (yellow sticks) with PRMT5 (green ribbons and amino acid residues as grey sticks). Hydrogen bonds are depicted as dotted lines. 2Fo-Fc electron density map (cyan) of Compound14 is contoured at 1σ.

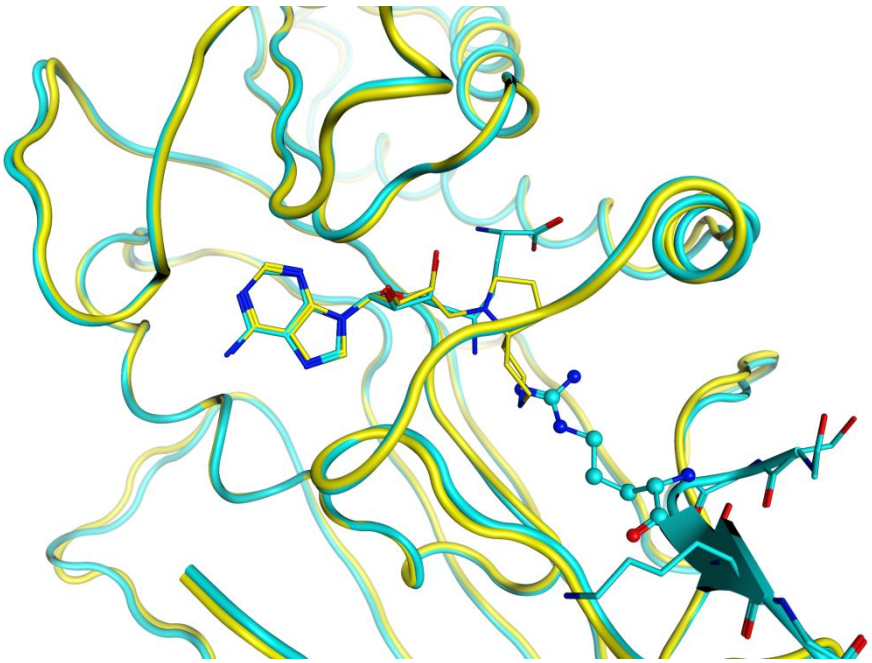


Figure S7.2 Superimposition of co-crystal structure from this study (PDB ID: 6RLL; yellow) with SAM analog **A9145C** and histone H4 peptide bound PRMT5 (PDB ID: 4GQB; cyan). Secondary structure of PRMT5 is depicted as ribbons while the amino acid residues of H4 peptide are visible. **Compound 14** and A9145C are depicted as sticks while the substrate arginine residue (R3) is depicted as balls-and-sticks

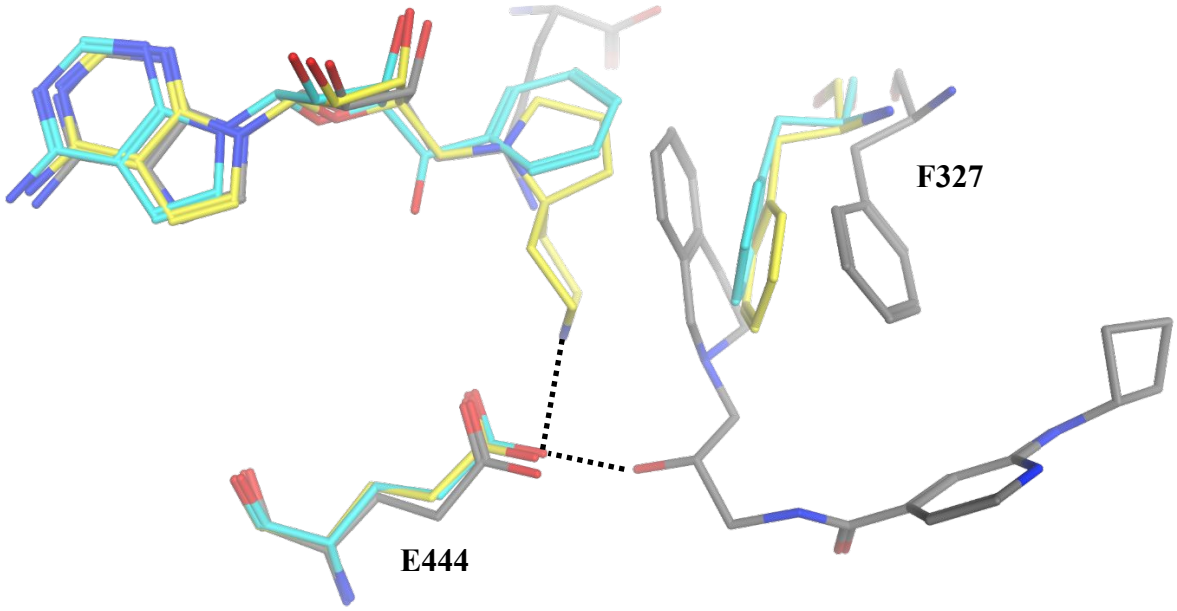


Figure S7.3 Superimposition of publicly available co-crystal structures: from this study (PDB ID: 6RLL; yellow) with LLY-283 (PDB ID: 6CKC; cyan) and a substrate competitive ligand bound to PRMT5 in the presence of Sinefungin (PDB ID: 5C9Z; gray).

S8. Selectivity profiling

Compound **15** was profiled in a commercially available panel of human Arg and Lys methyltransferases (Reaction Biology Corporation) to analyze selectivity, as reported.[10] Inhibitory effect of Compound **15** was determined via a single-dose at 10 μM, duplicate profiling assay against 21 methyltransferases.

Table S8. Selectivity profile of Compound **15** within the family of human arginine methyltransferases and lysine methyltransferase.

Methyl transferase	Substrate	Enzyme Activity (%)[a]
DOT1L	Nucleosomes	94.5
EZH2	Core Histone	93.6
G9A (EHMT2)	Histone H3 (1-21)	110
MLL1	Nucleosomes	94.7
MLL2	Core Histone	78.8
NSD1	Nucleosomes	89.3
PRDM9	Histone H3	125
PRMT1	Histone H4	101
PRMT3	Histone H4	99.6
PRMT4	Histone H3	103
PRMT6	Histone H3	93.3
PRMT7	GST-GAR	75.9
PRMT8	Histone H4	104
SET1B	Core Histone	104
SET7/9 (SETD7)	Core Histone	97.0
SETD2	Nucleosomes	92.9
SET8	Nucleosomes	95.9
SMYD2	Histone H4	102.8
SUV39H1	Nucleosomes	91.1
SUV39H2	Nucleosomes	81.2
SUV420H1TV2	Core Histone	97.5

[a] % Remaining activity in the presence of 10 μM compound compared to DMSO control (100%)

S9 References

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