Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 60 (2004) 8535–8546

Synthesis and biological activities of 5′-ethylenic and acetylenic modified L-nucleosides and isonucleosides

Jun-Feng Wang, Xiao-Da Yang, Liang-Ren Zhang, Zhen-Jun Yang and Li-He Zhang*

National Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100083, People's Republic of China

Received 30 March 2004; revised 22 June 2004; accepted 24 June 2004

Available online 3 August 2004

Abstract—Two series of 6′-halovinyl-adenosine stereoisomers including 5′-ethylenic and acetylenic substituted L-adenosine, 5′-ethylenic and acetylenic substituted isonucleosides were synthesized. In the L-nucleoside series, compounds **6b**, **8b**, **10b** and **13b** showed modest inhibition of SAH hydrolase (21, 44, 50 and 26% respectively) at 100 μM. The L-isomers of 5′-ethylenic and acetylenic modified isonucleoside **23**, **24** exhibited no activity for the inhibition of SAH hydrolase, however, the D-isomers **30** and **31** showed some activities in the same test (35 and 21%). It indicated clearly the strict stereochemical requirement for the substrate of SAH hydrolase. Compounds **6b**, **8b**, **8c**, **11b** exhibited modest to good inhibition effects on the growth of HeLa cells or Bel-7420 cells at 1 μM (64, 44, 53 and 82% respectively).

1. Introduction

Methylated 5′-cap structure plays an essential role for the stability of mRNA against phosphatases and ribonucleases, for proper binding to ribosomes, and for the promotion of splicing. Hence, an uncapped mRNA is much less likely to be translated into its respective protein. Since many types of viruses also require methylated 5′-capped mRNA for proper translation into proteins, interference with the formation of these 5′-caps could lead to the inhibition of replication.[1,2] The various methyltransferases which catalyze these reactions have been targeted for drug design. The normal cellular role of SAH hydrolase is regulating *S*-adenosyl-L-methionine dependent biological methylation reactions. *S*-Adenosyl-L-methionine is involved in the methylation of many biomolecules and SAH is a potent feedback inhibitor of crucial transmethylation enzymes.[3–5] De Clercq and Cools found that some adenosine analogues showed the inhibition of vaccinia virus replication and a good correlation between the antiviral effectiveness and their ability to inhibit SAH hydrolase.[6] According to the mechanism of the hydrolysis of SAH by SAH hydrolase, many adenosine analogues, including some acyclic sugar mimics, have been designed and displayed interesting broad-spectrum antiviral properties. These first-generation inhibitors act as substrates for the first step in the hydrolysis reaction, where, the enzyme oxidizes the 3′-hydroxyl group of SAH to ketone and converts the NAD^+ to NADH in the process.[7,8] However, some dihalohomovinyl adenosine analogues were reported as the inhibitors for the 'type II' inactivation of SAH hydrolase involving its '5′/6′-hydrolytic activity'. The type II inhibitors contain an electrophilic entity at 5′-position of adenosine which could bind covalently to the enzyme without prior oxidation at 3′-position.[9] This discovery may open a way to the rational design of antiviral and antitumour drugs. Many 5′-halovinyl adenosine analogues or its conjugated diene analogues and acetylenic analogues were reported.[10–15] It was suggested that enzyme-mediated addition of water across the 5′,6′-double bond could generate electrophilic acyl halids or α-halo ketone species that could undergo nucleophilic attack by proximal groups on the enzyme and addition of water across the 5′,6′-triple bond followed by tautomerization of the hydroxyvinyl intermediates could also generate similar electrophiles at the enzyme active site. L-Nucleosides are the enantiomers of the natural nucleosides. Among these

Keywords: Stereoisomers; Biological activity; L-Nucleoside series.

Abbreviations: Ado, adenosine; AIBN, 2,2′-azobisisobutyronitrile; BSA, *N*,*O*-bis(trimethylsilyl)acetamide; ACN, acetonitrile; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DCE, 1,2-dichloroethane; DCM, dichloromethane; DEAD, diethyl azodicarboxylate; DIBAL-H, diisobutylaluminum hydride; DMSO, dimethyl sulfoxide; EtOAc, ethyl acetate; Hcy, homocysteine; HBV, heptitis B virus; HIV, human immunodeficiency virus; HPLC, high-performance liquid chromatography; HRMS (TOF), high resolution mass spectrometry; NBS, *N*-bromosuccinimide; NIS, *N*-iodosuccinimide; NAD^+, the oxidized form of NAD; NADH, the reduced form of NAD; NAD, nicotinamide–adenine dinucleotide; NMR, nuclear magnetic resonance; PBE, 20 mM phosphate buffer and 5 mM EDTA; Py, pyridine; RP-HPLC, reverse phase high-performance liquid chromatography; SAH, *S*-adenosyl-L-homocysteine; 3TC, (−)-β-L-1,3-oxathiolanyl-cytosine; TFA, trifluoroacetic acid; TMSOTf, trimethylsilyl trifluromethanesulfonate; Ts, toluenesulfonyl; TSA, *p*-toluenesulfonic acid; THF, tetrahydrofuran.

* Corresponding author. Tel.: +86-10-82801700; fax: +86-10-828-02724; e-mail: zdszlh@bjmu.edu.cn

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.06.131

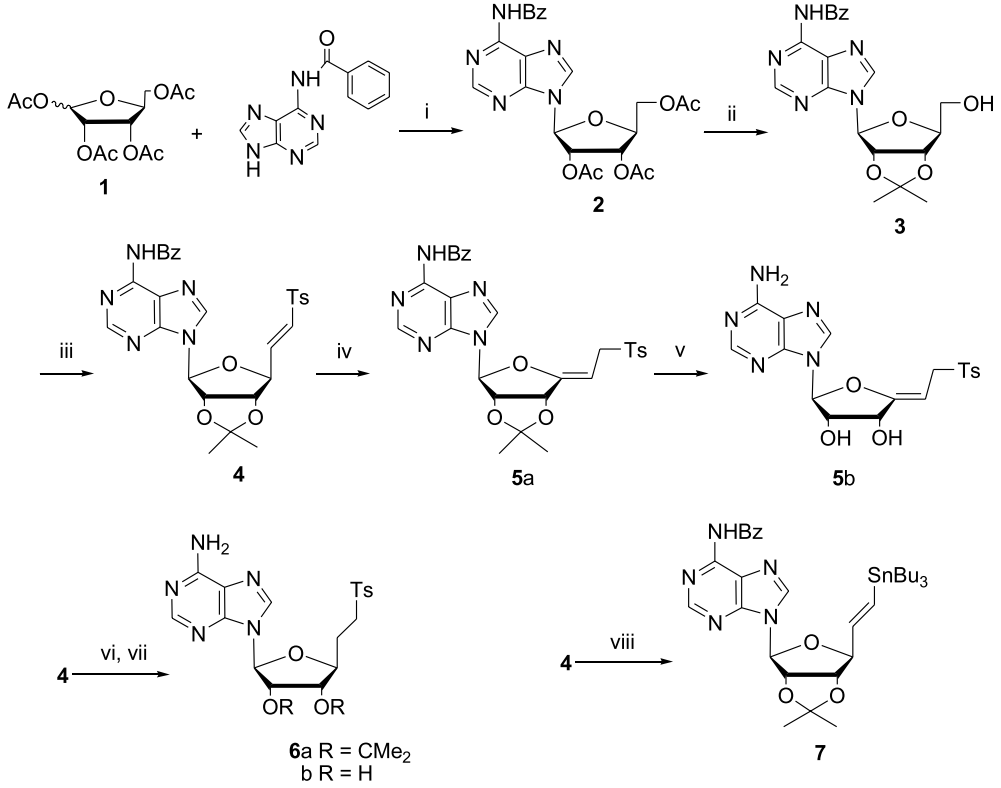
compounds, the separated enantiomer of L-2′-deoxy-3′-thiacytidine (3TC) demonstrated more potent activities against both HIV and HBV than their corresponding D-configuration counterparts with much less host toxicity.[16] Since then, a number of L-nucleoside analogues have been synthesized and biologically evaluated.[17–21] Isonucleosides represent a novel class of carbohydrate modified nucleosides in which the nucleobase is linked to various positions of ribose other than C-1′. We have reported a series of syntheses of isonucleosides.[22–25] The conformations of such L-nucleosides and isonucleosides exhibit profound changes compared to natural nucleosides. These alternations in conformation might be interesting to understand the recognition of L-nucleosides and isonucleosides as substrates by SAH hydrolase and explain the specific substrates requirement of SAH hydrolase. Herein, we report the synthesis of such L-nucleoside and isonucleoside analogues and their inactivation of SAH hydrolase.

2. Chemistry

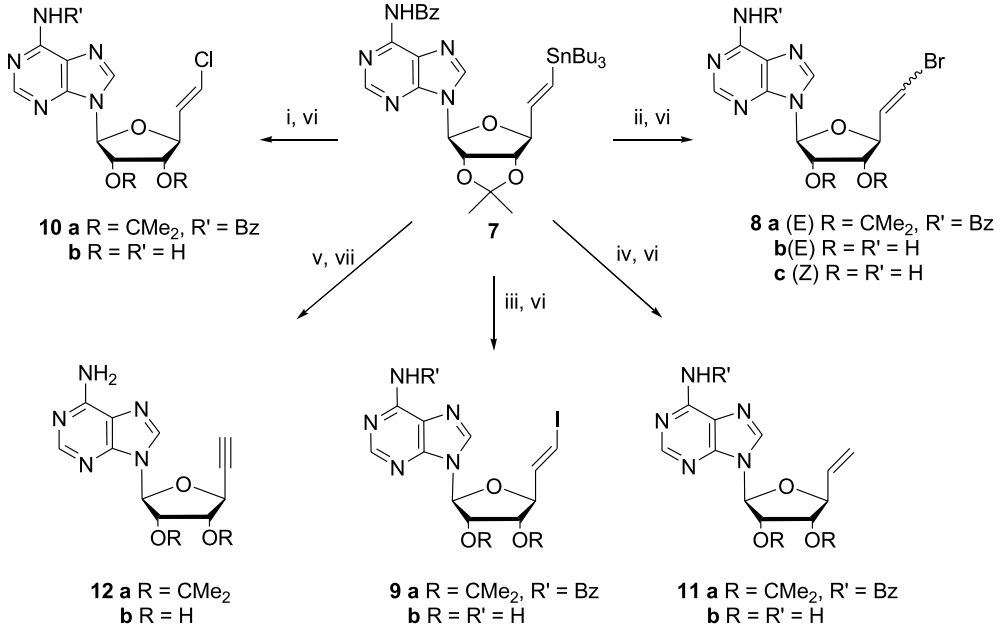
2.1. Synthesis of 5′-ethylenic and acetylenic substituted L-adenosine analogues

According to a known procedure, condensation of 1,2,3,5-tetra-*O*-acetyl-L-ribose **1** with benzoyl adenine provided the fully protected nucleoside **2** in 86% yield.[26] After removal of the acetyl groups, the 2′,3′-hydroxy groups of compound **2** were protected in the presence of $HC(OEt)_3$ and a catalytic amount of TSA at room temperature to give 6-*N*-benzoyl-2′,3′-*O*-isopropylidene-L-adenosine **3** in 81% yield. In order to synthesize the 5′-ethylenic adenosine, we tried first to isolate 5′-aldehyde adenosine after Moffatt oxidation of compound **3**. But many efforts failed due to the instability of 5′-aldehyde intermediate. However, the 5′-aldehyde intermediate in solution (without separation) could react with a stable Wittig reagent [(*p*-tolylsulfonyl)methylene]triphenyl phosphorane[27,28] to give compound **4** in 97% yield (Scheme 1). ^{1}H NMR data showed that compound **4** had *E*-form double bond signals at 5′ and 6′ position (δ=6.30, $H_{6'}$; δ=6.99, $H_{5'}$; $J_{5',6'}$=15.0 Hz). Isomerization of compound **4** was observed in basic condition. When compound **4** was treated with 1 M NaOH, a pure 6′-tosylallylic adenosine **5a** was obtained in 70% yield. **5a** was deprotected to give **5b** which can be confirmed by ^{13}C NMR, the double bond shifted to 4′,5′ position (δ=161.0, $C_{4'}$; δ=86.4, $C_{5'}$). According to the computer simulation, the *Z*-configuration of the double bond in compound **5b** is thermodynamicly more stable than the *E*-configuration. Treatment of **4** with sodium borohydride in aqueous methanol resulted in reduction of the tosylvinyl to give **6**a in 93% yield. Deprotection of **6a** gave **6b** in 54% yield.

Stannyldesulfonylation (Bu_3SnH/AIBN/toluene)[29] of **4** provided the 6-*N*-benzoyl-9-[6′-(*E*)-(tributylstannyl)-5′,6′-dideoxy-2′,3′-*O*-isopropylidene-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenine **7**. Compound **7** was reacted with *N*-bromosuccinimide (NBS), *N*-iodosuccinimide (NIS) and chlorine respectively to provide the 6-*N*-benzoyl-9-[6′-halo-5′,6′-dideoxy-2′,3′-*O*-isopropylidene-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenine **8a**, **9a**, **10a** (89, 98 and 77% respectively) and only *E*-form isomers were formed (Scheme 2).

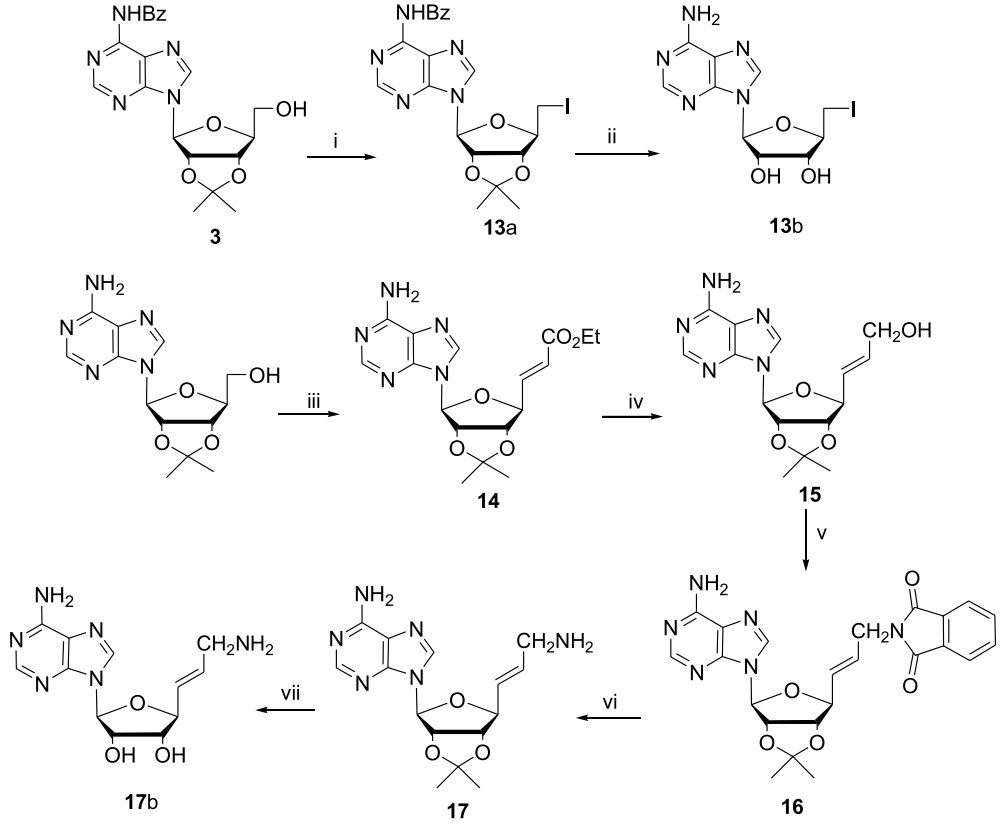


Scheme 1. Reagents and conditions: (i) BSA, 80 °C, DCE; TMSOTf, 80 °C, toluene. (ii) K_2CO_3/MeOH, rt; TSA, $HC(OEt)_3$, rt. (iii) DCC, $Cl_2CHCOOH$, DMSO; $TsCH{=}PPh_3$, rt. (iv) 1 N NaOH/H_2O, ACN, rt. (v) NH_3/MeOH, rt; CF_3COOH/H_2O, 0 °C. (vi) $NaBH_4$, MeOH/H_2O, rt. (vii) CF_3COOH/H_2O, 0 °C. (viii) Bu_3SnH, AIBN, toluene, reflux.



Scheme 2. Reagents and conditions: (i) Cl_2, DCM/CCl_4, −50 °C. (ii) NBS, DCM/CCl_4, −30 °C. (iii) NIS, DCM/CCl_4, −20 °C. (iv) NH_4F, EtOH, reflux. (v) $Pb(AcO)_4$, MeCN, rt. (vi) CF_3COOH/H_2O, 0 °C; NH_3/MeOH. (vii) NH_3/MeOH.

After deprotection, **8b**, **9b**, **10b** were obtained and in the case of compound **8a**, the deprotected product was a mixture of *E*- and *Z*-form isomers, *Z*-form isomer **8c** was separated in 16% yield. Destannnylation of **7** with ammonium fluoride in ethanol at reflux gave compound **11a** and treatment of **7** with lead tetraacetate in acetonitrile resulted in oxidative destannylation to give the 6-*N*-benzoyl-9-(5′,6′-dideoxy-2′,3′-*O*-isopropylidene-β-L-ribo-hex-5′-ynofuranosyl) adenine **12a**. Deprotection of **11a** and **12a** provided **11b** and **12b** respectively (Scheme 2). To extend the structure types of 5′-modified L-nucleosides, 5′-iodo-L-adenosine **13b** was synthesized from compound **3** by the substitution of iodine



Scheme 3. Reagents and conditions: (i) Ph_3P, I_2, 1,4-dioxane, py, rt. (ii) NH_3/MeOH, rt; CF_3COOH/H_2O, 0 °C. (iii) DCC, DMSO, $Cl_2CHCOOH$, $EtO_2CCH{=}PPh_3$. (iv) DIBAL-H, DCM, −78 °C. (v) Phthalimide, Ph_3P, DEAD, THF, rt. (vi) CH_3NH_2/EtOH, rt. (vii) CF_3COOH/H_2O, rt.

Table 1. [1]HNMR Spectral data[a,b] of 5′-modified L-nucleosides

Compound	H1′[c] ($J_{1'-2'}$)	H2′[d] ($J_{2'-3'}$)	H3′[d] ($J_{3'-4'}$)	H4′[d] ($J_{4'-5'}$)	H5′[d] ($J_{5'-6'}$)	H6′[c] ($J_{6'-4'}$)	H2[e]	H8[e]	NH_2[f]	Others[e]
3[g]	5.94 (4.8)	5.23[h] (5.7)	5.13 (2.1)	5.78[d] (1.8)	3.81[i], 4.03[j]		8.07	8.79	9.04[k]	1.28, 1.66 (CH_3), 7.54–7.63[i] (Bz)
4[g]	6.17 (1.5)	5.50 (6.0)	5.22 (3.6)	4.86[i] (4.2)	6.99 (15.0)	6.30 (1.8)	8.08	8.60	9.23[k]	1.38, 1.60, 2.39[l] (CH_3), 7.21–7.64[i] (Bz)
5a[g]	6.24[e]	5.61[c] (6.0)	5.24[c]		4.85[h] (8.1)	3.84[d]	8.02	8.75	8.91[k]	1.52, 1.61, 2.39[l] (CH_3), 7.18–8.00[i] (Bz)
5b	6.00 (4.0)	4.56 (7.5)	4.52[c]		4.85[h] (5.5)	3.90[i]	8.07	8.14	7.35	2.31[l] (CH_3), 5.62 (OH3′) 5.71 (OH2′), 7.21–7.64[i] (Bz)
6a[g]	5.95 (2.4)	5.43 (6.3)	4.90 (3.9)	4.21[i] (7.2)	2.16[i] (8.4)	3.12[i]	7.87	8.19	7.27	1.35, 1.57, 2.44[l] (CH_3) 7.27–7.68[i] (Bz)
6b	5.79 (4.5)	4.63 (5.0)	4.10 (5.5)	3.90[i] (6.0)	1.95[i] (8.0)	3.34[i]	8.02	8.27	7.30	2.38[l] (CH_3), 5.23 (OH3′), 5.45 (OH2′), 7.44–7.75[i] (Bz)
7[g]	6.19 (1.8)	5.57 (6.3)	5.04 (3.0)	4.72 (6.0)	6.03 (18.9)	6.27[d] (1.2)	8.10	8.35	8.96[k]	1.37, 1.63 (CH_3), 0.79–1.52[i] (Bu_3Sn), 7.57–8.05[i] (Bz)
8a[g]	6.15 (1.5)	5.57 (6.3)	5.12 (3.3)	4.69 (6.0)	6.34–6.36[i]		8.10	8.83	9.03[k]	1.41, 1.63 (CH_3), 7.58–8.03[i] (Bz)
8b	5.90 (5.0)	4.70 (10.0)	4.19 (5.0)	4.36 (8.0)	6.56 (13.5)	6.71[c]	8.15	8.37	7.31	
8c	5.91 (6.0)	4.85 (5.0)	4.14 (3.5)	4.73 (8.0)	6.72[h] (7.0)	6.75[c]	8.15	8.37	7.31	
9a[g]	6.15 (1.5)	5.59 (6.3)	5.13 (3.0)	4.69 (6.9)	6.66 (14.7)	6.38[d]	8.08	8.84	8.97[k]	1.43, 1.63 (CH_3), 7.58–8.03[i] (Bz)
9b	5.89 (5.1)	4.70 (5.4)	4.18 (4.5)	4.32 (7.5)	6.85 (14.4)	6.67[d]	8.15	8.35	7.31	
10a[g]	6.14 (2.1)	5.56 (6.3)	5.10 (3.3)	4.71 (7.8)	6.08 (13.2)	6.23[d]	8.24	8.81	9.14[k]	1.40, 1.64 (CH_3), 7.54–8.03[i] (Bz)
10b	5.89 (4.5)	4.38 (8.3)	3.36–3.41		6.31 (13.0)	6.60[d]	8.15	8.36	7.31	
11a[g]	6.19 (1.5)	5.59 (6.3)	5.05 (3.6)	4.72 (6.9)	5.89[m] (17.1, 10.8)	5.16, 5.27	8.11	8.84	8.92[k]	1.42, 1.63 (CH_3), 7.57–8.03[i] (Bz)
11b	6.25 (5.0)	4.68[h] (5.0)	4.12[h] (4.5)	4.32[h] (6.5)	6.08[m] (17.0, 10.5)	5.19, 5.30	8.16	8.31	7.29	
12a[g]	6.28[e]	5.75[c] (5.7)	5.13 (1.2)	5.07[c]		2.44	8.30	8.82	9.07[k]	1.41, 1.58 (CH_3), 7.47–8.00[i] (Bz)
12b	5.94 (5.0)	4.78[h] (5.0)	4.35[h] (4.0)	4.55		3.75[c] (2.0)	8.21	8.32	7.59	
13a[g]	6.21 (2.4)	5.52 (5.7)	5.11 (3.3)	4.45[j] (10.2)	3.29, 3.46		8.19	8.83	9.08[k]	1.42, 1.64 (CH_3), 7.58–8.03[i] (Bz)
13b	5.90 (6.0)	4.80 (6.0)	4.16 (5.0)	3.98[j] (10.5)	3.45, 3.60		8.14	8.36	7.30	5.50 (OH3′), 5.79 (OH2′)
14[g]	6.13 (1.8)	5.56 (6.3)	5.14 (3.6)	4.81[i]	6.96 (15.6)	5.81[d] (1.5)	7.86	8.33	5.59	1.40, 1.63 (CH_3), 1.22[h] (H9′), 4.11[n] (H8′)
15[g]	6.10 (2.1)	5.85–5.86		4.72[i]	5.02 (6.3)	5.54[d] (2.1[o])	7.87	8.36	5.60	1.40, 1.63 (CH_3), 3.97[c,p] (H7′,2.4)
16[g]	6.07 (2.0)	5.49 (6.0)	4.98 (3.5)	4.68 (7.0)	5.84 (15.5)	5.73[j] (5.5)	7.85	8.25	5.47	1.37, 1.60 (CH_3), 7.72–7.85[i] (Bz)
17a	6.09 (1.5)	5.52 (5.7)	5.00 (3.3)	4.70 (6.3)	5.76–5.86[j]		7.89	8.35	5.80	1.39, 1.62 (CH_3), 3.28[e] (H7′), 1.99[f] (CH_2NH_2)
17b	5.94 (5.1)	4.66[h] (5.1)	4.14[h] (4.5)	4.37[h] (5.7)	6.07 (15.3)	5.77[j,p] (6.9)	8.19	8.35	5.80	3.46[c] (H7′)

[a]Chemical shifts(δ) in DMSO-d_6 at 300 MHz (unless otherwise noted). [b]'Apparent' first-order coupling constants (Hz, in parentheses). [c]Doublet (unless otherwise noted). [d]Doublet of doublets (unless otherwise noted). [e]Singlet (unless otherwise noted). [f]Broad singlet. [g]In $CDCl_3$. [h]Triplet (unless otherwise noted). [i]Multiplet. [j]Doublet of triplets. [k]NH. [l]$PhCH_3$. [m]Doublet of doublets of doublets. [n]Quartet. [o]$^3J_{H6'-H7'}$.

(Scheme 3). For the synthesis of compound **17b**, the 2′,3′-*O*-isopropylidene-L-adenosine was oxidized by Moffat reaction and followed by the treatment of Wittig reagent to give the α,β-unsaturated ester **14** in 50% yield. Reduction of compound **14** with DIBAL-H gave the allylic alcohol **15**, which was converted into phthalimide **16** by a Mitsunobu reaction in 81% yield. After the cleavage with methylamine, and deprotection with TFA, compound **17b** was obtained in 81% yield (Scheme 3). The ^1H and ^{13}C NMR data of L-adenosine derivatives were listed in Tables 1 and 2.

2.2. Synthesis of 5′-ethylenic and acetylenic substituted isonucleosides

The [5-(*R*)-dimethoxy-4-(*R*)-hydroxy-3(*S*)-adenin-9-yl]tetrahydrofuran **18** was synthesized starting from D-xylose[22]

Table 2. ^{13}C NMR Spectral data[a,b] of 5′-modified L-nucleosides

Compound	C2	C4	C5	C6	C8	C1′	C2′[c]	C3′[c]	C4′	C5′	C6′
5b[d]	152.8	149.1	119.1	156.1	139.3	88.2	71.9	69.4	161.0	86.4	52.6
6b[e]	152.5	149.1	119.3	156.1	144.4	88.0	72.7	72.6	81.6	21.1	51.6
7[f,g]	152.8	149.6	123.4	152.8	142.3	90.8	84.6	84.2	90.5	143.8	133.6
8b	152.6	149.3	119.2	156.1	140.0	87.6	73.4	72.6	83.6	136.0	109.9
8c	152.6	149.4	119.4	156.1	140.3	87.7	74.2	72.7	82.2	133.6	111.1
9b	152.6	149.3	119.2	156.1	143.7	87.6	73.2	72.6	85.7	140.0	82.1
10b	152.7	149.3	119.2	156.1	140.0	87.6	73.7	72.7	82.3	132.2	121.5
11b	152.8	149.5	119.0	156.1	139.9	87.6	73.9	72.8	84.8	136.7	117.1
12b	152.0	149.5	119.0	155.4	139.6	87.4	73.5	73.1	81.1	78.7	75.2
13b	152.7	149.5	119.1	156.1	139.9	87.4	73.2	72.7	83.9	7.8	
14[g,h]	153.2	152.6	122.6	155.4	140.0	90.6	84.3	83.9	86.3	143.4	131.9
15[g,i]	153.2	151.3	122.4	155.4	140.0	90.6	84.6	84.2	87.5	133.7	127.4
16[g,j]	153.1	149.4	123.4	155.6	139.9	90.5	84.5	84.1	87.2	132.0	120.2
17a[k]	153.2	149.5	120.2	155.5	139.9	90.4	84.6	84.2	87.7	136.0	126.4
17b[l]	152.8	149.5	119.2	156.1	139.8	87.5	73.9	72.8	83.5	133.6	124.7

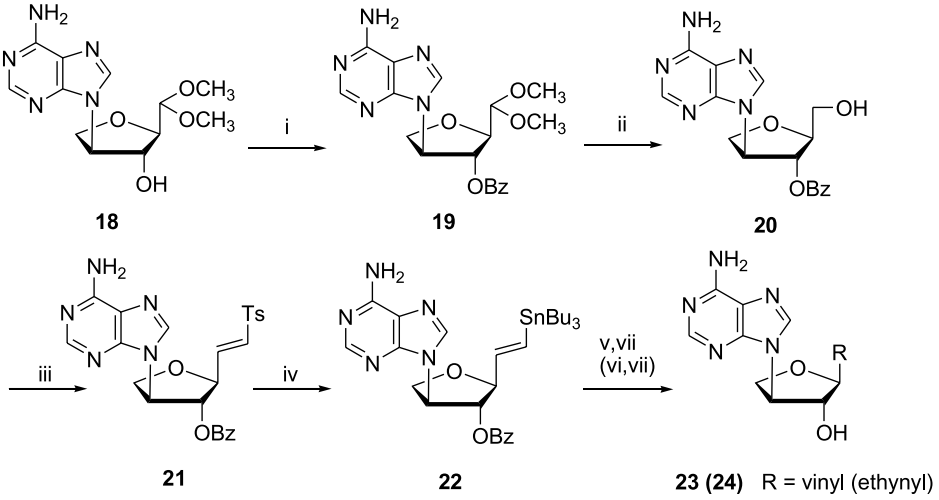
[a] Chemical shifts (δ) in DMSO-d_6 at 75 MHz.
[b] Proton-decoupled singlets.
[c] Assignment might be reversed.
[d] Peaks also at δ 21.5, 127.86, 129.3, 135.9, 144.0 ($PhCH_3$).
[e] Peaks also at δ 26.3, 127.7, 129.9, 135.9, 144.4 ($PhCH_3$).
[f] Peaks also at δ 9.4, 13.6, 27.2, 28.9 (Bu_3Sn); 25.4, 27.1, 114.5 (CMe_2); 127.8, 128.9, 132.8, 133.8, 164.3 (PhCO).
[g] In $CDCl_3$.
[h] Peaks also at δ 14.1, 60.6, 165.6 ($CO_2CH_2CH_3$); 25.3, 27.1, 114.7 (CMe_2).
[i] Peaks also at δ 25.4, 27.1, 114.5 (CMe_2); 62.4 (CH_2OH).
[j] Peaks also at δ 25.3, 27.0, 114.4 (CMe_2); 38.6 (CH_2NR); 123.4, 127.4, 130.4, 134.0 (Ph); 155.9, 167.0 (CO).
[k] Peaks also at δ 25.4, 27.1, 114.5 (CMe_2); 43.2 (CH_2NR).
[l] Peaks also at δ 63.1 (CH_2NR).

with a published procedure in our laboratory. Protection of **18** with benzoyl chloride gave compound **19**. One-pot reaction of **19** by treatment with 1% HCl/THF at 70–90 °C and followed by reduction with $NaBH_4$ provided the isonucleoside **20** in 52% yield. As the procedure described above, Moffatt oxidation was applied to **20** and followed by treatment with wittig reagent [(*p*-tolylsulfonyl)methylene]triphenylphosphorane to give the 6′-(*E*)-tosylvinyl derivative **21** in 85% yield. Stannyldesulfonylation (Bu_3SnH/AIBN/toluene) of **21** provided compound **22**. Compound **22** was treated in the presence of lead tetraacetate at ambient temperature to provide the 5′-vinyl isonucleoside **23**. Treatment of **22** with ammonium fluoride in ethanol at reflux gave the 5′-ethynyl isonucleoside **24** (Scheme 4). The compound **25**, enantiomer of isonucleoside **18**, were synthesized using our published procedure.[25]

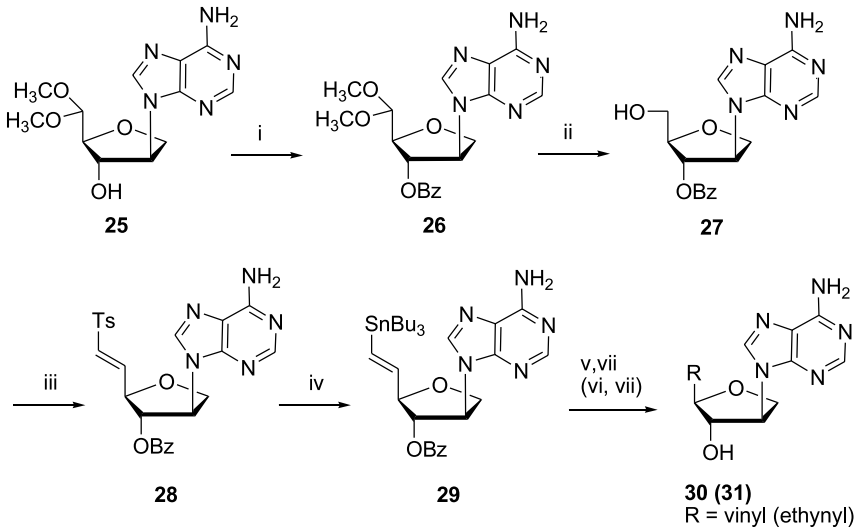
Using the similar procedure as described above, the syntheses of **30** and **31** were shown in Scheme 5. The ^1H and ^{13}C NMR data of iso-adenosine derivatives were listed in Tables 3 and 4.

2.3. Biological results and discussion

Compounds **5b**, **6b**, **8b**, **8c**, **9b**, **10b**, **11b**, **12b**, **13b**, **17b** and **23**, **24**, **30**, **31** were evaluated for the inactivation of SAH hydrolase and known active compound, 6′(*E*)-bromovinyl D-adenosine (**D–B**),[36] was used as control (Table 5). In the L-nucleoside series, compounds **6b**, **8b**, **10b** and **13b** showed modest inhibition of SAH hydrolase (21, 44, 50 and 26% respectively) at 100 μM. Compound **8c**, the *Z*-form isomer of **8b**, and compound **9b** exhibited no inhibition in this assay. Wnuk et al. reported the synthesis of 6′-(*E*) and



Scheme 4. Reagents and conditions: (i) BzCl, Py, 0–40 °C. (ii) 1% HCl, THF, 70–90 °C. (iii) DCC, DMSO, $Cl_2CHCOOH$; $TsCH{=}PPh_3$. (iv) Bu_3SnH, AIBN, toluene, reflux. (v) NH_4F, EtOH, reflux. (vi) $Pb(OAc)_4$, ACN, rt. (vii) $NaOCH_3$/MeOH, rt.



Scheme 5. Reagents and conditions: (i) BzCl, Py, 0–40 °C. (ii) 1% HCl, THF, 70–90 °C. (iii) DCC, DMSO, $Cl_2CHCOOH$; TsCH=PPh_3. (iv) Bu_3SnH, AIBN, toluene, reflux. (v) NH_4F, EtOH, reflux. (vi) $Pb(OAc)_4$, ACN, rt. (vii) $NaOCH_3$/MeOH, rt.

(*Z*)-halohomovinyl derivatives of adenosine and indicated that the order of inhibitory potency of these derivatives for the inactivation of *S*-adenosyl-L-homocysteine hydrolase was I > Br > Cl > F and *E* form > *Z* form.[14] However, in our L-nucleoside series, the inhibitory potency of SAH hydrolase was weaker than that of their D-isomers and the order of inhibitory potency seemed confusion. The activities of chloro-substituted derivative **10b** and the bromo partner **8b** were almost the same but iodo-substituted compound **9b** showed no activity. According to the mechanism studies on the inhibition of SAH hydrolase,[33] the acetylenic derivative of adenosine showed marked inhibition at 100 μM[14] but the L-isomer of 9-(5′,6′-dideoxy-β-D-ribo-hex-5′-ynofuranosyl)adenine **12b** was inactive at the same condition. The 5′-ethylenic and acetylenic modified isonucleoside analogues **23**, **24**, **30**, **31** were also evaluated in this assay. The L-isomers of 5′-ethylenic and acetylenic modified isonucleoside **23**, **24** exhibited no activity for the inhibition of SAH hydrolase, however, the D-isomers **30** and **31** showed some activities in the same test (35 and 21%). Computer modeling study showed that each pair of D- and L-enantiomer of 6′-halohomovinyl derivative of adenosine was fitted together by pairing base moiety and 5′-moiety (Fig. 1), but sugar ring was out from the model. L-5′-Ethylenic and acetylenic isonucleoside analogues could not fit to the 5′-acetylenic modified adenosine derivative but the D-isomer was more similar to the D-nucleoside partner (Fig. 2). In the cases of L-2′-deoxy-3′-thiacytidine (3TC) and other L-nucleoside antiviral drugs, the monophosphorylation of a nucleoside analogues was the crucial step for the biological activity and the certain kinase could recognize both of the D- and L-isomers without limitation of configuration,[30] however, this study indicated clearly the strict stereochemical requirement for the substrate of SAH hydrolase.[31]

Wnuk et al. reported the observation of a direct correlation of cytostatic activity with inhibition of SAH hydrolase and found that the most potent inhibitors of

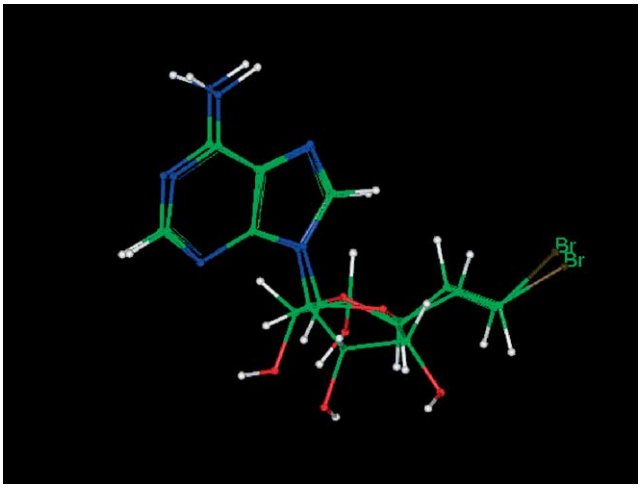


Figure 1. Overlap of structure of D- and L-6′(*E*)-bromovinyl adenosine. The modeling was performed based on the energy optimized structure. Enatiomers were fit together by pairing N3, N6, N7, N9, C5′ and C6′ (the modeling was performed on SGI Indy workstation and MSI Insight II was used).

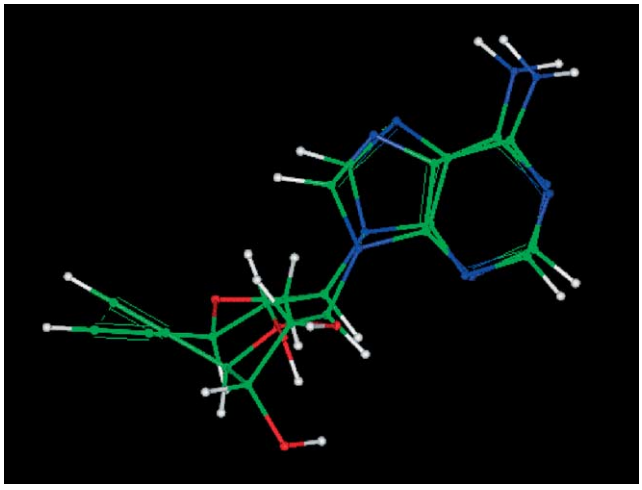


Figure 2. Overlap of structure of 6′-ethynyl D-adenosine. The modeling was performed based on the energy optimized structure. Enantiomers were fit together by pairing N3, N6, N7, N9, C5′ and C6′ (the modeling was performed on SGI Indy workstation and MSI Insight II was used).

Table 3. [1]HNMR Spectral data[a,b] of 5′-modified isonucleosides

Compound	H2′a[c,d] ($J_{2'-3'}$)	H2′b[e] ($J_{2'-3'}$)	H3′[e] ($J_{3'-4'}$)	H4′[e] ($J_{4'-5'}$)	H5′[e] ($J_{5'-6'}$)	H6′[e]	H2	H8	NH_2[f]	H7′	Others[g]
19	4.39 (6.0, 10.5)	4.29 (3.0)	5.72 (2.4)	5.32 (5.4)	4.28 (3.6)	4.77[c]	8.32	8.33	6.35[h]		3.51, 3.55[g] (OCH_3), 7.47–8.03[i] (Bz)
20	4.14 (5.7, 10.5)	4.04 (3.3)	5.75 (4.2)	5.34 (6.0)	4.08 (4.2)	4.36[c]	8.25	8.32	6.59[h]		7.42–8.01[i] (Bz)
21	4.44–4.46 (5.1, 10.5)		5.36 (7.5)	4.77[j] (4.5)	5.56 (3.0)	7.28[k] (15.0)	7.84	8.29	5.83[h]	6.80[d]	2.63[g] ($PhCH_3$), 7.27–8.03[i] (Bz)
22	4.44 (5.7, 10.5)	4.33 (2.7)	5.34[i] (7.8)	4.60[i] (4.5)	5.49 (4.5)	6.25[k]	8.01	8.39	5.69[h]	6.53[d]	0.94–1.48[i] (Bu_3Sn), 7.55–8.06[i] (Bz)
23[l]	4.19–4.20 (6.0, 10.5)		4.88 (6.0)	4.36 (6.5)	4.11[j] (6.5)	5.92[i,k] (17.5, 10.5)	8.13	8.14	7.24[h]	5.17	
24[l]	4.30 (5.7, 10.5)	4.20 (3.3)	4.59 (4.2)	4.85 (6.0)	4.40 (4.2)		8.14	8.15	7.25[h]	2.49[g]	6.20[g] (OH4′)
26	4.38 (6.0, 10.5)	4.29 (3.0)	5.73 (2.4)	5.33 (5.4)	4.28[c] (3.6)	4.79[c]	8.32	8.33	6.35[h]		7.47–8.03[i] (Bz)
28	4.44–4.46[c] (5.1, 10.5)		5.36 (7.8)	4.76 (4.5)	5.56 (3.0)	7.30[k] (15.0)	7.84	8.29	5.88[h]	6.83[e]	2.44[g] ($PhCH_3$), 7.27–8.03[i] (Bz)
29	4.44 (5.7, 10.5)	4.32 (2.7)	5.34 (7.8)	4.60[i] (4.5)	5.48 (4.8)	6.25[k] (19.2)	8.01	8.36	5.57[h]	6.53[e]	0.85–1.46[i] (Bu_3Sn), 7.27–8.05[i] (Bz)
30[l]	4.19[e] (6.0, 10.5)		4.88 (6.0)	4.36 (6.5)	4.11[j] (6.5)	5.92[i,k] (17.5, 10.5)	8.14	8.15	7.24[h]	5.17[e]	6.21[g] (OH4′)
31[l]	4.30 (5.0, 10.5)	4.20 (2.0)	4.59 (5.0)	4.85 (5.0)	4.40		8.14	8.15	7.25[h]	2.49[g]	

[a]Chemical shifts (δ) in $CDCl_3$ at 300 MHz (unless otherwise noted). [b]'Apparent' first-order coupling constants (Hz, in parentheses). [c]Doublet (unless otherwise noted). [d]$^2J_{H2'-H2'}$. [e]Doublet of doublets (unless otherwise noted). [f]Broad singlet. [g]Singlet (unless otherwise noted). [h]NH_2. [i]Multiplet. [j]Triplet (unless otherwise noted). [k]$^3J_{H6'-H7'}$. [l]In Me_2SO-d_6. [m]Doublet of triplets. [n]$^2J_{H6'-H6'}$. [o]$^2J_{H7'-H7'}$.

Table 4. [13]CNMR Spectral data[a,b] of 5′-modified isonucleosides

Compound	C2	C4	C5	C6	C8	CH_3	C2′	C3′	C4′	C5′	C6′	C7′
20[c,d]	152.5	149.3	118.6	156.0	139.3		70.4	60.5	79.1	84.4	59.9	
21[c,e]	153.3	149.8	119.6	155.5	140.2		70.9	59.9	81.6[f]	82.6[f]	128.3	144.7
22[c,g]	153.1	149.3	118.9	155.5	140.3		71.4	60.3	82.5	86.3	142.2	133.9
23	152.4	149.5	119.0	156.0	139.4		68.7	61.7	78.6	84.7	136.5	116.9
24	152.4	149.5	119.3	156.0	139.2		69.0	61.2	79.9	81.4	77.9	73.7
28[c,h]	153.3	149.8	119.6	155.6	140.2		70.9	59.9	81.5	82.6	128.3	144.7
29[c,i]	153.1	148.9	118.9	155.6	141.9		71.3	60.8	82.6	86.1	142.3	134.0
30	152.4	149.5	119.0	156.0	139.4		68.7	61.7	78.6	84.6	136.5	116.8
31	152.4	149.4	119.3	156.0	139.2		69.0	61.2	79.9	81.4	77.9	73.7

[a]Chemical shifts (δ) in DMSO-d_6 at 75 MHz. [b]Proton-decoupled singlets. [c]Peaks also at δ 128.5, 129.7, 133.8, 135.4, 166.1 (PhCO). [d]Peaks also at δ 128.8, 128.9, 129.5, 133.8, 165.2 (PhCO). [e]Peaks also at δ 12.6, 127.9, 128.6, 129.9, 130.0, 132.4, 134.0, 136.8, 138.3, 165.6 (PhCO). [f]Assignment might be reversed. [g]Peaks also at δ 9.5, 13.6, 17.5, 19.1, 26.8, 27.2 27.7, 27.8, 29.0, 29.7, (Bu_3Sn), 128.6, 129.9, 130.9, 133.8, 165.6 (PhCO). [h]Peaks also at δ 127.8, 128.6, 129.9, 130.0, 132.4, 134.0, 136.8, 138.3, 165.6 (PhCO). [i]Peaks also at δ 9.5, 13.6, 17.5, 19.2, 26.8, 27.2, 27.7, 29.0, 29.7 (Bu_3Sn), 128.8, 129.9, 130.9, 134.0, 165.6 (PhCO).

Table 5. Inhibition of *S*-adenosyl-L-homocysteine hydrolase by synthetic adenosine analogues[a]

Conc.\compound	D–B	6b	8b	10b	11b
100 μM	94%	21%	44%	50%	30%
10 μM	92%	—	10%	16%	—
1 μM	68%	—	—	—[b]	—
Conc\compound	**13b**	**23**	**24**	**30**	**31**
100 μM	26%	12%	20%	35%	21%
10 μM	24%	—	11%	16%	12%
1 μM	—	—	—	—	—

[a] The data shown are the average results of three experiments.
[b] Dash means no activity in this condition.

SAH hydrolase also showed the most potent cytostatic activities.[14] In our case, compounds **6b**, **8b**, **8c**, **9b**, **10b**, **11b**, **12b**, **13b**, **17b** and **23**, **24**, **30**, **31** were screened by culture of tumor cells (Table 6), only HeLa cells and Bel-7420 cells were sensitive to these compounds. Compounds **6b**, **8b**, **8c**, **11b** exhibited modest to good inhibition effects on the growth of HeLa cells or Bel-7420 cells at 1 μM (64, 44, 53 and 82% respectively), compounds **9b**, **12b** and **13b** were active at 10 μM (55, 64 and 73%). Therefore, the cytostatic activites of L-nucleoside and isonucleoside derivatives reported may correlate with inhibition of SAH hydrolase and other mechanisms.

3. Conclusions

The synthesis of stereoisomers of 6′-halovinyl modified adenosine analogues could explain the specific substrates requirement of SAH hydrolase. We designed and synthesized two series of 6′-halovinyl D-adenosine stereoisomers including 5′-ethylenic and acetylenic substituted L-adenosine and 5′-ethylenic substituted isonucleosides. In the L-nucleoside series, compounds **6b**, **8b**, **10b** and **13b** showed modest inhibition of SAH hydrolase (21, 44, 50 and 26% respectively) at 100 μM. The isonucleoside analogues **31**, **32** showed some weaker activities in the same test (35 and 21%). It indicated clearly the strict stereochemical requirement for the substrate of SAH hydrolase. Compounds **6b**, **8b**, **8c**, **11b** exhibited modest to good inhibition effects on the growth of HeLa cells or Bel-7420 cells at 1 μM (64, 44, 53 and 82% respectively), compounds **9b**, **12b** and **13b** were active at 10 μM (55, 64 and 73%). Therefore, the cytostatic activites of L-nucleoside and isonucleoside derivatives reported may correlate with inhibition of SAH hydrolase and other mechanisms.

Table 6. The inhibitory effect of 5′-modified L-nucleosides on Hela cells and Bel-7420 cells in vitro[a]

Compound	Tumor cell line	Conc. (μM)	Inhibition rate (%)
6b	Bel-7420	1.0	64
		10.0	81
8b	Hela	1.0	44
		10.0	67
8c	Hela	0.1	23
		1.0	53
		10.0	67
9b	Hela	1.0	22
		10.0	55
13b	Hela	1.0	10
		10.0	64
11b	Bel-7420	0.1	27
		1.0	82
12b	Bel-7420	1.0	35
		10.0	73

[a] The data shown are the average results of three experiments.

4. Experimental

4.1. General

Uncorrected melting points were determined on a XT-4A melting point apparatus. NMR spectra were recorded on a Varian 300 MHz spectrometer. Mass spectra were obtained on PE SCLEX QSTAR mass spectrometer. Elemental analyses were performed on Varian ELIII analyzer. Solvents were dried by reflux over CaH_2 and distilled before use, chromatographic purifications were carried out using silica gel (200–300 mesh, Qingdao chemicals). Preparative RP-HPLC was performed with spectra physics SP 8800 ternary pump system and Pynamax C_{18} columns.

4.1.1. 6-*N*-Benzoyl-2′,3′-*O*-isopropylidene-L-adenosine (3). Compound **2** (240 mg, 0.48 mmol) was dissolved in K_2CO_3/CH_3OH (20 mL), the resulting solution was stirred at ambient temperature for 1.2 h. The solution was neutralized to pH=7 with HCl/H_2O and then was evaporated, the residue was dissolved into acetone (20 mL) and TSA (400 mg, 2.1 mmol), triethyl orthoformate (0.5 mL, 2.9 mmol) were added. The mixture was stirred at ambient temperature overnight. The solution was neutralized with $NaHCO_3/H_2O$ and evaporated. The residue was partitioned ($CHCl_3/H_2O$), dried ($MgSO_4$) and evaporated. Purified on column chromatograph (EtOAc/acetone=4/1), **3** (161 mg, 81%) was obtained as a white foam. TOF-MS ($M^+ + H$): 412.

4.1.2. 6-*N*-Benzoyl-9-[5′,6′-dideoxy-2′,3′-*O*-isopropylidene-6′-(*p*-toluenesulfonyl)-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (4). A solution of **3** (375 mg, 0.91 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (DCC, 564 mg, 2.7 mmol) in dried DMSO (2 mL) was cooled (0 °C) under argon, $Cl_2CHCOOH$ (38 μL, 0.47 mmol) was added, and stirred for 2 h at ambient temperature, then [(*p*-tolylsulfonyl)methylene]triphenyl phosphorane was added to the solution and stirred overnight. Oxalic acid dihydrate (453 mg, 3.6 mmol) in MeOH (4 mL) was added. After 30 min the dicyclohexylurea was filtered and washed with cold MeOH, and the combined filtrates were evaporated (in vacuo). The residue was partitioned ($EtOAc/H_2O$), the organic layer was washed with H_2O (3×10 mL), $NaHCO_3/H_2O$, and $NaCl/H_2O$, dried ($MgSO_4$) and evaporated. Purified on column chromatography, **4** (500 mg, 97%) was obtained as a white

solid. Mp 108–109 °C. HRMS (TOF) calcd for $C_{28}H_{28}N_5O_6S$ ($M^+ + H$): 562.1760; found: 562.1770.

4.1.3. 6-*N*-Benzoyl-9-[5′,6′-dideoxy-6′-(*p*-toluenesulfonyl)-β-L-ribo-hex-5′(*Z*)-enofuranosyl] adenosine (5a). To a stirred solution of **4** (181 mg, 0.32 mmol) in CH_3CN/H_2O (4:1, 10 mL) was added 1 M NaOH/H_2O(1 mL) and stirring was continued at ambient temperature for 4 h, the solution was concentrated to half volume and EtOAc (10 mL) and 0.05 M HCl/H_2O (2 mL) were added. The organic layer was washed with saturated $NaHCO_3$/H_2O, brine, dried (Na_2SO_4) and purified on column chromatograph to give **5a** (127 mg, 70%) as a white foam. Mp 103–104 °C. TOF-MS ($M^+ + H$): 562.

4.1.4. 9-[4′,5′-Dideoxy-6′-(*p*-toluenesulfonyl)-β-L-ribo-hex-5′(*Z*)-enofuranosyl] adenosine (5b). Compound **5a** (25 mg, 0.044 mmol) was dissolved in 15 mL saturated NH_3/CH_3OH solution, the resulting solution was stirred overnight at ambient temperature and evaporated, CF_3COOH/H_2O (9:1, 3 mL) was added to the residue and stirring was continued for 1 h at 0 °C (ice bath). After co-evaporation with EtOH (2×3 mL), the residue was separated with a short silica gel column (EtOAc→EtOAc/*i*-PrOH/H_2O, 20:1:2, upper layer), compound **5b** (12 mg, 64%) was collected as a white solid. Mp 115–117 °C. HRMS (TOF) calcd for $C_{18}H_{20}N_5O_5S$ ($M^+ + H$): 418.1185; found: 418.1152

4.1.5. 9-[2′,3′-*O*-Isopropylidene-6′-(*p*-toluenesulfonyl)-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (6a). To a stirred solution of **4** (12 mg, 0.021 mmol) in MeOH/H_2O (1:1, 3 mL) was added sodium borohydride (3 mg, 0.08 mmol). After 18 h at ambient temperature, the solution was concentrated to half volume and the residue was partitioned ($CHCl_3$/H_2O). The organic layer was washed with brine, H_2O, dried ($MgSO_4$), and evaporated. Purified on column chromatography (EtOAc/petroleum ether, 2:1), compond **6a** (9 mg, 93%) was obtained as a white foam. TOF-MS ($M^+ + H$): 460.

4.1.6. 9-[6′-(*p*-Toluenesulfonyl)-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (6b). The deprotection of **6a** was performed (as described for 5b) gave a white solid **6b** (11 mg, 55%). Mp 124–126 °C. HRMS (TOF) calcd for $C_{18}H_{22}N_5O_5S$ ($M^+ + H$): 420.1342; found: 420.1329.

4.1.7. 6-*N*-Benzoyl-9-[6′-(*E*)-(tributylstannyl)-5′,6′-dideoxy-2′,3′-*O*-isopropylidene-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (7). The oxygen in the solution of **4** (93 mg, 0.165 mmol) in toluene (10 mL) was removed by argon for 30 min, and Bu_3SnH (0.2 mL, 0.66 mmol) was added. Argon was passed through the solution continually for 15 min, and AIBN (70 mg, 0.33 mmol) was added. The solution was refluxed for 5 h and evaporated, and the residue was purified on column chromatography (EtOAc/petroleum ether, 1:3). Compound **7** (70 mg, 61%) was obtained as a colorless oil. Analysis calcd for $C_{33}H_{47}N_5O_4Sn$: C, 56.91; H, 6.80; N, 10.06; found: C, 57.07; H, 7.01; N, 9.66.

4.1.8. 6-*N*-Benzoyl-9-[6′-bromo-5′,6′-dideoxy-2′,3′-*O*-isopropylidene-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (8a). A solution of NBS (59 mg, 0.329 mmol) in CH_2Cl_2/CCl_4 (1:1, 10 mL) was added dropwise to a stirred solution of **7** (169 mg, 0.243 mmol) in CH_2Cl_2/CCl_4 (1:1, 6 mL) at −30 °C. After 30 min, the mixture was poured into saturated $NaHCO_3$/H_2O and extracted by $CHCl_3$. The combined organic phase was washed (brine), dried ($MgSO_4$), and evaporated, and the residue was purified on column chromotography (EtOAc/petroleum ether, 1:1) to provide **8a** (106 mg, 89%). TOF-MS ($M^+ + H$): 486.

4.1.9. 9-[6′-Bromo-5′,6′-dideoxy-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (8b). 9-[6′-Bromo-5′,6′-dideoxy-β-L-ribo-hex-5′(*Z*)-enofuranosyl] adenosine (8c). Deprotection of **8a** (35 mg, 0.072 mmol) (as described for **5b**) gave a residue that was purified by RP-HPLC (preparative column; program: 17% CH_3CN/H_2O for 1 h at 3 mL/min) to give **8b** (*E*) (15 mg, 61%, t_R=47 min. Mp 141–142 °C) and **8c** (*Z*) (4 mg, 16%, t_R=31 min. Mp 135–136 °C). **8b**. HRMS (TOF) calcd for $C_{11}H_{13}BrN_5O_3$ ($M^+ + H$): 342.0202; found: 342.0218; **8c**. TOF-MS ($M^+ + H$): 342.

4.1.10. 6-*N*-Benzoyl-9-[6′-iodo-5′,6′-dideoxy-2′,3′-*O*-isopropylidene-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (9a). A solution of NIS (70 mg, 0.312 mmol) in CH_2Cl_2/CCl_4 (1:1, 10 mL) was added dropwise to a stirred solution of **7** (150 mg, 0.216 mmol) in CH_2Cl_2/CCl_4 (1:1, 10 mL) at ∼−20 °C. After 1.5 h the slightly pink mixture was poured into saturated $NaHCO_3$/H_2O and extracted by $CHCl_3$, the combined organic phase was washed with 2% $NaHSO_3$/H_2O and brine, dried ($MgSO_4$), and evaporated. Purified on column chromatography (EtOAc/petroleum ether, 1:1) gave **9a** (113 mg, 98%). TOF-MS ($M^+ + H$): 534.

4.1.11. 9-[6′-Iodo-5′,6′-dideoxy-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (9b). Deprotection of **9a** (20 mg, 0.0375 mmol) (as described for **5b**) gave a residue, which was purified by RP-HPLC (preparative column; program: 17% CH_3CN/H_2O for 100 min at 3 mL/min) to give a white solid **9b** (8 mg, 54%, t_R=93 min. Mp 113–114 °C). HRMS (TOF) calcd for $C_{11}H_{13}N_5O_3I$ ($M^+ + H$): 390.0063; found: 390.0076.

4.1.12. 6-*N*-Benzoyl-9-[6′-chloro-5′,6′-dideoxy-2′,3′-*O*-isopropylidene-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (10a). Cl_2 was gently bubbled through a solution of **7** (35 mg, 0.05 mmol) in CH_2Cl_2/CCl_4 (1:1, 2 mL) at ∼−50 °C, and stirring was continued for 5 min. The solution was carefully washed ($NaHCO_3$/H_2O, 2% $NaHSO_3$/H_2O, and brine), dried ($MgSO_4$), and evaporated. Purified on column chromatography [EtOAc/petroleum ether, 1:1] gave **10a** (17 mg, 77%) as a white foam. Mp 80–83 °C. TOF-MS ($M^+ + H$): 512.

4.1.13. 9-[6′-Chloro-5′,6′-dideoxy-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (10b). Deprotection of **10a** (17 mg, 0.0385 mmol) (as described for **5b**) gave a residue which was purified by RP-HPLC (preparative column; program: 17% CH_3CN/H_2O for 60 min at 3 mL/min) to give a white solid **10b** (5 mg, 43%, t_R=54 min. Mp 140–141 °C). HRMS (TOF) calcd for $C_{11}H_{13}N_5O_3Cl$ ($M^+ + H$): 298.0707; found: 298.0690

4.1.14. 6-*N*-Benzoyl-9-[5′,6′-dideoxy-2′,3′-*O*-isopropylidene-β-L-ribo-hex-5′-enofuranosyl] adenosine (11a). A solution of **7** (200 mg, 0.287 mmol) and NH_4F (190 mg,

5.75 mmol) in anhydrous EtOH (15 mL) was refluxed for 19 h and evaporated. The residue was partitioned ($NaHCO_3$/H_2O/$CHCl_3$), and the organic layer was washed (brine), dried ($MgSO_4$), and concentrated. Purified on column chromatography (EtOAc/petroleum ether, 1:1) gave **11a** (116 mg, 99%) as a foam. TOF-MS ($M^+ + H$): 408.

4.1.15. 9-[5′,6′-Dideoxy-β-L-ribo-hex-5′-enofuranosyl] adenosine (11b). Deprotection of **11a** (20 mg, 0.049 mmol) (as described for 5b) gave a residue which purified by a short silica gel column (EtOAc→EtOAc/*i*-PrOH/H_2O, 20:1:2) to give **11b** (7 mg, 54%) as a white solid. Mp 160–161 °C. HRMS (TOF) calcd for $C_{11}H_{14}N_5O_3$ ($M^+ + H$): 264.1097; found: 264.1111

4.1.16. 9-[2′,3′-*O*-Isopropylidene-5′,6′-dideoxy-β-L-ribo-hex-5′-ynofuranosyl] adenosine (12a). A deoxygenated solution of **7** (341 mg, 0.49 mmol) in anhydrous CH_3CN (25 mL) under argon was treated with $Pb(OAc)_4$ (272 mg, 0.61 mmol), and stirred at ~0 °C (ice bath) for 5 h and then for 2 h at ambient temperature, the mixture was evaporated, the residue was partitioned ($NaHCO_3$/H_2O/$CHCl_3$) and the organic phase was washed ($NaHCO_3$/H_2O and brine), dried ($MgSO_4$), and evaporated. Purified on column chromatography (EtOAc/petroleum ether, 2:1) gave **12a** (105 mg, 71%) as a foam. TOF-MS ($M^+ + H$): 406.

4.1.17. 9-[5′,6′-Dideoxy-β-L-ribo-hex-5′-ynofuranosyl] adenosine (12b). Compound **12a** (20 mg, 0.066 mmol) was added to a solution of CF_3COOH/H_2O (9:1, 3 mL), the mixture was stirred for 1 h at ~0 °C and evaporated, co-evaporated with EtOH (2×3 mL), the residue was separated with a short silica gel column (EtOAc→EtOAc/*i*-PrOH/H_2O, 20:1:2). **12b** (8 mg, 46%) was collected as a solid. Mp 114–116 °C. HRMS (TOF) calcd for $C_{11}H_{12}N_5O_3$ ($M^+ + H$): 262.0940; found: 262.0922

4.1.18. 6-*N*-Benzoyl-9-[5′-deoxy-5′-iodo-2′,3′-*O*-isopropylidene-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (13a). To a stirred solution of **3** (233 mg, 0.56 mmol) in anhydrous dioxane (15 mL) containing anhydrous pyridine (0.1 mL, 1.12 mmol), triphenylphosphine (222 mg, 0.84 mmol), I_2 (220 mg, 0.84 mmol) were added and stirring was continued for 20 h at ambient temperature, methanol (1.0 mL) was added, and then the solvents were removed by evaporation. The residue was partitioned (EtOAc/H_2O), and the organic phase was washed (10% $Na_2S_2O_3$ and brine), dried (Na_2SO_4), and evaporated. Purified on column chromatography (EtOAc/petroleum ether, 1:1) gave **13a** (263 mg, 89%) as a foam. TOF-MS ($M^+ + H$): 522.

4.1.19. 9-[5′-Deoxy-5′-iodo-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (13b). Deprotection of **13a** (25 mg, 0.046 mmol) (as described for **5b**) gave a residue that purified by RP-HPLC (preparative column; program: 0–10% CH_3CN/H_2O for 3 min followed by a gradient of 10–40% for 50 min) to give **13b** as a white foam (8 mg, 45%, t_R= 34 min). HRMS (TOF) calcd for $C_{10}H_{13}N_5O_3I$ ($M^+ + H$): 378.0063; found: 378.0071.

4.1.20. 9-[5′,6′-Dideoxy-2′,3′-*O*-isopropylidene-6′-ethoxycarbonyl-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (14). 2′,3′-*O*-Isopropylidene-L-adenosine (500 mg, 1.63 mmol), DCC (1 g, 4.89 mmol) were added to a stirred solution of DMSO (4.5 mL) under argon, $Cl_2CHCOOH$ (78 μl, 0.96 mmol) was added at ~0 °C, and stirring was continued for 2 h at ambient temperature. $Ph_3P{=}CHCO_2Et$ (680 mg, 1.95 mmol) was added and the resulting mixture was stirred overnight. Oxalic acid dihydrate (1.1 g, 8.7 mmol) in MeOH (10 mL) was added, after 30 min the dicyclohexylurea was filtered and the filtrate was evaporated (in vacuo). The residue was partitioned (EtOAc/H_2O), the organic layer was washed with H_2O (3×40 mL), $NaHCO_3$/H_2O, and brine, dried (Na_2SO_4) and the solution was evaporated. Purified on column chromatography (EtOAc/petroleum ether, 2:1) gave **14** (306 mg, 50%) as a white solid. Mp 105–107 °C. HRMS (TOF) calcd for $C_{17}H_{22}N_5O_5$ ($M^+ + H$): 376.1621; found: 376.1608.

4.1.21. 9-[5′,6′-Dideoxy-2′,3′-*O*-isopropylidene-6′-hydroxymethyl-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (15). To **14** (306 mg, 0.816 mmol) in CH_2Cl_2 (10 mL), a 20% solution of DIBAL-H in toluene (5.25 mL, 8.16 mmol) was added drop wise. The mixture was stirred at −78 °C for 2 h, and then quashed with MeOH (10 mL). A saturated aqueous solution of potassium sodium tartrate monohydrate (80 mL) was added, and the resulting suspension was stirred vigorously for 16 h at ambient temperature, then extracted with EtOAc (3×100 mL). The combined organic phase were dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 25:1) to give **15** (180 mg, 66%) as a white foam. HRMS (TOF) calcd for $C_{15}H_{20}N_5O_4$ ($M^+ + H$): 334.1515; found: 334.1538.

4.1.22. 9-[5′,6′-Dideoxy-2′,3′-*O*-isopropylidene-6′-phthalimido-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (16). DEAD (40% wt in toluene, 0.23 mL, 0.51 mmol) was added dropwise to a stirred suspension of **15** (170 mg, 0.51 mmol), phthalimide (75 mg, 0.51 mmol) and Ph_3P (133 mg, 0.51 mmol) in THF (3 mL). After stirring for 3 h at ambient temperature, the solvents were evaporated. Purified on column chromatography gave **16** (160 mg, 67%) as a white solid. Mp 198–200 °C. HRMS (TOF) calcd for $C_{23}H_{23}N_6O_5$ ($M^+ + H$): 463.1730; found: 463.1714.

4.1.23. 9-[5′,6′-Dideoxy-2′,3′-*O*-isopropylidene-6′-aminomethyl-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (17a). To phthalimide **16** (150 mg, 0.32 mmol), a solution of 30% $MeNH_2$ in EtOH (20 mL) was added and the mixture was stirred at 20 °C for 24 h. After evaporation in vacuo, the residue was separated with a silica gel column (CH_2Cl_2/MeOH, 35:1), compound **17** (101 mg, 93%) was collected as a colorless oil. HRMS (TOF) calcd for $C_{15}H_{21}N_6O_3$ ($M^+ + H$): 333.1675; found: 333.1693.

4.1.24. 9-[5′,6′-Dideoxy-6′-aminomethyl-β-L-ribo-hex-5′(*E*)-enofuranosyl] adenosine (17b). A solution of **17a** (28 mg, 0.084 mmol) in TFA-H_2O (5:2, 1.5 mL), was stirred at 20 °C for 2 h and then evaporated to dryness. The residue was dissolved with H_2O and purified by RP-HPLC (preparative column; program: a gradient of 0–10% CH_3CN/H_2O for 30 min at 3 mL/min) to give **17b** (20 mg, 81%, t_R=22 min) as a colorless foam. HRMS (TOF) calcd for $C_{12}H_{17}N_6O_3$ ($M^+ + H$): 293.1362; found: 293.1381.

4.1.25. [5-(*R*)-Dimethoxymethyl-4-(*R*)-benzoyloxy-3(*S*)-(adenine-9′-yl)]-tetrahydrofuran (19). A stirred solution of compound **18** (2.3 g, 8.0 mmol) in anhydrous pyridine (50 mL), benzoyl chloride (1.0 mL, 8.8 mmol) was added dropwise at 0 °C and stirring was continued for 3 h at 0 °C. The solvents were evaporated and the residue was partitioned (saturated $NaHCO_3/H_2O$/EtOAc), the organic layer was washed with brine, dried (Na_2SO_4) and evaporated. Purified on column chromatography (EtOAc/petroleum ether, 2:1) gave **19** (2.1 g, 66%) as a colorless foam, and recovered **18** (0.56 g, 23%). HRMS (TOF) calcd for $C_{19}H_{22}N_5O_5$ ($M^+ + H$): 400.1621; found: 400.1618.

4.1.26. [5-(*S*)-Hydroxymethyl-4-(*R*)-benzoyloxy-3(*S*)-(adenine-9′-yl)]-tetrahydrofuran (20). Compound **19** (1.2 g, 3.0 mmol) was added to a mixed solution of THF (12 mL) and 1% HCl (12 mL). The resulting solution was stirred for 8 h at 90 °C, 2 N $NaOH/H_2O$ was used to neutralize the solution to pH = 7, $NaBH_4$ (370 mg, 9.2 mmol) was added and stirred at ambient temperature for 1 h. After neutralization with 1 N HCl/H_2O, the solution was evaporated to dryness and the residue was purified on silica gel column to give compound **20** (851 mg, 80%) as a colorless foam. HRMS (TOF) calcd for $C_{17}H_{18}N_5O_4$ ($M^+ + H$): 356.1359; found: 356.1349.

4.1.27. {5-(*S*)-[2(*E*)-*p*-Toluenesulfonylethylene]-4-(*R*)-benzoyloxy-3(*S*)-(adenine-9′-yl)}-tetrahydrofuran (21). A stirred solution of compound **20** (476 mg, 1.3 mmol), DCC (1.08 g, 5.9 mmol) in DMSO (3.5 mL), $Cl_2CHCOOH$ (66 μl, 0.7 mmol) was added dropwise at 0 °C (ice bath), stirring was continued for 2 h at ambient temperature, [(*p*-tolylsulfonyl)methylene]triphenyl phosphorane (900 mg, 7.2 mmol) was added and stirred overnight. MeOH (8 mL) was added and stirred for 30 min at room temperature, the resulted dicyclohexylurea was filtered and washed with cold MeOH, and the combined filtrates were evaporated (in vacuo). The residue was partitioned (EtOAc/H_2O), the organic layer was washed with H_2O (3×30 mL), $NaHCO_3$/H_2O, and brine, dried ($MgSO_4$) and evaporated. Purified on column chromatography gave **21** (350 mg, 51%) as a white solid. Mp 136–138 °C. HRMS (TOF) calcd for $C_{25}H_{24}N_5O_5S$ ($M^+ + H$): 506.1498; found: 506.1484.

4.1.28. {5-(*S*)-[2(*E*)-Tributylstannylvinyl]-4-(*R*)-benzoyloxy-3(*S*)-(adenine-9′-yl)}-tetrahydrofuran (22). A solution of **21** (320 mg, 0.6 mmol) in toluene (50 mL) was deoxygenated (argon, 30 min), and Bu_3SnH (0.67 mL, 2.2 mmol) was added. Deoxygenation was continued for 15 min, and AIBN (40 mg, 0.2 mmol) was added. The solution was refluxed for 6 h and evaporated, and the residue was purified by column chromatograph (EtOAc/petroleum ether, 2:1). The elution gave **22** (219 mg, 54%) as a colorless oil, and recovered **21** (85 mg, 26%). HRMS (TOF) calcd for $C_{30}H_{44}N_5O_3Sn$ ($M^+ + H$): 642.2466; found: 642.2449.

4.1.29. [5-(*S*)-(2-Vinyl)-4-(*R*)-hydroxy-3(*S*)-(adenine-9′-yl)]-tetrahydrofuran (23). A solution of compound **22** (76 mg, 0.12 mmol) in dried EtOH (10 mL), NH_4F (200 mg, 6.0 mmol), was added, the resulting solution was refluxed for 3 days, and then evaporated. The residue was purified by silica gel column (EtOAc/petroleum ether, 3:1) to give protected **23**. The product was dissolved in CH_2Cl_2 (10 mL) and catalytic amount of 30% NaOMe/MeOH was added under stirring. After stirred at room temperature for 1 h, the mixture was evaporated and purified by a silica gel column, to give **23** (9 mg, 68%) as a white solid. Mp 179–181 °C. HRMS (TOF) calcd for $C_{11}H_{14}N_5O_2$ ($M^+ + H$): 248.1147; found: 248.1152.

4.1.30. [5-(*S*)-(2-Ethynyl)-4-(*R*)-hydroxy-3(*S*)-(adenine-9′-yl)]-tetrahydrofuran (24). The procedure of reaction is the same as the synthesis for **12a**, but $Pb(OAc)_4$ (4 equiv to compound **22**) was added and stirring was continued for 3 days at ambient temperature. The procedure gave the residue no further purification for the removal of the benzoyl group. The procedure of deprotection was described as compound **23** that gave **24** (18 mg, 67%) as a colorless foam. HRMS (TOF) calcd for $C_{11}H_{12}N_5O_2$ ($M^+ + H$): 246.0991; found: 246.1005.

4.1.31. [5-(*S*)-Dimethoxymethyl-4-(*S*)-benzoyloxy-3(*R*)-(adenine-9′-yl)]-tetrahydrofuran (26). Similar to the synthesis of **19**, compound **26** was obtained in a yield of 85% as a white solid. Mp 183–185 °C.

4.1.32. [5-(*R*)-Hydroxymethyl-4-(*S*)-benzoyloxy-3(*R*)-(adenine-9′-yl)]-tetrahydrofuran (27). Similar to the synthesis of **20**, compound **27** was obtained in a yield of 70% as a colorless oil.

4.1.33. {5-(*R*)-[2(*E*)-*p*-Toluenesulfonylethylene]-4-(*S*)-benzoyloxy-3(*R*)-(adenine-9′-yl)}-tetrahydrofuran (28). Similar to the synthesis of **21**, compound **28** was obtained in a yield of 54% as a white solid. Mp 141–143 °C. HRMS (TOF) calcd for $C_{25}H_{24}N_5O_5S$ ($M^+ + H$): 506.1498; found: 506.1485.

4.1.34. {5-(*R*)-[2(*E*)-tributylstannylvinyl]-4-(*S*)-benzoyloxy-3(*R*)-(adenine-9′-yl)}-tetrahydrofuran (29). Similar to the synthesis of **22**, compound **29** was obtained in a yield of 63% as a white foam, with recovered **28** (29%). HRMS (TOF) calcd for $C_{30}H_{44}N_5O_3Sn$ ($M^+ + H$): 642.2466; found: 642.2427.

4.1.35. [5-(*R*)-(2-Vinyl)-4-(*S*)-hydroxy-3(*R*)-(adenine-9′-yl)]-tetrahydrofuran (30). Similar to the synthesis of **23**, compound **30** was obtained in a yield of 41% as a white solid. Mp 172–174 °C. HRMS (TOF) calcd for $C_{11}H_{14}N_5O_2$ ($M^+ + H$): 248.1147; found: 248.1143.

4.1.36. [5-(*R*)-(2-Ethynyl)-4-(*S*)-hydroxy-3(*R*)-(adenine-9′-yl)]-tetrahydrofuran (31). Similar to the synthesis of **24**, compound **31** was obtained in a yield of 64% as a white foam. HRMS (TOF) calcd for $C_{11}H_{12}N_5O_2$ ($M^+ + H$): 246.0991; found: 246.1002.

4.2. Purification of AdoHcy hydrolase and evaluation of the effectiveness of synthetic compounds

Recombination human placental AdoHcy hydrolase was purified from cell free extracts of *Escherichia coli* transformed with plasmid pPROK-19.[32] To evaluate the inhibitory potential of the compounds, different concentrations (0.01–100 μM) were preincubated with 10 μL (10 μg)

enzyme at 37 °C for 10 min at pH 7.4 in 240 μL PBE buffer. The mixture was then incubated with 50 μL Ado (10 mM) and 200 μL Hcy (10 mM) at 37 °C for 10 min. This reaction was terminated by addition of 20 μL sulfuric acid, and the mixture was cooled with ice bath. After centrifugalization (10,000 rpm for 5 min), the AdoHcy formed was analyzed by HPLC on a C-18 reversed-phase column (Diamonsil™; 250×4.6 mm). Elution was performed with a gradient: (94%A: 6% B, 1 mL/min) over 10 min. (mobile phase A was 0.1% CF_3COOH and mobile phase B was acetonitrile). Quantitative analysis of AdoHcy was monitored (UV) at 258 nm.

4.3. Assays for the inhibition on the growth of various tumor cells

Growth inhibition of the synthetic compounds to various tumor cells were determined by MTT and SRB assays.[34,35] Briefly, tumor cells ($1–2.5\times10^4$ cells mL^{-1}) were inoculated in 96-well culture plates (180 μL/well). After 24 h culture, 20 μL of culture medium containing synthetic compound of various concentrations were added to the wells, and RPMI-1640 medium in control cells, then the cells were incubated for 48 h. The growth inhibition of HL-60 cells were determined by MTT method, and the other cell lines were assayed by SRB method. The absorbance of each well was measured using a microculture plate reader at 570 nm (MTT) and 540 nm (SRB).

Acknowledgements

We thank National Natural Science Foundation of China for financial support (20132030, 20272032, 20332010).

References

1. Konaraka, M. M.; Padgett, R. A.; Sharp, P. A. *Cell* **1984**, *38*, 731–736.
2. Banerjee, A. K. *Microbiol. Rev.* **1980**, *44*, 175–205.
3. Walfe, M. S.; Borchardt, R. T. *J. Med. Chem.* **1991**, *34*, 1521–1530.
4. Borchardt, R. T. *J. Med. Chem.* **1980**, *23*, 347–357.
5. Yuan, C.-S.; Liu, S.; Wnuk, S. F.; Robins, M. J.; De Clercq, E., Ed.; *Advances in Antiviral Drug Design*; Vol. 2. JAI: Greenwich; 1996. p 41–88.
6. Cools, M.; De Clercq, E. *Biochem. Pharmacol.* **1989**, *38*, 1061–1067.
7. Palmer, J. L.; Abeles, R. H. *J. Biol. Chem.* **1979**, *254*, 1217–1225.
8. Narayanan, S. R.; Borchardt, R. T. *Biochim. Biophys. Acta* **1988**, *965*, 22–28.
9. Wnuk, S. F.; Mao, Y.; Yuan, C.-S.; Borchardt, R. T.; Andrei, G.; Balzarini, J.; De Clercq, E.; Robin, M. J. *J. Med. Chem.* **1998**, *41*, 3078–3083.
10. Yuan, C.-S.; Liu, S.; Wnuk, S. F.; Robin, M. J.; Borchardt, R. T. *Biochemistry* **1994**, *33*, 3758–3765.
11. Yuan, C.-S.; Wnuk, S. F.; Liu, S.; Robin, M. J.; Borchardt, R. T. *Biochemistry* **1994**, *33*, 12305–12311.
12. Liu, S.; Wnuk, S. F.; Yuan, C.-S.; Robins, M. J.; Borchardt, R. T. *J. Med. Chem.* **1993**, *36*, 883–887.
13. Wnuk, S. F.; Dalley, N. K.; Robins, M. J. *J. Org. Chem.* **1993**, *58*, 111–117.
14. Wnuk, S. F.; Yuan, C.-S.; Borchardt, R. T.; Balzarini, J.; De Clercq, E.; Robins, M. J. *J. Med. Chem.* **1994**, *37*, 3579–3587.
15. Wnuk, S. F.; Ro, B.-O.; Valdez, C. A.; Lewandowska, E.; Valdez, N. X.; Sacasa, P. R.; Yin, D.; Zhang, J.; Borchardt, R. T.; De Clercq, E. *J. Med. Chem.* **2002**, *45*, 2651–2658.
16. Coates, J. A. V.; Cammack, N.; Jenkinson, H. J.; et al *Antimicrob. Agents Chemother.* **1992**, *36*, 202–205.
17. Wang, P.; Hong, J. H.; Cooperwood, J. S.; Chu, C. K. *Antiviral Res.* **1998**, *40*, 19–44.
18. Gumina, G.; Song, G.-Y.; Chu, C. K. *FEMS Microbiol. Lett.* **2001**, *202*, 9–15.
19. Zhu, W.; Gumina, G.; Schinazi, R. F.; Chu, C. K. *Tetrahedron* **2003**, *59*, 6423–6431.
20. Choo, H.; Chong, Y.; Choi, Y.; Mathew, J.; Schinazi, R.; Chu, C. K. *J. Med. Chem.* **2003**, *46*, 389–398.
21. Mugnaini, C.; Botta, M.; Coletta, M.; Corelli, F.; Focher, F.; Marini, S.; Renzulli, M. L.; Verri, A. *Bioorg. Med. Chem.* **2003**, *11*, 357–366.
22. Yu, H. W.; Zhang, L. R.; Ma, L. T.; Zhang, L. H. *Bioorg. Med. Chem.* **1996**, *4*, 609–614.
23. Yang, Z. J.; Yu, H. W.; Min, J. M.; Ma, L. T.; Zhang, L. H. *Tetrahedron: Asymmetry* **1997**, *8*, 2739–2747.
24. Lei, Z.; Min, J. M.; Zhang, L. H. *Tetrahedron: Asymmetry* **2000**, *11*, 2899–2906.
25. Tian, X. B.; Min, J. M.; Zhang, L. H. *Tetrahedron: Asymmetry* **2000**, *11*, 1877–1889.
26. Robins, M. J.; Zou, R. M.; Guo, Z. Q.; Wnuk, S. F. *J. Org. Chem.* **1996**, *61*, 9207–9212.
27. Ziegler, W. M.; Connor, R. *J. Am. Chem. Soc.* **1940**, *62*, 2596–2599.
28. Van Leusen, A. M.; Reith, B. A. *Recueil* **1972**, *91*, 37–49.
29. Watanabe, Y.; Ueno, Y.; Araki, T.; Endo, T.; Okawara, M. *Tetrahedron Lett.* **1986**, *27*, 215–218.
30. Ma, T. W.; Pai, S. B.; Zhu, Y.-L. *J. Med. Chem.* **1996**, *39*, 2835–2843.
31. Hu, Y.; Komoto, J.; Huang, Y. *Biochemisty* **1999**, *38*, 8323–8333.
32. Yuan, C.-S.; Yeh, J.; Liu, S.; Borchart, R. T. *J. Biol. Chem.* **1993**, *268*, 17030–17037.
33. Parry, R. J.; Muscate, A.; Askonas, L. J. *Biochemistry* **1991**, *30*, 9988–9997.
34. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, T.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer* **1990**, *82*, 1107–1112.
35. Carmichael, J.; DeGraff Gazdar, A. F. *Cancer Res.* **1987**, *47*, 936–942.
36. 6′(*E*)-bromovinyl D-adenosine (**D–B**) was synthesized and identified using the procedure published in *J. Med. Chem.* **1994**, *37*, 3579–3587.