SUPPLEMENTARY INFORMATION

SMALL-MOLECULE INHIBITORS THAT SELECTIVELY BLOCK DENGUE VIRUS METHYLTRANSFERASE

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Running title: Selective inhibitors of dengue virus methyltransferase

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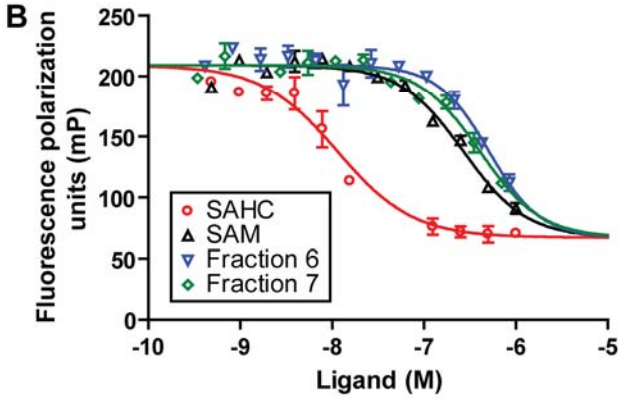
Supplementary Fig. 1. Identification of SAM bound to DENV MTase. (A) DENV-3 MTase was denatured in buffer (50mM Tris/HCl, pH 7.5, 100 mM NaCl) containing 6 M urea to separate co-purified bound ligand. The protein solution was passed through a desalting PD10 column. The initial flow-through and each subsequent 1-ml eluate were collected, and their absorbance readings at OD_{260} and OD_{280} nm were determined. DENV-3 MTase was mainly found in fractions 1-3, whilst an unknown ligand (likely SAH or SAM) that strongly absorbed at A_{260} eluted mostly in fractions 5-8. Molar concentrations of SAM/SAH present in the fractions were calculated from the molar extinction of SAM (molar extinction coefficient SAM at OD_{260} = 15400 ; ie 1M SAM absorbs 15400 units at OD 260nm) to convert the A260 absorbance values to molar concentration. Quantification of their concentrations suggest that 7 molecules of ligand bind to every 10 molecules of MTase. (B) Fractions 6 and 7 were diluted to give SAM/SAH concentrations ranging from 10^{-5} to 10^{-9} M and used to compete against SAH-tracer for binding to the anti-SAH antibody (IMx homocysteine FPIA kit; Abbott Laboratories, IL,USA) in a competitive fluorescence polarization assay as described in Graves et al (2008). SAH and SAM were used as controls. The anti-SAH antibody binds SAM with 20-100-fold poorer affinity compared to SAH (Graves et al., 2008). Competitive binding of the various ligands to the antibody-tracer complex was used to determine their IC_{50} values which was calculated from the four parameter logistic equation (Finney, 1976; Cheng and Prusoff, 1973). (C) Summary table showing the IC_{50} values of SAH, SAM and the ligand present in fractions 6 and 7. SAH gave an IC_{50} value of 12.1 nM, whilst the IC_{50} value of SAM was much higher (259.5 nM). These results are comparable to previous observations (Graves et al., 2008). Both fractions 6 and 7 contained a ligand that showed similar competitive properties as SAM, with high IC_{50} values of 479.5 and 382.5 nM, respectively. This indicates that they contained SAM and not SAH, and that DENV-3 MTase purified from bacteria cells is bound with SAM.

A

Samples	Absorbance A_{260}	Absorbance A_{280}	Ratio A_{280}/A_{260}	MTase or Ligand (μM)	Total amount (nmoles)
Flow-through	0.998	1.252	1.25		
Fraction 1	0.5	0.855	1.71	18.6	
Fraction 2	0.729	1.245	1.71	27.1	
Fraction 3	0.626	1.073	1.71	23.3	68.9 (MTase)
Fraction 4	0.08	0.06			
Fraction 5	0.169	0.053	0.31	11.0	
Fraction 6	0.262	0.072	0.27	17.0	
Fraction 7	0.216	0.063	0.29	14.0	
Fraction 8	0.111	0.038	0.34	7.2	49.2 (SAM)
Fraction 9	0.03	0.02	0.67		
Fraction 10	0	0			

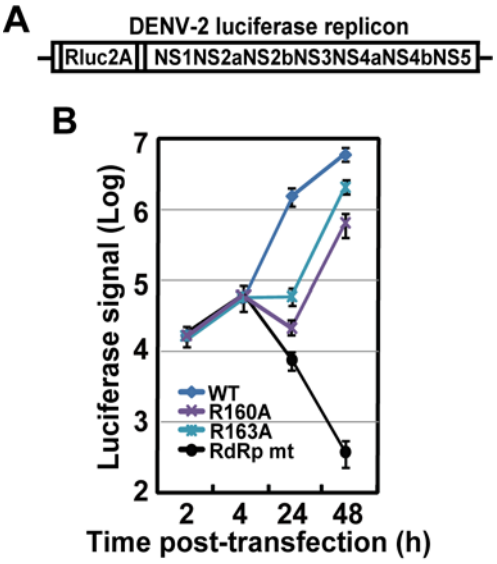
B



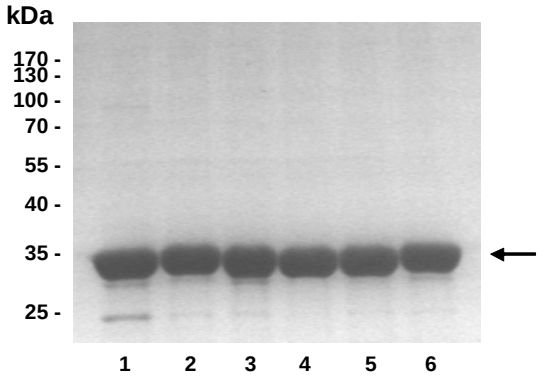
C

Ligand	IC_{50} (nM)
SAH	12.06
SAM	259.5
Fraction 6	479.5
Fraction 7	382.8

Supplementary Fig. 2. Transient replication of wild-type (WT) and mutant replicons of DENV-2. (A) A luciferase-reporting replicon of DENV-2. The viral structural CprME genes were replaced with a *Renilla* luciferase reporter. (B) Replication of WT and mutant replicons of DENV-2. Two mutations (R160A and R163A) were individually engineered into the luciferase-reporting replicon of DENV-2. A mutant replicon containing an inactive viral RNA-dependent RNA polymerase (RdRp mt) was included as a negative control. The replicons were transfected into BHK-21 cells and assayed for luciferase activities at indicated time points post-transfection. Average results of triplicate experiments are shown. The assay was performed as previously described (Shi et al., 2002).



Supplementary Fig. 3. Recombinant WT and mutant DENV4 MTases. Recombinant MTase proteins were expressed and purified as described in Methods. The proteins were analyzed on a 10% SDS-PAGE gel stained with coomassie blue. Lanes 1-6 denote WT, F133A, G148A, R160A, R163A, and L182A MTase, respectively. Molecular mass standards were labeled on the left side of the gel. Arrow indicates the purified DENV MTases.



Supplementary Table. Primer sequences

Primer	Sequence
hRNMT-RT-REV	5'-GAGTTGTTCAAATTTGTGCA-3'
hRNMT-Xho-FOR	5'-TACACTCGAGATGGCAAATTCTGCAAAAGC-3'
hRNMT-Bam-REV	5'-GCCGGATCCTCACTGCTGTTTCTCAAAGG-3'
hDNMT sense	5'-biotin-GATCCGAC*GAC*GAC*GC*GC*GC*GC*GAC*GAC*GAGATC-3' (Symbol * indicates that the adjacent C is methylated)
hDNMT anti-sense	5'-GATCTCGTCGTCGCGCGCGCGTCGTCGTCGGATC-3'

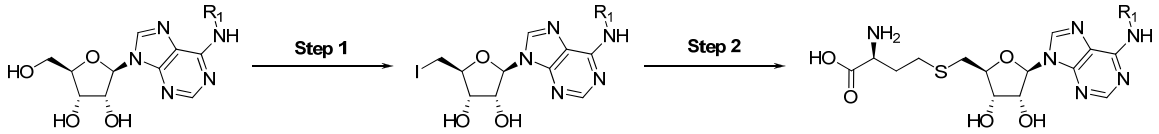
COMPOUND SYNTHESIS

Synthesis of compounds 1-12

General Methods. Reagents and building blocks from commercial sources were used without further purification. Reactions were routinely run under a dry nitrogen atmosphere using standard techniques. Reaction progress was monitored using silica gel plates. Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F254 (2.5 × 7.5 cm) using UV light (λ = 254nm) or $KMnO_4$ stain for visualization. ^1H NMR and ^{13}C NMR spectra were determined on a Varian 300 Mercury spectrometer. Chemical shifts (δ) are expressed in ppm. LC-MS analyses were performed with an Agilent LC1100 coupled with an Applied Biosystems API2000, using the following conditions: monolithic-C18, 50 × 4.6 mm column; mobile system of acetonitrile/water with 0.1% of formic acid with a gradient of 5-95% acetonitrile; run time of 4 min; UV 254 and 214 nm; and flow rate of 3 mL/min.

HPLC purity determinations were performed using an Agilent LC1100 HPLC, and the following conditions: SymmetryShield RP-18 3.5µm, 150 × 4.6 mm column; mobile system of acetonitrile/water with 0.1% of formic acid with a gradient of 5-95% acetonitrile; run time of 20 min; UV 254 and 214 nm; and flow rate of 0.8 mL/min. UPLC-MS was performed using a Waters Acquity UPLC-MS (ZQ2000) with an Acquity C18 Column, 2.1x50mm, 1.7µm, with 0.1% of formic acid with a gradient of 5-95% acetonitrile; run time of 3 min; UV 210-400nm; and flow rate of 0.8 mL/min. HPLC purification was carried out on a Waters Prep LC with an Atlantis C18, 10µm, 19 × 250 mm column; mobile system of acetonitrile/water with 0.1% of formic acid; run time of 40 min; UV 254 and 214 nm; and flow rate of 20 mL/min.

Synthesis of compounds 1 and 3



A

General procedure for Step 1:

The 6-substituted adenosine (0.44mmol, 1 equiv.) and triphenylphosphine (0.88mmol, 2 equiv.) were dissolved in anhydrous dimethylformamide (3mL), cooled to 0°C and stirred under a blanket of argon. N-Iodosuccinimide (0.88mmol, 2 equiv.) was added to the solution at 0°C. The reaction mixture was warmed to 50°C and heated for 8 hours at this temperature, then allowed to cool to room temperature and stirred overnight. The resulting suspension was filtered and purified directly by reverse phase preparative column chromatography.

General procedure for Step 2:

L-Homocysteine monosodium salt (0.44mmol, 10 equiv.) was added to **A** (0.044mmol, 1 equiv.) in water (3mL) and 1,4-dioxane (1mL) to give a suspension. The pH value of the suspension was adjusted to pH10 with sodium hydroxide (1M in H_2O) and was heated to reflux at 90°C for 4 hours. The reaction mixture was filtered and purified directly by reverse phase preparative column chromatography.

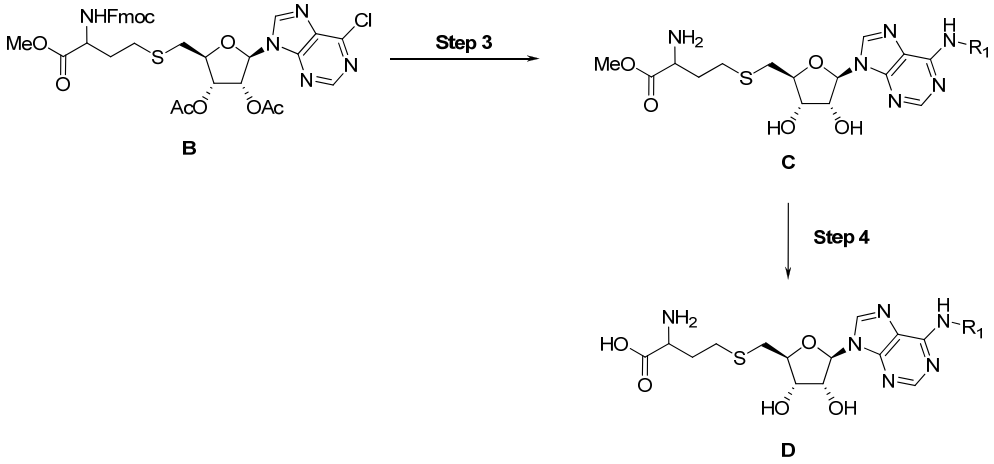
(S)-2-Amino-4-[(2S,3S,4R,5R)-3,4-dihydroxy-5-(6-methylamino-purin-9-yl)-tetrehydro-furan-2-ylmethylsulfanyl]-butyric acid (1, R_1= Me). The title compound was prepared following the general procedures for step 1 and step 2. It was purified by reverse phase preparative column chromatography using acetonitrile and water with 0.1% formic acid as solvents to give compound **1** (0.0104g, 15.4%).

^{1}H NMR (300 MHz, D_2O) δ ppm 8.27 (s, 1 H), 8.21 (s, 1 H), 6.04 (d, *J*=5.3 Hz, 1 H), 4.82 (t, *J*=5.3 Hz, 1 H), 4.40 (t, *J*=5.1 Hz, 1 H), 4.27 - 4.36 (m, 1 H), 3.75 - 3.84 (m, 1 H), 3.07 (s, 3 H), 2.91 - 3.05 (m, 2 H), 2.68 (t, *J*=7.6 Hz, 2 H), 1.97 - 2.22 (m, 2 H). ES-MS: calcd. for $C_{15}H_{22}N_6O_5S$ (398.44); found (pos.): 399.30 [M+H].

(S)-2-Amino-4-[(2S,3S,4R,5R)-3,4-dihydroxy-5-(6-phenylamino-purin-9-yl)-tetrehydro-furan-2-ylmethylsulfanyl]-butyric acid (3, R_1=Ph). The compound was prepared following the general procedures for step 1 and step 2. It was purified by reverse phase preparative column chromatography using acetonitrile and water with 0.1% formic acid as solvents to give compound **3** (0.002g, 10%).

^{1}H NMR (300 MHz, METHANOL-d_4) δ ppm 8.41 (s, 1 H), 8.36 (s, 1 H), 7.64 (m, 2 H), 7.46 (m, 2 H), 7.25 (m,1 H), 6.08 (d, *J*=5.1 Hz, 1 H), 4.82 - 4.86 (m, 1 H), 4.40 (t, *J*=5.1 Hz, 1 H), 4.31 (m, 1 H), 3.78 (dd, *J*=7.1, 5.4 Hz, 1 H), 2.93 - 3.14 (m, 2 H), 2.78-2.70 (m, 2 H), 2.00 - 2.29 (m, 2 H). ES-MS: calcd. for $C_{20}H_{24}N_6O_5S$ (460.52); found (pos.): 461.40 [M+H].

Synthesis of compounds SAH, 2, and 4-12



General procedure for Step 3:
Triethylamine (8.60mmol, 61 equiv.) was added to a solution of 6-chloro-adenosine analogue **B** (0.14mmol, 1 equiv.) and the amine (2.74mmol, 20 equiv.) in anhydrous 1,4-dioxane (3mL). The reaction mixture was subjected to microwave irradiation at 90^{o}C and heated for 20 min. The reaction mixture was filtered and purified directly by reverse phase preparative column chromatography to give product **C**.

General procedure for Step 4:
Lithium hydroxide (4M in H_2O, 1mL) was added to **C** (0.04mmol, 1 equiv.) and the suspension was stirred at room temperature for 1 hour. The reaction mixture was neutralized to pH7.0 using formic acid. The neutralized solution was filtered and purified directly by reversed phase preparative column chromatography.

2-Amino-4-[(2S,3S,4R,5R)-5-[6-cyclopropylamino-purin-9-yl]-3,4-dihydroxy-tetrehydro-furan-2-ylmethylsulfanyl]-butyric acid (2, R1=cyclopropyl).
The title compound was prepared from **B** according to the general procedure for step 3 and step 4 providing 0.0026g, 68.1% of **2**.

^{1}H NMR (300 MHz, D_2O) δ ppm 8.27 - 8.30 (m, 2 H), 6.05 (d, *J*=5.1 Hz, 1 H), 4.82 (t, *J*=5.3 Hz, 1 H), 4.39 (t, *J*=4.9 Hz, 1 H), 4.26 - 4.34 (m, 1 H), 3.73 - 3.81 (m, 1 H), 2.89 - 3.08 (m, 2 H), 2.78

- 2.89 (m, 1 H), 2.60 - 2.70 (m, 2 H), 1.96 - 2.20 (m, 2 H), 0.85 - 0.95 (m, 2 H), 0.63 - 0.72 (m, 2 H). ES-MS: calcd. for $C_{17}H_{24}N_6O_5S$ (424.48); found (pos.): 425.17 [M+H].

2-Amino-4[(2S,3S,4R,5R)-5-(6-benzylamino-purin-9-yl)-3,4-dihydroxy-tetrahydro-furan-2-ylmethylsulfanyl]-butyric acid (4, R_1=Bn). The compound was synthesized from **B** following the general procedure for step 3 and step 4. Purification by reversed phase preparative column chromatography using acetonitrile and water with 0.1% formic acid as solvents provided compound **4** (0.011g, 50%) as a white solid. ES-MS: calcd. for $C_{21}H_{26}N_6O_5S$ (474.54); found (pos.): 476.25 [M+H]

2-Amino-4-[(2S,3S,4R)-3,4-dihydroxy-5-(6-phenethylamino-purin-9-yl)-tetrehydro-furan-2-ylmethylsulfanyl]-butyric acid (5, R=2-phenylethyl).
The title compound (0.0023g, 18.9%) was prepared from **B** following the general procedures in step 3 and step 4. ES-MS: calcd. for $C_{22}H_{28}N_6O_5S$ (488.57); found (pos.): 489.28 [M+H].

2-Amino-4-{(2S,3S,4R)-3,4-dihydroxy-5-[6-(2-methyl-benzylamino)-purin-9-yl]-tetrehydro-furan-2-ylmethylsulfanyl]-butyric acid (6, R=2-methylbenzyl).
The title compound (0.014g, 43.2%) was prepared from **B** following the general procedures in step 3 and step 4.
^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.40 (s, 1 H), 8.30 (br. s., 1 H), 8.20 (s, 1 H), 6.95 - 7.28 (m, 4 H), 5.90 (d, *J*=5.9 Hz, 1 H), 4.59 - 4.81 (m, 2 H), 4.18 (dt, *J*=14.2, 3.6 Hz, 1 H), 3.97 - 4.09 (m, 1 H), 2.86 - 3.02 (m, 1 H), 2.73 - 2.86 (m, 1 H), 2.63 (t, *J*=7.4 Hz, 2 H), 2.34 (s, 3 H), 1.91 - 2.10 (m, 1 H), 1.70 - 1.91 (m, 1 H). ES-MS: calcd. for $C_{22}H_{28}N_6O_5S$ (488.57); found (pos.): 489.28 [M+H].

2-Amino-4-{(2S,3S,4R)-3,4-dihydroxy-5-[6-(3-methyl-benzylamino)-purin-9-yl]-tetrehydro-furan-2-ylmethylsulfanyl]-butyric acid (7, R=3-methylbenzyl).
The title compound (0.0077g, 23.9%) was prepared from B following the general procedures in step 3 and step 4.
^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.39 (s, 1 H), 8.22 (s, 1 H), 7.06 - 7.28 (m, 3 H), 6.97 - 7.06 (m, 1 H), 5.90 (d, *J*=5.6 Hz, 1 H), 4.60 - 4.81 (m, 2 H), 4.08 - 4.31 (m, *J*=14.2, 4.0, 4.0 Hz, 1 H), 3.96 - 4.08 (m, 1 H), 2.87 - 3.02 (m, 1 H), 2.73 - 2.86 (m, 1 H), 2.62 (t, *J*=7.6 Hz, 2 H), 2.25 (s, 3 H), 1.92 - 2.08 (m, 1 H), 1.72 - 1.90 (m, 1 H). ES-MS: calcd. for $C_{22}H_{28}N_6O_5S$ (488.57); found (pos.): 489.41 [M+H].

2-Amino-4-{(2S,3S,4R)-3,4-dihydroxy-5-[6-(4-methyl-benzylamino)-purin-9-yl]-tetrehydro-furan-2-ylmethylsulfanyl]-butyric acid (8, R=4-methylbenzyl)).
The title compound (0.017g, 34.6%) was prepared from **B** following the general procedures in step 3 and step 4.
^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.37 (d, *J*=1.2 Hz, 1 H), 8.21 (s, 1 H), 7.21 (d, *J*=7.9 Hz, 2 H), 7.08 (d, *J*=7.9 Hz, 2 H), 5.89 (d, *J*=5.9 Hz, 1 H), 4.73 (t, *J*=5.1 Hz, 1 H), 4.65 (br. s., 1 H), 4.10 - 4.23 (m, 1 H), 3.94 - 4.09 (m, 1 H), 3.17 - 3.35 (m, 2 H), 2.86 - 3.00 (m, 1 H), 2.71 - 2.84 (m, 1 H), 2.62 (t, *J*=7.6 Hz, 2 H), 2.26 (s, 3 H), 1.91 - 2.07 (m, 1 H), 1.69 - 1.88 (m, 1 H). ES-MS: calcd. for $C_{22}H_{28}N_6O_5S$ (488.57); found (pos.): 489.48 [M+H].

2-Amino-4-{(2S,3S,4R)-5-[6-(3-bromo-benzylamino)-purin-9-yl]-3,4-dihydroxy-tetrehydro-furan-2-ylmethylsulfanyl]-butyric acid (9, R=3-bromobenzyl).
The title compound (0.0143g, 35.1%) was prepared from **B** following the general procedures in step 3 and step 4 (reaction time for step 4 was 2 hours at 100^{o}C).

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.47 (br. s., 1 H), 8.40 (d, *J*=1.5 Hz, 1 H), 8.24 (d, *J*=3.2 Hz, 1 H), 7.49 - 7.57 (m, 1 H), 7.38 - 7.45 (m, 1 H), 7.31 - 7.38 (m, 1 H), 7.21 - 7.30 (m, 1 H), 5.90 (d, *J*=5.6 Hz, 1 H), 4.63 - 4.80 (m, 2 H), 4.18 (dt, *J*=15.1, 4.4 Hz, 1 H), 3.97 - 4.09 (m, 1 H), 2.86 - 3.04 (m, 1 H), 2.71 - 2.86 (m, 1 H), 2.62 (t, *J*=7.6 Hz, 2 H), 1.93 - 2.06 (m, 1 H), 1.68 - 1.88 (m, 1 H). ES-MS: calcd. for $C_{21}H_{25}BrN_6O_5S$ (553.44); found (pos.): 555.28 [M+H].

2-Amino-4-{(2S,3S,4R)-5-[6-(3-chloro-benzylamino)-purin-9-yl]-3,4-dihydroxy-tetrehydro-furan-2-ylmethylsulfanyl]-butyric acid (10, R=3-chlorobenzyl).

The title compound (0.0151g, 41.9%) was prepared from **B** following the general procedures in step 3 and step 4 (reaction time for step 4 was 2 hours at 100^{o}C).

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.47 (br. s., 1 H), 8.42 (d, *J*=1.2 Hz, 1 H), 8.24 (s, 1 H), 7.38 - 7.42 (m, 1 H), 7.26 - 7.37 (m, 3 H), 5.92 (d, *J*=5.6 Hz, 1 H), 4.65 - 4.85 (m, 2 H), 4.19 (dt, *J*=14.9, 4.4 Hz, 1 H), 3.99 - 4.10 (m, 1 H), 2.88 - 3.03 (m, 1 H), 2.73 - 2.88 (m, 1 H), 2.64 (t, *J*=7.6 Hz, 2 H), 1.92 - 2.08 (m, 1 H), 1.71 - 1.90 (m, 1 H). ES-MS: calcd. for $C_{21}H_{25}ClN_6O_5S$ (508.99); found (pos.): 509.25 [M+H].

(S)-2-Amino-4-{(2S,3S,4R,5R)-5-[6-(3-chloro-benzylamino)-purin-9-yl]-3,4-dihydroxy-tetrehydro-furan-2-ylmethylsulfanyl]-butyric acid (10*, R=3-chlorobenzyl).

The title compound (0.0169g, 47.1%) was prepared from (S)-4-[(2S,3S,4R,5R)-3,4-diacetoxy-5-(6-chloro-purin-9-yl)-tetrahydro-furan-2-ylmethylsulfanyl]-2-(9H-fluoren-9-ylmethoxycarbonylamino)-butyric acid methyl ester according to the general procedures for step 3 and step 4.

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.49 (br. s., 1 H), 8.41 (s, 1 H), 8.23 (s, 1 H), 7.23 - 7.42 (m, 4 H), 5.90 (d, *J*=5.9 Hz, 1 H), 4.66 - 4.80 (m, 2 H), 4.15 (t, *J*=4.5 Hz, 1 H), 3.96 - 4.07 (m, 1 H), 2.92 (dd, *J*=13.8, 6.4 Hz, 1 H), 2.80 (dd, *J*=13.8, 7.0 Hz, 1 H), 2.63 (t, *J*=7.6 Hz, 2 H), 1.93 - 2.08 (m, 1 H), 1.78 - 1.91 (m, 1 H). ES-MS: calcd. for $C_{21}H_{25}ClN_6O_5S$ (508.99); found (pos.): 509.40 [M+H], 507.5 [M-1].

2-Amino-4-{(2S,3S,4R,5R)-5-[6-(3-fluoro-benzylamino)-purin-9-yl]-3,4-dihydroxy-tetrehydro-furan-2-ylmethylsulfanyl]-butyric acid (11, R=3-fluorobenzyl).

The title compound (0.021g, 59.3%) was prepared from **B** according to the general procedures for step 3 and step 4 (reaction time for step 4 was 2 hour at 100^{o}C).

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.46 (br. s., 1 H), 8.41 (d, *J*=1.2 Hz, 1 H), 8.24 (s, 1 H), 7.28 - 7.41 (m, 1 H), 7.10 - 7.24 (m, 2 H), 6.98 - 7.09 (m, 1 H), 5.92 (d, *J*=5.9 Hz, 1 H), 4.68 - 4.81 (m, 2 H), 4.19 (dt, *J*=15.2, 4.2 Hz, 1 H), 3.83 - 4.20 (m, 1 H), 2.87 - 3.04 (m, 1 H), 2.73 - 2.87 (m, 1 H), 2.64 (t, *J*=7.5 Hz, 2 H), 1.93 - 2.08 (m, 1 H), 1.72 - 1.90 (m, 1 H). ES-MS: calcd. for $C_{21}H_{25}FN_6O_5S$ (492.53); found (pos.): 493.26 [M+H].

2-Amino-4-{(2S,3S,4R)-3,4-dihydroxy-5-[6-(3-iodo-benzylamino)-purin-9-yl]- tetrehydro-furan-2-ylmethylsulfanyl]-butyric acid (12, R=3-iodobenzyl).

The title compound (0.0282g, 65.7%) was prepared from **B** according to the general procedures for step 3 and step 4 (reaction time for step 4 was 2 hour at 100^{o}C).

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.46 (br. s., 1 H), 8.40 (d, *J*=1.5 Hz, 1 H), 8.23 (s, 1 H), 7.72 (s, 1 H), 7.58 (d, *J*=7.9 Hz, 1 H), 7.36 (d, *J*=7.9 Hz, 1 H), 7.10 (t, *J*=7.8 Hz, 1 H), 5.90 (d, *J*=5.9 Hz, 1 H), 4.74 (t, *J*=4.8 Hz, 1 H), 4.66 (br. s., 1 H), 4.18 (dt, *J*=15.6, 4.2 Hz, 1 H), 3.98 - 4.08 (m, 1 H), 2.86 - 3.02 (m, 1 H), 2.73 - 2.85 (m, 1 H), 2.58 (t, *J*=7.9 Hz, 2 H), 1.92 - 2.07 (m, 1 H), 1.71 - 1.89 (m, 1 H). ES-MS: calcd. for $C_{21}H_{25}IN_6O_5S$ (600.44); found (pos.): 601.38 [M+H].

2-Amino-4-[(2S,3S,4R,5R)-5-[6-amino-purin-9-yl]-3,4-dihydroxy-tetrehydro-furan-2-ylmethylsulfanyl]-butyric acid (SAH).

Aqueous ammonia (30% v/v, 2mL) was added to **B** (0.035g, 0.05mmol, 1equiv.) in 1,4-dioxane (2mL) in a pressure tube. The reaction mixture was stirred at room temperature for 5 min before heating to 80°C for 3 hour. The resulting solution was concentrated, taken up in water and purified directly by reversed phase preparative column chromatography using acetonitrile and water with 0.1% formic acid as solvents to give compound the title compound **SAH** (0.011g, 6%).

^{1}H NMR (300 MHz, D_2O) δ ppm 8.41 (s, 1 H), 8.36 (d, *J*=0.9 Hz, 1 H), 8.27 (s, 1 H), 6.08 (d, *J*=5.1 Hz, 1 H), 4.85 (t, *J*=5.3 Hz, 1 H), 4.42 (t, *J*=5.1 Hz, 1 H), 4.29 - 4.36 (m, 1 H), 3.76 - 3.83 (m, 1 H), 2.90 - 3.12 (m, 2 H), 2.68 (t, *J*=7.4 Hz, 2 H), 1.98 - 2.20 (m, 2 H). ES-MS: calcd. for $C_{14}H_{20}N_6O_5S$ (384.12); found (pos.): 385.22 [M+H].

Supplementary information references

Graves, T.L., Zhang, Y. and Scott, J.E. (2008). A universal competitive fluorescence polarization activity assay for S-adenosylmethionine utilizing methyltransferases. *Anal Biochem*, **373**, 296-306.

Finney, D.J. (1976). Radioligand Assay. *Biometrics* **32**, 721-740.

Cheng Y., Prusoff W.H. (1973). Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol* **22**, 3099-3108.

Shi, P.Y., Tilgner, M., Lo, M.K., Kent, K.A. and Bernard, K.A. (2002). Infectious cDNA clone of the epidemic West Nile virus from New York City. *J. Virol.*, **76**, 5847-5856.