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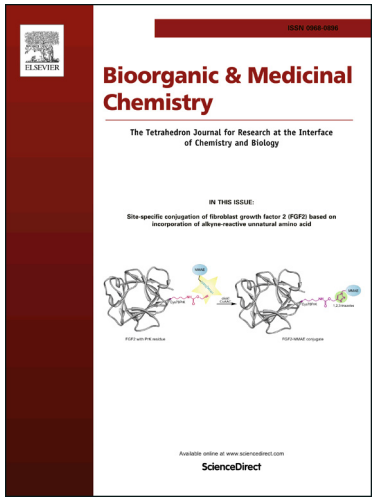

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PII: S0968-0896(16)31064-1
DOI: http://dx.doi.org/10.1016/j.bmc.2017.06.032
Reference: BMC 13815

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 27 October 2016
Revised Date: 15 June 2017
Accepted Date: 19 June 2017

Please cite this article as: Liu, Q., Cai, X., Yang, D., Chen, Y., Shao, L., Wang, M-W., Cycloalkane analogues of sinefungin as EHMT1/2 inhibitors, *Bioorganic & Medicinal Chemistry* (2017), doi: http://dx.doi.org/10.1016/j.bmc.2017.06.032

Cycloalkane analogues of sinefungin as EHMT1/2 inhibitors

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ABSTRACT

A series of cycloalkyl substituted analogues of the natural product sinefungin lacking the amino-acid moiety was designed and synthesized. Two stereoisomers (6-*R* and 6-*S*) were separated and their bioactivities examined against EHMT1/2. Of which, compound **14d** showed an inhibitory activity against EHMT1/2 (88.9%, IC_{50} = 21.8 μM for EHMT1 and 77.6%, IC_{50} = 39.6 μM for EHMT2, respectively) similar to that of sinefungin (100.0%, IC_{50} = 28.4 μM for EHMT1 and 79.5%, IC_{50} = 30.1 μM for EHMT2, respectively). Further studies against other methyltransferases such as PRMT1 showed no activity except that **12d** displayed about 20 % inhibition.

Keywords

Methyltransferase inhibitor; cycloalkyl substituted analogue; natural product; sinefungin

INTRODUCTION

Epigenetic regulation of gene transcription is mediated by a group of regulatory enzymes, including DNA methyltransferases, protein methyltransferases, protein demethylases, histone acetyltransferases, histone deacetylases and ubiquitin ligases.[1,2] Among them, histonelysine methyltransferases (HKMTs) are recognized as an important family playing key roles in cell

differentiation, gene regulation, DNA recombination and damage repair and carcinogenesis.[3-6] HKMTs transfer the methyl group from the cofactor (*S*)-adenosylmethionine (SAM), which contains a highly reactive methylthiol group, to the tailed nitrogen of the substrate lysine residue, producing mono-, di- or tri-methylated products and its analogue (*S*)-adenosylhomocysteine (SAH) (Figure 1).[7-9]

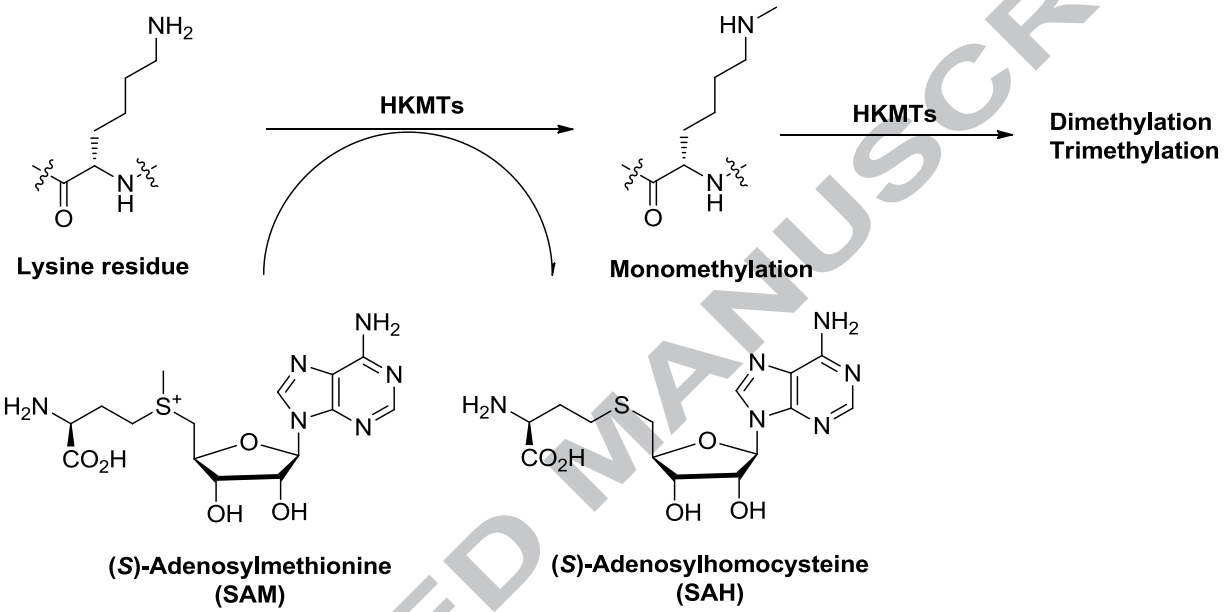


Figure 1. Methylation of lysine residue by histone lysine methyltransferases (HKMTs) utilizing (*S*)-adenosylmethionine (SAM) as cofactor.

Euchromatin histone methyltransferases[10-11] (EHMTs), including G9a encoded by *EHMT2* and GLP (G9a-like protein) encoded by *EHMT1*, regulate transcriptional repression and activation in the process of germ cell formation, embryogenesis and cardiac morphogenesis in the form of heterodimeric complex.[12-13] G9a is highly expressed in a variety of human cancers such as leukemia, prostate cancer, lung cancer and hepatocellular carcinoma with a poor prognosis.[14] Several small molecules have been reported as inhibitors of EHMTs and were divided into two categories, substrate-competitive and AdoMet-competitive inhibitors, according to their different mechanisms of action (Figure 2). Substrate-competitive inhibitors such as BIX01294[15-16] and UNC0638[17-18] showed IC_{50} values of 1.9 μM and <15 nM for G9a, and 0.7 μM and 19 nM for GLP, respectively. UNC0638

also dose-dependently increased the expression of human γ-globin and HbF.[18] SAM-competitive inhibitors include SAH[19] and sinefungin[20-22] which is a natural product isolated from *Streptomyces incamatus* and *Streptomyces griseolus*. Both compounds have similar structures and displayed no selectivity over a wide range of methyltransferases. We have previously reported a series of sinefungin analogues capable of inhibiting EHMTs at micromolar potency with little effect on three other methyltransferase (DNMT1, PRMT1 and SET7/9) at 200 μM.[23] However, the lead compound **1** was a mixture of two epimers (6-*R* and 6-*S*) and no further structure-activity relationship (SAR) analysis was conducted.

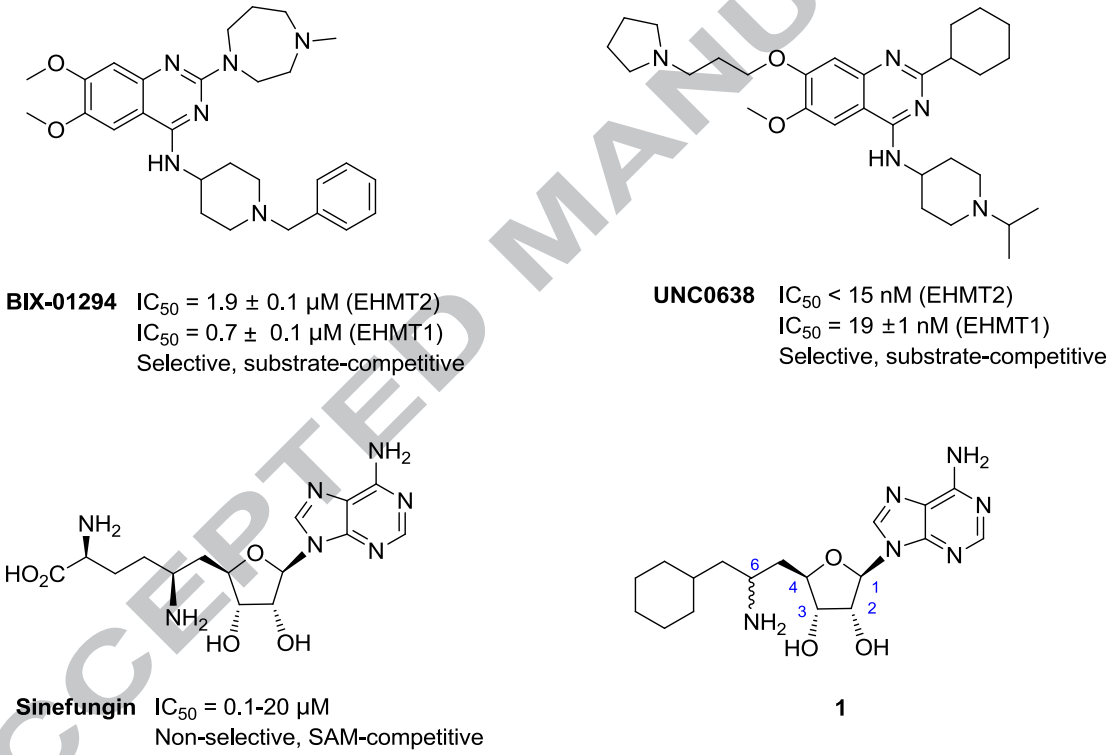


Figure 2. Reported small molecule EHMT inhibitors.

Our follow-up studies presented here were focused on the continuous modification around the cyclohexyl moiety and stereochemistry at C-6 (Figure 3). We designed and synthesized a series of 7-cycloalkyl (C3-6) and 8-cyclohexyl substituted analogues, followed by isolation of two 6-*R*/*S* epimers, to examine the effects of ring size, chain elongation and C-6 conformation on their ability to inhibit EHMT1/2. Also, we synthesized a series of N-cyclohexylmethyl-N-alkyl-5'-deoxy-5'-amino-adenosine analogues to inspect the effects of

nitrogen-position change on the inhibitory effect on EHMT1/2.

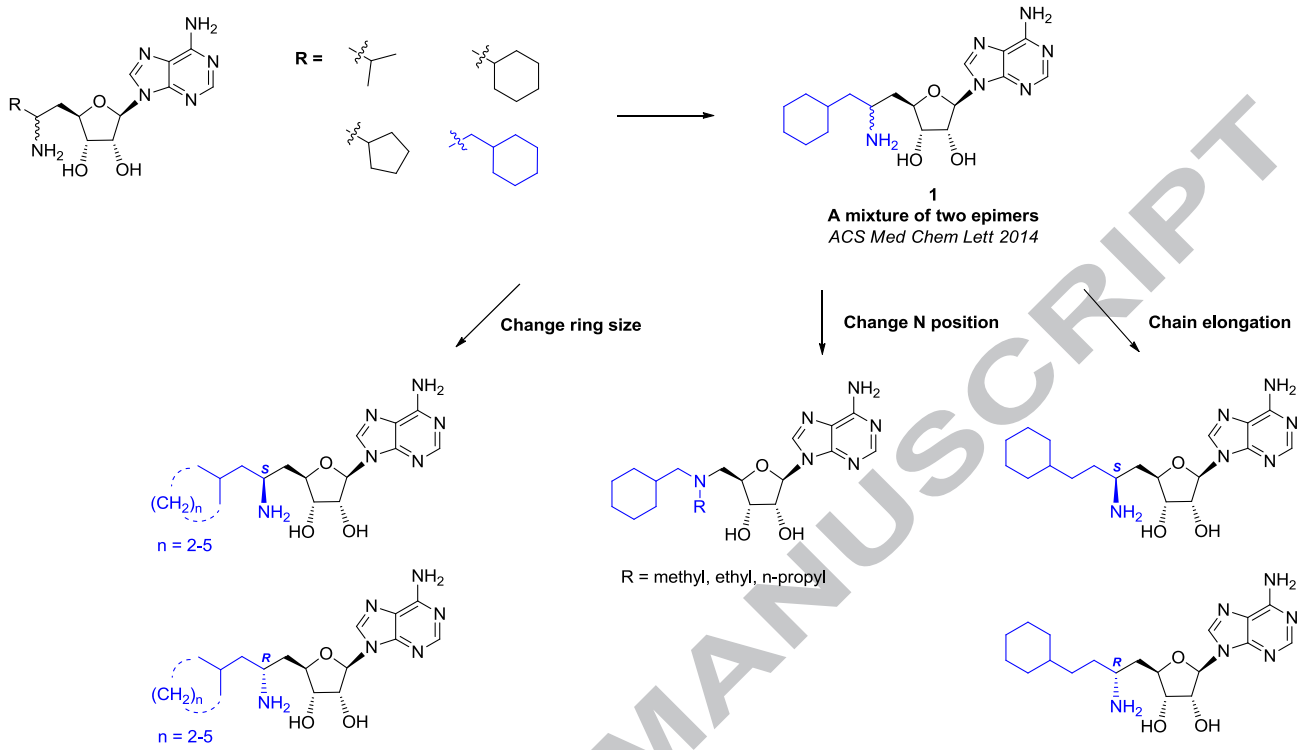


Figure 3. Structural modifications based on compound 1.

RESULTS AND DISCUSSION

For the series of cycloalkyl substituted analogues, our synthetic strategy consisted of three parts: the construction of different substituent-containing *β*-furanoside moiety derived from 5,6-deoxy-6-cyano-6-diethoxyphosphono-2,3-*O*-isopropylidene-*β*-D-hexo-furanoside **2**, the coupling of N^6-benzoyl protected adensine **7** with 1,2,3-*O*-triacetyl protected *β*-D-furanoside moiety **6** and the rearrangement of amide to amino group as outlined in Scheme 1.

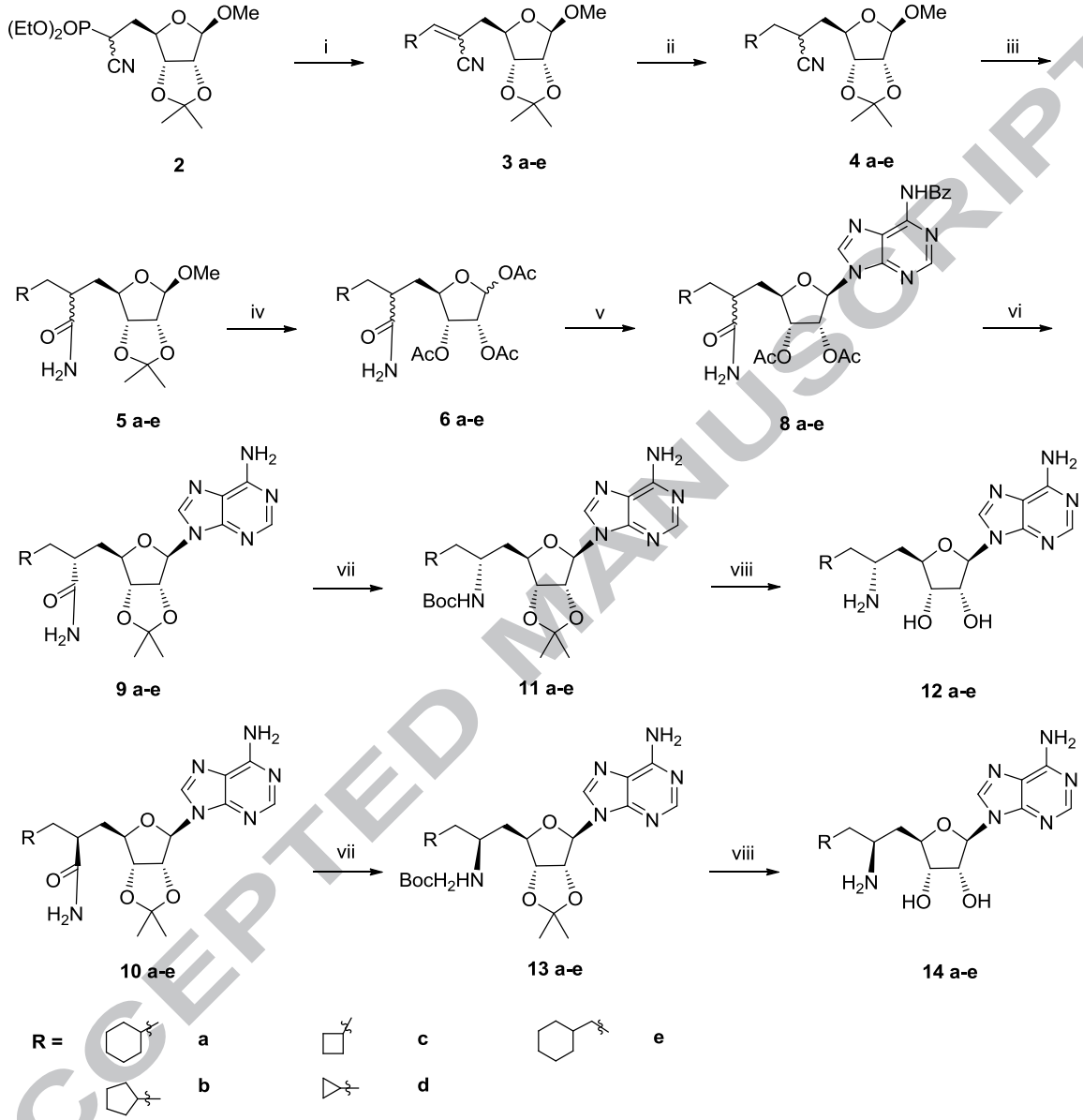
Condensation of different aldehydes with cyanophosphonate **2** led to unsaturated nitriles **3**. ^{1}H NMR spectrum showed two groups of alkene hydrogen signals indicating the existence of *E* and *Z* configuration at a ratio of 1:3. Reduction of **3** gave 7-cycloalkyl (C3-C6) or 8-cyclohexyl substituted nitriles **4**. It was worthy to mention that **4d** (7-cyclopropyl substituted analogue) was hydrogenated under higher pressure (0.6 MPa) compared with other compounds (**4a-c**, **e**: 0.3 MPa). Compound **4** was oxidatively hydrolyzed under basic condition to its corresponding amide **5**, which was subsequently refluxed in 0.2 N sulphuric acid to obtain its triol. Without further purification, this triol

was protected with acetyl group in the mixture of acetic anhydride and pyridine to obtain the 1,2,3-*O*-triacetate **6**. Intermediate **6** was a mixture of anomers, which was difficult to separate by silica gel chromatography. It was utilized for the subsequent adenosylation directly.

Next, the key *Vorbrüggen* reaction was conducted by two steps: 1) persilylation of N^6-benzoyladenine **7** with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) in acetonitrile at reflux for 2 h to afford N^6,N^9-bis(trimethylsilyl)-N^6-benzoyladenine; 2) anomeric adenosylation of **6** with the resulting N^6,N^9-bis(trimethylsilyl)-N^6-benzoyladenine in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in dichloroethane at reflux for 2 h to afford *β*-nucleoside **8a-e**[22] in 40-92% yield after silica gel chromatography. Interestingly, the amount of TMSOTf was different depending on cycloalkyl substitutes (**8a**,**b**: 1 eq; **8c**: 3 eq; **8d**: 2 eq; **8e**: 2.5 eq) and the 9-ribo product **8** was the only product, which was in agreement with the previous reports[22, 24] that 7- and 1-regioisomers were kinetic products and transglycosylation was undergone after a prolonged reaction time. Two stereoisomers existed in compound **8a-e** as suggested by ^{1}H NMR and ^{13}C NMR. For compound **8a**, two groups of peaks existed in ^{1}H NMR and ^{13}C NMR around C-1, 2, 3, 4 and 6, indicating the existence of two epimers (6-*R* and 6-*S*).

Finally, we tried to transform 6-amide **8** to the corresponding amine by *Hoffmann* rearrangement in the presence of [bis(trifluoroacetoxy)iodo]benzene and found unsuccessful. Alternative strategy was thus carried out by saponification of acetate **8** with 2N NH_3 in methanol and followed by isopropylidene protection catalyzed by TMSOTf in acetone to obtain two 6-epimers of **9** and **10** at a ratio of about 1:1, which were easy to separate by prep-TLC (0.4-0.5 mm). *Hofmann* rearrangement of **9** and **10** gave the corresponding amines **11** and **13** as their N-(*tert*-butyloxy)carbonyl derivatives, respectively. The conformation at C-6 was retained during the *Hofmann* rearrangement as indicated by ^{1}H NMR signals between the two epimers (**9** *vs.* **11** and **10** *vs.* **13**). For 7-cyclohexyl substituted analogues, it was found that H-6 of **9a** and **11a** (2.32 ppm for **9a** and 3.55 ppm for **11a**) showed up in a relative upfield than that of **10a** and **13a** (2.52 ppm for **10a** and 3.72 ppm for **13a**). Deprotection of

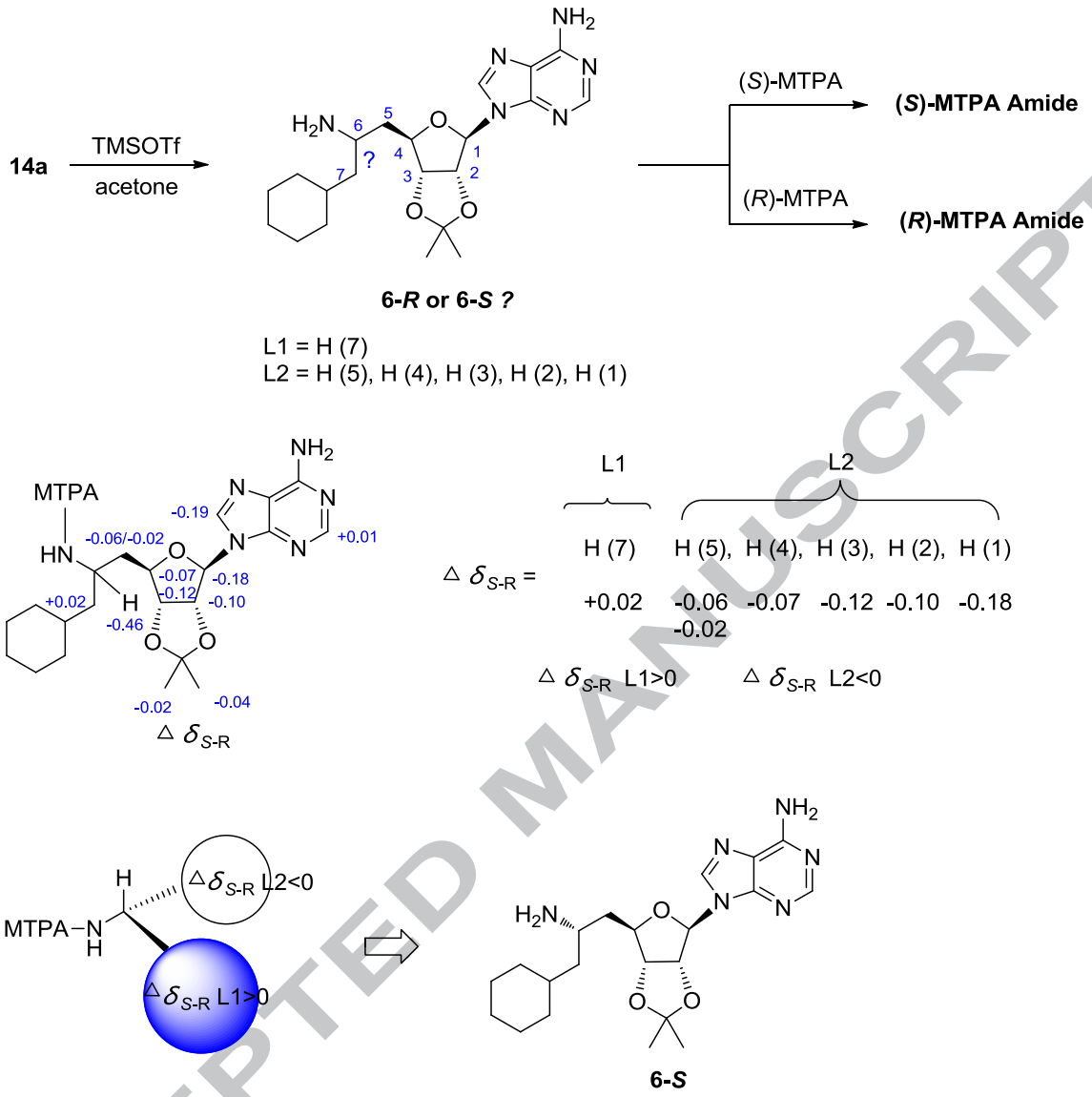
11 or **13** was accomplished in the mixture of trifluoroacetic acid and water (1:1) to obtain the final product **12** or **14** (6-*S* or 6-*R*) as their trifluoroacetates.



Scheme 1. Synthetic route of 12 a-e and 14 a-e. (i) RCHO, NaH/THF at room temperature for 4 h, 96.0-97.6% yield; (ii) 10% Pd/C/MeOH hydrogenated at 30°C under 0.3 or 0.6 MPa for 5-6 h, 82.0-98.0% yield; (iii) 2N NaOH/H_2O_2/DMSO/MeOH at reflux for 1 h, 69.6-99.1% yield; (iv) 1) 0.2N H_2SO_4/dioxane/H_2O at reflux for 5 h, 2) Ac_2O/Py at room temperature overnight, 69.3-99.1% yield; (v) 1) N^6-benzoyl-adenine **7** in BSA/CH_3CN at reflux for 2 h, 2) **6**, TMSOTf in dichloroethane at reflux for 2 h, 40.5-91.7% yield; (vi) 1) 2N NH_3 in MeOH at 40°C overnight, 2) acetone/TMSOTf at room temperature for 1 h, 90.5-99.7% yield (*R* and *S*); (vii) 1) $(CF_3CO_2)_2IC_6H_5$ in CH_3CN/H_2O at

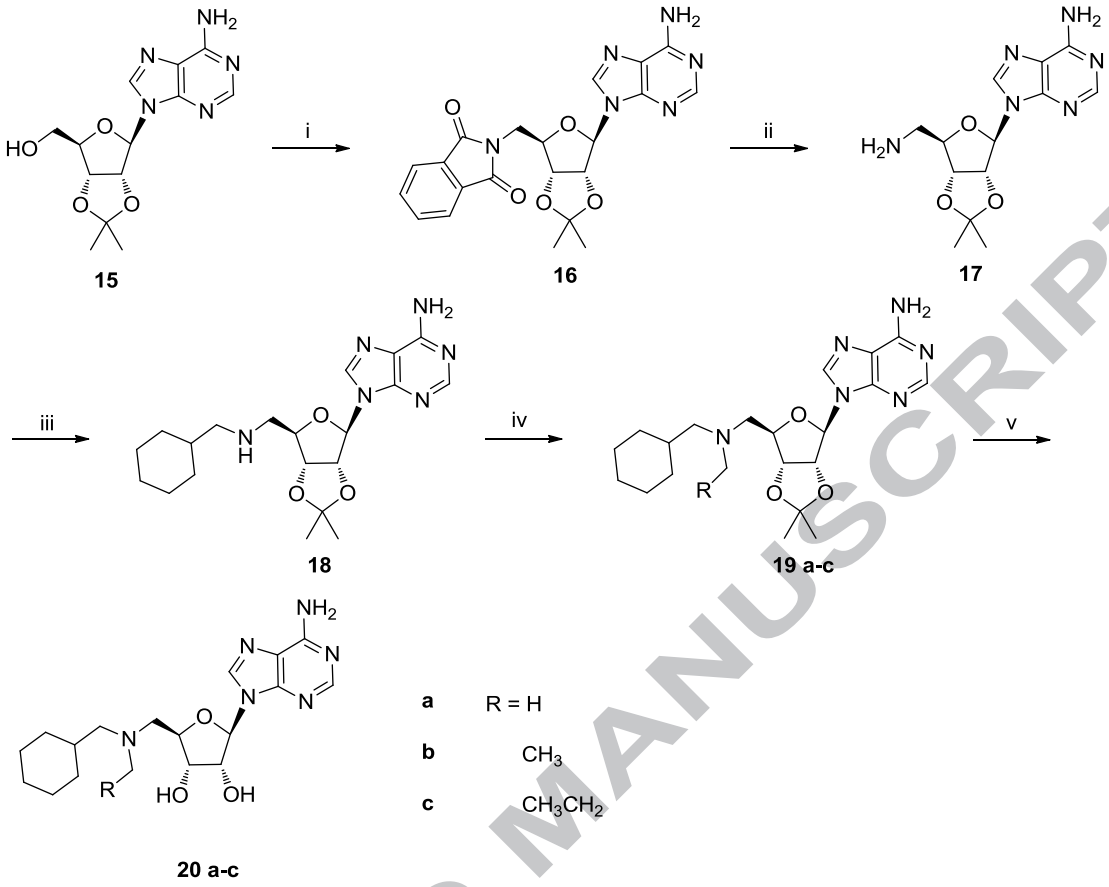
room temperature for 2 h, 2) Boc_2O/TEA, 10.1-83.7% yield; (viii) TFA/H_2O at room temperature for 30 min, 34.0-97.2% yield.

The Mosher's method was used to determine the absolute stereochemistry of C-6. We prepared the 2,3-*O*-isopropylidene derivative of **14a** by catalysis with TMSOTf in acetone, followed by acylation with both (*R*)- and (*S*)-Mosher's acid chloride (MTPA-Cl) to form a pair of diastereomeric amides. ^{1}H NMR data for the two diastereomers, (*R*)-MTPA and (*S*)-MTPA amide were compared. The protons in the L_1 portion of (*S*)-MTPA amide are shifted downfield relative to that of (*R*)-MTPA amide, while the protons in the L_2 portion of (*S*)-MTPA amide are shifted upfield relative to that of (*R*)-MTPA amide due to the anisotropic shielding effect of phenyl ring. $\Delta\delta_{S-R}$ values for the shifts of protons located near the C-6 stereo-center in the amide diastereomers were calculated, and the results were analyzed against the models. In the end, the conformation of **14** was assigned to be 6-*S*, while **12** was designated as 6-*R* (Scheme 2).



Scheme 2. Procedure for the C-6 conformation assignment of 14a.

Next, to investigate the effect of nitrogen-position, we synthesized a series of 5'-deoxy-5'-amino-adenosine derivatives starting from 2,3-*O*-isopropylideneadenosine **15**, which underwent *Mitsunobu* reaction followed by hydrazinolysis to obtain **17**. Two rounds of reductive amination of **17** with cyclohexanecarbaldehyde and C1-3 alkyl substituted aldehyde were carried out sequentially to obtain **19a-c**, which were deprotected in the mixture of trifluoroacetic acid and water (1:1) to afford the final products **20a-c** (Scheme 3).



Scheme 3. Synthetic route of 20a-c. (i) Phthalimide/DIAD/Ph_3P/THF at room temperature overnight, 95.1% yield; (ii) $N_2H_4 \cdot H_2O/C_2H_5OH$ at reflux overnight, 90.5% yield; (iii) $C_6H_{11}CH_2CHO$/HAc/$NaCNBH_3$/MeOH at room temperature overnight, 49.0% yield; (iv) RCHO/HAc/$NaCNBH_3$/MeOH at room temperature overnight, 50.0-91.9% yield; (v) TFA/H_2O at room temperature for 3 h, 69.4-98.1% yield.

Initial characterization of these compounds was performed at a concentration of 200 μM on EHMT1 and 400 μM on EHMT2, and the results were shown in Figures 4, 5 and Table 1. Most cycloalkyl (C3-6) substituted analogues demonstrated moderate to strong inhibition on EHMT1/2 while N-cyclohexylmethyl-N-alkyl-5'-deoxy-5'-amino-adenosine analogues showed weak inhibitory activities, indicating the importance of nitrogen position. Compounds with >50% inhibition on EHMT1 (**12a, c-d**, **14a-d**) and EHMT2 (**12d**, **14b-d**) were selected and further assessed for dose-response characteristics against EHMT1/2.

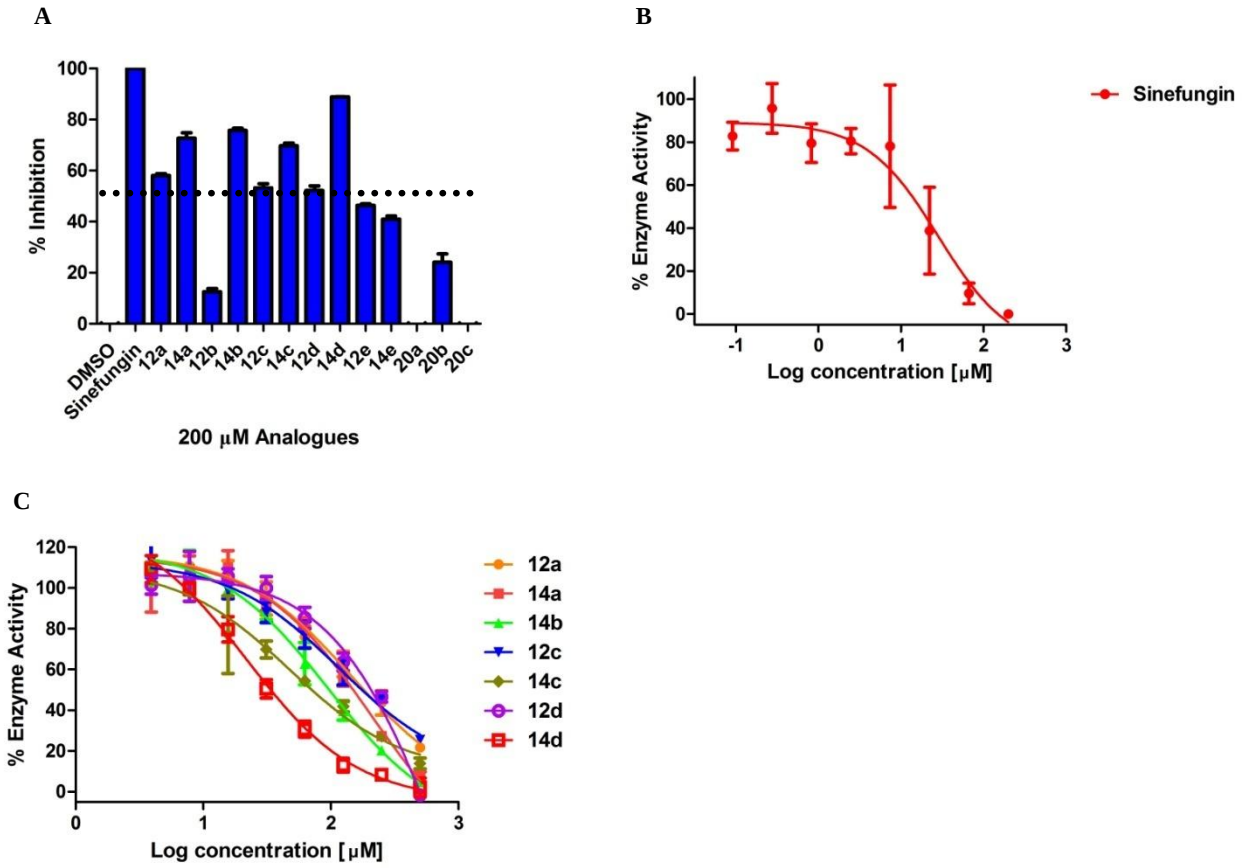


Figure 4. Bioactivities of sinefungin and its analogues on EHMT1. (A) Inhibition of EHMT1 by sinefungin and its analogues at 200 μM in the HTRF assay. (B) Dose-response characteristics of sinefungin on EHMT1. (C) Dose-response characteristics of the analogues that showed more than 50% inhibition on EHMT1. Each value represents mean±SEM of 3-4 independent experiments performed in triplicate.

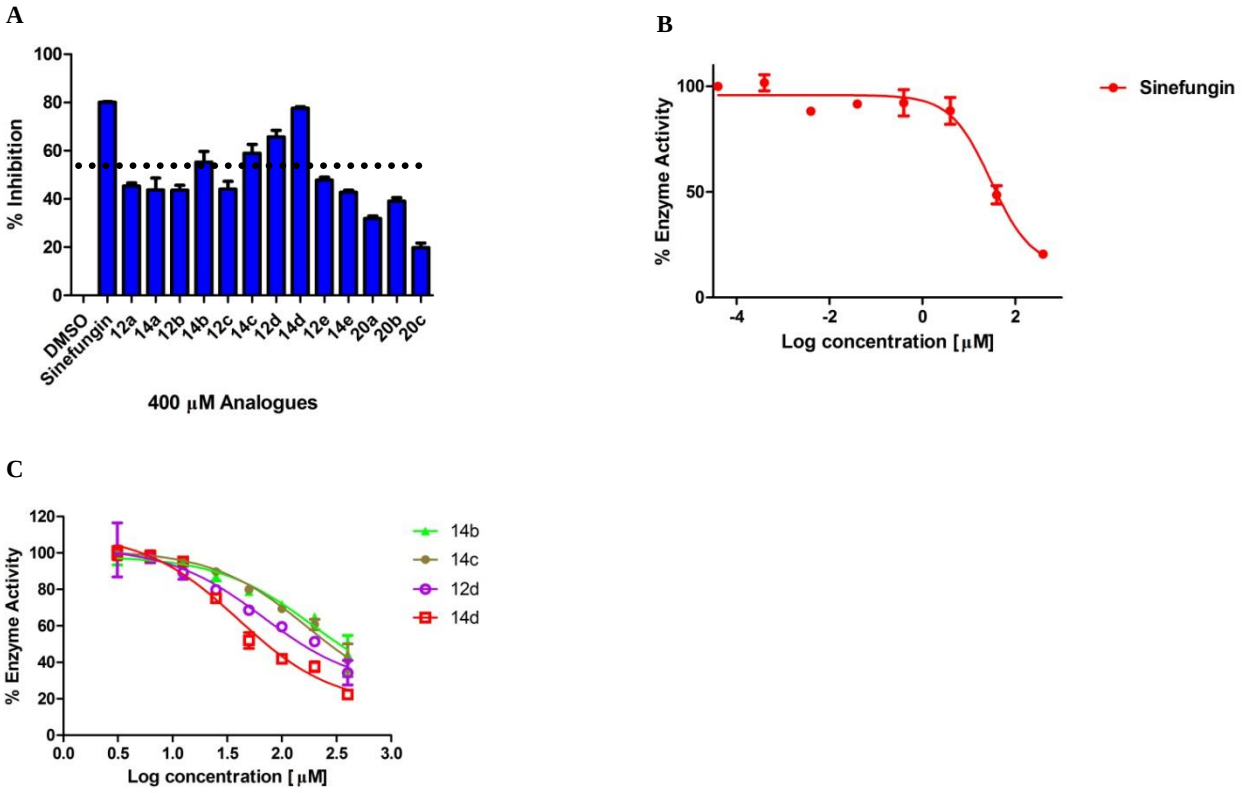


Figure 5. Bioactivities of sinefungin and its analogues on EHMT2. (A) Inhibition of EHMT2 by sinefungin and its analogues at 400 μM in the TR-FRET assay. (B) Dose-response characteristics of sinefungin on EHMT2. (C) Dose-response characteristics of the analogues that showed more than 50% inhibition on EHMT2. Each value represents mean±SEM of 3-4 independent experiments performed in triplicate.

The results on EHMT1 showed that the 6-*S* epimers were markedly more effective than 6-*R* epimers with the same substitutes at C-6 (**14a** *vs.* **12a**, **14b** *vs.* **12b**, **14c** *vs.* **12c** and **14d** *vs.* **12d**) and this conformation difference was weakened when the side chain was elongated (**12e** *vs.* **14e**). The length of side chain also affected their bioactivities on EHMT1. For analogues with the same cyclohexyl substitutes (**14a** *vs.* **14e** and **12a** *vs.* **12e**), both elongation and reduction of the length (data not shown) weakened or totally destroyed their activities. For the 6-*S* epimers **14a-d**, different cycle size also influenced their inhibitory activities with cyclopropyl substituted analogue **14d** as the most effective inhibitor of EHMT1, which exhibited a similar potency as the natural product sinefungin (IC_{50} = 21.8 μM for **14d** *vs.* IC_{50} = 28.4 μM for sinefungin). It was noted that the 6-NH_2

conformation affected the analogues substituted with 7-cyclopentyl group (**14b** *vs.* **12b**) most, with 6-fold higher inhibitory activity for 6-*S* epimer than that of 6-*R* epimer. The results on EHMT2 were slightly different from that on EHMT1. The same tendency was observed that 6-*S* epimers displayed stronger inhibitory activities than 6-*R* epimers except for 7-cyclohexyl (**14a** and **12a**) and 8-cyclohexyl (**14e** and **12e**) analogues, which exhibited almost the same inhibitory activities between different epimers. Among all the 6-*S* epimers **14a-d**, cyclopropyl substituted analogue **14d** was the most potent one, showing a similar potency as the natural product sinefungin (IC_{50} = 39.6 μM for **14d** *vs.* IC_{50} = 30.1 μM for sinefungin). None of the compounds discriminated significantly between EHMT1 and EHMT2. However, when we selected compounds **12a**, **12d** and **14a-d** demonstrating good inhibitory activities towards EHMTs and tested their ability to inhibit other methyltransferases, e.g. PRMT1, **12a** and **14a-d** did not exhibit any inhibition at the concentration of 400 μM, only **12d** showed 20 % inhibition on PRMT1 (Figure 6). The effect of the sinefungin analogues were examined in HL60 (human promyelocytic leukemia) and MDA-MB-231 (human breast adenocarcinoma) cell lines at 200 μM. The results showed that compound **14d** and sinefungin had similar growth inhibitory effects (38%-43%) on these cancer cells (Table S1).

Table 1. Inhibition by sinefungin and its analogues on EHMT1/2.

Compound	EHMT1 IC_{50} (μM)[a]	Inhibition (%)[b]	EHMT2 IC_{50} (μM)[a]	Inhibition (%)[b]
Sinefungin	28.4 (12.4–65.2)	100.0±0.0	30.1 (19.4-46.7)	79.5±0.9
12a	145.8 (100.8–210.8)	58.1±0.7	N.D.[c]	45.3±1.3
12b	N.D.[c]	12.5±1.3	N.D.[c]	43.6±2.0
12c	115.0 (83.6–158.1)	53.2±1.7	N.D.[c]	44.0±5.6
12d	794.3 (405.1–1557.0)	52.2±1.9	67.2 (41.8-108.2)	65.7±4.8
12e	N.D.[c]	46.3±0.6	N.D.[c]	47.8±1.2
14a	203.7 (142.2–291.9)	72.7±2.1	N.D.[c]	43.7±4.9
14b	95.1 (71.6–126.2)	75.7±1.0	191.3 (106.8-342.8)	55.3±7.1

14c	47.7 (29.2–78.0)	69.7±0.9	169.0 (110.3-258.8)	58.9±6.4
14d	21.8 (17.5–27.1)	88.9±0.1	39.6 (28.8-54.5)	77.6±0.7
14e	N.D.[c]	40.9±1.3	N.D.[c]	42.7±1.0
20a	N.A.[d]	N.A.[d]	N.D.[c]	31.9±1.0
20b	N.D.[c]	24.1±3.3	N.D.[c]	39.1±1.5
20c	N.A.[d]	N.A.[d]	N.D.[c]	19.8±1.9

[a]IC_{50} value shown represents an average of 4 independent experiments. Numbers in the brackets indicate 95% confidence interval data.

[b]Inhibition of methyltransferase EHMT1/2 activities by sinefungin and its analogues at concentrations of 200 μM and 400 μM, respectively. Each value represents mean±SEM of 3 independent experiments performed in triplicate.

[c]N.D., not determined.

[d]N.A., no activity.

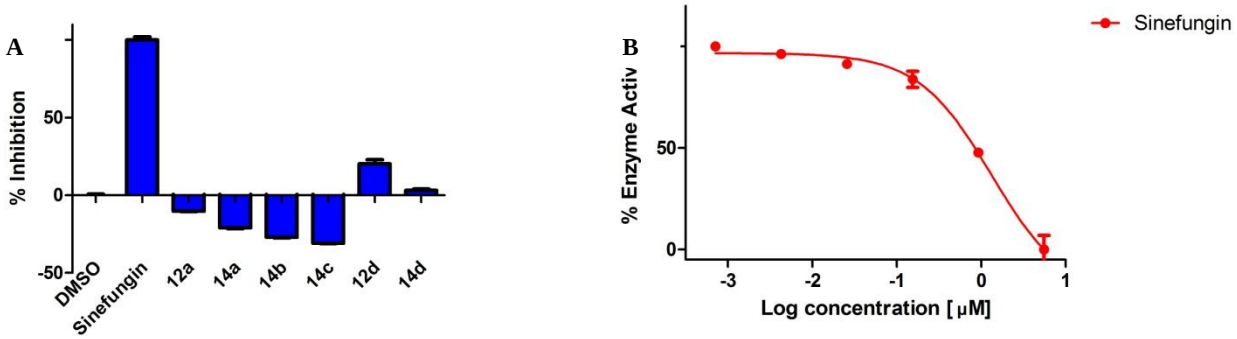


Figure 6. Bioactivities of sinefungin and its analogues on PRMT1. (A) Inhibition of PRMT1 by sinefungin (10 μM) and its analogues (400 μM) in the HTRF assay. (B) Dose-response characteristics of sinefungin on PRMT1. Each value represents mean±SEM of 3 independent experiments performed in triplicate.

CONCLUSION

In this paper we reported a series of cycloalkyl substituted sinefungin analogues as inhibitors of

EHMT1/2. Two epimers (6-*R* and 6-*S*) were separated. Their structures were determined by ^{1}H NMR, ^{13}C NMR, LR-MS and HR-MS, and the conformation at C-6 was assigned by Mosher's method. The inhibitory activities of these compounds on methyltransferases EHMT1/2 were subsequently examined and cyclopropyl substituted analogue **14d** showed the most potent effect, similar to that of the natural product sinefungin. Further studies are ongoing to improve synthetic routes and to understand the SAR around the adenine part of the compounds. Nevertheless, this scaffold of cycloalkyl substituted sinefungin analogues provides a sound starting point for follow-up investigations on EHMTs.

EXPERIMENT

Chemistry.

General Statement. Reagents were commercial grades and used as received unless otherwise noted. The structures of all new compounds were consistent with their ^{1}H, ^{13}C NMR, and mass spectra, and were judged to be ≥95% pure by HPLC. NMR spectra were recorded on Varian Mercury 300, AVANCE III 500 spectrometers. Chemical shifts were reported in parts per million (ppm), with the solvent resonance as the internal standard (CD_3OD 3.31 ppm for ^{1}H NMR,49.15 ppm for ^{13}C NMR; $CDCl_3$7.27 ppm for ^{1}H NMR,77.23 ppm for ^{13}C NMR). Low resolution mass spectral data (electrospray ionization) were acquired on a Finnigan LCQ-DECA mass spectrometer. High resolution mass spectral data (TOF) were acquired on an Agilent 6224 mass spectrometer. Samples were analyzed for purity on a HP1100 series equipped with a Zorbax SB-C18 column (5 μm, 4.6 mm×250 mm). Purities of final compounds were determined using a 5 μL injection with quantitation by AUC at 210 and 254 nm. Reversed-phase silica gel (20−45 μm) was used for column chromatography. Specific optical rotation was determined on Autopol VI-Rudolph polarimeter.

5,6-Deoxy-6-cyano-6-diethoxyphosphono-2,3-*O*-isopropylidene-*β*-D-hexo-furanoside (2)

5,6-Deoxy-6-cyano-6-diethoxyphosphono-2,3-*O*-isopropylidene-*β*-D-hexo-furanoside **2** was synthesized according to the same procedures described in the previous paper[23]. HRMS (TOF) *m*/*z*

calcd for C15H27NO7P $[M+H]^+$ 364.1525, found 364.1529. *R*:*S* = 1:1. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.31 (s, 6H), 1.39 (t, *J* = 6.6 Hz, 12H), 1.47 (s, 6H), 1.80 (m, 4H), 2.88 (m, 2H), 3.33 (s, 6H), 3.36 (s, 6H), 4.25 (q, *J* = 7.2 Hz, 8H), 4.39 (dd, *J* = 3.3 Hz, *J* = 8.1 Hz, 2H), 4.59 (t, *J* = 5.4 Hz, 2H), 4.60 (t, *J* = 6.0 Hz, 2H), 4.97 (d, *J* = 3.3 Hz, 2H).^{13}C NMR ($CDCl_3$, 75 MHz) 15.7, 16.5, 16.6, 25.2, 26.6, 32.8, 55.8, 64.1, 64.2, 83.7, 83.9, 85.4, 110.2, 110.4, 113.0.

1-Methyl-5,6,7-deoxy-6-cyano-7-cyclohexyl-2,3-*O*-isopropylidene-*β*-D-hepta-6-enofuranoside (3a)

Sodium hydride (60%, 0.51 g, 12.8 mmol, 4 eq) was added into the solution of 5,6-deoxy-6-cyano-6-diethoxyphosphono-2,3-*O*-isopropylidene-*β*-D-hexo-furanoside (**2**, 1.16 g, 3.2 mmol, 1 eq) in anhydrous tetrahydrofuran (20 mL) under nitrogen and the mixture was stirred for 10 min followed by addition of cyclohexanecarbaldehyde (1.43 g, 12.7 mmol, 4 eq) . The reaction was stirred at room temperature for 3 h. It was terminated by addition of 10% oxalic acid/methanol solution (2 mL) and evaporated *in vacuo*. The residue was extracted with ethyl acetate (3×10 mL) and the organic phase was combined, washed with water (3×10 mL) and dried over anhydrous sodium sulphate. After filtration, the solvent was removed *in vacuo* and the residue was purified on the Biotage SNAP Cartridge KP-Sil 100 g eluting with petroleum ether, 20% ethyl acetate/petroleum ether to give the product as yellowish oil (1.0 g, yield: 97.1 %). The purity was 96% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C18H28NO4$[M+H]^+$ 322.2018, found 322.2016. *E*:*Z* = 1:3. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.21 (m, 10H), 1.24 (s, 6H), 1.40 (s, 6H), 1.65 (m, 10H), 2.28 (m, 1H), 2.37 (m, 4H), 2.50 (m, 1H), 3.26 (s, 3H), 3.30 (s, 3H), 4.29 (m, 2H), 4.48 (d, *J* = 6.0 Hz, 1H), 4.55 (m, 1H), 4.54 (d, *J* = 5.7 Hz, 1H), 4.57 (d, *J* = 6.0 Hz, 1H), 4.87 (s, 1H), 4.90 (s, 1H), 6.02 (d, *J* = 9.9 Hz, 1H), 6.23 (d, *J* = 9.9 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) 25.0 (4C), 25.2 (2C), 25.7 (2C), 26.2 (2C), 34.0 (2C), 37.7 (4C), 39.5, 40.8, 55.3, 55.5, 83.4, 84.9, 85.4, 109.9, 110.1, 112.6, 112.7, 117.2 (2C), 119.8 (2C), 155.5, 155.6.

1-Methyl-5,6,7-deoxy-6-cyano-7-cyclopentyl-2,3-*O*-isopropylidene-*β*-D-hepta-6-enofuranoside

(3b)

Compound **3b** was prepared according to the same procedure of **3a**, in which cyclopentanecarbaldehyde was used and **3b** was obtained as yellowish oil in 96.9 % yield. The purity was 97% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C17H26NO4[M+H]$^+$ 308.1862, found 308.1865. *E*:*Z* = 1:3. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.25 (m, 8H), 1.27 (s, 3H), 1.31(s, 3H), 1.44 (s, 3H), 1.47 (s, 3H), 1.66 (m, 8 H), 2.27 (m, 1H), 2.43 (m, 2H), 2.57 (m, 1H), 2.74 (m, 1H), 2.98 (m, 1H), 3.33 (s, 3H), 3.36 (s, 3H), 4.10 (m, 1H), 4.39 (t, *J* = 7.8 Hz, 1H), 4.55 (d, *J* = 5.7 Hz, 2H), 4.61 (d, *J* = 5.4 Hz, 2H), 4.91(s, 1H), 4.95 (s, 1H), 6.13 (d, *J* = 10.2 Hz, 1H), 6.38 (d, *J* = 10.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) 25.2 (2C), 25.7 (4C), 26.7 (2C), 33.3 (4C), 39.3, 39.6, 42.5, 42.7, 55.6, 55.7, 83.5, 83.6, 85.2 (2C), 85.6 (2C), 110.5 (2C), 112.8 (2C), 116.5 (2C), 155.9, 155.6.

1-Methyl-5,6,7-deoxy-6-cyano-7-cyclobutyl-2,3-*O*-isopropylidene-*β*-D-hepta-6-enofuranoside(3c)

Compound **3c** was prepared according to the same procedure of **3a**, in which cyclobutanecarbaldehyde was used and **3c** was obtained as yellowish oil in 95.7% yield. The purity was 96% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C16H24NO4[M+H]$^+$ 294.1705, found 294.1708.*E*:*Z* = 1:3. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.30 (s, 6H), 1.46 (s, 6H), 1.96 (m, 8H), 2.28 (m, 4H), 2.42 (m, 4H), 3.33 (s, 3H), 3.35 (s, 3H), 3.45 (m, 2H), 4.37 (t, *J* = 7.8 Hz, 2H), 4.54 (d, *J* = 6.0 Hz, 2H), 4.60 (d, *J* = 6.3 Hz, 2H), 4.94 (s, 2H), 6.32 (d, *J* = 9.6 Hz, 1H), 6.57 (d, *J* = 9.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) 19.1 (2C), 25.2 (2C), 26.6 (2C), 28.9 (4C), 37.4 (2C), 39.5 (2C), 55.6 (2C), 83.5, 83.6, 85.2 (2C), 85.4, 85.6, 108.7 (2C), 110.1 (2C), 112.8 (2C), 117.5 (2C), 154.6, 154.9.

1-Methyl-5,6,7-deoxy-6-cyano-7-cyclopropyl-2,3-*O*-isopropylidene-*β*-D-hepta-6-enofuranoside (3d)

Compound **3d** was prepared according to the same procedure of **3a**, in which cyclopropanecarbaldehyde was used and **3d** was obtained as yellowish oil in 97.6 % yield. The purity was 98% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C15H22NO4[M+H]$^+$ 280.1549,

found 280.1546. *E*:*Z* = 1:3. ^{1}H NMR ($CDCl_3$, 300 MHz) 0.59 (m, 4H), 0.65 (m, 4H), 1.01 (dd, *J* = 2.4 Hz, *J* = 7.5 Hz, 2H), 1.31 (s, 6H), 1.48 (s, 6H), 2.43 (m, 2H), 2.51 (m, 1H), 2.63 (m, 1H), 3.33 (s, 3H), 3.37 (s, 3H), 4.37 (t, *J* = 7.2 Hz, 1H), 4.44 (t, *J* = 7.5 Hz, 1H), 4.57 (t, *J* = 6.0 Hz, 1H), 4.60 (t, *J* = 5.7 Hz, 1H), 4.63 (d, *J* = 7.8 Hz, 2H), 4.95 (s, 1H), 4.98 (s, 1H), 5.57 (d, *J* = 10.2 Hz, 1H), 5.77 (d, *J* = 10.2 Hz, 1H).^{13}C NMR ($CDCl_3$, 75 MHz) 8.7 (2C), 9.3 (2C), 14.5 (2C), 25.3 (2C), 26.7 (2C), 39.4 (2C), 55.5, 56.0, 83.6 (2C), 85.4 (2C), 85.6 (2C), 98.6 (2C), 110.1, 110.7, 113.0 (2C), 121.8 (2C), 154.3, 155.0.

1-Methyl-5,6,7,8-deoxy-6-cyano-8-cyclohexyl-2,3-*O*-isopropylidene-*β*-D-octa-6-enofuranoside (3e)

Compound **3e** was prepared according to the same procedure of **3a**, in which 2-cyclohexaneacetaldehyde was used and **3e** was obtained as yellowish oil in 96.0% yield. The purity was 96% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C19H30NO4[M+H]$^{+}$ 336.2175, found 336.2174. *E*:*Z* = 1:3. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.01 (m, 3H), 1.20 (m, 6H), 1.31 (s, 6H), 1.48 (s, 6H), 1.69 (m, 13H), 2.09 (m, 2H), 2.30 (t, *J* = 7.2 Hz, 2H), 2.47 (dd, *J* = 5.7 Hz, *J* = 11.7 Hz, 2H), 2.47 (m, 2H), 3.36 (s, 6H), 4.39 (t, *J* = 6.6 Hz, 2H), 4.56 (d, *J* = 5.7 Hz, 2H), 4.62 (d, *J* = 6.3 Hz, 2H), 4.96(s, 1H), 4.97 (s, 1H), 6.27 (t, *J* = 7.5 Hz, 1H), 6.52 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) 25.2 (4C), 26.4 (4C), 26.7 (2C), 33.1 (4C), 37.9 (2C), 39.6 (2C), 39.8 (2C), 55.5, 55.7, 83.6 (2C), 85.3 (2C), 85.6 (2C), 110.1, 110.3, 111.9, 112.9, 117.7 (2C), 122.6 (2C), 149.6, 150.1.

1-Methyl-5,6,7-deoxy-6-cyano-7-cyclohexyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (4a)

10% Palladium/carbon (0.1 g) was added into the solution of 1-methyl-5,6,7-deoxy-6-cyano-7-cyclohexyl-2,3-*O*-isopropylidene-*β*-D-hepta-6-enofuranoside (**3a**, 1.03 g) in methanol (100 mL). The mixture was hydrogenated under the pressure of 0.3 MPa at 30°C for 5–6 h. After filtration, the solution was evaporated *in vacuo* and the product was used in the next step directly (1.0 g, yield: 97.1 %). The purity was 95% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C18H30NO4[M+H]$^{+}$ 324.2175, found 324.2169. *R*:*S* or *S*:*R* = 1:1.1. ^{1}H NMR ($CDCl_3$, 300 MHz)

0.96 (m, 4H), 1.28 (m, 10H), 1.31 (s, 6H), 1.48 (s, 6H), 1.52 (m, 12H), 1.73 (m, 4H), 2.80 (m, 2H), 3.33 (s, 3H), 3.38 (s, 3H), 4.26 (dd, *J* = 5.7 Hz, *J* = 9.9 Hz, 1H), 4.42 (dd, *J* = 5.1 Hz, *J* = 10.2 Hz, 1H), 4.53 (t, *J* = 5.7 Hz, 2H), 4.61 (t, J = 5.7 Hz, 2H), 4.96 (s, 2H). ^{13}C NMR ($CDCl_3$, 75MHz) 21.8, 25.1, 25.3, 25.8, 26.2, 27.2, 29.9, 32.2, 32.5, 34.0, 35.6, 55.7, 67.5, 84.2, 85.6, 110.1, 110.4, 112.9.

1-Methyl-5,6,7-deoxy-6-cyano-7-cyclopentyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (4b)

Compound **4b** was prepared according to the same procedure of **4a**, in which 1-methyl-5,6,7-deoxy-6-cyano-7-cyclopentyl-2,3-*O*-isopropylidene-*β*-D-hepta-6-enofuranoside (**3b**) was used and **4b** was obtained as yellowish oil in 98.0% yield. The purity was 98% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C17H28NO4[M+H]$^+$ 310.2018, found 310.2013. *R*:*S* or *S*:*R* = 1:1.5. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.31 (s, 6H), 1.48 (s, 6H), 1.60 (m, 18H), 1.74 (m, 4H), 2.03 (m, 4H), 2.75 (m, 2H), 3.34 (s, 3H), 3.38 (s, 3H), 4.27 (dd, *J* = 5.4 Hz, *J* = 9.9 Hz, 1H), 4.42 (t, *J* = 8.1 Hz, 1H), 4.53 (t, *J* = 5.7 Hz, 2H), 4.61 (t, *J* = 4.8 Hz, 2H), 4.96 (s, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) 22.9, 25.1, 25.2 (2C), 27.8, 29.9, 32.1, 32.8, 33.0, 33.2, 55.7, 68.7, 84.1, 85.6, 110.1, 110.4, 112.8.

1-Methyl-5,6,7-deoxy-6-cyano-7-cyclobutyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (4c)

Compound **4c** was prepared according to the same procedure of **4a**, in which 1-methyl-5,6,7-deoxy-6-cyano-7-cyclobutyl-2,3-*O*-isopropylidene-*β*-D-hepta-6-enofuranoside (**3c**) was used and **4c** was obtained as yellowish oil in 91.5% yield. The purity was 97% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C16H26NO4[M+H]$^+$ 296.1862, found 296.1859. *R*:*S* or *S*:*R* = 1:1.1. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.31 (s, 6H), 1.48 (s, 6H), 1.60 (m, 4H), 1.80 (m, 9H), 2.13 (m, 9H), 2.50 (m, 1H), 2.62 (m, 1H), 3.34 (s, 3H), 3.37 (s, 3H), 4.26 (dd, *J* = 5.7 Hz, *J* = 9.9 Hz, 1H), 4.39 (dd, *J* = 6.0 Hz, *J* = 10.2 Hz, 1H), 4.52 (t, *J* = 5.7 Hz, 2H), 4.61 (t, *J* = 5.7 Hz, 2H), 4.96 (s, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) 18.7, 22.9, 25.3, 27.3, 28.4, 28.7, 29.9, 34.0, 37.6, 55.7, 84.2, 84.6, 86.9, 109.5, 110.4, 112.9.

1-Methyl-5,6,7-deoxy-6-cyano-7-cyclopropyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (4d)

10% Palladium/carbon (0.26 g) was added into the solution of

1-methyl-5,6,7-deoxy-6-cyano-7-cyclopropyl-2,3-*O*-isopropylidene-*β*-D-hepta-6-enofuranoside (2.3 g) in methanol (100 mL). The mixture was hydrogenated under the pressure of 0.6 MPa at 30°C for 5–6 h. After filtration, the solvent was removed *in vacuo* and the residue was separated on the silico gel column eluting with 5-10% ethyl acetate/petroleum ether. The product was obtained as yellowish oil (1.9 g, yield: 82.0%). The purity was 95% by HPLC analysis. HRMS (TOF) *m*/z calcd for C15H24NO4 $[M+H]^+$ 282.1705, found 282.1709. *R*:*S* or *S*:*R* = 1:1.2. ^{1}H NMR ($CDCl_3$, 300 MHz) 0.11 (m, 4H), 0.20 (m, 2H), 0.55 (m, 4H), 1.30 (s, 6H), 1.46 (s, 6H), 1.58 (m, 4H), 1.83 (m, 4H), 2.83 (m, 2H), 3.32 (s, 3H), 3.37 (s, 3H), 4.20 (m, 1H), 4.38 (m, 1H), 4.55 (m, 2H), 4.59 (m, 2H), 4.90 (s, 1H), 4.94 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) 4.3, 4.6, 5.0, 5.2, 8.6, 8.8, 21.2 (2C), 25.1, 25.2, 26.6 (2C), 28.8, 29.5, 37.3, 37.8, 55.6 (2C), 84.1, 84.3, 84.6 (2C), 85.3, 85.5, 110.1 (2C), 110.3 (2C), 112.8 (2C).

1-Methyl-5,6,7,8-deoxy-6-cyano-8-cyclohexyl-2,3-*O*-isopropylidene-*β*-D-octa-furanoside (4e)

Compound **4e** was prepared according to the same procedure of **4a**, in which 1-methyl-5,6,7,8-deoxy-6-cyano-8-cyclohexyl-2,3-*O*-isopropylidene-*β*-D-octa-6-enofuranoside (**3e**) was used and **4e** was obtained as yellowish oil in 88.7% yield. The purity was 98% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C19H32NO4$[M+H]^+$ 338.2331, found 338.2333. *R*:*S* = 1:1. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.25 (m, 14H), 1.31 (s, 6H), 1.48 (s, 6H), 1.71 (m, 20 H), 2.71 (m, 2H), 3.33 (s, 3H), 3.38 (s, 3H), 4.26 (dd, *J* = 5.4 Hz, *J* = 10.2 Hz, 1H), 4.40 (dd, *J* = 5.7 Hz, *J* = 9.6 Hz, 1H), 4.54 (dd, *J* = 6.0 Hz, *J* = 9.3 Hz, 2H), 4.61 (t, *J* = 4.5 Hz, 2H), 4.96 (s, 2H).

1-Methyl-5,6,7-deoxy-6-amide-7-cyclohexyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (5a)

1-Methyl-5,6,7-deoxy-6-cyano-7-cyclohexyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (**4a**, 2.65 g, 8.2 mmol) was dissolved in the mixture of DMSO (710 μL), 30% hydroperoxide (3.82 mL), 2N NaOH solution (61.2 mL, 122 mmol) and methanol (200 mL). The mixture was refluxed for 1 h and cooled. The solvent was removed *in vacuo* and the residue was extracted with dichloromethane (3×50 mL). The organic phase was combined, washed with water and dried over anhydrous sodium

sulphate. After filtration, the solvent was removed *in vacuo* and the residue was separated on the silico gel column eluting with 10-50% ethyl acetate/petroleum ether. The product was obtained as colorless oil (2.2 g, yield: 78.6 %). The purity was 96% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C18H32NO5[M+H]$^+$ 342.2281, found 342.2285. LR-ESI: 364.2 (M+Na). *R*:*S* or *S*:*R* = 1:1.1. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.22 (m, 12H), 1.29 (s, 3H), 1.31 (s, 3H), 1.45 (s, 3H), 1.47 (s, 3H), 1.62 (m, 18H), 2.46 (m, 1H), 2.61 (m, 1H), 3.36 (s, 3H), 3.37 (s, 3H), 4.19 (t, *J* = 6.9 Hz, 2H), 4.50 (t, *J* = 5.4 Hz, 2H), 4.60 (d, *J* = 5.7 Hz, 2H), 4.94 (s, 2H), 5.38 (brs, $CONH_2$), 5.63 (brs, $CONH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz) 24.8, 25.0, 26.1 (2C), 26.2 (2C), 26.4 (2C), 26.5 (2C), 32.8, 33.0, 33.7, 34.0, 35.2, 35.4, 38.4 (2C), 39.9 (2C), 40.5, 40.9, 55.1, 55.3, 84.0, 84.3, 84.9, 85.0, 85.3, 85.4, 109.5, 110.0, 112.1, 112.4, 178.3, 178.5.

1-Methyl-5,6,7-deoxy-6-amide-7-cyclopentyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (5b)

Compound **5b** was prepared according to the same procedure of **5a**, in which 1-methyl-5,6,7-deoxy-6-cyano-7-cyclopentyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (**4b**) was used and **5b** was obtained as colorless oil in 69.6 %yield. The purity was 99% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C17H30NO5[M+H]$^+$ 328.2124, found 328.2122. LR-ESI: 350.2 (M+Na). *R*:*S* or *S*:*R* = 1:1.5. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.29 (s, 3H), 1.31 (s, 3H), 1.45 (s, 3H), 1.47 (s, 3H), 1.63 (m, 14H), 1.80 (m, 12H), 2.34 (m, 1H), 2.45 (m, 1H), 3.36 (s, 3H), 3.37 (s, 3H), 4.20 (dd, *J* = 8.1 Hz, *J* = 12.3 Hz, 2H), 4.50 (t, *J* = 5.7 Hz, 2H), 4.60 (d, *J* = 6.0 Hz, 2H), 4.94 (s, 2H), 5.41 (brs, $CONH_2$), 5.62 (brs, $CONH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz) 24.7 (2C), 24.9 (2C), 25.0 (2C), 26.2, 26.4, 32.2, 32.4, 32.9, 33.1, 37.8 (2C), 37.9 (2C), 38.1, 38.6, 39.0, 39.7, 54.9, 55.2, 83.9, 84.2, 84.9 (2C), 85.2, 85.3, 109.4, 109.9, 112.0, 112.3, 178.3, 178.5.

1-Methyl-5,6,7-deoxy-6-amide-7-cyclobutyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (5c)

Compound **5c** was prepared according to the same procedure of **5a**, in which 1-methyl-5,6,7-deoxy-6-cyano-7-cyclobutyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (**4c**) was used and **5c** was obtained as white needles in 78.7 % yield. The purity was 96% by HPLC analysis.

HRMS (TOF) *m*/*z* calcd for C16H28NO5[M+H]$^+$ 314.1968, found 314.1963. LR-ESI: 336.2 (M+23). *R*:*S* or *S*:*R* = 1:1.2. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.29 (s, 3H), 1.31 (s, 3H), 1.45 (s, 3H), 1.47 (s, 3H), 1.62 (m, 12H), 1.81 (m, 10H), 2.31(m, 2H), 3.35 (s, 3H), 3.37 (s, 3H), 4.21 (t, *J* = 8.1 Hz, 2H), 4.50 (t, *J* = 5.1 Hz, 2H), 4.60 (d, *J* = 6.0 Hz, 2H), 4.93 (s, 2H), 5.35 (brs, $CONH_2$), 5.58 (brs, $CONH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz) 18.0, 18.2, 24.5, 24.7, 26.1, 26.2, 28.2 (2C), 28.3 (2C), 33.8, 34.0, 37.5, 37.7, 39.3, 39.6, 41.2, 41.3, 54.7, 54.9, 83.7, 84.1, 84.8, 84.9, 85.1, 85.2, 109.1, 109.7, 111.9, 112.1, 178.1, 178.2.

1-Methyl-5,6,7-deoxy-6-amide-7-cyclopropyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (5d)

Compound **5d** was prepared according to the same procedure of **5a**, in which 1-methyl-5,6,7-deoxy-6-cyano-7-cyclopropyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (**4d**) was used and **5d** was obtained as colorless oil in 71.9 % yield. The purity was 97% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C15H26NO5[M+H]$^+$ 300.1811, found 300.1808. *R*:*S* or *S*:*R* = 1:1.2. ^{1}H NMR ($CDCl_3$, 300 MHz) 0.03 (m, 4H), 0.41 (d, *J* = 7.5 Hz, 2H), 0.69 (m, 2H), 0.85 (m, 2H), 1.26 (s, 3H), 1.28 (s, 3H), 1.42 (s, 3H), 1.43 (s, 3H), 1.62 (m, 4H), 1.80 (m, 2H), 1.89 (m, 2H), 2.39 (m, 1H), 2.52 (m, 1H), 3.32 (s, 3H), 3.33 (s, 3H), 4.18 (m, 2H), 4.48 (t, *J* = 5.1 Hz, 2H), 4.56 (d, *J* = 5.7 Hz, 2H), 4.90 (s, 1H), 4.93 (s, 1H), 5.91 (brs, $CONH_2$), 5.98 (brs, $CONH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz) 4.3, 4.6, 4.8, 5.0, 9.2, 9.3, 25.0, 25.2, 26.5, 26.7, 37.2, 37.7, 37.9, 38.3, 43.9, 44.1, 55.2, 55.4, 84.2, 84.5, 85.1, 85.3, 85.5, 85.6, 109.6, 110.2, 112.4, 112.6, 178.0, 178.3.

1-Methyl-5,6,7,8-deoxy-6-amide-8-cyclohexyl-2,3-*O*-isopropylidene-*β*-D-octa-furanoside (5e)

Compound **5e** was prepared according to the same procedure of **5a**, in which 1-methyl-5,6,7,8-deoxy-6-cyano-8-cyclohexyl-2,3-*O*-isopropylidene-*β*-D-octa-furanoside (**4e**) was used and **5e** was obtained as colorless oil in 99.1% yield. The purity was 98% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C19H34NO5[M+H]$^+$ 356.2437, found 356.2435. *R*:*S* = 1:1. ^{1}H NMR (CD_3OD, 300 MHz) 1.20 (m, 14H), 1.29 (s, 3H), 1.30 (s, 3H), 1.41 (s, 3H), 1.42 (s, 3H), 1.70 (m, 20 H), 2.35 (m, 1H), 2.49 (m, 1H), 3.34 (s, 3H), 3.36 (s, 3H), 4.05 (dd, *J* = 7.2 Hz, *J* = 11.7 Hz, 1H),

4.12 (d, *J* = 7.5 Hz, 1H), 4.57 (t, *J* = 5.7 Hz, 2H), 4.60 (brs, 2H), 4.88 (s, 1H), 4.89 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) 25.2 (2C), 26.9 (2C), 27.6 (2C), 27.9 (2C), 31.0 (2C), 31.9 (2C), 34.4 (2C), 34.8 (2C), 36.3 (2C), 39.0 (2C), 39.1, 39.2, 42.6, 44.8, 55.5, 55.7, 85.4, 85.8, 86.5, 86.7, 86.9, 87.0, 111.1, 111.4, 113.4, 113.5, 181.0, 181.1.

5,6,7-Deoxy-6-amide-7-cyclohexyl-1,2,3-*O*-triacetyl-*β*-D-hepta-furanoside (6a)

0.2 N Sulphuric acid (3 mL) was added into a solution of 1-methyl-5,6,7-deoxy-6-amide-7-cyclohexyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside **5a** (0.2 g) in the mixture of water and dioxane (1:1, 40 mL) and the resulted solution was refluxed for 5 h. After cooling, the mixture was adjusted to pH = 7 with saturated sodium carbonate solution and the solvent was removed *in vacuo*. The residue was dissolved in pyridine (6 mL), followed by addition of acetic anhydride (3 mL). The solution was stirred at room temperature overnight. Water was added and the mixture was extracted with dichloromethane (25 mL×3). The organic phase was combined, washed with water, and dried over anhydrous sodium sulphate. After filtration, the solution was evaporated *in vacuo* to obtain the product as yellowish oil, which was used directly in the next step (0.24 g, yield: 99.1%).

5,6,7-Deoxy-6-amide-7-cyclopentyl-1,2,3-*O*-triacetyl-*β*-D-hepta-furanoside (6b)

Compound **6b** was prepared according to the same procedure of **6a**, in which 1-methyl-5,6,7-deoxy-6-amide-7-cyclopentyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (**5b**) was used and **6b** was obtained as yellowish oil in 98.4% yield.

5,6,7-Deoxy-6-amide-7-cyclobutyl-1,2,3-*O*-triacetyl-*β*-D-hepta-furanoside (6c)

Compound **6c** was prepared according to the same procedure of **6a**, in which 1-methyl-5,6,7-deoxy-6-amide-7-cyclobutyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (**5c**) was used and **6c** was obtained as yellowish oil in 97.6% yield.

5,6,7-Deoxy-6-amide-7-cyclopropyl-1,2,3-*O*-triacetyl-*β*-D-hepta-furanoside (6d)

Compound **6d** was prepared according to the same procedure of **6a**, in which

1-methyl-5,6,7-deoxy-6-amide-7-cyclobutyl-2,3-*O*-isopropylidene-*β*-D-hepta-furanoside (**5d**) was used and **6d** was obtained as yellowish oil in 96.7% yield.

5,6,7,8-Deoxy-6-amide-8-cyclohexyl-1,2,3-*O*-triacetyl-*β*-D-octa-furanoside (6e)

Compound **6e** was prepared according to the same procedure of **6a**, in which 1-methyl-5,6,7,8-deoxy-6-amide-8-cyclohexyl-2,3-*O*-isopropylidene-*β*-D-octa-furanoside (**5e**) was used and **6e** was obtained as yellowish oil in 69.3% yield.

N^6-Benzoyl-adenine (7)

Benzoyl chloride (3.41 mL, 29.6 mmol) was added into the solution of adenine (2 g, 14.8 mmol) in anhydrous pyridine (25 mL) and the resulted mixture was stirred at 60°C overnight. The solvent was removed *in vacuo* and the residue was dissolved in methanol (30 mL). It was adjusted to pH = 9-10 with 2N NaOH solution and stirred for another 30 min. After neutralization with 20% HCl solution, the solvent was removed *in vacuo* and the crude powder was obtained, which was recrystallized with ethanol to obtain N^6-benzoyl-adenineas white powder (3.198 g, yield: 90.3%).The purity was 95% by HPLC analysis. HRMS (TOF) *m/z* calcd for C12H10N5O$[M+H]^+$ 240.0885, found 240.0888. ^{1}H NMR (CD_3OD, 300 MHz) 7.57 (t, *J* = 7.2 Hz, 2H), 7.65 (t, *J* = 7.5 Hz, 1H), 8.10 (d, *J* = 6.9 Hz, 2H), 8.48 (s, 1H), 8.71 (s, 1H). ^{13}C NMR (DMSO-d_6) 93.3, 128.5 (2C), 128.6 (2C), 132.1, 132.7, 133.0, 145.9, 151.2, 161.0, 166.6.

N^6-Benzoyl-9-(5',6',7'-deoxy-6'-amide-7'-cyclohexyl-2',3'-*O*-diacetyl-*β*-D-hepta-furanoside-1')adenine (8a)

A mixture of N^6-benzoyl-adenine **7** (192 mg, 0.8 mmol, 1 eq) in acetonitrile (10 mL) and *N*,*O*-bis(trimethylsilyl)acetamide (10 mL) was refluxed for 2 h. After evaporation *in vacuo*, the residue was dissolved in dichloroethane (30 mL), followed by addition of the mixture of 5,6,7-deoxy-6-amide-7-cyclohexyl-1,2,3-*O*-triacetyl-*β*-D-hepta-furanoside (0.48 g, 1.16 mmol, 1.45 eq) in dichloroethane (30 mL). Trimethylsilyl trifluoromethanesulfonate (144 μL, 0.8 mmol, 1 eq) was added and the reaction was refluxed for 2 h. After cooling, water was added and the mixture was

extracted with dichloromethane (25 mL×3). The organic phase was combined, washed with saturated Na_2CO_3 solution and water, and dried over anhydrous sodium sulphate. After filtration, the solvent was removed *in vacuo* and the residue was separated on the prep-TLC eluting with 5% methanol/dichloromethane. The product was obtained as yellowish oil (192 mg, yield: 40.5 %). The purity was 97% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C30H37N6O7$[M+H]^+$ 593.2724, found 593.2720. *R*:*S* or *S*:*R* = 1:1.1. ^{1}H NMR (CD_3OD, 300 MHz) 0.89 (m, 4H), 1.20 (m, 22H), 1.65 (m, 4H), 2.06 (s, 6H), 2.14 (s, 3H), 2.16 (s, 3H), 2.58 (m, 2H), 4.20 (m, 1H), 4.29 (m, 1H), 5.53 (t, *J* = 5.4 Hz, 1H), 5.55 (t, *J* = 5.4 Hz, 1H), 6.04 (t, *J* = 5.4 Hz, 1H), 6.13 (t, *J* = 5.4 Hz, 1H), 6.27 (d, *J* = 2.1 Hz, 1H), 6.29 (d, *J* = 2.1 Hz, 1H), 7.56 (t, *J* = 7.5 Hz, 4H), 7.66 (t, *J* = 7.5 Hz, 2H), 8.08 (d, *J* = 7.2 Hz, 4H), 8.54 (s, 1H), 8.56 (s, 1H), 8.74 (s, 1H), 8.76 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 20.5 (2C), 20.7 (2C), 27.4 (4C), 27.5 (2C), 27.7 (2C), 34.1, 34.2, 34.9, 35.1, 36.7 (2C), 37.5 (2C), 41.5, 41.6, 74.4, 74.6, 75.1 (2C), 81.9, 82.2, 88.6, 88.9, 125.4, 125.5, 129.6 (4C), 129.9 (4C), 134.1 (2C), 135.0 (2C), 145.1, 145.3, 151.3, 151.4, 153.2 (2C), 153.6 (2C), 168.4 (2C), 171.4 (2C), 171.7 (2C), 180.9, 181.2.

N^6-Benzoyl-9-(5',6',7'-deoxy-6'-amide-7'-cyclopentyl-2',3'-*O*-diacetyl-*β*-D-hepta-furanoside-1')adenine (8b)

Compound **8b** was prepared according to the same procedure of **8a**, in which N^6-benzoyl-adenine (1 eq), 5,6,7-deoxy-6-amide-7-cyclopentyl-1,2,3-*O*-triacetyl-*β*-D-hepta-furanoside (**6b**, 1.5 eq) and trimethylsilyl trifluoromethanesulfonate (1 eq) were used. The product **8b** was obtained as yellowish oil in 91.7% yield. The purity was 98% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C29H35N6O7$[M+H]^+$ 579.2567, found 579.2569. LR-ESI: 579.3 (M+1), 601.3 (M+Na). *R*:*S* or *S*:*R*= 1:1.5. ^{1}H NMR (CD_3OD, 300 MHz) 1.57 (m, 18H), 1.79 (m, 8H), 2.06 (s, 6H), 2.17 (s, 3H), 2.18 (s, 3H), 2.53 (m, 2H), 4.20 (m, 1H), 4.31 (dt, *J* = 5.1 Hz, *J* = 9.6 Hz, 1H), 5.54 (t, *J* = 5.4 Hz, 1H), 5.55 (t, *J* = 5.4 Hz, 1H), 6.05 (t, *J* = 5.4 Hz, 1H), 6.16 (t, *J* = 4.8 Hz, 1H), 6.28 (d, *J* = 4.8 Hz, 1H), 6.29 (d, *J* = 5.1 Hz, 1H), 7.57 (t, *J* = 6.9 Hz, 4H), 7.66 (t, *J* = 7.2 Hz, 2H), 8.09 (d, *J* = 7.2 Hz, 4H), 8.55 (s,

1H), 8.57 (s, 1H), 8.74 (s, 1H), 8.76 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) 20.5 (2C), 20.7 (2C), 25.1 (2C), 25.2 (2C), 32.6 (2C), 33.0 (2C), 36.4 (2C), 37.9 (2C), 39.9 (2C), 42.0 (2C), 72.8 (2C), 73.6 (2C), 80.7 (2C), 86.9 (2C), 124.1 (2C), 128.3 (4C), 128.9 (4C), 133.0 (2C), 133.5 (2C), 142.4 (2C), 150.1 (2C), 151.8 (2C), 152.7 (2C), 165.8 (2C), 169.6 (2C), 169.8 (2C), 178.0 (2C).

N^6-Benzoyl-9-(5',6',7'-deoxy-6'-amide-7'-cyclobutyl-2',3'-*O*-diacetyl-*β*-D-hepta-furanoside-1')adenine (8c)

Compound **8c** was prepared according to the same procedure of **8a**, in which N^6-benzoyl-adenine (1 eq), 5,6,7-deoxy-6-amide-7-cyclobutyl-1,2,3-*O*-triacetyl-*β*-D-hepta-furanoside (**6c**, 1.6 eq) and trimethylsilyl trifluoromethanesulfonate (3 eq) were used. The product **8c** was obtained as yellowish oil in 60.7% yield. The purity was 96% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for $C28H33N6O7[M+H]^+$ 565.2411, found 565.2408. *R*:*S* or *S*:*R* = 1:1.1. ^{1}H NMR (CD_3OD, 300 MHz) 1.63 (m, 14H), 1.80 (m, 8H), 2.03 (s, 3H), 2.06 (s, 3H), 2.14 (s, 3H), 2.15 (s, 3H), 2.35 (m, 2H), 4.13 (m, 1H), 4.28 (m, 1H), 5.52 (t, *J* = 5.4 Hz, 2H), 6.04 (t, *J* = 5.1 Hz, 1H), 6.13 (t, *J* = 4.8 Hz, 1H), 6.27 (t, *J* = 4.8 Hz, 2H), 7.56 (t, *J* = 7.2 Hz, 4H), 7.66 (t, *J* = 7.2 Hz, 2H), 8.09 (d, *J* = 7.2 Hz, 4H), 8.54 (s, 1H), 8.56 (s, 1H), 8.74 (s, 1H), 8.77 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHZ) 19.4 (2C), 20.5 (2C), 20.7 (2C), 29.5 (2C), 29.7 (2C), 35.5, 35.9, 37.0, 37.1, 41.0, 41.3, 42.4, 42.5, 74.4, 74.6, 75.1 (2C), 81.9, 82.2, 88.5, 88.8, 125.3, 125.5, 129.6 (4C), 129.9 (4C), 134.1 (2C), 135.0 (2C), 145.1, 145.3, 151.3 (2C), 151.4 (2C), 153.2, 153.6, 168.4 (2C), 171.5 (2C), 171.7, 171.8, 180.6, 180.9.

N^6-Benzoyl-9-(5',6',7'-deoxy-6'-amide-7'-cyclopropyl-2',3'-*O*-diacetyl-*β*-D-hepta-furanoside-1') adenine (8d)

Compound **8d** was prepared according to the same procedure of **8a**, in which N^6-benzoyl-adenine (1 eq), 5,6,7-deoxy-6-amide-7-cyclopropyl-1,2,3-*O*-triacetyl-*β*-D-hepta-furanoside (**6d**, 1.6 eq) and trimethylsilyl trifluoromethanesulfonate (2 eq) were used. The product **8d** was obtained as yellowish oil in 65.0% yield. The purity was 95% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for $C27H31N6O7[M+H]^+$ 551.2254, found 551.2251. *R*:*S* or *S*:*R* = 1:1.2. ^{1}H NMR (CD_3OD, 300 MHz)

0.04 (m, 4H), 0.41 (m, 4H), 0.69 (m, 1H), 0.88 (m, 1H), 1.29 (m, 4H), 1.50 (m, 4H), 2.05 (s, 6H), 2.13 (s, 3H), 2.15 (s, 3H), 2.59 (m, 2H), 4.19 (m, 1H), 4.29 (m, 1H), 5.53 (dd, *J* = 5.4 Hz, *J* = 7.5 Hz, 1H), 5.55 (dd, *J* = 3.6 Hz, *J* = 5.7 Hz, 1H), 6.04 (t, *J* = 4.8 Hz, 1H), 6.10 (t, *J* = 5.1 Hz, 1H), 6.26 (d, *J* = 6.0 Hz, 1H), 6.28 (d, *J* = 4.8 Hz, 1H), 7.53 (t, *J* = 6.9 Hz, 4H), 7.63 (t, *J* = 6.9 Hz, 2H), 8.07 (d, *J* = 7.8 Hz, 4H), 8.54 (s, 1H), 8.56 (s, 1H), 8.71 (s, 1H), 8.72 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 5.0, 5.1, 5.3, 5.4, 9.9, 10.0, 20.5 (2C), 20.7 (2C), 36.6, 36.7, 38.8, 39.7, 44.4 (2C), 74.5, 74.6, 75.0 (2C), 81.8, 82.1, 88.4, 88.7, 125.3, 125.4, 129.5 (4C), 129.9 (4C), 134.1 (2C), 134.9 (2C), 145.0, 145.2, 151.3 (2C), 153.1 (2C), 153.5 (2C), 167.7, 168.2, 171.4 (2C), 171.6 (2C), 180.6, 180.9.

N^6-Benzoyl-9-(5',6',7',8'-deoxy-6'-amide-8'-cyclohexyl-2',3'-*O*-diacetyl-*β*-D-octa-furanoside-1') adenine (8e)

Compound **8e** was prepared according to the same procedure of **8a**, in which N^6-benzoyl-adenine (1 eq), 5,6,7,8-deoxy-6-amide-8-cyclohexyl-1,2,3-*O*-triacetyl-*β*-D-octa-furanoside (**6e**, 1.17 eq) and trimethylsilyl trifluoromethanesulfonate (2.5 eq) were used. The product **8e** was obtained as yellowish oil in 70.5% yield. The purity was 96% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C31H39N6O7[M+H]$^+$ 607.2880, found 607.2884. *R*:*S* or *S*:*R* = 1:1. ^{1}H NMR (CD_3OD, 300 MHz) 0.90 (m, 4H), 1.20 (m, 18H), 1.68 (m, 12H), 2.06 (s, 6H), 2.14 (s, 3H), 2.16 (s, 3H), 2.42 (m, 2H), 4.22 (m, 1H), 4.32 (m, 1H), 5.54 (t, *J* = 6.0 Hz, 1H), 5.55 (t, *J* = 6.6 Hz, 1H), 6.04 (t, *J* = 5.7 Hz, 1H), 6.13 (t, *J* = 5.4 Hz, 1H), 6.28 (d, *J* = 5.1 Hz, 1H), 6.32 (d, *J* = 4.8 Hz, 1H), 7.56 (t, *J* = 7.2 Hz, 4H), 7.66 (t, *J* = 7.2 Hz, 2H), 8.09 (d, *J* = 7.2 Hz, 4H), 8.56 (s, 1H), 8.58 (s, 1H), 8.74 (s, 1H), 8.76 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 20.5 (2C), 20.7 (2C), 27.5 (4C), 27.9 (2C), 34.4 (2C), 34.5 (2C), 34.6, 34.7, 36.1, 36.9, 39.0 (2C), 39.1 (2C), 44.3, 44.4, 74.4, 74.6, 75.1, 75.2, 82.0, 82.1, 88.6, 88.8, 125.3 (2C), 129.6 (4C), 129.9 (4C), 134.2 (2C), 135.0 (2C), 145.1, 145.4, 151.3, 151.4, 153.2 (2C), 153.6 (2C), 168.4 (2C), 171.4, 171.6, 171.7, 172.0, 180.7, 181.0.

6'(*R*)-9-(5',6',7'-Deoxy-6'-amide-7'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1') adenine (9a) and

6'(*S*)-9-(5',6',7'-deoxy-6'-amide-7'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (10a)

2N NH_3 in methanol (26 mL) was added into the solution of N^6-benzoyl-9-(5',6',7'-deoxy-6'-amide-7'-cyclohexyl-2',3'-*O*-diacetyl-*β*-D-hepta-furanoside-1')adenine (**8a**, 81 mg, 0.14 mmol) in methanol (26 mL) and the resulted solution was stirred at 40°C overnight. After evaporation *in vacuo*, the residue was dissolved in acetone (25 mL) followed by addition of trimethylsilyl trifluoromethanesulfonate in dichloroethane (83 μL TMSOTf in 250 μL DCE). After stirring for 1 h, the solvent was removed *in vacuo* and the residue was separated on the prep-TLC eluting with 5% methanol/dichloromethane. Two epimers were obtained as yellowish oil (**9a**: 30 mg, **10a**: 32 mg, total: 62 mg, yield: 99.7 %). **9a:** The purity was 95% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for $C_{22}H_{33}N_6O_4[M+H]^+$ 445.2563, found 445.2558. ^{1}H NMR (CD_3OD, 300 MHz) 0.65 (m, 3H), 1.01 (m, 8H), 1.31 (m, 2H), 1.38 (s, 3H), 1.56 (s, 3H), 1.78 (m, 2H), 2.32 (m, 1H), 4.17 (dt, *J* = 2.7 Hz, *J* = 9.9 Hz, 1H), 4.92 (dd, *J* = 2.7 Hz, *J* = 6.3 Hz, 1H), 5.64 (dd, *J* = 1.8 Hz, *J* = 6.3 Hz, 1H), 6.17 (d, *J* = 2.1 Hz, 1H), 8.24 (s, 1H), 8.30 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 25.6, 27.3, 27.4, 27.6, 30.8, 34.3, 34.4, 36.4, 37.7, 41.2, 42.1, 85.0, 86.3, 86.9, 91.8, 115.3, 120.7, 142.1, 150.5, 154.1, 157.6, 181.1. **10a:** The purity was 95% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for $C_{22}H_{33}N_6O_4[M+H]^+$ 445.2563, found 445.2559. ^{1}H NMR (CD_3OD, 300 MHz) 0.89 (m, 3H), 1.26 (m, 8H), 1.38 (s, 3H), 1.58 (s, 3H), 1.77 (m, 2H), 1.94 (m, 2H), 2.52 (m, 1H), 4.17 (dt, *J* = 3.3 Hz, *J* = 10.2 Hz, 1H), 4.92 (dd, *J* = 3.6 Hz, *J* = 6.3 Hz, 1H), 5.46 (dd, *J* = 2.4 Hz, *J* = 6.3 Hz, 1H), 6.12 (d, *J* = 2.7 Hz, 1H), 8.22 (s, 1H), 8.27 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 25.8, 27.4, 27.5, 27.6, 29.7, 34.1, 35.0, 36.7, 37.7, 41.4, 41.6, 85.4, 85.6, 86.2, 91.1, 116.0, 120.7, 142.0, 150.4, 154.1, 157.5, 181.2.

6'(*R*)-9-(5',6',7'-Deoxy-6'-amide-7'-cyclopentyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1') adenine (9b) and 6'(*S*)-9-(5',6',7'-deoxy-6'-amide-7'-cyclopentyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')

adenine (10b)

Compounds **9b** and **10b** were prepared according to the same procedure of **9a** and **10a**, in which N^6-benzoyl-9-(5',6',7'-deoxy-6'-amide-7'-cyclopentyl-2',3'-*O*-diacetyl-*β*-D-hepta-furanoside-1')adenine (**8b**) was used and the two epimers were obtained as yellowish oil (**9b**:**10b** = 1:1.76, yield: 99.2%). **9b:** The purity was 97% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C21H31N6O4$[M+H]^+$ 431.2407, found 431.2410. LR-ESI: 431.3 (M+1), 453.2 (M+Na). ^{1}H NMR (CD_3OD, 300 MHz) 1.16 (m, 2H), 1.38 (s, 3H), 1.46 (m, 7H), 1.56 (s, 3H), 1.78 (m, 4H), 2.25 (m, 1H), 4.17 (dt, *J* = 2.4 Hz, *J* = 10.5 Hz, 1H), 4.92 (dd, *J* = 2.4 Hz, *J* = 6.3 Hz, 1H), 5.67 (dd, *J* = 1.8 Hz, *J* = 6.0 Hz, 1H), 6.17 (d, *J* = 2.1 Hz, 1H), 8.23 (s, 1H), 8.29 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 25.6, 26.0, 26.1, 27.4, 30.1, 33.6, 37.5, 39.0, 40.9, 43.2, 85.0, 86.4, 86.8, 91.9, 115.3, 120.7, 142.2, 150.5, 154.1, 157.6, 181.1. **10b:** The purity was 97% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C21H31N6O4$[M+H]^+$ 431.2407, found 431.2412. LR-ESI: 431.3 (M+1), 453.2 (M+Na). ^{1}H NMR (CD_3OD, 300 MHz) 1.38 (s, 3H), 1.58 (s, 3H), 1.63 (m, 6H), 1.80 (m, 5H), 1.95 (m, 2H), 2.44 (m, 1H), 4.17 (dt, *J* = 3.6 Hz, *J* = 10.8 Hz, 1H), 4.92 (dd, *J* = 3.9 Hz, *J* = 6.3 Hz, 1H), 5.47 (dd, *J* = 2.7 Hz, *J* = 6.3 Hz, 1H), 6.12 (d, *J* = 2.7 Hz, 1H), 8.21 (s, 1H), 8.27 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 25.8, 26.1, 26.2, 27.6, 33.5, 34.1, 37.6, 39.3, 40.4, 43.5, 85.5, 85.6, 86.3, 91.1, 115.9, 120.7, 142.0, 150.4, 154.1, 157.5, 181.2.

6'(*R*)-9-(5',6',7'-Deoxy-6'-amide-7'-cyclobutyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine(9c) and 6'(*S*)-9-(5',6',7'-deoxy-6'-amide-7'-cyclobutyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (10c)

Compounds **9c** and **10c** were prepared according to the same procedure of **9a**and **10a**, in which N^6-benzoyl-9-(5',6',7'-deoxy-6'-amide-7'-cyclobutyl-2',3'-*O*-diacetyl-*β*-D-hepta-furanoside-1')adenine (**8c**) was used and the two epimers were obtained as white powder (**9c**:**10c** = 1:1.06, yield: 94.6%). **9c:** The purity was 95% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C20H29N6O4$[M+H]^+$ 417.2250, found 417.2257. ^{1}H NMR (CD_3OD, 300 MHz) 1.25 (m, 2H), 1.38 (s, 3H), 1.56 (s, 3H),

1.72 (m, 5H), 1.90 (m, 4H), 2.14 (m, 1H),4.15 (dt, *J* = 3.9 Hz, *J* = 10.5 Hz, 1H), 4.89 (dd, *J* = 3.0 Hz, *J* = 6.6 Hz, 1H), 5.65 (dd, *J* = 2.1 Hz, *J* = 6.0 Hz, 1H), 6.16 (d, *J* = 2.1 Hz, 1H), 8.25 (s, 1H), 8.30 (s, 1H). ^{13}C NMR (CD_3OD, 75MHz) 19.3, 25.6, 27.4, 29.3, 29.4, 35.3, 37.3, 41.7, 42.1, 85.0, 86.3, 86.6, 91.8, 115.4, 120.7, 142.2, 150.5, 154.2, 157.6,180.8. **10c:** The purity was 95% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C20H29N6O4[M+H]$^+$ 417.2250, found 417.2255. ^{1}H NMR (CD_3OD, 300 MHz) 1.38 (s, 3H), 1.58 (s, 3H), 1.65 (m, 2H), 1.76 (m, 5H), 2.02 (m, 4H), 2.30 (m, 1H),4.15 (dt, *J* = 3.9 Hz, *J* = 11.1Hz, 1H), 4.90 (dd, *J* = 3.9 Hz, *J* = 6.3 Hz, 1H), 5.46 (dd, *J* = 2.4 Hz, *J* = 6.3 Hz, 1H), 6.12 (d, *J* = 2.4 Hz, 1H), 8.22 (s, 1H), 8.26 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 19.4, 25.8, 27.6, 29.5, 29.6, 35.5, 37.3, 41.2, 42.4, 85.5, 85.6, 86.2, 91.1, 115.9,120.7, 142.0, 150.4, 154.1, 157.5, 180.9.

6'(*R*)-9-(5',6',7'-Deoxy-6'-amide-7'-cyclopropyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (9d) and 6'(*S*)-9-(5',6',7'-deoxy-6'-amide-7'-cyclopropyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1') adenine (10d)

Compounds **9d** and **10d** were prepared according to the same procedure of **9a**and **10a**, in which N^6-benzoyl-9-(5',6',7'-deoxy-6'-amide-7'-cyclopropyl-2',3'-*O*-diacetyl-*β*-D-hepta-furanoside-1')adenine (**8d**) was used and the two epimers were obtained as yellowish oil (**9d**:**10d** = 1:1.44, yield: 91.1%). **9d:** The purity was 98% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C19H27N6O4[M+H]$^+$ 403.2094, found 403.2089. ^{1}H NMR (CD_3OD, 300 MHz) -0.11 (m, 2H), 0.28 (m, 2H), 0.42 (m, 1H), 1.15 (m, 2H), 1.39 (s, 3H), 1.56 (s, 3H), 1.82 (m, 2H), 2.36 (m, 1H),4.18 (dt, *J* = 3.3 Hz, *J* = 6.6 Hz, 1H), 4.90 (m, 1H), 5.62 (dd, *J* = 2.1 Hz, *J* = 6.3 Hz, 1H), 6.16 (d, *J* = 2.4 Hz, 1H), 8.22 (s, 1H), 8.29 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 5.0, 5.1, 9.9, 25.6, 27.5, 37.2, 39.5, 44.5, 85.0, 86.2, 86.6, 91.7, 115.5, 120.8, 142.1, 150.6, 154.2, 157.6, 180.8. **10d:** The purity was 96% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C19H27N6O4[M+H]$^+$ 403.2094, found 403.2093. ^{1}H NMR (CD_3OD, 300 MHz) 0.04 (m, 2H), 0.40 (m, 2H), 0.65 (m, 1H), 1.38 (s, 3H), 1.58 (s, 3H), 1.81 (m, 2H), 1.96 (m, 2H),

2.54 (m, 1H),4.16 (dt, *J* = 3.9 Hz, *J* = 11.1Hz, 1H), 4.92 (dd, *J* = 3.9 Hz, *J* = 6.6 Hz, 1H), 5.46 (dd, *J* = 2.4 Hz, *J* = 6.3 Hz, 1H), 6.12 (d, J = 2.7 Hz, 1H), 8.21 (s, 1H), 8.26 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 5.0, 5.3, 10.0, 25.8, 27.6, 37.1, 39.0, 44.6, 85.5, 85.6, 86.2, 91.0, 116.0, 120.8, 142.0, 150.5, 154.1, 157.5, 181.0.

6'(*R*)-9-(5',6',7',8'-Deoxy-6'-amide-8'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-octa-furanoside-1') adenine (9e) and 6'(*S*)-9-(5',6',7',8'-deoxy-6'-amide-8'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-octa-furanoside-1') adenine (10e)

Compounds **9e** and **10e** were prepared according to the same procedure of **9a**and **10a**, in which N^6-benzoyl-9-(5',6',7',8'-deoxy-6'-amide-8'-cyclohexyl-2',3'-*O*-diacetyl-*β*-D-octa-furanoside-1')adenine (**8e**) was used and the two epimers were obtained as yellowish oil (**9e**:**10e** = 1:1, yield: 90.5%). **9e:** The purity was 97% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C23H35N6O4$[M+H]^+$ 459.2720, found 459.2718. LR-ESI: 459.3 (M+1), 481.3 (M+Na). ^{1}H NMR (CD_3OD, 400 MHz) 0.74 (m, 1H), 0.90 (m, 2H), 1.12 (m, 2H), 1.33 (m, 8H), 1.38 (s, 3H), 1.56 (s, 3H), 1.63 (m, 2H), 1.75 (m, 1H), 1.85 (m, 1H), 2.18 (m, 1H), 4.17 (dt, *J* = 2.8 Hz, *J* = 8.8 Hz, 1H), 4.90 (d, *J* = 4.8 Hz, 1H), 5.65 (dd, *J* = 2.0 Hz, *J* = 5.2 Hz, 1H), 6.16 (d, *J* = 2.4 Hz, 1H), 8.23 (s, 1H),8.30 (d, *J* = 4.0 Hz, 1H). ^{13}C NMR (CD_3OD, 100 MHz)27.4, 27.5, 27.8, 27.9, 31.6, 34.4, 34.5, 36.0, 37.3, 38.8,43.7, 44.2, 85.0, 86.3, 86.7, 91.8, 115.4, 120.7, 142.2, 150.5, 154.1, 157.6, 181.0. **10e:** The purity was 97% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C23H35N6O4$[M + H]^+$ 459.2720, found 459.2725. LR-ESI: 459.3 (M+1), 481.3 (M+Na). ^{1}H NMR (CD_3OD, 400 MHz) 0.85 (m, 1H), 0.98 (m, 2H), 1.16 (m, 2H), 1.31 (m, 8H), 1.38 (s, 3H), 1.58 (s, 3H), 1.66 (m, 2H),1.78 (m, 1H), 1.98 (m, 1H),2.34 (m, 1H), 4.17 (dt, *J* = 3.2 Hz, *J* = 8.8 Hz, 1H), 4.91 (dd, *J* = 2.8 Hz, *J* = 4.8 Hz, 1H), 5.46 (dd, *J* = 2.0 Hz, *J* = 5.2 Hz, 1H), 6.12 (d, *J* = 2.4 Hz, 1H), 8.21 (s, 1H),8.27 (d, *J* = 4.0 Hz, 1H). ^{13}C NMR (CD_3OD, 100 MHz) 27.6, 27.6, 27.8, 27.9, 31.2, 34.5, 34.7, 36.2, 37.3, 39.1, 43.7, 44.3, 85.4, 85.6, 86.2, 91.1,116.0, 120.7, 142.1, 150.5, 154.1, 157.5, 181.1.

6'(*R*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (11a)

[Bis(trifluoroacetoxy)iodo]benzene (133 mg, 0.31 mmol, 1.5 eq) was added into the solution of 6'(*R*)-9-(5',6',7'-deoxy-6'-amide-7'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (**9a**, 90 mg, 0.2 mmol, 1 eq) in the mixture of acetonitrile (30 mL) and water (30 mL). The reaction was stirred at room temperature for 2 h. The solvent was removed *in vacuo* and the residue was dissolved in the mixture of dioxane: water: triethylamine (4 mL, 2 mL, 1 mL) followed by addition of Boc_2O (90 μL, 0.39 mmol, 2 eq). The resulted solution was stirred at room temperature for 1 h and terminated by addition of water. It was extracted with dichloromethane (25 mL×3). The organic phase was combined, washed with water and dried over anhydrous sodium sulphate. After filtration, the solvent was removed *in vacuo* and the residue was separated on the prep-TLC eluting with 5% methanol/dichloromethane. The product was obtained as yellowish oil (24 mg, yield: 23.2 %). The purity was 98% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for $C_{26}H_{41}N_6O_5[M+H]^+$ 517.3138, found 517.3142. LR-ESI: 517.3 (M+1), 539.3 (M+Na). ^{1}H NMR (CD_3OD,300 MHz) 0.77 (m, 2H), 1.05 (m, 5H), 1.38 (s, 3H), 1.41 (s, 9H), 1.57 (s, 3H), 1.60 (m, 6H), 1.75 (m, 2H), 3.55 (m, 1H), 4.28 (dt, *J* = 3.3 Hz, *J* = 10.2 Hz, 1H), 4.95 (dd, *J* = 3.0 Hz, *J* = 6.3 Hz, 1H), 5.62 (dd, *J* = 2.1 Hz, *J* = 6.3 Hz, 1H), 6.16 (d, *J* = 2.1 Hz, 1H), 6.37 (d, *J* = 9.3 Hz, 1H, NHBoc), 8.23 (s, 1H), 8.28 (s, 1H).

6'(*R*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclopentyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (11b)

Compound **11b** was prepared according to the same procedure of **11a**, in which [bis(trifluoroacetoxy)iodo]benzene (3 eq) and 6'(*R*)-9-(5',6',7'-deoxy-6'-amide-7'-cyclopentyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (**9b**, 1 eq) were used and the product **11b** was obtained as yellowish oil in 32.1% yield. The purity was 98% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for $C_{25}H_{39}N_6O_5[M+H]^+$ 503.2982, found 503.2988. ^{1}H NMR (CD_3OD, 300 MHz) 1.38 (s, 3H), 1.41 (s, 9H), 1.48 (m, 11H), 1.57 (s, 3H), 1.87 (m, 2H), 3.46 (m, 1H), 4.29 (d, *J* = 9.6 Hz, 1H),

4.94 (m, 1H), 5.64 (d, *J* = 4.5 Hz, 1H), 6.16 (s, 1H), 6.38 (d, *J* = 9.3 Hz, 1H, NH), 8.22 (s, 1H), 8.27 (s, 1H).

6'(*R*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclobutyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (11c)

Compound **11c** was prepared according to the same procedure of **11a**, in which [bis(trifluoroacetoxy)iodo]benzene (3.3 eq) and 6'(*R*)-9-(5',6',7'-deoxy-6'-amide-7'-cyclobutyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (**9c**, 1 eq) were used and the product **11c** was obtained as yellowish oil in 61.3% yield. The purity was 96% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for $C_{24}H_{37}N_6O_5[M+H]^+$ 489.2825, found 489.2829. ^{1}H NMR (CD_3OD, 400 MHz) 1.38 (s, 3H), 1.41 (s, 9H), 1.57 (s, 3H), 1.51 (m, 2H), 1.75 (m, 4H), 2.05 (m, 3H), 2.33 (m, 2H), 3.48 (m, 1H), 4.26 (m, 1H), 4.93 (dd, *J* = 3.0 Hz, *J* = 6.4 Hz, 1H), 5.63 (dd, *J* = 2.0 Hz, *J* = 6.4 Hz, 1H), 6.14 (d, *J* = 2.0 Hz, 1H),6.37 (d, *J* = 9.2 Hz, 1H, NHBoc), 8.23 (s, 1H), 8.27 (s, 1H).

6'(*R*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclopropyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (11d)

Compound **11d** was prepared according to the same procedure of **11a**, in which [bis(trifluoroacetoxy)iodo]benzene (1.5 eq) and 6'(*R*)-9-(5',6',7'-deoxy-6'-amide-7'-cyclopropyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (**9d**, 1 eq) were used and the product **11d** was obtained as white powder in 20.9 % yield. The purity was 96% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for $C_{23}H_{35}N_6O_5[M+H]^+$ 475.2669, found 475.26671. LR-ESI: 475.3 (M+1), 497.3 (M+Na). ^{1}H NMR (CD_3OD, 300 MHz) -0.10 (m, 2H), 0.25 (m, 2H), 0.40 (m, 1H), 0.90 (m, 2H), 1.38 (s, 3H), 1.41 (s, 9H), 1.58 (s, 3H), 1.85 (m, 2H), 3.56 (m, 1H),4.28 (m, 1H), 5.27 (m, 1H), 5.62 (d, *J* = 6.3 Hz, 1H), 6.15 (d, *J* = 2.4 Hz, 1H), 6.43 (d, *J* = 8.1 Hz, NHBoc), 8.22 (s, 1H), 8.28 (s, 1H).

6'(*R*)-N-Boc-9-(5',6',7',8'-deoxy-6'-amine-8'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-octa-furano

side-1')adenine (11e)

Compound **11e** was prepared according to the same procedure of **11a**, in which [bis(trifluoroacetoxy)iodo]benzene (2.5 eq) and 6'(*R*)-9-(5',6',7',8'-deoxy-6'-amide-8'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-octa-furanoside-1')adenine (**9e**, 1 eq) were used and the product **11e** was obtained as yellowish oil in 81.3 % yield. The purity was 97% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for $C_{27}H_{43}N_6O_5[M+H]^+$ 531.3295, found 531.3290. LR-ESI: 531.0 (M+1), 553.0 (M+Na). ^{1}H NMR (CD_3OD, 400 MHz) 0.89 (m, 2H), 1.16 (m, 7H), 1.32 (s, 9H), 1.38 (s, 3H), 1.56 (s, 3H), 1.60 (m, 6H), 1.81 (m, 2H), 3.63 (m, 1H), 4.16 (m, 1H), 4.98 (m, 1H), 5.89 (d, *J* = 4.4 Hz, 1H), 6.16 (s, 1H), 8.22 (s, 2H).

6'(*S*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (13a)

Compound **13a** was prepared according to the same procedure of **11a**, in which [bis(trifluoroacetoxy)iodo]benzene (1.5 eq) and 6'(*S*)-9-(5',6',7'-deoxy-6'-amide-7'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (**10a**, 1 eq) were used and the product **13a** was obtained as yellowish oil in 29.0 % yield. The purity was 96% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for $C_{26}H_{41}N_6O_5[M+H]^+$ 517.3138, found 517.3143. LR-ESI: 517.3 (M+1), 539.3 (M+Na). ^{1}H NMR (CD_3OD,300 MHz) 0.90 (m, 2H), 1.22 (m, 5H), 1.37 (s, 3H), 1.39 (s, 9H), 1.50 (m, 6H), 1.58 (s, 3H), 1.77 (m, 2H), 3.72 (m, 1H), 4.26 (m, 1H), 4.92 (dd, *J* = 3.3 Hz, *J* = 6.3 Hz, 1H), 5.44 (dd, *J* = 2.1 Hz, *J* = 6.0 Hz, 1H), 6.12 (d, *J* = 2.4 Hz, 1H), 6.39 (d, *J* = 9.3 Hz, 1H, NHBoc), 8.22 (s, 1H), 8.32 (s, 1H).

6'(*S*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclopentyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (13b)

Compound **13b** was prepared according to the same procedure of **11a**, in which [bis(trifluoroacetoxy)iodo]benzene (1.5 eq) and 6'(*S*)-9-(5',6',7'-deoxy-6'-amide-7'-cyclopentyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adeni

ne (**10b**, 1 eq) were used and the product **13b** was obtained as yellowish oil in 83.7% yield. The purity was 96% by HPLC analysis. HRMS (TOF) *m/z* calcd for C25H39N6O5[M+H]$^+$ 503.2982, found 503.2987. LR-ESI: 503.3 (M+1), 525.3 (M+Na). ^{1}H NMR (CD_3OD, 300 MHz) 1.11 (m, 2H), 1.37 (s, 3H), 1.39 (s, 9H), 1.50 (m, 6H), 1.58 (s, 3H), 1.74 (m, 5H), 3.61 (m, 1H), 4.26 (m, 1H), 4.92 (m, 1H), 5.45 (dd, *J* = 2.7 Hz, *J* = 6.3 Hz, 1H), 6.13 (d, *J* = 2.7 Hz, 1H), 6.43 (d, *J* = 9.0 Hz, 1H, NHBoc), 8.21 (s, 1H), 8.32 (s, 1H).

6'(*S*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclobutyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1'-)adenine (13c)

Compound **13c** was prepared according to the same procedure of **11a**, in which [bis(trifluoroacetoxy)iodo]benzene (3.0 eq) and 6'(*S*)-9-(5',6',7'-deoxy-6'-amide-7'-cyclobutyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (**10c**, 1 eq) were used and the product **13c** was obtained as yellowish oil in 43.9% yield. The purity was 99% by HPLC analysis. HRMS (TOF) *m/z* calcd for C24H37N6O5[M+H]$^+$ 489.2825, found 489.2829. ^{1}H NMR (CD_3OD, 400 MHz) 1.39 (s, 9H),1.41 (s, 3H), 1.58 (s, 3H), 1.49 (m, 2H), 1.77 (m, 4H), 2.05 (m, 3H),2.30 (m, 2H), 3.56 (m, 1H), 4.25 (m, 1H), 4.86 (m, 1H), 5.44 (dd, *J* = 2.8 Hz, *J* = 6.4 Hz, 1H), 6.12 (d, *J* = 2.8 Hz, 1H), 6.39 (d, *J* = 8.8 Hz, 1H, NHBoc), 8.22 (s, 1H), 8.31 (s, 1H).

6'(*S*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclopropyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (13d)

Compound **13d** was prepared according to the same procedure of **11a**, in which [bis(trifluoroacetoxy)iodo]benzene (2.6 eq) and 6'(*S*)-9-(5',6',7'-deoxy-6'-amide-7'-cyclopropyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine (**10d**, 1 eq) were used and the product **13d** was obtained as yellowish oil in 10.1% yield. The purity was 96% by HPLC analysis. HRMS (TOF) *m/z* calcd for C23H35N6O5[M+H]$^+$ 475.2669, found 475.2670. LR-ESI: 475.3 (M+1), 497.3 (M+Na). ^{1}HNMR (CD_3OD, 300 MHz) 0.1 (m, 2H),

0.40 (m, 2H), 0.65 (m, 1H), 1.33 (s, 9H), 1.38 (s, 3H), 1.59 (s, 3H), 1.60 (m, 2H), 1.94 (m, 2H), 3.67 (m, 1H),4.27 (m, 1H), 4.60 (m, 1H), 5.34 (t, *J* = 4.2 Hz, 1H), 6.12 (d, *J* = 2.7 Hz, 1H), 8.21 (s, 1H), 8.32 (s, 1H).

6'(*S*)-N-Boc-9-(5',6',7',8'-deoxy-6'-amine-8'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-octa-furanoside-1')adenine (13e)

Compound **13e** was prepared according to the same procedure of **11a**, in which [bis(trifluoroacetoxy)iodo]benzene (2.5 eq) and 6'(*S*)-9-(5',6',7',8'-deoxy-6'-amide-8'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-octa-furanoside-1')adenine (**10e**, 1 eq) were used and the product **13e** was obtained as yellowish oil in 72.0% yield. The purity was 98% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for $C_{27}H_{43}N_6O_5[M+H]^+$ 531.3295, found 531.3297. LR-ESI: 531.1 (M+1). ^{1}H NMR (CD_3OD, 400 MHz) 0.88 (m, 2H), 1.18 (m, 7H), 1.29 (s, 9H), 1.38 (s, 3H), 1.57 (s, 3H), 1.69 (m, 6H), 1.89 (m, 2H), 3.80 (m, 1H), 4.13 (m, 1H), 5.02 (m, 1H), 5.76 (d, *J* = 5.2 Hz, 1H), 6.13 (s, 1H), 8.22 (s, 1H), 8.32 (s, 1H).

6'(*R*)-9-(5',6',7'-Deoxy-6'-amine-7'-cyclohexyl-*β*-D-hepta-furanoside-1')adenine (12a)

6'(*R*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine **11a** (24 mg, 0.05 mmol) was dissolved in the mixture of trifluoroacetic acid and water (0.5 mL, 0.5 mL) and stirred for 30 min. The solvent was removed *in vacuo* and the residue was separated on the reversed-phase silica gel column eluting with 0-30% acetonitrile/water. The product was obtained as its trifluoroacetate (16 mg, yield: 91.5%). The purity was 96% by HPLC analysis. $[\alpha]_D^{20}$ +9.3°(c 0.075 in methanol). HRMS (TOF) *m*/*z* calcd for $C_{18}H_{29}N_6O_3[M+H]^+$ 377.2301, found 377.2303. LR-ESI: 377.3 (M+1), 399.2 (M+Na). ^{1}H NMR (CD_3OD, 300 MHz) 0.94 (m, 2H), 1.22 (m, 5H), 1.67 (m, 6H), 2.07 (m, 1H), 2.20 (m, 1H), 3.52 (m, 1H), 4.21 (m, 1H), 4.34 (t, *J* = 6.0 Hz, 1H), 4.76 (t, *J* = 5.4 Hz, 1H), 5.94 (d, *J* = 4.5 Hz, 1H), 8.22 (s, 2H). ^{13}C NMR (CD_3OD, 75 MHz) 27.2 (2C), 27.5, 33.9, 34.5 (2C), 34.8, 36.5, 41.7, 74.5, 74.8, 81.8, 91.5, 121.1, 142.1, 147.9, 150.5, 154.1.

6'(*R*)-9-(5',6',7'-Deoxy-6'-amine-7'-cyclopentyl-*β*-D-hepta-furanoside-1')adenine (12b)

Compound **12b** was prepared according to the same procedure of **12a**, in which 6'(*R*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclopentyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine **11b** was used and **12b** was obtained as its trifluoroacetate in 52.0% yield. The purity was 96% by HPLC analysis. $[\alpha]^{20}_{D}$ +8.7°(c 0.05 in methanol). HRMS (TOF) *m*/*z* calcd for C17H27N6O3[M+H]$^{+}$ 363.2145, found 363.21446. LR-ESI: 363.2 (M+1), 385.2 (M+Na). ^{1}H NMR (CD_3OD, 300 MHz) 1.16 (m, 2H), 1.63 (m, 7H), 1.92 (m, 2H), 2.07 (m, 1H), 2.25 (m, 1H), 3.42 (m, 1H), 4.22 (m, 1H), 4.32 (t, *J* = 5.7 Hz, 1H), 4.76 (t, *J* = 5.4 Hz, 1H), 5.97 (d, *J* = 4.5 Hz, 1H), 8.27 (s, 1H), 8.29 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 26.0, 26.1, 33.3, 33.8, 36.3, 37.4, 40.2, 51.5, 74.4, 74.8, 81.8, 91.6, 121.1, 142.2, 149.2, 154.0, 157.6.

6'(*R*)-9-(5',6',7'-Deoxy-6'-amine-7'-cyclobutyl-*β*-D-hepta-furanoside-1')adenine (12c)

Compound **12c** was prepared according to the same procedure of **12a**, in which 6'(*R*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclobutyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine **11c** was used and **12c** was obtained as its trifluoroacetate in 87.6% yield. The purity was 95% by HPLC analysis. $[\alpha]^{20}_{D}$ +4.2°(c 0.1 in methanol). HRMS (TOF) *m*/*z* calcd for C16H25N6O3[M+H]$^{+}$ 349.1988, found 349.1986. ^{1}H NMR (CD_3OD, 400 MHz) 1.60 (m, 4H), 2.03 (m, 7H), 3.48 (m, 1H), 4.18 (m, 1H), 4.28 (t, *J* = 5.6 Hz, 1H), 4.75 (t, *J* = 4.8 Hz, 1H), 5.94 (d, *J* = 4.0 Hz, 1H), 8.22 (s, 1H), 8.23 (s, 1H). ^{13}C NMR (CD_3OD, 125 MHz) 19.5, 29.5, 29.1, 33.4, 36.3, 40.8, 45.5, 74.8 (2C), 81.7, 91.6, 121.1, 143.4, 147.2, 150.0, 155.1.

6'(*R*)-9-(5',6',7'-Deoxy-6'-amine-7'-cyclopropyl-*β*-D-hepta-furanoside-1')adenine (12d)

Compound **12d** was prepared according to the same procedure of **12a**, in which 6'(*R*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclopropyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine **11d** was used and **12d** was obtained as its trifluoroacetate in 42.9% yield. The purity was 96% by HPLC analysis. $[\alpha]^{20}_{D}$ +2.0°(c 0.1 in methanol). HRMS (TOF) *m*/*z* calcd for C15H23N6O3[M+H]$^{+}$ 335.1832, found 335.1831. ^{1}H NMR (CD_3OD, 300 MHz) 0.15 (d, *J* = 4.8 Hz,

1H), 0.55 (d, *J* = 6.3 Hz, 1H), 0.77 (m, 1H), 0.93 (m, 2H), 1.59 (m, 2H),2.19 (m, 2H),3.41 (m, 1H),4.21 (m, 1H), 4.33 (t, *J* = 6.0 Hz, 1H), 4.75 (t, *J* = 5.4 Hz, 1H), 5.95 (d, *J* = 4.5 Hz, 1H), 8.22 (s, 1H), 8.24 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 5.3 (2C), 14.3, 33.7, 36.0, 38.8, 74.7, 74.9, 81.8, 91.4, 121.9, 142.3, 148.4, 150.1, 154.1.

6'(*R*)- 9-(5',6',7',8'-Deoxy-6'-amine-8'-cyclohexyl-*β*-D-octa-furanoside-1')adenine (12e)

Compound **12e** was prepared according to the same procedure of **12a**, in which 6'(*R*)-N-Boc-9-(5',6',7',8'-deoxy-6'-amine-8'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-octa-furanoside-1')adenine **11e** was used and **12e** was obtained as its trifluoroacetate in 34.0% yield. The purity was 96% by HPLC analysis. $[\alpha]^{20}_{D}$ +3.3°(c 0.05 in methanol). HRMS (TOF) *m*/*z* calcd for C19H31N6O3$[M+H]^+$ 391.2458, found 391.2457. ^{1}H NMR (CD_3OD, 400 MHz) 0.89 (m, 2H), 1.26 (m, 5H), 1.69 (m, 8H), 2.06 (m, 1H), 2.24 (m, 1H), 3.39 (m, 1H), 4.19 (m, 1H), 4.31 (t, *J* = 5.6 Hz, 1H), 4.77 (t, *J* = 5.2 Hz, 1H), 5.94 (d, *J* = 4.4 Hz, 1H), 8.22 (s, 1H), 8.23 (s, 1H). ^{13}C NMR (CD_3OD, 150 MHz) 27.5, 27.8, 28.3, 31.4, 33.8, 34.4 (2C), 36.2, 38.9, 51.1, 74.4, 74.9, 82.0, 91.5, 121.2, 142.1, 150.5, 154.1, 157.7.

6'(*S*)-9-(5',6',7'-Deoxy-6'-amine-7'-cyclohexyl-*β*-D-hepta-furanoside-1')adenine (14a)

Compound **14a** was prepared according to the same procedure of **12a**, in which 6'(*S*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine **13a** was used and **14a** was obtained as its trifluoroacetate in 97.2% yield. The purity was 96% by HPLC analysis. $[\alpha]^{20}_{D}$ -2.3°(c 0.03 in methanol). HRMS (TOF) *m*/*z* calcd for C18H29N6O3$[M+H]^+$ 377.2301, found 377.2303. LR-ESI: 377.3 (M+1), 399.2 (M+Na). ^{1}H NMR (CD_3OD, 300 MHz) 0.97 (m, 2H), 1.29 (m, 5H), 1.70 (m, 6H), 2.01 (m, 1H), 2.17 (m, 1H), 3.46 (m, 1H), 4.15 (dt, *J* = 2.7 Hz, *J* = 6.3 Hz, 1H), 4.33 (t, *J* = 5.7 Hz, 1H), 4.67 (t, *J* = 5.4 Hz, 1H), 5.99 (d, *J* = 3.6 Hz, 1H), 8.24 (s, 2H). ^{13}C NMR (CD_3OD, 75 MHz) 27.2 (2C), 27.5, 30.9, 34.2, 34.3, 34.9, 37.6, 42.2, 74.7, 75.3, 83.1, 91.5, 120.9, 142.0, 148.2, 151.0, 154.0.

6'(*S*)-9-(5',6',7'-Deoxy-6'-amine-7'-cyclopentyl-*β*-D-hepta-furanoside-1')adenine (14b)

Compound **14b** was prepared according to the same procedure of **12a**, in which 6'(*S*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclopentyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine **13b** was used and **14b** was obtained as its trifluoroacetate in 94.9% yield. The purity was 95% by HPLC analysis. $[\alpha]^{20}_{D}$ -4.0°(c 0.1 in methanol). HRMS (TOF) *m*/*z* calcd for $C_{17}H_{27}N_6O_3[M+H]^+$ 363.2145, found 363.2144. LR-ESI: 363.2 (M+1), 385.2 (M+Na). ^{1}H NMR (CD_3OD, 300 MHz) 1.16 (m, 2H), 1.63 (m, 7H), 1.86 (m, 2H), 2.00 (m, 1H), 2.23 (m, 1H), 3.38 (m, 1H), 4.16 (m, 1H), 4.33 (t, *J* = 6.0 Hz, 1H), 4.67 (dd, *J* = 3.9 Hz, *J* = 5.4 Hz, 1H), 5.98 (d, *J* = 3.9 Hz, 1H), 8.22 (s, 1H), 8.24 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 26.0, 26.1, 33.5, 33.7, 37.4, 37.6, 40.9, 51.9, 74.7, 75.3, 83.3, 91.5, 121.0, 141.8, 150.5, 154.2, 157.6.

6'(*S*)-9-(5',6',7'-Deoxy-6'-amine-7'-cyclobutyl-*β*-D-hepta-furanoside-1')adenine (14c)

Compound **14c** was prepared according to the same procedure of **12a**, in which 6'(*S*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclobutyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1') adenine **13c** was used and **14c** was obtained as its trifluoroacetate in 82.0% yield. The purity was 95% by HPLC analysis. $[\alpha]^{20}_{D}$ -2.0°(c 0.05 in methanol). HRMS (TOF) *m*/*z* calcd for $C_{16}H_{25}N_6O_3[M+H]^+$ 349.1988, found 349.1989. ^{1}H NMR (CD_3OD, 400 MHz) 1.75 (m, 4H), 2.13 (m, 7H), 3.26 (m, 1H), 4.13 (dt, *J* = 5.2 Hz, *J* = 11.6 Hz, 1H), 4.31 (t, *J* = 5.6 Hz, 1H), 4.66 (dd, *J* = 3.6 Hz, *J* = 5.2 Hz, 1H), 5.97 (d, *J* = 3.6 Hz, 1H), 8.22 (s, 2H). ^{13}C NMR (CD_3OD, 125 MHz) 19.5, 29.3, 29.5, 33.3, 37.4, 41.2, 51.0, 75.2, 75.3, 83.3, 91.8, 122.6, 144.1, 146.8, 150.0, 153.0.

6'(*S*)-9-(5',6',7'-Deoxy-6'-amine-7'-cyclopropyl-*β*-D-hepta-furanoside-1')adenine (14d)

Compound **14d** was prepared according to the same procedure of **12a**, in which 6'(*S*)-N-Boc-9-(5',6',7'-deoxy-6'-amine-7'-cyclopropyl-2',3'-*O*-isopropylidene-*β*-D-hepta-furanoside-1')adenine **13d** was used and **14d** was obtained as its trifluoroacetate in 42.9% yield. The purity was 95% by HPLC analysis. $[\alpha]^{20}_{D}$ -2.0°(c 0.1 in methanol). HRMS (TOF) *m*/*z* calcd for $C_{15}H_{23}N_6O_3[M+H]^+$ 335.1832, found 335.1831. ^{1}H NMR (CD_3OD, 300 MHz) 0.16 (m, 2H), 0.56 (m, 2H), 0.78 (m, 1H), 1.60 (m, 2H), 2.20 (m, 2H), 3.40 (m, 1H), 4.16 (m, 1H), 4.32 (m, 1H), 4.67

(dd, *J* = 3.9 Hz, *J* = 9.0 Hz, 1H),5.98 (d, *J* = 3.9Hz, 1H), 8.25 (s, 2H). ^{13}C NMR (CD_3OD, 75 MHz) 5.3 (2C), 7.7, 33.8, 37.3, 38.6, 73.5, 75.2, 83.4, 91.6, 121.0, 144.1, 147.4, 150.1, 154.9.

6'(*S*)-9-(5',6',7',8'-Deoxy-6'-amine-8'-cyclohexyl-*β*-D-octa-furanoside-1')adenine (14e)

Compound **14e** was prepared according to the same procedure of **12a**, in which 6'(*S*)-N-Boc-9-(5',6',7',8'-deoxy-6'-amine-8'-cyclohexyl-2',3'-*O*-isopropylidene-*β*-D-octa-furanoside-1')adenine **13e** was used and **14e** was obtained as its trifluoroacetate in 93.9% yield. The purity was 95% by HPLC analysis. $[\alpha]^{20}_{D}$ -2.0°(c 0.05 in methanol). HRMS (TOF) *m*/*z* calcd for C19H31N6O3$[M+H]^+$ 391.2458, found 391.2455. ^{1}H NMR (CD_3OD, 500 MHz) 0.90 (m, 2H), 1.23 (m, 5H), 1.69 (m, 8H), 2.01 (m, 1H), 2.19 (m, 1H), 3.35 (m, 1H), 4.15 (m, 1H), 4.32 (t, *J* = 6.0 Hz, 1H), 4.69 (dd, *J* = 4.0 Hz, *J* = 5.5 Hz, 1H), 5.97 (d, *J* = 4.0 Hz, 1H), 8.22 (s, 1H), 8.23 (s, 1H). ^{13}C NMR (CD_3OD, 125 MHz) 27.5 (2C), 27.8, 31.8, 33.8, 34.3, 34.4, 37.1, 38.9, 52.7, 74.6, 75.3, 83.2, 91.4, 122.6, 141.8, 150.6, 154.2, 157.6.

(*R*)-Mosher amide

6' (*S*)-9-(5',6',7'-Deoxy-6'-amine-7'-cyclohexyl-*β*-D-hepta-furanoside-1'-)adenine (**14a**, 30 mg) was dissolved in acetone (20 mL), followed by the addition of trimethylsilyl trifluoromethanesulfonate in dichloroethane (5 uL TMSOTf in 15 uL DCE). After stirring for 1 h, conc NH_3 solution was added to neutralize. The mixture was evaporated i*n vacuo* and the residue was separated on the prep-TLC eluting with 10% methanol/dichloromethane. The product was obtained as white powder (31 mg, yield: 93.4%). ^{1}H NMR (CD_3OD, 300 MHz) 0.91 (m, 2H), 1.18 (m, 5H), 1.38 (s, 3H), 1.61 (s, 3H), 1.66 (m, 6H), 2.10 (m, 2H), 3.35 (m, 1H), 4.32 (m, 1H), 5.02 (dd, *J* = 4.2 Hz, *J* = 6.6 Hz, 1H), 5.41 (dd, *J* = 2.7 Hz, *J* = 6.6 Hz, 1H), 6.21 (d, *J* = 2.4 Hz, 1H), 8.24 (s, 1H), 8.32 (s, 1H). This intermediate (10 mg, 0.024 mmol) was then dissolved in dichloromethane (10 mL), followed by addition of triethylamine (1 mL) and (*S*)-Mosher's acid chloride (0.024 mmol). The mixture was stirred at room temperature overnight. Water was added and the mixture was extracted with dichloromethane. The organic phase was combined, washed with water and dried over anhydrous

sodium sulphate. After filtration, the solvent was removed *in vacuo* and the residue was separated on the prep-TLC eluting with 5% methanol/ dichloromethane to obtain the product as yellowish powder (5 mg, yield: 32.9%). ^{1}H NMR ($CDCl_3$, 300 MHz) 0.86 (m, 2H), 1.06 (m, 5H), 1.37 (s, 3H), 1.60 (s, 3H), 1.62 (m, 6H), 1.94 (m, 1H), 2.02 (m, 1H), 3.26 (s, 3H), 3.72 (m, 1H), 4.22 (m, 1H), 4.88 (dd, *J* = 4.8 Hz, *J* = 7.2 Hz, 1H), 5.42 (dd, *J* = 2.1 Hz, *J* = 5.7 Hz, 1H), 6.01 (d, *J* = 2.1 Hz, 1H), 6.61 (d, *J* = 9.3 Hz, 1H, NH), 7.37 (m, 3H), 7.46 (m, 2H), 7.97 (s, 1H), 8.33 (s, 1H).

(*S*)-Mosher Amide

(*S*)-Mosher amide was prepared according to the same procedure of (*R*)-Mosher amide and (*R*)-Mosher's acid chloride was used to obtain the product as yellowish powder. ^{1}H NMR ($CDCl_3$, 300 MHz) 0.88 (m, 2H), 1.25 (m, 5H), 1.34 (s, 3H), 1.58 (s, 3H), 1.63 (m, 6H), 1.88 (m, 1H), 2.00 (m, 1H), 3.26 (m, 1H), 3.30 (s, 3H), 4.15 (dd, *J* = 4.2 Hz, *J* = 6.6 Hz, 1H), 4.76 (dd, *J* = 3.9 Hz, *J* = 6.3 Hz, 1H), 5.32 (dd, *J* = 2.4 Hz, *J* = 6.6 Hz, 1H), 5.83 (d, *J* = 2.4 Hz, 1H), 6.69 (d, *J* = 8.4 Hz, 1H, NH), 7.39 (m, 3H), 7.50 (m, 2H), 7.79 (s, 1H), 8.34 (s, 1H).

2',3'-*O*-Isopropylidene-5'-deoxy-5'-(isoindoline-1'',3''-dione-2'')adenosine (16)

Phthalimide (96 mg, 0.65 mmol) and tirphenylphosphine (171 mg, 0.65 mmol) were added into the solution of 2,3-*O*-isopropylideneadenosine (0.2 g, 0.65 mmol) in anhydrous THF (20 mL) followed by addition of diisopropyl azodicarboxylate (0.129 mL, 0.65 mmol). The mixture was stirred at room temperature overnight. The reaction was detected by TLC and terminated by water. It was extracted with dichloromethane (25 mL×3). The organic phase was combined, washed with water and dried over anhydrous sodium sulphate. After filtration, the solvent was removed *in vacuo* and the residue was separated on the prep-TLC eluting with 10% methanol/dichloromethane. The product was obtained as white powder (0.27 g, yield: 95.1%). The purity was 95% by HPLC analysis. LR-ESI: 437.4 (M+1), 459.4 (M+Na). HRMS (TOF) *m/z* calcd for C21H21N6O5 $[M+H]^+$437.1573, found 437.1577. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.37 (s, 3H), 1.58 (s, 3H), 3.72 (dd, *J* = 14.1 Hz, *J* = 7.2 Hz, 1H), 4.01 (dd, *J* = 6.3 Hz, *J* = 12.0 Hz, 1H), 4.54 (m, 1H), 5.25 (dd, *J* = 3.3 Hz, *J* = 6.3 Hz, 1H), 5.53

(dd, *J* = 2.1 Hz, *J* = 6.3 Hz, 1H), 5.61 (brs, NH_2), 6.03 (d, *J* = 1.8 Hz, 1H), 7.70 (m, 2H), 7.79 (m, 2H), 7.86 (s, 1H), 8.06 (s, 1H).

2',3'-*O*-Isopropylidene-5'-deoxy-5'-amino-adenosine (17)

Compound **16** (0.284 g, 0.65 mmol) was dissolved in ethanol (20 mL), followed by addition of hydrazine hydrate (2.4 mL, 50 mmol). The resulted solution was refluxed overnight. After cooling, it was filtered and the solvent was removed *in vacuo*. The residue was dissolved in ethanol (20 mL) and some white powder appeared, which was removed by filtration. The resulted solution was evaporated *in vacuo* and the product was obtained as white powder (0.18 g, yield: 90.5%). The purity was 97% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C13H19N6O3 $[M+H]^+$307.1519, found 307.1517. ^{1}H NMR (CD_3OD, 300 MHz) 1.36 (s, 3H), 1.58 (s, 3H), 2.99 (d, *J* = 5.7 Hz, 2H), 4.27 (m, 1H), 5.03 (dd, *J* = 3.3 Hz, *J* = 6.3 Hz, 1H), 5.45 (dd, *J* = 3.0 Hz, *J* = 6.3 Hz, 1H), 6.16 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.27 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 25.7, 27.6, 44.4, 83.3, 85.0, 87.9, 91.8, 115.8, 127.1, 133.9, 142.1, 150.3, 154.1.

N-Cyclohexylmethyl-2',3'-*O*-isopropylidene-5'-deoxy-5'-amino-adenosine (18)

To the solution of compound **17** (200 mg, 0.65 mmol, 1 eq) and cyclohexanecarbaldehyde (0.54 g, 4.8 mmol, 7.4 eq) in methanol (50 mL), acetic acid (0.285 mL, 4.98 mmol, 7.6 eq) and sodium cyanoborohydride (0.33 g, 4.98 mmol, 7.6 eq) were added. The mixture was stirred at room temperature overnight. Water was added and the mixture was extracted with dichloromethane (25 mL×3). The organic phase was combined, washed with water and dried over anhydrous sodium sulphate. After filtration, the solvent was removed *in vacuo* and the residue was separated on the prep-TLC eluting with 10% methanol/dichloromethane. The product was obtained as white powder (0.128 g, yield: 49.0%). The purity was 96% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C20H31N6O3 $[M+H]^+$403.2458, found 403.2455. ^{1}H NMR (CD_3OD, 300 MHz) 0.77 (m, 2H), 1.12 (m, 4H), 1.38 (s, 3H), 1.59 (s, 3H), 1.62 (m, 5H), 2.47 (m, 2H), 3.05 (dd, *J* = 4.2 Hz, *J* = 11.1 Hz, 2H), 4.44 (dt, *J* = 3.6 Hz, *J* = 7.5 Hz, 1H), 5.09 (dd, *J* = 3.3 Hz, *J* = 6.3 Hz, 1H), 5.52 (dd, *J* = 2.1 Hz,

J = 6.0 Hz, 1H), 6.22 (d, *J* = 2.4 Hz, 1H), 8.23 (s, 1H), 8.30 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 25.7, 26.7, 26.8, 27.3, 27.5, 31.6, 31.8, 36.6, 51.3, 55.8, 84.0, 85.2, 85.3, 92.1, 115.9, 120.9, 142.3, 150.1, 154.2, 157.6.

N-Cyclohexylmethyl-N-methyl-2',3'-*O*-isopropylidene-5'-deoxy-5'-amino-adenosine (19a)

Compound **19a** was prepared according to the same procedure of **18**, in which N-cyclohexylmethyl-2',3'-*O*-isopropylidene-5'-deoxy-5'-amino-adenosine **18** and formaldehyde were used and **19a** was obtained as white powder in 91.9% yield. The purity was 95% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C21H33N6O3[M+H]$^+$ 417.2614, found 417.2619. ^{1}H NMR (CD_3OD, 300 MHz) 0.86 (m, 2H), 1.15 (m, 4H), 1.38 (s, 3H), 1.61 (s, 3H), 1.56 (m, 5H), 2.57 (d, *J* = 1.5 Hz, 2H), 2.59 (s, 3H), 3.09 (dd, *J* = 3.6 Hz, *J* = 13.5 Hz, 1H), 3.36 (dd, *J* = 9.9 Hz, *J* = 13.5 Hz, 1H), 4.56 (dt, *J* = 3.6 Hz, *J* = 9.6 Hz, 1H), 5.15 (dd, *J* = 3.6 Hz, *J* = 6.3 Hz, 1H), 5.48 (dd, *J* = 1.8 Hz, *J* = 6.3 Hz, 1H), 6.28 (d, *J* = 1.8 Hz, 1H), 8.26 (s, 1H), 8.30 (s, 1H).

N-Cyclohexylmethyl-N-ethyl-2',3'-*O*-isopropylidene-5'-deoxy-5'-amino-adenosine (19b)

Compound **19b** was prepared according to the same procedure of **18**, in which N-cyclohexylmethyl-2',3'-*O*-isopropylidene-5'-deoxy-5'-amino-adenosine **18** and acetaldehyde were used and **19b** was obtained as white powder in 50.0% yield. The purity was 96% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C22H35N6O3 [M+H]$^+$ 431.2771, found 431.2775. ^{1}H NMR (CD_3OD, 300 MHz) 0.86 (m, 3H), 1.11 (m, 2H), 1.18 (t, *J* = 7.2 Hz, 3H), 1.39 (s, 3H), 1.62 (brs, 9H), 2.78 (d, *J* = 6.6 Hz, 2H), 3.15 (q, *J* = 7.2 Hz, 2H),3.43 (dd, *J* = 3.0 Hz, *J* = 14.1 Hz, 1H),3.62 (dd, *J* = 10.2 Hz, *J* = 14.1 Hz, 1H),4.61 (m, 1H), 5.22 (dd, *J* = 3.9 Hz, *J* = 6.6 Hz, 1H), 5.47 (dd, *J* = 1.8 Hz, *J* = 6.3 Hz, 1H), 6.33 (d, *J* = 1.8 Hz, 1H), 8.29 (s, 1H), 8.33 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 9.5, 25.6, 26.5, 26.7, 27.0, 27.5, 31.6, 31.9, 34.8, 51.1, 55.9, 61.5, 84.4, 84.7, 85.5, 92.3, 116.1, 120.9, 142.6, 150.1, 154.4, 157.7.

N-Cyclohexylmethyl-N-propyl-2',3'-*O*-isopropylidene-5'-deoxy-5'-amino-adenosine (19c)

Compound **19c** was prepared according to the same procedure of **18**, in which

N-cyclohexylmethyl-2',3'-*O*-isopropylidene-5'-deoxy-5'-amino-adenosine **18** and propionaldehyde were used and **19c** was obtained as white powder in 61.7% yield. The purity was 95% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C23H37N6O3 $[M + H]^+$ 445.2927, found 445.2931. ^{1}H NMR (CD_3OD, 300 MHz) 0.79 (m, 3H), 0.84 (t, *J* = 7.5 Hz, 3H), 1.08 (m, 2H), 1.38 (s, 3H), 1.40 (m, 2H), 1.59 (s, 3H), 1.67 (m, 6H), 2.33 (d, *J* = 6.9Hz, 2H), 2.54 (m, 2H), 2.89 (d, *J* = 6.9 Hz, 2H), 4.39 (dd, *J* = 3.6 Hz, *J* = 6.6 Hz, 1H), 5.08 (dd, *J* = 3.0 Hz, *J* = 6.3 Hz, 1H), 5.55 (dd, *J* = 1.8 Hz, *J* = 6.3 Hz, 1H), 6.21 (d, *J* = 1.8 Hz, 1H), 8.23 (s, 1H), 8.27 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 11.9, 20.4, 25.6, 27.0, 27.1, 27.5, 27.7, 32.5, 32.7, 36.6, 57.7, 58.3, 63.1, 85.0, 85.1, 86.3, 92.2, 115.5, 120.9, 142.4, 150.3, 154.1, 157.6.

N-Cyclohexylmethyl-N-methyl-5'-deoxy-5'-amino-adenosine (20a)

N-Cyclohexylmethyl-N-methyl-2',3'-*O*-isopropylidene-5'-deoxy-5'-amino-adenosine **19a** (44 mg, 0.11 mmol) was dissolved in the mixture of trifluoroacetic acid and water at the ratio of 1:1 (2 mL). The reaction was stirred at room temperature for 3 h. The solvent was removed *in vacuo* and the product was obtained as its trifluoroacetate (39 mg, yield: 98.1%). The purity was 95% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C18H29N6O3 $[M+H]^+$377.2301, found 377.2298. ^{1}H NMR (CD_3OD, 300 MHz) 0.95 (m, 2H), 1.23 (m, 4H), 1.66 (m, 5H), 2.95 (s, 3H), 3.00 (m, 2H), 3.60 (m, 1H), 3.76 (m, 1H), 4.53 (m, 3H), 6.11 (d, *J* = 3.9 Hz, 1H), 8.30 (s, 1H), 8.33 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 26.3, 26.5, 26.9, 31.4, 31.7, 34.4, 42.8, 57.6, 59.2, 73.6, 74.5, 79.9, 92.1, 120.3, 142.8, 150.2, 152.7, 156.8.

N-Cyclohexylmethyl-N-ethyl-5'-deoxy-5'-amino-adenosine (20b)

Compound **20b** was prepared according to the same procedure of **20a**, in which N-cyclohexylmethyl-N-ethyl-2',3'-*O*-isopropylidene-5'-deoxy-5'-amino-adenosine **19b** was used and **20b** was obtained as its trifluoroacetate in 97.7% yield. The purity was 96% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C19H31N6O3 $[M+H]^+$ 391.2458, found 391.2459. ^{1}H NMR (CD_3OD, 300 MHz) 0.94 (m, 3H), 1.16 (m, 2H), 1.21 (t, *J* = 6.9 Hz, 3H), 1.72 (m, 6H), 2.76 (d, *J* = 5.4 Hz,

2H), 3.07 (q, *J* = 6.3 Hz, 2H), 3.32 (m, 2H), 4.33 (t, *J* = 5.1 Hz, 1H), 4.47 (t, *J* = 5.7 Hz, 1H), 4.77 (t, *J* = 4.8 Hz, 1H), 6.03 (d, *J* = 3.9 Hz, 1H), 8.23 (s, 1H), 8.26 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 9.05, 26.6 (2C), 26.9, 31.8 (2C), 34.6, 51.3, 56.3, 61.1, 73.6, 74.8, 79.8, 92.4, 121.1, 144.4, 146.8, 149.8, 153.0.

N-Cyclohexylmethyl-N-propyl-5'-deoxy-5'-amino-adenosine (20c)

Compound **20c** was prepared according to the same procedure of **20a**, in which N-cyclohexylmethyl-N-propyl-2',3'-*O*-isopropylidene-5'-deoxy-5'-amino-adenosine **19c** was used and **20c** was obtained as its trifluoroacetate in 69.4% yield. The purity was 97% by HPLC analysis. HRMS (TOF) *m*/*z* calcd for C20H33N6O3 $[M+H]^+$ 405.2614, found 405.2612. ^{1}H NMR (CD_3OD, 300 MHz) 0.92 (t, *J* = 7.2 Hz, 3H), 1.18 (m, 6H), 1.63 (m, 7H), 2.81 (m, 2H), 2.97 (m, 2H), 3.41 (m, 2H), 4.37 (m, 1H), 4.50 (t, *J* = 5.1 Hz, 1H), 4.78 (m, 1H), 6.05 (d, *J* = 4.2 Hz, 1H), 8.24 (s, 1H), 8.28 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 11.5, 19.0, 26.7, 26.8, 27.2, 32.1, 32.3, 35.4, 57.2, 58.1, 62.2, 73.9, 74.5, 81.2, 91.8, 121.1, 142.2, 150.5, 154.1, 157.6.

Biology

EHMT1/2 activity assessment

Activities of the enzymes were tested using HTRF® histone H3K9 dimethylation assay kit (Cisbio, Bedford, MA) for EHMT1 and LANCE® Ultra Europium-anti-methyl-histone H3K9 assay kit (PerkinElmer, Waltham, MA) for EHMT2. For EHMT1, the final concentrations used for screening were: 0.4 nM EHMT1, 150 nM H3 (1-21) lysine 9 unmethylated biotinylated peptide and 6 μM SAM. The detection mixture was prepared by mixing Eu-Ab diluted by 50-fold and XL-665 conjugated streptavidin (SA-XL665) diluted by 100-fold in detection buffer (5 μL for each well, in 20 μL total assay volume). Assay buffer used was 50 mM Tris-HCl, pH 8.8, 50 mM NaCl, 1 mM DTT and 0.01% Tween-20. Initial inhibition screening was carried out using 200 μM samples dissolved in DMSO (1% DMSO final concentration). Dose-response characteristics study was performed at concentrations of 500, 250, 125, 62.5, 31.25, 15.63, 7.81 and 3.91 μM for the analogues,

and 200, 66.67, 22.22, 7.41, 2.47, 0.82, 0.27 and 0.09 μM for sinefungin, respectively. EHMT1 enzyme mixture was incubated with the samples for 5 min prior to the addition of SAM and H3 (1-21) lysine 9 unmethylated biotinylated peptide. Methylation reaction was allowed to proceed for 120 min. Then the samples were incubated with the detection buffer for 60 min and fluorescence readings were taken in an HTRF mode by exciting at 320 or 340 nm and emitting at 665 nm and 620 nm wavelengths using an EnSpire® Multimode Plate Reader (PerkinElmer, Boston, MA). For EHMT2, the final concentrations used for screening were: 0.6 nM EHMT2, 0.5 μM H3 (1-21) lysine 9 unmethylated biotinylated peptide and 3 μM SAM. The detection mixture was prepared by diluting Eu-Ab to 4 nM, ULight-Streptavidin to 100 nM and poly-L-lysine to 0.0002% in 1×LANCE Detection Buffer (final concentrations: 2 nM, 50 nM and 0.0001%, respectively, in 20 μL total assay volume). Assay buffer used was 50 mM Tris-HCl, pH 9.0, 50 mM NaCl, 1mM DTT and 0.01% Tween-20. Initial inhibition screening was conducted using 400 μM samples dissolved in DMSO (1% DMSO final concentration). Dose-response characteristics study was performed at concentrations of 400, 200, 100, 50, 25, 12.5, 6.25 and 3.13 μM for the analogues, and 400, 40, 4, 0.4, 0.04, 0.004, 0.0004 and 0.00004 μM for sinefungin, respectively. EHMT2 enzyme mixture was incubated with the samples for 15 min prior to the addition of SAM and H3 (1-21) lysine 9 unmethylated biotinylated peptide. Methylation reaction was allowed to proceed for 30 min. Then the samples were incubated with the detection buffer for 60 min and fluorescence readings were taken in TR-FRET mode by exciting at 320 or 340 nm and emitting at 665 and 620 nm wavelengths using an EnVision® Multilabel Reader (PerkinElmer). Data analysis was done using Graphpad Prism® software version 5.0 (San Diego, CA).

PRMT1 activity assessment

Activities of the PRMT1 were examined using the EPI Geneous Methyltransferase Assay Kit (Cisbio). Final concentrations for screening were: 2.5 nM PRMT1, 2 μM H4 (1-25) peptide and 1 μM SAM. Assay buffer used was 50 mM Tris-HCl, pH 8.5, 10 mM NaCl, 1 mM EDTA, 1mM DTT,

0.01% Tween and 0.01% BSA. Initial inhibition screening was conducted with 400 μM analogues and 10 μM sinefungin dissolved in DMSO (1% DMSO final concentration). Dose-response characteristics experiment of sinefungin was done at the concentrations of 5.5556, 0.9259, 0.1543, 0.0257, 0.0043 and 0.0007 μM. PRMT1 enzyme mixture was incubated with sinefungin and the analogues for 5 min prior to the addition of SAM and H4 (1-25) peptide. Methylation reaction was allowed to proceed for 1 h. Then the sample mixture was incubated with Detection Buffer One for 10 min, and then with SAH-d2 and anti-SAH-Lumi4-Tb for 1 h. Fluorescence readings were taken in an HTRF mode by exciting at 320 or 340 nm and emitting at 665 nm and 620 nm wavelengths on an EnVision® Multilabel Reader (PerkinElmer). Data analysis was performed using Graphpad Prism® software version 5.0.

ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support from the National Health and Family Planning Commission of China (2012ZX09304-011, 2013ZX09401003-005, 2013ZX09507001, 2013ZX09507-002 and 2014ZX09507002-001), the National Natural Science Foundation of China (21302202), Shanghai Science and Technology Development Fund (15DZ2291600) and the Thousand Talents Program in China.

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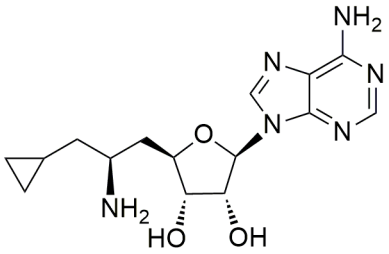
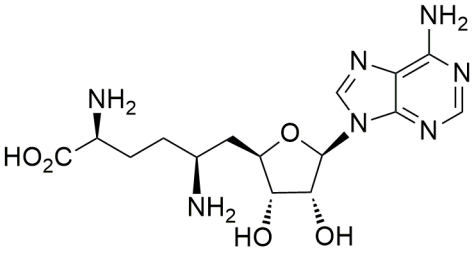


14 d

88.9%, IC_{50} = 21.8 μ M for EHMT1
77.6%, IC_{50} = 39.6 μ M for EHMT2



Sinefungin

100.0%, IC_{50} = 28.4 μ M for EHMT1
79.5%, IC_{50} = 30.1 μ M for EHMT2