Organic & Biomolecular Chemistry

Dynamic Article Links

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 5766

www.rsc.org/obc

PAPER

Microwave-assisted synthesis of dinucleoside analogues containing a thiazolidin-4-one linkage *via* one-pot tandem Staudinger/aza-Wittig/cyclization†

Fengjuan Shen,[a,b] Xiaoliu Li,*[a] Xiaoyuan Zhang,[a] Qingmei Yin,[a] Zhanbin Qin,[a] Hua Chen,[a] Jinchao Zhang[a] and Zhaipu Ma[c]

Received 29th April 2011, Accepted 16th May 2011
DOI: 10.1039/c1ob05675a

Dinucleosides containing a thiazolidin-4-one linkage were prepared by one-pot tandem Staudinger/aza-Wittig/intermolecular cyclization under microwave irradiation and their structures were confirmed. Preliminary examination of HIV-RT inhibition showed that the dinucleosides containing (*R*)-thiazolidin-4-one linkage are significantly more active than those containing (*S*)-thiazolidin-4-one linkage.

Introduction

Dinucleoside analogues have been demonstrated as possible therapeutic agents for DNA repair or mutation in functional genomics.[1] The dinucleoside tetraphosphate has been applied as an excellent chain-terminating substrate for resistant RT,[2] and the modified dinucleosides having purine and pyrimidine heterocyclic bases have been used as inhibitors of de novo RNA polymerases for treatment or prevention of viral infections.[3] However, dinucleoside analogues have very limited applications as therapeutic agents because of their high toxicity, low stability to nucleases and poor cell penetration. To overcome these problems, chemical modifications at the sugar ring, the internucleotidic linkage and the base have been under development.[4] Especially, efforts in linking the dinucleosides with the different functional groups, illustrated in Fig. 1, shows an enduring interest in this area.[5] These functional groups are likely to make the dinucleasides resistant to nucleases, be membrane permeable, and to increase the binding affinity. On the other hand, the heterocycle bridged dinucleosides and their biological activity have received less attention.[6]

The thiazolidin-4-one ring is a core substructure in various synthetic pharmaceuticals that are associated with diverse biological activities such as antibacterial, antifungal, anticancer, antiviral, anti-inflammatory, analgesic, and calcium antagonistic.[7]

Conventionally, the thiazolidin-4-one derivatives can be synthesized by multi component condensation using aldehydes, amines and mercaptoacetic acid through key imine intermediates,[8] which can be readily prepared *in situ* from azides and aldehydes by a Staudinger/aza-Wittig reaction[9] (Scheme 1). Following this synthetic strategy, we have developed a convenient pathway to access to glycoside derivatives and disaccharides containing a thiazolidin-4-one moiety from azido sugars and aromatic (or sugar) aldehydes.[10] In order to get backbone modified nucleoside analogues as therapeutic agents for HIV, we have synthesized a series of dinucleosides containing a novel thiazolidin-4-one linkage from the azido nucleoside to the nucleoside aldehyde (Scheme 2) using this methodology and checked the effect of the configurations of the thiazolidin-4-one on inhibitory activity.

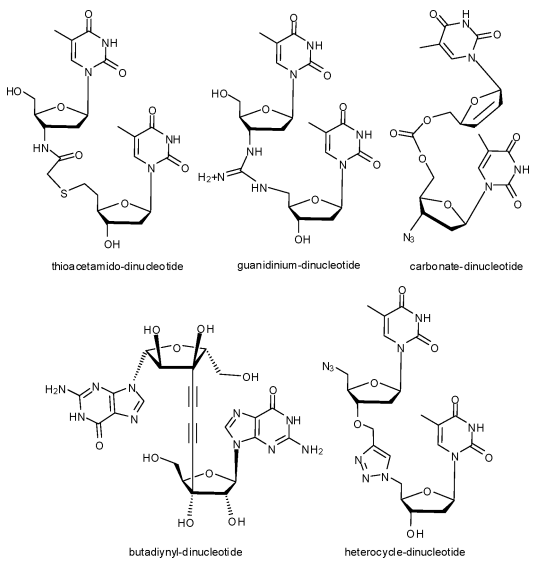
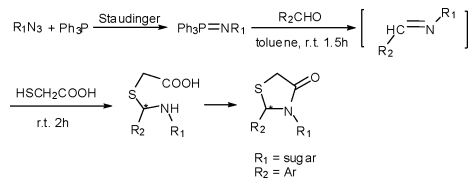


Fig. 1 Dinucleoside analogues with different linkages.

[a]*Key Laboratory of Chemical Biology of Hebei Province, College of Chemistry and Environmental Science, Hebei University, Baoding, Hebei, China. E-mail: lixl@hbu.edu.cn; Fax: 86 0312 5971116; Tel: 86 0312 5971116*
[b]*College of Science, Hebei University of Science & Technology, Shijiazhuang, Hebei, China. E-mail: shenfjliu@126.com*
[c]*College of Life Sciences, Hebei University, Baoding, Hebei, 071002, China*
† CCDC reference number 786926. For crystallographic data in CIF or other electronic format see DOI: 10.1039/c1ob05675a



Scheme 1 One-pot tandem Staudinger/aza-Wittig reactions and three–component synthesis of thiazolidin-4-one derivatives.

Results and discussion

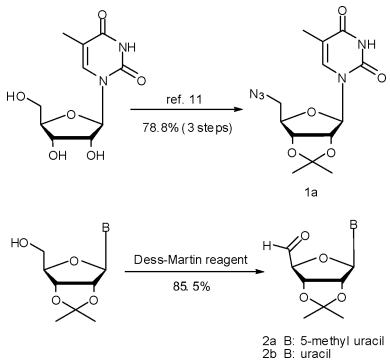
On the basis of our successful synthesis of the thiazolidin-4-one derivatives containing a sugar moiety,[10] our synthetic plan is shown in Scheme 2.

The key azido nucleosides **1** were obtained from 5-methyl uridine according to a procedure described preciously[11](Scheme 3). The 2′,3′-isopropylidene-5-methyl uridine and 2′,3′-isopropylidene-uridine were oxidized, using the Dess–Martin reagent, to the aldehydes **2a** and **2b** in high yield (Scheme 3). The initial attempt to carry out a Staudinger/aza-Wittig reaction at room temperature by stirring the mixture of azido nucleoside **1a**, triphenylphosphane (Ph_3P), and nucleoside aldehyde **2a** failed to yield the imine intermediate. After careful examination for the reaction conditions, the tandem reactions were found to proceed smoothly under microwave irradiation and afforded the thiazolidin-4-one linked dinucleoside analogues **3aa** and **4aa**. In this study, the synthesis of dinucleoside analogues was carried out by a one-pot tandem Staudinger/aza-Wittig/intermolecular cyclization under microwave irradiation. Thus, the mixture of azido nucleoside **1** and triphenylphosphane (Ph_3P) in dry THF was irradiated with microwave radiation for 5 min to generate an iminophosphorane, then the nucleoside aldehyde **2** was added and the reaction mixture was irradiated for another 10 min to form the key imine intermediate, followed by the addition of mercaptoacetic acid and microwave irradiation reaction for 10 min to afford the corresponding dinucleoside analogues containing a thiazolidin-4-one linkage **3aa–3bb** and **4aa–4bb** as shown in Scheme 2. Usually, the thiazolidin-4-one derivatives were obtained as a mixture of (*R*,*S*)-diastereomers in nearly equal concentrations affording an unresolvable mixture of both isomers. The ratios of the diastereomers were not equal for dinucleosides containing a thiazolidin-4-one linkage, and the two diastereomers were separated, as shown in Table 1. It was found that the yields

Table 1 Synthesis of novel dinucleosides linked by thiazolidin-4-one

	[a]Yield (%)				[a]Yield (%)	
	3	**4**	**3** + **4**	Ratio of **3**/**4**	**5**	**6**
aa	18.2	53.9	72.1	1 : 3.0	76.8	76.4
ab	14.4	47.9	62.3	1 : 3.3	78.6	75.3
ba	5.8	40.2	46.0	1 : 6.9	85.2	84.1
bb	7.3	36.6	43.9	1 : 5.0	74.7	74.2

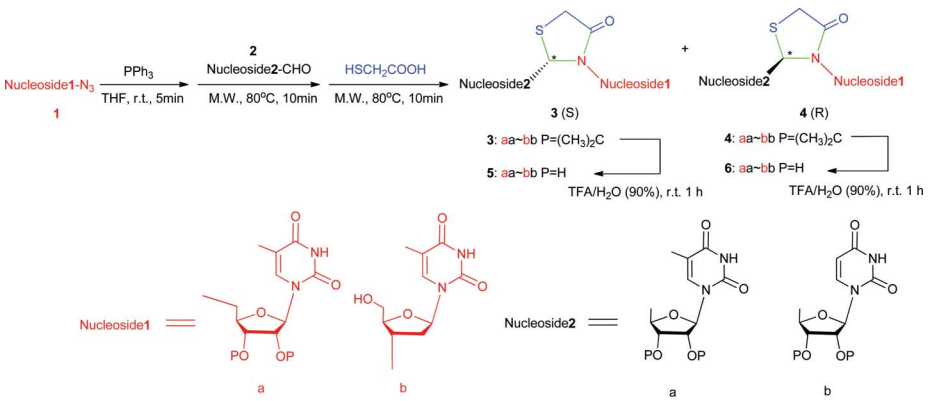
[a] Isolated yield.



Scheme 3 The synthesis of the raw materials.

of dinucleosides from **1a** were higher than those from **1b**, and the isomer **4**, which has been determined to be of *R*-configuration at the new generated chiral carbon (C-2) on the thiazolidin-4-one, was the dominant product in both diastereomers. The removal of the protecting isopropylidene of **3** and **4** was effectively carried out in TFA–H_2O (90%) at room temperature and provided the corresponding products **5** and **6** in good yields, respectively (Table 1).

The structure of **4ba** was confirmed to be of *R* configuration at C-2 by X-ray crystallography[12] (Fig. 2). Accordingly, its diastereomer **3ba** should have an *S*-configuration at C-2. Based on the crystallographic structure of **4ba** and the comparison of the chemical shifts of H-2 and C-2 of compounds **3** and **4**, the structures of the other compounds of **3** and **4** could be tentatively determined. As shown in Table 2, the chemical shifts of H-2 and C-2 of **3** appeared more downfield than those of **4**, implying that the C-2 configurations in each **3** and **4** would be the same and assigned as *S* and *R*, respectively. Consequently, the deprotected



Scheme 2 The tandem reactions for the synthesis of **5** and **6**.

Table 2 The chemical shifts of H-2 and C-2 of compounds **3**, **4**, **5**, and **6**

	δ of H-2 (ppm)				δ of C-2 (ppm)			
	3(*S*)	**4**(*R*)	**5**(*S*)	**6**(*R*)	**3**(*S*)	**4**(*R*)	**5**(*S*)	**6**(*R*)
aa	4.97	4.88	5.07	4.87	62.3	60.1	49.1	59.8
ab	4.92	4.89	5.02	4.88	62.5	59.9	45.8	46.2
ba	5.03	4.73	5.17	4.82	64.9	61.8	35.9	37.7
bb	5.02	4.74	4.92	4.81	63.5	61.9	34.1	37.8

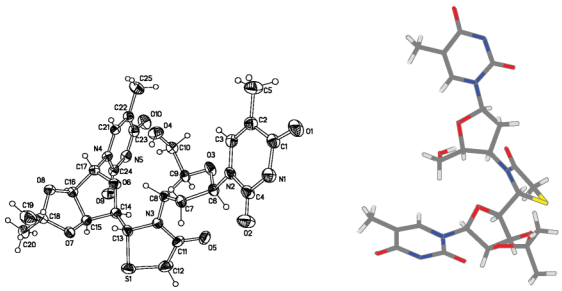


Fig. 2 Perspective view of compound **4ba**.

products **5** and **6** should keep the same *S* and *R* forms at C-2, respectively.

The biological activities of the target compounds (**5** and **6**) were preliminary evaluated for HIV-RT inhibitory activities. The HIV-RT inhibition was measured with a HIV-RT kit[13] using AZT as the comparison. As shown in Table 3, compounds **6aa**, **6ab**, and **6ba** have a good HIV-RT inhibitory activity, even better than that of the positive control AZT, and compounds **5aa**, **5ab**, and **5ba** have a moderate anti-HIV-RT activity. Comparing the inhibition of **6** with the *R* configuration at C-2 with that of the diastereoisomer **5** with an *S* configuration, it was suggested that the configuration of the C-2 effects the inhibition against HIV-RT.

To understand the difference in the anti-HIV-RT activity between the configuration of (*R*) and (*S*), molecular modeling studies using the crystal structure of HIV-1 RT (PDB code: 1RTD) were conducted by replacing TTP with **5ba** and **6ba**. The best position of each inhibitor was found with a Monte Carlo simulation and molecular mechanic optimization using a CHARMM (Chemistry at Harvard Macromolecular Mechanics) force field. According to the results of molecular docking, the azido function on AZT formed two H-bonds, one with Ala72 and another with Try115, and the hydroxyl of **6ba** formed two H-bonds with the amide backbone of Asp185 (the amino acid constituting the dNTP-binding site), whereas the function on **5ba** formed no H-bonds at all, which may partially explain the (*R*) configuration showing a better anti-HIV-RT activity than the (*S*) form (Fig. 3).

Table 3 HIV-RT kit assay for compounds (**5**) and (**6**)

Compounds (*S*)	IC_{50} (μM)	Compounds (*R*)	IC_{50} (μM)
5aa	35.7	**6aa**	10.3
5ab	26.3	**6ab**	12.9
5ba	45.3	**6ba**	6.5
5bb	>100	**6bb**	>100
AZT	21.4		

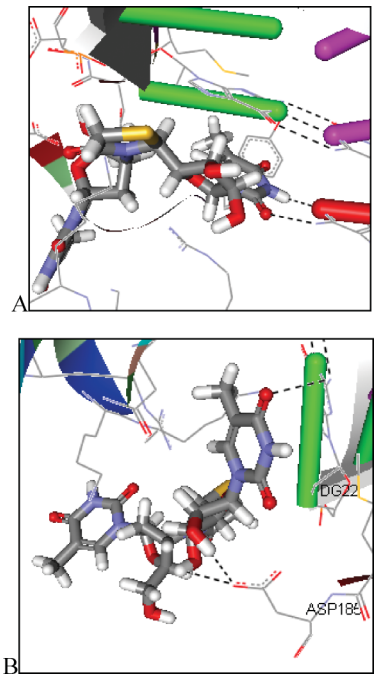


Fig. 3 In silico simulation of compounds **5ba** or **6ba** docking to the RNase H domain of HIV-1 RT. (A) Binding mode of **5ba** at the active site of HIV-RT. (B) Binding mode of **6ba** at the active site of HIV-RT. Compounds are presented as stick models with carbon in grey, oxygen in red, nitrogen in blue, sulfur in yellow. Amino acid residues forming hydrogen bonds with compounds **6ba** or **5ba** are depicted by black dashed lines. Figures were generated by DS 2.5.

Conclusion

In conclusion, novel dinucleoside analogues containing thiazolidin-4-one have been synthesized by a convenient one-pot tandem Staudinger/aza-Wittig/cyclization, starting from nucleoside azides, nucleoside aldehydes, and mercaptoacetic acid, under microwave irradiation. Preliminary examination of the HIV-RT inhibition showed that the dinucleosides containing (*R*)-thiazolidin-4-one linkages **6** are significantly more active than those containing (*S*)-thiazolidin-4-on linkages **5**, even better than that of the positive control AZT. Further synthesis and biological study of new dinucleosides is underway in this laboratory.

Experimental

Synthesis

General methods. Melting points were determined with a SGW@ X-4 micro melting point apparatus and are uncorrected. Optical rotations were determined on a SGW@-1 automatic polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker AVANCE 600 (600 MHz) NMR spectrometer, and chemical shifts are reported in parts per million relative to tetramethylsilane or a residual solvent peak (DMSO-d_6: 1H: δ 2.50, 13C: δ 39.52). Mass spectra (MS) and high resolution mass spectra (HRMS) were recorded using negative electrospray ionization (ESI-) on a FTICR-MS (Ionspec 7.0T) mass spectrometer. X-ray crystallographic measurements were made on a Bruker SMART CCD diffractometer. The optical densities for examining the

activity of HIV-RT inhibition was measured on a BioRad Model 3550 microplate spectrophotometer. Anticancer activity was evaluated using MTT assay. The microwave assisted reaction was carried out on a CEM DISCOVER S-Class Chemical Synthesis System in a 10 mL (or 35 mL) seamless pressure vial. Thin-layer chromatography (TLC) was performed on precoated plates (Qingdao GF254) with detection by UV light or with phosphomolybdic acid in EtOH–H_2O followed by heating. Column chromatography was performed using SiO_2 (Qingdao 200–300 mesh or 300-400 mesh). All other commercial reagents were used as received.

General procedure for the synthesis of compounds 3 and 4

A solution of nucleoside azide **1** (1 mmol), triphenylphosphane (Ph_3P 1.2 mmol) was irradiated (80 °C, 150 W, 5 min), then aldehyde **2** (1.2 mmol) was added and the mixture was irradiated with microwave radiation for 10 min, followed by addition of mercaptoacetic acid and irradiated at 80 °C for 10 min. The reaction mixture was cooled to room temperature and then submitted to silica gel column chromatography to afford the corresponding products **3** and **4**, respectively.

Compound 3aa. White solid, 18%; mp: 176.7–178.4 °C; $[\alpha]_D^{30}$ –12.0 (*c* 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 10.39 (s, 1H, NH (T)), 10.36 (s, 1H, NH (T)), 7.15 (d, J = 1.2 Hz, 1H, CH-6 (T)), 7.08 (d, J = 1.2 Hz, 1H, CH-6 (T)), 5.62 (d, J = 2.4 Hz, 1H, CH-1′ (nucleoside^2)), 5.38 (d, J = 1.8 Hz, 1H, CH-1′ (nucleoside^1)), 5.10 (dd, J = 1.8, 6.6 Hz, 1H, CH-2′ (nucleoside^1)), 5.02 (dd, J = 2.4, 6.6 Hz, 1H, CH-2′ (nucleoside^2)), 4.97 (dd, J = 1.2, 7.8 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.75 (q, J = 3.6, 6.6 Hz, 1H, CH-3′ (nucleoside^2)), 4.67 (dd, J = 4.8, 6.6 Hz, 1H, CH-3′ (nucleoside^1)), 4.41 (m, 1H, CH-4′ (nucleoside^1)), 4.32 (q, J = 4.2, 7.8 Hz, 1H, CH-4′ (nucleoside^2)), 3.99 (dd, J = 4.8, 14.4 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.68 (dd, J = 1.2, 15.6 Hz, 1H, CH_2-5′ (thiazolidin-4-one)), 3.51–3.47 (m, 2H, CH_2-5′ (thiazolidin-4-one), CH_2-5′ (nucleoside^1)), 1.94 (d, J = 0.6 Hz, 3H, CH_3-5), 1.92 (d, J = 0.6 Hz, 3H, CH_3-5), 1.59 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.55, 164.95, 164.76, 150.28, 150.04, 139.82, 138.62, 114.96, 114.57, 111.45, 110.89, 96.47, 95.13, 90.08, 84.29, 83.90, 83.61, 82.63, 81.76, 62.32, 47.11, 31.62, 27.13, 26.99, 25.33, 25.12, 12.34, 12.22; HR-ESI-MS: calcd for $[M - H]^-$ $C_{28}H_{36}N_5O_{11}S$, 649.2053; found 649.2043.

Compound 4aa. White solid, 54%; mp: 164.1–165.9 °C; $[\alpha]_D^{31}$ 81.4 (*c* 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 10.15 (s, 1H, NH(T)), 9.66 (s, 1H, NH(T)), 7.10 (d, J = 1.2 Hz, 1H, CH-6(T)), 7.03 (d, J = 0.6 Hz, 1H, CH-6(T)), 5.74 (d, J = 2.4 Hz, 1H, CH-1′ (nucleoside^1)), 5.33 (s, 1H, CH-1′ (nucleoside^2)), 5.23 (d, J = 6.6 Hz, 1H, CH-2′ (nucleoside^2)), 5.18 (dd, J = 3.6, 6 Hz, 1H, CH-3′ (nucleoside^2)), 5.01 (dd, J = 2.4, 6.6 Hz, 1H, CH-2′ (nucleoside^1)), 4.88 (dd, J = 1.2, 9.6 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.79 (q, J = 3.6, 6.6 Hz, 1H, CH-3′ (nucleoside^1)), 4.68 (q, J = 3.6, 9.6 Hz, 1H, CH-4′ (nucleoside^2)), 4.276 (m, 1H, CH-4′ (nucleoside^1)), 4.24 (m, 1H, CH_2-5 (thiazolidin-4-one)), 3.73 (d, J = 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.69 (dd, J = 3.6, 14.4 Hz, 1H, CH_2-5′ (thiazolidin-4-one)), 3.41 (d, J = 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 1.93 (s, 6H, two CH_3), 1.43 (s, 6H, two CH_3), 1.34 (s, 3H, CH_3), 1.33 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.59, 164.71, 164.24, 150.28, 150.53, 140.48, 137.59, 114.62, 113.14, 111.69, 111.36, 98.67, 92.34, 91.01, 83.98, 83.83, 83.12, 81.54, 60.11, 45.21, 31.50, 27.13, 26.90, 26.04, 25.34, 12.25, 12.22; HR-ESI-MS: calcd for $[M - H]^-C_{28}H_{36}N_5O_{11}S$, 649.2053; found, 649.2045.

Compound 3ab. White solid, 14%; mp: 162.5–164 °C; $[\alpha]_D^{22}$ –24.1 (*c* 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 10.55 (s, 1H, NH), 10.45 (s, 1H, NH), 7.33 (d, J = 7.8 Hz, 1H, CH-6 (U)), 7.05 (s, 1H, CH-6 (T)), 5.87 (d, J = 7.8 Hz, 1H, CH-5 (U)), 5.59 (d, J = 1.8 Hz, 1H, CH-1′ (nucleoside^2)), 5.32 (d, J = 1.8 Hz, 1H, CH-1′ (nucleoside^1)), 5.10 (dd, J = 1.8, 6.6 Hz, 1H, CH-2′ (nucleoside^1)), 5.02 (dd, J = 2.4, 6.6 Hz, 1H, CH-2′ (nucleoside^2)), 4.92 (dd, J = 1.2, 7.8 Hz, CH-2 (thiazolidin-4-one)), 4.75 (q, J = 4.2, 7.2 Hz, CH-3′ (nucleoside^2)), 4.64 (q, J = 4.8, 6 Hz, 1H, CH-3′ (nucleoside^1)), 4.41 (m, 1H, CH-4′ (nucleoside^1)), 4.29 (q, J = 3.6, 7.8 Hz, 1H, CH-4′ (nucleoside^2)), 3.97 (dd, J = 3.6, 14.4 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.68 (dd, J = 1.2, 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.47–3.43 (m, 2H, CH_2-5′ (nucleoside^1), CH_2-5 (thiazolidin-4-one)), 1.92 (s, 3H, CH_3 (T)), 1.59 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.30 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.42, 165.02, 164.55, 149.96, 149.92,143.07, 140.04, 114.94, 114.58, 110.78, 102.88, 96.72, 95.79, 90.96, 84.24, 83.90, 83.75, 82.52, 81.10, 62.51, 47.47, 31.64, 27.17, 27.00, 25.36, 25.09, 12.24; HR-ESI-MS: calcd for $[M - H]^-$ $C_{27}H_{34}N_5O_{11}S$, 635.1799; found, 635.1810.

Compound 4ab. White solid, 47.9%; mp: 201.5–202.8 °C; $[\alpha]_D^{30}$ 109.8 (*c* 0.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 10.24 (s, 1H, NH), 9.75 (s, 1H, NH), 7.25 (d, J = 8.4 Hz, 1H, CH-6 (U)), 7.11 (d, J = 0.6 Hz, 1H, CH-6 (T)), 5.78 (dd, J = 1.8, 8.4 Hz, 1H, CH-5 (U)), 5.69 (d, J = 1.8 Hz, 1H, CH-1′ (nucleoside^2)), 5.40 (s, 1H, CH-1′ (nucleoside^1)), 5.25 (d, J = 6 Hz, 1H, CH-2′ (nucleoside^2)), 5.19 (dd, J = 4.2, 6 Hz, 1H, CH-3′ (nucleoside^2)), 5.06 (dd, J = 2.4, 6.6 Hz, 1H, CH-2′ (nucleoside^1)), 4.89 (dd, J = 1.2, 9.6 Hz, CH-2 (thiazolidin-4-one)), 4.84 (q, J = 3.6, 6.6 Hz, 1H, CH-3′ (nucleoside^1)), 4.68 (q, J = 4.2, 9.6 Hz, 1H, CH-4′ (nucleoside^2)), 4.26–4.22 (m, 2H, CH-4′ (nucleoside^1), CH_2-5′ (nucleoside^1)), 3.73 (d, J = 15.6 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.68 (d, J = 10.8 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.43 (d, J = 15.6 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 1.93 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.35 (s, 6H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.51, 164.24, 164.08, 151.10, 150.47, 144.53, 138.04, 114.51, 113.27, 111.59, 102.91, 98.81, 93.12, 91.18, 84.31, 83.98, 83.03, 81.79, 59.94, 53.44, 45.37, 31.54, 27.13, 26.06, 25.32, 24.41, 12.26; HR-ESI-MS: calcd for $[M - H]^-C_{27}H_{34}N_5O_{11}S$, 635.1799; found, 635.1806.

Compound 3ba. White solid, 5.8%; mp: 173.5–174.4 °C; $[\alpha]_D^{15}$ –19.8 (*c* 1, DMSO); ^{1}H NMR (600 MHz, CD_3OD): δ 7.67 (d, J = 0.6 Hz, 1H, CH-6 (T)), 7.53 (d, J = 1.2 Hz, 1H, CH-6(T)), 6.33 (q, J = 5.4, 7.2 Hz, 1H, CH-1′ (nucleoside^1)), 5.77 (d, J = 1.8 Hz, 1H, CH-1′ (nucleoside^2)), 5.16 (dd, J = 1.8, 6 Hz, 1H, CH-2′ (nucleoside^2)), 5.03 (dd, J = 1.2, 9 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.85 (q, J = 3.6, 6.6 Hz, 1H, CH-3′ (nucleoside^2)), 4.37 (m, 2H, CH-3′ (nucleoside^1), CH-4′ (nucleoside^2)), 4.30 (m, 1H, CH-4′ (nucleoside^1)), 3.83 (dd, J = 1.2, 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.79 (dd, J = 3, 12 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.64 (dd, J = 3, 12 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.46 (d, J = 16.2 Hz, 1H, CH_2-5′ (nucleoside^1)), 2.89 (m, 1H, CH_2-2′ (nucleoside^1)), 2.17 (m, 1H, CH_2-2′ (nucleoside^1)), 1.90 (d,

J = 1.2 Hz, 3H, CH_3 (T)), 1.86 (d, J = 1.2 Hz, 3H, CH_3 (T)), 1.56 (s, 3H, CH_3), 1.37 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 174.05, 166.46, 166.37, 152.26, 152.10, 141.03, 138.42, 115.45, 112.11, 111.36, 97.27, 92.92, 87.28, 85.26, 84.07, 82.40, 64.92, 62.56, 58.32, 36.70, 32.72, 27.24, 25.24, 12.49, 12.27; HR-ESI-MS: calcd for $[M - H]^-$ $C_{25}H_{32}N_5O_{10}S$, 593.1791; found, 593.1785.

Compound 4ba. White solid, 40.2%; mp: 241.1–241.9 °C; $[\alpha]_D^{21}$ 76.7 (c 1, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.41 (s, 1H, NH (T)), 11.25 (s, 1H, NH (T)), 7.75 (d, J = 1.2 Hz, 1H, CH-6 (T)), 7.55 (d, J = 1.2 Hz, 1H, CH-6 (T)), 6.39 (t, J = 4.8, 14.4 Hz, 1H, CH-1′ (nucleoside^1)), 5.69 (s, 1H, CH-1′ (nucleoside^2), 5.22 (d, J = 6 Hz, 1H, CH-2′ (nucleoside^2)), 5.05 (dd, J = 4.2, 6 Hz, 1H, CH-3′ (nucleoside^2)), 4.93 (s, 1H, OH), 4.73 (dd, J = 1.2, 9 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.60 (q, J = 3.6, 9 Hz, 1H, CH-4′ (nucleoside^2)), 4.38 (m, 1H, CH-3′ (nucleoside^1)), 4.27 (m, 1H, CH-4′ (nucleoside^1)), 3.79 (dd, J = 1.8, 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.53–3.46 (m, 2H, CH_2-5 (thiazolidin-4-one)), 3.41 (d, J = 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 2.32 (m, 1H, CH_2-2′ (nucleoside^1)), 2.20 (m, 1H, CH_2-2′ (nucleoside^1)), 1.77 (d, J = 0.6 Hz, 3H, CH_3 (T)), 1.77 (d, J = 1.2 Hz, 3H, CH_3 (T)), 1.47 (s, 3H, CH_3), 1.30 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.41, 164.61, 164.20, 151.39, 150.85, 140.67, 136.93, 112.63, 109.87, 109.68, 95.65, 89.16, 85.13, 84.06, 82.76, 82.48, 61.84, 61.46, 56.79, 37.96, 31.67, 26.50, 24.76, 12.68, 12.40; HR-ESI-MS: calcd for $[M - H]^-$ $C_{25}H_{32}N_5O_{10}S$, 593.1791; found, 593.1794.

Compound 3bb. White solid, 7.3%; mp: 168.4–169.6 °C; $[\alpha]_D^{27}$ –7.9 (c 1, DMSO); ^{1}H NMR (600 MHz, CD_3OD): δ 7.70 (dd, J = 1.2, 7.8 Hz, 1H, CH-6 (U)), 7.66 (d, J = 1.2 Hz, 1H, CH-6 (T)), 6.31 (t, J = 7.2, 12.6 Hz, 1H, CH-1′ (nucleoside^1)), 5.79 (s, 1H, CH-1′ (nucleoside^2)), 5.73 (dd, J = 1.2, 7.8 Hz, 1H, CH-5 (U)), 5.18 (dd, J = 1.8, 6.6 Hz, 1H, CH-2′ (nucleoside^1)), 5.02 (dd, J = 1.2, 8.4 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.85 (q, J = 3.6, 6.6 Hz, 1H, CH-3′ (nucleoside^2)), 4.38 (m, 2H, CH-3′ (nucleoside^1), CH-4′ (nucleoside^2)), 4.29 (m, 1H, CH-4′ (nucleoside^1)), 3.83 (d, J = 16.2 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.80 (dd, J = 3, 12.6 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.65 (dd, J = 3, 12 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.45 (d, J = 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 2.92 (m, 1H, CH_2-2′ (nucleoside^1)), 2.20 (m, 1H, CH_2-2′ (nucleoside^1)), 1.87 (s, 3H, CH_3(T)), 1.56 (s, 3H, CH_3), 1.37 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 172.61, 165.01, 164.68, 150.75, 150.67, 143.99, 137.10, 114.07, 109.98, 101.94, 96.10, 91.77, 85.96, 83.88, 82.69, 80.82, 63.49, 61.09, 56.89, 35.07, 31.35, 25.85, 23.87, 11.13; HR-ESI-MS: calcd for $[M - H]^-$ $C_{24}H_{30}N_5O_{10}S$, 579.1634; found, 579.1645.

Compound 4bb. White solid, 36.6%; mp: 202.5–203.8 °C; $[\alpha]_D^{28}$ 53.5 (c 1, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.44 (d, J = 6 Hz, 1H, NH (U)), 11.44 (s, 1H, NH (T)), 7.76 (d, J = 0.6 Hz, 1H, CH-6 (T)), 7.71 (d, J = 8.4 Hz, 1H, CH-6 (U)), 6.41 (t, J = 7.2, 14.4 Hz, 1H, CH-1′ (nucleoside^1)), 5.74 (s, 1H, CH-1′ (nucleoside^2)), 5.61 (dd, J = 2.4, 8.4 Hz, 1H, CH-5 (U)), 5.22 (d, J = 6 Hz, 1H, CH-2′ (nucleoside^2)), 5.04 (dd, J = 4.2, 11.4 Hz, 1H, CH-3′ (nucleoside^2)), 4.96 (s, 1H, OH), 4.74 (dd, J = 1.2, 9 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.62 (q, J = 4.2, 9.6 Hz, 1H, CH-4′ (nucleoside^2)), 4.39 (m, 1H, CH-3′ (nucleoside^1)), 4.29 (m, 1H, CH-4′ (nucleoside^1)), 3.80 (dd, J = 1.8, 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.55 (q, J = 11.4, 39 Hz, 2H, CH_2-5 (thiazolidin-4-one)), 3.41 (d, J = 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 2.33 (m, 1H, CH_2-2′ (nucleoside^1)), 2.19 (m, 1H, CH_2-2′ (nucleoside^1)), 1.77 (s, 3H, CH_3 (T)), 1.47 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.42, 164.21, 164.00, 151.41, 150.86, 144.95, 136.93, 112.65, 109.86, 102.00, 95.88, 89.35, 85.21, 84.20, 82.69, 82.64, 61.86, 61.54, 56.89, 38.12, 31.70, 26.47, 24.72, 12.71; HR-ESI-MS: calcd for $[M - H]^-$ $C_{24}H_{30}N_5O_{10}S$, 579.1634; found, 579.1648.

General procedure for deprotection of compounds 3 and 4

The deprotection of **3** and **4** was carried out effectively in TFA–H_2O (90%) at room temperature for 1 h and provided the corresponding products **5** and **6** in good yields.

Compound 5aa. White solid, 77%; mp: 270 °C (decomposition); $[\alpha]_D^{21}$ –11.5 (c 1, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.40 (s, 1H, NH (T)), 11.35 (s, 1H, NH (T)), 7.58 (s, 1H, CH-6 (T)), 7.49 (s, 1H, CH-6 (T)), 5.83 (d, J = 7.2 Hz, 1H, CH-1′ (Nucleoside^2)), 5.82 (d, J = 6.6 Hz, 1H, CH-1′ (Nucleoside^1)), 5.49 (d, J = 6 Hz, 1H, OH), 5.38 (d, J = 5.4 Hz, 1H, OH), 5.31 (d, J = 4.8 Hz, 1H, OH), 5.23 (d, J = 4.8 Hz, 1H, OH), 5.07 (dd, J = 1.2, 6 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.25 (m, 3H, CH-2′ (nucleoside^2), CH-2′ (nucleoside^1), CH-4′ (nucleoside^2)), 4.03 (m, 1H, CH-4′ (nucleoside^1)), 3.96 (m, 2H, CH-3′ (nucleoside^2), CH-5 (thiazolidin-4-one)), 3.87 (m, 1H, CH-3′ (nucleoside^1)), 3.74 (d, J = 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.50 (d, J = 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.40 (dd, J = 7.2, 14.4 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 1.77 (s, 3H, CH_3), 1.75 (s, 3H, CH_3); ^{1}H NMR (600 MHz, DMSO-d_6, D_2O): δ 7.53 (s, 1H, CH-6(T)), 7.46 (s, 1H, CH-6(T)), 5.80 (d, J = 6.6 Hz, 1H, CH-1′ (nucleoside^2)), 5.70 (d, J = 6.6 Hz, 1H, CH-1′ (nucleoside^1)), 5.03 (d, J = 6 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.23 (m, 3H, CH-2′ (nucleoside^2), CH-2′ (nucleoside^1), CH-4′ (nucleoside^2)), 4.01 (m, 1H, CH-4′ (nucleoside^1)), 3.97 (q, J = 3.6, 5.4 Hz, 1H, CH-3′ (nucleoside^2)), 3.93 (dd, J = 4.8, 14.4 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.86 (t, J = 4.2 Hz, 1H, CH-3′ (nucleoside^1)), 3.70 (covered by H_2O, s, 1H, CH_2-5′ (nucleoside^1)), 3.48 (d, J = 9.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.40 (q, J = 7.8, 14.4 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 1.76 (s, 3H, CH_3), 1.75 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.48, 164.16, 164.02, 151.29, 137.26, 136.48, 110.60, 110.34, 88.45, 87.79, 85.56, 82.03, 72.17, 71.85, 71.73, 70.25, 61.78, 49.07, 46.33, 31.38, 12.49, 12.33; HR-ESI-MS: calcd for $[M - H]^-$ $C_{28}H_{36}N_5O_{11}S$, 569.1427; found, 569.1435.

Compound 6aa. White solid, 76%; mp: >300 °C; $[\alpha]_D^{20}$ 55.5 (c 1, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.32 (s, 2H, NH (T)), 7.62 (d, J = 0.6 Hz, 1H, CH-6 (T)), 7.42 (d, J = 0.6 Hz, 1H, CH-6 (T)), 5.95 (d, J = 7.8 Hz, 1H, CH-1′ (nucleoside^2)), 5.76 (d, J = 5.4 Hz, 1H, CH-1′ (nucleoside^1)), 4.89 (dd, J = 0.6, 9 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.60 (dd, J = 3, 9.6 Hz, 1H, CH-4′ (nucleoside^2)), 4.33 (q, J = 4.2, 7.8 Hz, 1H, CH-2′ (nucleoside^2)), 4.16 (t, J = 3.6, 7.2 Hz, 1H, CH-3′ (nucleoside^2)), 4.08 (m, 1H, CH-4′ (nucleoside^1)), 3.99 (t, J = 5.4, 10.8 Hz, 1H, CH-2′ (nucleoside^1)), 3.99 (t, J = 8.4, 14.4 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.90 (q, J = 4.8, 9 Hz, 1H, CH-3′ (nucleoside1)), 3.68 (d, J = 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.51 (dd, J = 3.6, 14.4 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.46 (d, J = 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 1.81 (s, 6H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 12.42, 12.50, 31.42, 46.27, 59.82, 71.45, 72.57, 73.20, 73.95, 80.48, 86.70, 88.04, 89.14, 110.23, 110.41, 136.42, 137.39, 151.19,

151.31, 164.17, 164.20, 172.16; HR-ESI-MS: calcd for $[M - H]^-$ $C_{28}H_{36}N_5O_{11}S$, 569.1427; found, 569.1433.

Compound 5ab. White solid, 79%; mp: 214–215 °C; $[\alpha]_D^{21}$ –58.8 (*c* 1, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.38 (s, 2H, NH (T)), 7.62 (d, J = 8.4 Hz, 1H, CH-6 (U)), 7.59 (s, 1H, CH-6 (T)), 5.81 (d, J = 6.6 Hz, 1H, CH-1′ (nucleoside^2)), 5.72 (d, J = 6 Hz, 1H, CH-1′ (nucleoside^1)), 5.70 (d, J = 8.4 Hz, 1H, CH-4 (U)), 5.56 (d, J = 6 Hz, 1H, OH), 5.41 (d, J = 6 Hz, 1H, OH), 5.33 (d, J = 5.4 Hz, 1H, OH), 5.28 (d, J = 4.8 Hz, 1H, OH), 5.02 (dd, J = 1.2, 5.4 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.30 (q, J = 3.6, 4.8 Hz, 1H, CH-2′ (nucleoside^2)), 4.25 (q, J = 6, 12 Hz, 1H, CH-2′ (nucleoside^1)), 4.19 (q, J = 6, 12 Hz, 1H, CH-3′ (nucleoside^1)), 4.15 (q, J = 5.4, 10.2 Hz, 1H, CH-4′ (nucleoside^2)), 3.99 (m, 1H, CH-4′ (nucleoside^1)), 3.94 (dd, J = 4.2, 14.4 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.87 (q, J = 4.8, 9 Hz, 1H, CH-3′ (nucleoside^2)), 3.71 (d, J = 15.6 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.51 (d, J = 15.6 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.41 (d, J = 9 Hz, 1H, CH_2-5′ (nucleoside^1)), 1.78 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.84, 163.69, 162.85, 150.76, 150.72, 140.57, 136.89, 109.87, 102.49, 88.46, 87.48, 84.38, 81.78, 72.02, 71.43, 71.13, 69.28, 61.57, 45.84, 30.83, 11.84; HR-ESI-MS: calcd for $[M - H]^-$ $C_{21}H_{26}N_5O_{11}S$, 555.1271; found, 555.1263.

Compound 6ab. White solid, 75%; mp: 215 °C; $[\alpha]_D^{20}$ 44.2 (*c* 1, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.31 (s, 2H, NH (T), NH (U)), 7.78 (d, J = 7.8 Hz, 1H, CH-6 (U)), 7.42 (d, J = 1.2 Hz, 1H, CH-6 (T)), 5.93 (d, J = 7.8 Hz, 1H, CH-1′ (nucleoside^2)), 5.76 (d, J = 5.4 Hz, 1H, CH-1′ (nucleoside^1)), 5.68 (d, J = 7.8 Hz, 1H, CH-5 (U)), 5.59 (d, J = 6 Hz, 1H, OH), 5.56 (d, J = 4.8 Hz, 1H, OH), 5.47 (d, J = 6 Hz, 1H, OH), 5.22 (d, J = 5.4 Hz, 1H, OH), 4.88 (dd, J = 1.2, 9.6 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.60 (dd, J = 3, 9.6 Hz, 1H, CH-4′ (nucleoside^2)), 4.33 (q, J = 7.8, 10.2 Hz, 1H, CH-2′ (nucleoside^2)), 4.17–4.12 (m, 1H, CH-3′ (nucleoside^2)), 4.08–4.05 (m, 1H, CH-4′ (nucleoside^1)), 3.99 (q, J = 6, 11.4 Hz, 1H, CH-2′ (nucleoside^1)), 3.94–3.87 (m, 2H, CH-3′ (nucleoside^1), CH_2-5′ (nucleoside^1)), 3.68 (d, J = 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.50 (dd, J = 3.6, 14.4 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.45 (d, J = 16.2 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 1.81 (d, 1.2 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.15, 164.18, 163.54, 151.23, 151.19, 142.15, 136.44, 110.24, 102.65, 89.66, 88.07, 86.80, 80.48, 74.12, 73.19, 72.59, 71.46, 59.79, 46.23, 31.39, 12.41; HR-ESI-MS: calcd for $[M - H]^-$ $C_{21}H_{26}N_5O_{11}S$, 555.1271; found, 555.1266.

Compound 5ba. White solid, 85%; mp: 206.5–207 °C; $[\alpha]_D^{21}$ –46.5 (*c* 1, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.35 (s, 2H, NH (T)), 7.80 (s, 1H, CH-6 (T)), 7.45 (s, 1H, CH-6 (T)), 6.32 (t, J = 0.6, 13.8 Hz, 1H, CH-1′ (nucleoside^1)), 5.86 (d, J = 7.2 Hz, 1H, CH-1′ (nucleoside^2)), 5.17 (d, J = 7.2 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.32 (t, J = 6, 13.2 Hz, 1H, CH-2′ (nucleoside^2)), 4.28 (m, 1H, CH-3′ (nucleoside^1)), 4.16 (m, 1H, CH-4′ (nucleoside^1)), 4.12 (dd, J = 3, 7.2 Hz, 1H, CH-4′ (nucleoside^2)), 3.97 (q, J = 3, 6 Hz, 1H, CH_2-3′ (nucleoside^2)), 3.77 (dd, J = 1.8, 16.2 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.58–3.58 (d, 2H, CH_2-5′ (nucleoside^1)), 3.46 (d, J = 15.6 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 2.60 (m, 1H, CH_2-2′ (nucleoside^1)), 1.95 (m, 1H, CH_2-2′ (nucleoside^1), 1.84 (d, J = 1.2 Hz, 3H, CH_3 (T)), 1.74 (d, J = 1.2 Hz, 3H, CH_3(T)); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.55, 164.15 (two), 151.47, 150.79, 136.62, 136.20, 110.55, 110.15, 87.42, 86.30, 84.21, 81.25, 72.04, 70.56, 63.46, 61.49, 57.07, 35.87, 32.11, 12.67, 12.40; HR-ESI-MS: calcd for $[M - H]^-$ $C_{22}H_{28}N_5O_{10}S$, 553.1478; found, 553.1480.

Compound 6ba. White solid, 84%; mp: 206.9–207.7 °C; $[\alpha]_D^{22}$ 6.15 (*c* 1, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.28 (s, 1H, NH (T)), 11.22 (s, 1H, NH (T)), 7.73 (d, J = 1.2 Hz, 1H, CH-6 (T)), 7.64 (d, J = 1.2 Hz, 1H, CH-6 (T)), 6.36 (t, J = 7.2, 14.4 Hz, 1H, CH-1′ (nucleoside^1)), 5.97 (d, J = 7.8 Hz, 1H, CH-1′ (nucleoside^2)), 5.53 (s, 2H, OH), 4.82 (dd, J = 1.8, 9 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.59 (dd, J = 3, 9 Hz, 1H, CH-4′ (nucleoside^2)), 4.34–4.29 (m, 3H, CH-3′ (nucleoside^1), CH-2′ (nucleoside^2),CH-3′ (nucleoside^2)), 4.16 (m, 1H, CH-4′ (nucleoside^1)), 3.68 (dd, J = 1.8, 15.6 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 3.54–3.42 (m, 2H, CH_2-5′ (nucleoside^1)), 3.44 (d, J = 15.6 Hz, 1H, CH_2-5 (thiazolidin-4-one)), 2.32 (m, 1H, CH_2-2′ (nucleoside^1)), 2.18 (m, 1H, CH_2-2′ (nucleoside^1)), 1.80 (d, J = 0.6 Hz, 3H, CH_3(T)), 1.75 (d, J = 0.6 Hz, 3H, CH_3 (T)); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.22, 164.29, 164.20, 151.36, 150.82, 137.26, 136.96, 110.37, 109.78, 88.90, 86.02, 85.05, 82.32, 74.09, 72.69, 62.02, 61.94, 56.81, 37.66, 31.67, 12.61, 12.46; HR-ESI-MS: calcd for $[M - H]^-$ $C_{22}H_{28}N_5O_{10}S$, 553.1478; found, 553.1483.

Compound 5bb. White solid, 75%; mp: 194.7–196.2 °C; $[\alpha]_D^{28}$ –94.0 (*c* 1, DMSO); ^{1}H NMR (600 MHz, D_2O): δ 7.67 (d, J = 7.8 Hz, 1H, CH-6 (U)), 7.52 (s, 1H, CH-6 (T)), 6.20 (t, J = 5.4, 12.6 Hz, 1H, CH-1′), 5.81 (d, J = 7.8 Hz, 1H, CH-5 (U)), 5.77 (d, J = 6.6 Hz, 1H, CH-1′ (nucleoside^2)), 4.92 (dd, J = 1.8, 7.2 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.42–4.37 (m, 2H, CH-4′ (nucleoside^1), CH-2′ (nucleoside^2)), 4.23–4.18 (m, 3H, CH-3′ (nucleoside^1), CH-4′ (Nucleoside^2), CH-3′ (Nucleoside^2)), 3.78–3.74 (m, 2H, CH_2-5′ (nucleoside^1), CH_2-5 (thiazolidin-4-one)), 3.68 (dd, J = 4.2, 12.6 Hz, CH_2-5 (thiazolidin-4-one)), 3.49 (d, J = 16.2 Hz, 1H, CH_2-5′ (nucleoside^1)), 2.86 (m, 1H, CH_2-2′ (nucleoside^1)), 2.25 (m, 1H, CH_2-2′ (nucleoside^1)), 1.75 (s, 3H, CH_3); ^{13}C NMR (125 MHz, D_2O): δ 174.10, 166.31, 165.80, 151.60, 151.38, 142.31, 137.71, 111.13, 102.73, 89.66, 86.09, 85.88, 80.35, 72.19, 70.03, 64.06, 60.97, 56.53, 34.09, 32.14, 11.55; HR-ESI-MS: calcd for $[M - H]^-$ $C_{21}H_{26}N_5O_{10}S$, 539.1321; found, 539.1333.

Compound 6bb. White solid, 74%; mp: 228.4–229.7 °C; $[\alpha]_D^{27}$ 20.6 (c 1, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.33 (d, J = 1.2 Hz, 1H, NH (U)), 11.24 (s, 1H, NH (T)), 7.82 (d, J = 8.4 Hz, 1H, CH-6 (U)), 7.75 (s, 1H, CH-6 (T)), 6.36 (t, J = 5.4, 12.6 Hz, 1H, CH-1′ (nucleoside^1)), 5.97 (d, J = 7.8 Hz, 1H, CH-1′ (nucleoside^2)), 5.67 (d, J = 8.4 Hz, 1H, CH-5 (U)), 4.81 (d, J = 9 Hz, 1H, CH-2 (thiazolidin-4-one)), 4.60 (dd, J = 2.4, 9 Hz, 1H, CH-4′ (nucleoside^2)), 4.35 (m, 2H, CH-3′ (nucleoside^1), CH-2′ (nucleoside^2)), 4.29 (m, 1H, CH-4′ (nucleoside^1)), 4.17 (m, 1H, CH-3′ (nucleoside^2)), 3.68 (dd, J = 1.2, 15.6 Hz, 1H, CH_2-5′ (nucleoside^1)), 3.54 (m, 3H, CH_2-5′ (nucleoside^1), CH_2-5 (thiazolidin-4-one)), 2.30 (m, 2H, CH_2-2′ (nucleoside^1)), 1.75 (s, 3H, CH_3 (T)); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.26, 164.22, 163.64, 151.29, 150.83, 141.99, 136.98, 109.80, 102.65, 89.25, 86.03, 85.04, 82.36, 74.29, 72.62, 61.93, 56.86, 37.79, 31.68, 12.62; HR-ESI-MS: calcd for $[M - H]^-$ $C_{21}H_{26}N_5O_{10}S$, 539.1321; found, 539.1336.

X-ray crystallographic measurement of single crystal of 4ba

The single crystal of the compound **4ba** was obtained by recrystallization from the solution of H_2O–MeOH–THF and applied

on a Bruker Axssmart apexII Mo diffractometer for analysis. The intensity data were collected on a Bruker SMART CCD diffractometer with graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) using the $\theta/2\omega$ scan technique from a single-crystal of 0.68 mm × 0.16 mm × 0.15 mm, and a semi-empirical absorption correction was applied for all complexes. The crystal system was orthorhombic, and the space group was P212121. The structures were solved by direct methods and refined by full-matrix least-squares on F2. The absolute structure parameter was –0.13 (13). All non-hydrogen atoms were refined anisotropically. The crystallographic structure is shown in Fig. 1.

Biological activity assay

HIV-RT kit assay. The HIV-RT inhibition assay was performed by using an RT assay kit (Roche), and the procedure for assaying RT inhibition was performed as described in the kit protocol. Briefly, the reaction mixture consists of a template–primer complex, 2′-deoxy-nucleotide-5′-triphosphates (dNTPs) and reverse transcriptase (RT) enzyme in the lysis buffer with or without inhibitors. After 1 h incubation at 37 °C the reaction mixture was transferred to a streptavidin-coated microtitre plate (MTP). The biotin labeled dNTPs that are incorporated in the template due to the activity of RT were bound to streptavidin. The unbound dNTPs were washed using wash buffer and antidigoxigenin-peroxidase (DIG-POD) was added in MTP. The DIG-labeled dNTPs incorporated in the template was bound to anti-DIG-POD antibody. The unbound anti-DIG-POD was washed and the peroxide substrate (ABST) was added to the MTP. A colored reaction product was produced during the cleavage of the substrate catalyses by a peroxide enzyme. The absorbance of the sample was determined at OD 405 nm using a microtiter plate ELISA reader. The resulting color intensity is directly proportional to the actual RT activity. The percentage inhibitory activity of RT inhibitors was calculated by comparing to a sample that does not contain an inhibitor. The percentage inhibition was calculated by formula as given below:

$$\% \text{ Inhibition} = 100 - [(\text{OD 405 nm with inhibitor}/\text{OD 405 nm without inhibitor}) \times 100]$$

.

Dock modeling

An X-ray structure of HIV-1 RT was downloaded from the Protein Data Bank (PDB code 1RTD). This structure contains a DNA template, primer, dTTP containing two Mg^{2+} ions, and two Mg^{2+} ions in the RNase H active site in addition to the p66 and p51 domains. Molecular docking of a ligand into the dNTP-binding site of HIV-1 RT was carried out using discovery studio 2.5 (software from Accelrys, USA) and libdock was used.

Acknowledgements

The financial support from the National Natural Science Foundations of China (NSFC) (20672027 and 20972039), the National Basic Research 973 Pre-research Program of China (2010CB534913), and the program of Science and Technology (S & T) of Hebei (09276418D-13) is gratefully acknowledged.

Notes and references

1 (*a*) L. Q. Chen, R. Petrelli, M. Olesiak, D. J. Wilson, N. P. Labello and K. W. Pankiewicz, *Bioorg. Med. Chem.*, 2008, **16**, 7462; (*b*) R. M. C. Cattaneo-Pangrazzi, H. Schott and R. A. Schwendener, *Prostate*, 2000, **45**, 8; (*c*) J. Hanuš, I. Barvík, K. Ruszová-Chmelová, J. ŠtÆpánek, P. Y. Turpin, J. Bok, I. Rosenberg and M. Petrová-Endová, *Nucleic Acids Res.*, 2001, **29**, 5182; (*d*) A. Grajkowski, J. Pedras-Vasconcelos, V. Wang, C. Ausín, S. Hess, D. Verthelyi and S. L. Beaucage, *Nucleic Acids Res.*, 2005, **33**, 3550.

2 (*a*) Q. W. Han, S. G. Sarafianos, E. Arnold, M. A. Parniak, B. L. Gaffney and R. A. Jones, *Org. Lett.*, 2007, **9**, 5243; (*b*) S. Dharmasena, Z. Pongracz, E. Arnold, S. G. Sarafianos and M. A. Parniak, *Biochemistry*, 2007, **46**, 828.

3 Z. Hong, A. Viejo, W. D. Zhong, L. Niguel, H. Y. An Carlsbad, D. Barawkar, F. Ranch, US patent 2006/0074035 A1. http://ip.com/patapp/US20060074035.

4 (*a*) E. Uhlmann and A. Peyman, *Chem. Rev.*, 1990, **90**, 543; (*b*) P. D. Cook, *Anti-Cancer Drug Des.*, 1991, **6**, 585; (*c*) J. F. Milligan, M. D. Matteucci and J. C. Martin, *J. Med. Chem.*, 1993, **36**, 1923; (*d*) A. D. Mesmaeker, R. Haner, P. Martin and H. E. Moser, *Acc. Chem. Res.*, 1995, **28**, 366.

5 (*a*) E. T. Kool, *Chem. Rev.*, 1997, **97**, 1473; (*b*) Micklefield, *Curr. Med. Chem.*, 2001, **8**, 1157; (*c*) S. M. Freier and K. H. Altmann, *Nucleic Acids Res.*, 1997, **25**, 4429 and references therein; (*d*) F. Jung, A. Burger and J. F. Biellmann, *Org. Lett.*, 2003, **5**, 383; (*e*) K. Gogoi, A. D. Gunjal, U. D. Phalgune and V. A. Kumar, *Org. Lett.*, 2007, **9**, 2697; (*f*) D. A. Barawkar, B. Linkletter and T. C. Bruice, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 1517; (*g*) M. Taourirte, L. A. Mohamed, A. Rochdi, J. J. Vasseur, S. Fernández, M. Ferrero, V. Gotor, C. Pannecouque, E. D. Clercq and H. B. Lazrek, *Nucleosides, Nucleotides Nucleic Acids*, 2004, **23**, 701; (*h*) B. A. Linkletter, I. E. Szabo and T. C. Bruice, *J. Am. Chem. Soc.*, 1999, **121**, 3888.

6 (*a*) R. Lucas, P. H. Elchinger, P. A. Faugeras and R. Zerrouki, *Nucleosides, Nucleotides Nucleic Acids*, 2010, **29**, 168; (*b*) R. Lucas, R. Zerrouki, R. Granet, P. Krausz and Y. Champavier, *Tetrahedron*, 2008, **64**, 5467.

7 (*a*) C. F. Brown, *Chem. Rev.*, 1961, **61**, 463; (*b*) S. P. Singh, S. S. Parmar, K. Raman and V. I. Stenberg, *Chem. Rev.*, 1981, **81**, 175, and references cited therein; (*c*) A. Verma and S. K. Saraf, *Eur. J. Med. Chem.*, 2008, **43**, 897, and references cited therein.

8 (*a*) S. P. Singh, S. S. Parmar, K. Raman and V. I. Stenberg, *Chem. Rev.*, 1981, **81**, 175; (*b*) H. Chen, J. Bai, L. L. Jiao, Z. H. Guo, Q. M. Yin and X. L. Li, *Bioorg. Med. Chem.*, 2009, **17**, 3980.

9 (*a*) A. Scondo, F. Dumarçay-Charbonnier, D. Barth and A. Marsura, *Tetrahedron Lett.*, 2009, **50**, 5582; (*b*) S. Porwanski, B. Kryczka and A. Marsura, *Tetrahedron Lett.*, 2002, **43**, 8441; (*c*) S. Porwanski and A. Marsura, *Eur. J. Org. Chem.*, 2009, **13**, 2047.

10 H. Chen, H. Z. Zhang, J. N. Feng, X. L. Li, L. L. Jiao, Z. B. Qin, Q. M. Yin and J. C. Zhang, *Eur. J. Org. Chem.*, 2009, **35**, 6100.

11 (*a*) K. A. Winans and C. R. Bertozzi, *Chem. Biol.*, 2002, **9**, 113; (*b*) A. Babič, S. Gobec, C. Gravier-Pelletier, Y. L. Merrer and S. Pečar, *Tetrahedron*, 2008, **64**, 9093; (*c*) E. J. Corey and B. Samuelsson, *J. Org. Chem.*, 1984, **49**, 4735; (*d*) R. K. Boeckman, P. C. Shao and J. J. Mullins, *Org. Synth.*, 2004, Coll. Vol. **10**, 696; 2000, **77**, 141. http://www.orgsyn.org/orgsyn/pdfs/v77p0141.pdf.

12 CCDC (786926) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

13 Reverse Transcriptase Assay, Colorimetric kit, Roche Diagnostics GmbH, Roche Applied Science, Sandhofer strasse 116, D-68305 Mannheim, Germany.

