Synthesis and Anti-HIV Activity of [ddN]-[ddN] Dimers and Benzimidazole Nucleoside Dimers

by **Guo-Rui Li**[a])[1]), **Jun Liu**[b])[1]), **Qin Pan**[b])[1]), **Zhi-Bin Song**[a]), **Feng-Ling Luo**[b]), **Shao-Ru Wang**[a]), **Xiao-Lian Zhang***[b]), and **Xiang Zhou***[a])

[a]) College of Chemistry and Molecular Sciences, Wuhan University, Hubei, Wuhan, 430072, P. R. China (phone: +86-27-61056559; fax: +86-27-87336380; e-mail: xzhou@whu.edu.cn)

[b]) State Key Laboratory of Virology, Department of Immunology, Hubei Province Key Laboratory of Allergy and Immunology, Wuhan University School of Medicine, Wuhan, 430071, P. R. China (phone: +86-27-61056559; fax: +86-27-87336380; e-mail: zhangxl65@whu.edu.cn)

In an attempt to combine the HIV-inhibitory capacity of different 2′,3′-dideoxynucleoside (ddN) analogs, we have designed and synthesized several dimers of [AZT]-[AZT] and [AZT]-[d4T]. In addition, we also synthesized the dimers of 1-(1*H*-benzimidazol-1-yl)-1-deoxy-β-D-ribofuranose. The *in vitro* anti-HIV activity of these compounds on a pseudotype virus, pNL4-3.Luc.R-E-, in the 293T cells has been determined. Among these compounds, 2,2′-(propane-1,3-diyl)bis[1-(β-D-ribofuranosyl)-1*H*-benzimidazole] (**3**) showed the highest anti-HIV activity with similar effect as AZT.

Introduction. – Since the human immunodeficiency virus (HIV) was identified as the etiological agent for AIDS in 1983 [1], many anti-HIV drugs have been discovered and evaluated based on different targets, including nucleoside reverse-transcriptase inhibitors (NRTIs), non-nucleoside reverse-transcriptase inhibitors (NNRTIs), protease inhibitors, entry inhibitors, and so on [2]. Among them, 2′,3′-dideoxynucleosides (ddN) such as 3′-azido-2′,3′-dideoxythymidine (=1-(3-azido-2,3-dideoxypentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione; AZT; *Fig. 1*) [3], 2′,3′-didehydro-2′,3′-dideoxythymidine (=1-[5-hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione; d4T; *Fig. 1*) [4] have been found to be effective in the treatment of HIV-infected patients and are the early drugs approved by the FDA [5]. Unfortunately, drug resistance gradually emerges to these nucleoside based inhibitors (nucleoside reverse-transcriptase inhibitors, NRTIs) [6], and unexpected side effects become increasingly obvious [7]. So, there is still continued interest in developing novel strategies and new drugs for the treatment of HIV/AIDS.

A combination of anti-HIV agents is now being one kind of clinical application as therapeutic modalities to prevent emergence of virus-drug resistance [8]. *Camarasa* and co-workers [9–11] have reported the synthesis and anti-HIV activity of a series of heterodimers which combine an NRTI analogue and an NNRTI (non-nucleoside reverse-transcriptase inhibitor) through appropriate spacers. In their studies, the nature of the spacer and the position of the linker on the NRTI had little effect on the antiviral activity. In contrast, the nature of the NRTI was shown to be important for the anti-

[1]) These authors contributed equally to this work.

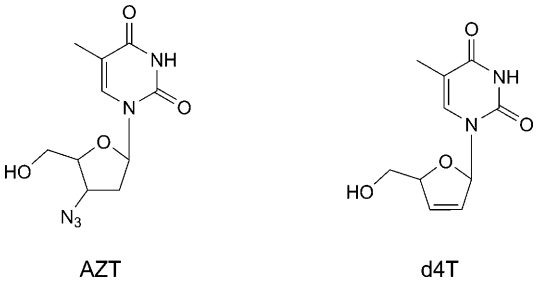


Fig. 1. *Structures of AZT and d4T*

HIV activities [11]. In order to fulfill the work in this field and evaluate the synergistic effect of different 2′,3′-dideoxynucleoside (ddN) analogues, we have designed and synthesized several dimers of the general formula [ddN]-spacer-[ddN] (*Fig. 2*). As ddN inhibitors, we chose AZT and d4T (the drugs used in the clinical treatment of AIDS). The ddN inhibitors were linked at the N(3) of the thymidine base of each compound by an appropriate spacer. We also performed several modifications in the spacer to study the effect of spacers on anti-HIV activity.

The derivatives of benzimidazole nucleoside have also been synthesized and exhibit antiviral activities [12–14]. In this article, we describe the synthesis of dimers of benzimidazole nucleoside (*Fig. 2*) for the first time and evaluated their anti-HIV activities *in vitro*.

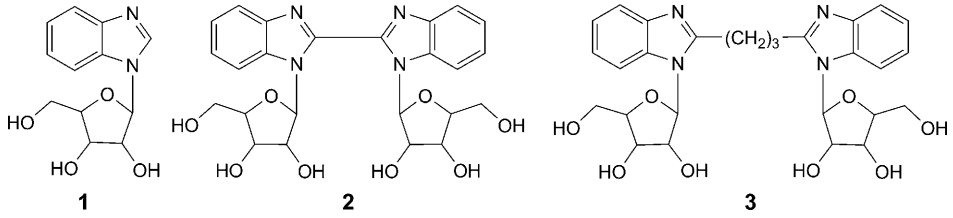
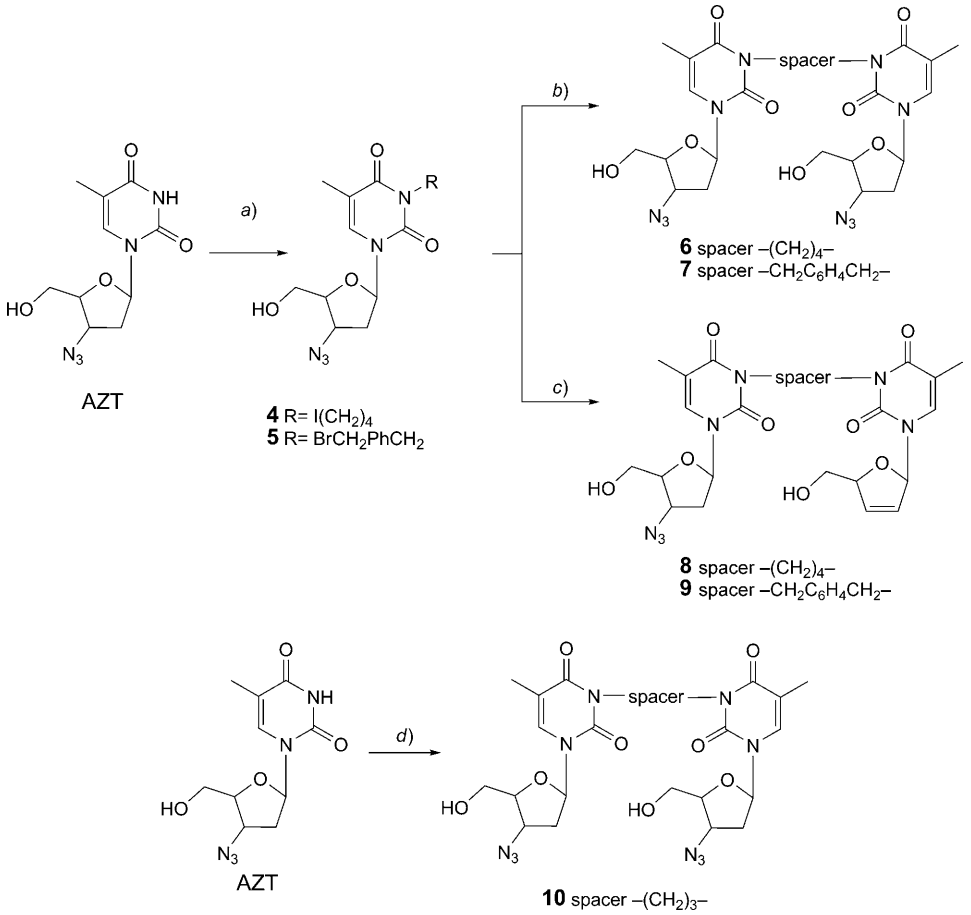


Fig. 2. *Structures of 1′-deoxy-1′-(benzimidazol-1-yl)-β-*D*-ribofuranose and its dimers*

Results and Discussion. – 1. *Chemistry.* The [ddN]-[ddN] dimers were synthesized based on the method reported in [11], shown in *Scheme 1*. In this method, firstly AZT was treated with the appropriate diiodoalkyl or dibromoalkyl reagent to yield the nucleoside intermediate **4** and **5**, then the nucleoside intermediate reacted with the ddN (AZT, d4T) to give the N(3),N(3)-alkyl dimers **6**–**9**. For the synthesis of [AZT]-[AZT] dimer, we also tried a one-step method. Using this method, we synthesized compound **10** in moderate yield.

The monomer 1-(benzimidazol-1-yl)-1-deoxy-β-D-ribofuranose was synthesized according to the literature [15]. The compounds **2** and **3** were conveniently synthesized in three steps (*Scheme 2*). The bis[benzimidazoles] **11** and **12** were synthesized by the acid-catalyzed condensation of *o*-phenylenediamine with an appropriate biscarboxylic acid. The synthesis of protected bisbenzimidazole nucleosides followed the glycosylation procedure described in [15]. Refluxing bisbenzimidazoles with 2 equiv. of *N*,*O*-

Scheme 1



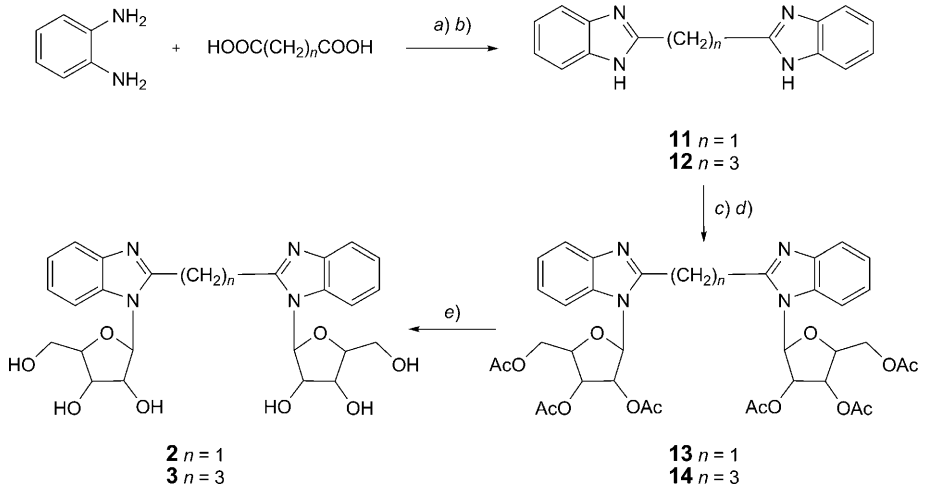
a) K_2CO_3, acetone, $(CH_2)_4I_2$ for **4**, or K_2CO_3, acetone/DMF (1:1), $BrCH_2PhCH_2Br$ for **5**, reflux. *b*) AZT, K_2CO_3, MeCN, reflux. *c*) d4T, K_2CO_3, MeCN, reflux. *d*) K_2CO_3, acetone, $(CH_2)_3Br_2$, reflux.

bis(trimethylsilyl)acetamide and subsequent reaction of the persilylated base with 2 equiv. of 1,2,3,5-tetra-*O*-acetyl-*β*-D-ribofuranose in the presence of the *Lewis* acid trimethylsilyl trifluoromethanesulfonate afforded the desired compound **13** and **14**. Deprotection of the acetylated nucleoside furnished the nucleosides **2** and **3**.

The structures of all the target compounds were identified by ^{1}H-NMR, ^{13}C-NMR, and high-resolution mass spectroscopy (HR-MS).

2. *Anti-HIV Activity.* The anti-HIV activity of these compounds was tested using a pseudotype virus, pNL4-3.Luc.R-E-, in HEK 293T cells. The HIV-1 recombinant clone has been widely investigated by biologists [16–18], and recently has also been used by medicinal chemists for exploration of anti-HIV drugs [19].

Scheme 2



a) 4N HCl, 135°. *b*) Conc. NH_4OH. *c*) *N*,*O*-bis(trimethylsilyl)acetamide (BSA), MeCN. *d*) 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (TAR), trimethylsilyl trifluoromethanesulfonate (TMSOTf), MeCN. *e*) MeONa, MeOH.

In our study, an HIV pseudotype virus was produced by transfection of 293T cells with plasmids pNL4-3.Luc.R-E- containing the env deficient HIV proviral genome and an intact luciferase gene, and cotransfected with pGL4.73 [hRluc/SV40] as internal control reporters. The replications of the HIV pseudotype in 293T cells were measured through normalizing the transfection efficacy with luciferase and Renilla luciferase. The relative luciferase activities represented the viral replication efficacy of HIV pseudotype. To detect the effects of the different compounds on the HIV replication, different compounds (with the same concentration 65 μM) were added to each HIV proviral genome transfected cells, respectively. The preliminary results were given. As shown in *Fig. 3*, for the [ddN]-[ddN] dimers, they all had some inhibition effects on the HIV replications comparing with the control group without drugs but are less potent than their parent compounds AZT and d4T (the result is consistent with the literature [20]). What interests us most is, for the benzimidazole nucleosides, although the monomer **1** and the dimer **2** showed 37% inhibition effect, the dimer **3**, only different in the length of the linker with **2**, showed a *ca.* 60% inhibitory effect. It had a higher activity than d4T and can inhibit the HIV replication almost at the same level as AZT.

To validate these results, we selected several compounds for further study. We measured the expression of HIV p24 by ELISA using anti-p24 monoclonal antibody. As shown in *Fig. 4*, for the [ddN]-[ddN] dimer **7** and **9**, the expression of HIV p24 was higher than AZT and d4T. And the benzimidazole nucleoside dimer **3** showed lower expression than d4T. The results were consistent with that of relative luciferase activities.

In conclusion, we have synthesized the [ddN]-[ddN] dimers and several benzimidazole nucleosides and tested their anti-HIV activity. The benzimidazole nucleoside dimer **3** showed potent anti-HIV activity and has the potential as a new kind of anti-

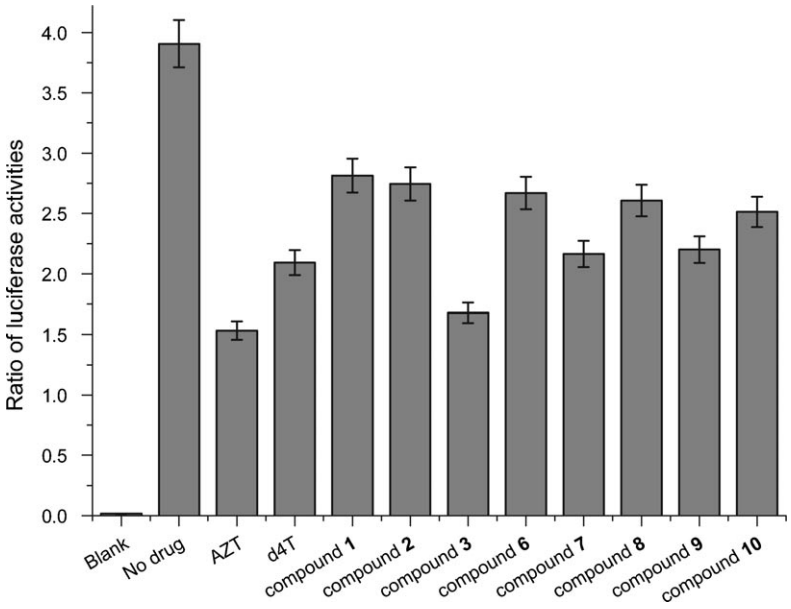


Fig. 3. *Relationship between drugs and luciferase activity of the pseudotype reporter viruses.* Blank refers to no virus inoculated. The experiments have been repeated three times. Data are means of triplicate wells of one representative experiment and the error bars represent the standard errors of the means.

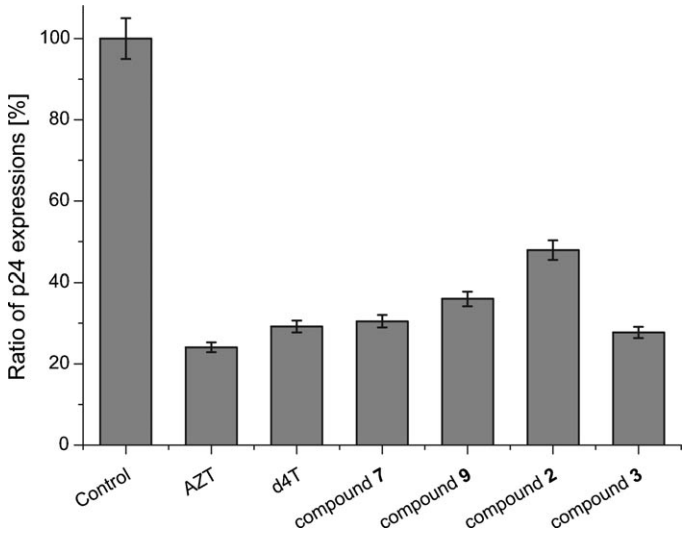


Fig. 4. *Relationship between drugs and p24 expression*. Control refers to no drug added. P24 Expression of control was arbitrarily assigned a value of 100%. Ratio of p24 expression was compared with that of control. The experiments have been repeated three times. Data are means of triplicate wells of one representative experiment and the error bars represent the standard errors of the means.

HIV agent. Further structure modification on compound **3** and an in-depth study on the HIV replication inhibitory activity of it are currently in progress.

Experimental Part

General. AZT and d4T were purchased from *Tianzun Co.* (P. R. China). All other chemicals and solvents were commercially available. Anh. solvents (acetone, DMF, MeCN) were prepared by standard methods. NMR Spectra: *Varian Mercury-VX300* spectrometer, at 300 and 75 MHz, resp. MS: *Bruker Daltonics APE XII 47e* and *VG-707VHF* mass spectrometer.

General Procedure for Preparation of **4** *and* **5**. To a soln. of the nucleoside AZT in acetone was added K_2CO_3 and $(CH_2)_4I_2$ or $BrCH_2C_6H_4CH_2Br$ (2 equiv.). The mixture was refluxed for 20 h. After evaporation of the solvent, the residue was dissolved in AcOEt, washed with H_2O, dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified by silica gel (SiO_2) column chromatography (CC) using a mixture of $CHCl_3$/MeOH (30 : 1, *v*/*v*) as the eluent to afford the compounds **4** and **5**.

3′-Azido-3-N-*(4-iodobutyl)-3′-deoxythymidine* (=*1-(3-Azido-2,3-dideoxy-β*-erythro-*pentofuranosyl)-3-(4-iodobutyl)-5-methylpyrimidine-2,4(1*H,*3*H*)-dione*; **4**). Yield: 650 mg (77%). Yellow syrup. ^{1}H-NMR ($CDCl_3$): 7.35 (*s*, H−C(6)); 6.04 (*t*, *J* = 6.0, H−C(1′)); 4.39 (*m*, H−C(3′)); 4.00–3.77 (*m*, CH_2(5′), H−C(4′), HO−C(5′), CH_2N); 3.20 (*t*, *J* = 6.2, CH_2I); 2.59–2.35 (*m*, CH_2(2′));1.92 (*s*, Me−C(5)); 1.88–1.83 (*m*, CH_2); 1.74 (*m*, CH_2). ESI-HR-MS: 472.0452 ($[M+Na]^+$, $C_{14}H_{20}IN_5NaO_4^+$; calc. 472.0458).

3′-Azido-3-N-*(α-bromo*-p-*xylenyl)-3′-deoxythymidine* (=*1-(3-Azido-2,3-dideoxy-β*-erythro-*pentofuranosyl)-3-[4-(bromomethyl)benzyl]-5-methylpyrimidine-2,4(1*H,*3*H*)-dione*; **5**). Yield: 107 mg (24%). White syrup. ^{1}H-NMR ($CDCl_3$): 7.46 (*d*, *J* = 8.1, 2 arom. H); 7.36 (*s*, H−C(6)); 7.32 (*d*, *J* = 8.1, 2 arom. H); 6.07 (*t*, *J* = 6.6, H−C(1′)); 5.09 (*s*, $PhCH_2$); 4.46 (*s*, CH_2I); 4.40–4.38 (*m*, H−C(3′)); 3.96–3.80 (*m*, CH_2(5′), H−C(4)); 2.64–2.41 (*m*, CH_2(2′), HO−C(5′)); 1.93 (*s*, Me−C(5)).

General Procedure for Preparation of **6**–**9**. To a soln. of the nucleoside intermediates **4** and **5** in dry MeCN was added K_2CO_3 and ddN (AZT, d4T). The mixture was refluxed for 26 h. After evaporation of the solvent, the residue was dissolved in AcOEt, washed with H_2O, dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified by SiO_2 CC using a mixture of $CHCl_3$/MeOH 20 : 1 as the eluent.

[AZT]N³-(CH₂)₄-N³[AZT] (**6**). Yield: 514 mg (87%). White solid. ^{1}H-NMR ($CDCl_3$): 7.28 (*s*, CH_2(6)); 6.01 (*t*, *J* = 6.5, CH_2(1′)); 4.45–4.39 (*m*, CH_2(3′)); 4.01–3.78 (*m*, CH_2(5′), H−C(4), CH_2N); 2.66–2.38 (*m*, 2 CH_2(2′)); 1.93 (*s*, 2 Me−C(5)); 1.68 (br., CH_2CH_2). ^{13}C-NMR ($CDCl_3$): 163.5 (C=O); 151.0 (C=O); 135.1 (C(6)); 110.7 (C(5)); 87.9 (C(1′)); 84.7 (C(4′)); 62.2 (C(5′)); 60.1 (C(3′)); 41.3 (CH_2N); 37.4 (C(2′)); 25.3 (CH_2); 13.6 (*Me*−C(5)). ESI-HR-MS: 611.2297 ($[M+Na]^+$, $C_{24}H_{32}N_{10}NaO_8^+$; calc. 611.2302).

[AZT]N³-CH₂C₆H₄CH₂-N³[AZT] (**7**). Yield: 100 mg (71%). White solid. ^{1}H-NMR ($CDCl_3$): 7.38 (*s*, 4 arom. H); 7.30 (*s*, CH_2(6)); 6.03 (*t*, *J* = 6.3, CH_2(1′)); 5.06 (*s*, 2 $C_6H_4CH_2$); 4.37 (*m*, CH_2(3′)); 3.99–3.79 (*m*, 2 CH_2(5′), 2 H−C(4)); 2.53–2.38 (*m*, 2 CH_2(2′), 2 HO−C(5′)); 1.91 (*s*, 2 Me−C(5)). ^{13}C-NMR ($CDCl_3$): 163.0 (C=O); 150.6 (C=O); 135.8 (arom. C); 134.5 (C(6)); 129.0 (arom. C); 110.3 (C(5)); 87.1 (C(1′)); 84.2 (C(4′)); 61.7 (C(5′)); 59.6 (C(3′)); 43.9 (CH_2N); 37.1 (C(2′)); 13.1 (*Me*−C(5)). ESI-HR-MS: 659.2297 ($[M+Na]^+$, $C_{28}H_{32}N_{10}NaO_8^+$; calc. 659.2302).

[AZT]N³-(CH₂)₄-N³[d4T] (**8**). Yield: 159 mg (86%). White solid. ^{1}H-NMR ($CDCl_3$): 7.36 (*s*, H−C(6)-d4T); 7.27 (*s*, H−C(6)-AZT); 7.02 (br., H−C(1′)-d4T); 6.32 (*d*, *J* = 6.0, H−C(3′)-d4T); 5.98 (*t*, *J* = 6.3, H−C(1′)-AZT); 5.87 (*d*, *J* = 5.4, H−C(2′)-d4T); 4.91 (br., H−C(4)-d4T); 4.43–4.39 (*m*, H−C(3′)-AZT); 3.99–3.77 (*m*, 2 CH_2(5′), H−C(4)-AZT, 2 CH_2N); 2.65–2.37 (*m*, CH_2(2′)-AZT); 1.92 (*s*, Me−C(5)); 1.89 (*s*, Me−C(5)); 1.68 (*m*, CH_2CH_2). ^{13}C-NMR ($CDCl_3$): 163.9, 151.7, 150.9 (C=O); 135.0 (C(6)); 126.6 (C(2′)); 110.3 (C(5)); 90.8 (C(3′)); 87.3 (C(1′)); 84.7 (C(4′)); 63.6, 61.9 (C(5′)); 60.1 (C(3′)); 41.2 (CH_2N); 37.6 (C(2′)-AZT); 25.2 (CH_2); 13.4 (*Me*−C(5)). ESI-HR-MS: 568.2126 ($[M+Na]^+$, $C_{24}H_{31}N_7NaO_8^+$; calc. 568.2132).

[AZT]N³-CH₂C₆H₄CH₂-N³[d4T] (**9**). Yield: 165 mg (77%). White solid. ^{1}H-NMR ($CDCl_3$): 7.38 (*s*, 4 arom. H); 7.30 (*s*, CH_2(6)); 7.02 (br, H−C(1′)-d4T); 6.29 (*d*, *J* = 6.0, H−C(3′)-d4T); 6.03 (*t*, *J* = 6.3,

CH_2(1′)-AZT); 5.83 (*d*, *J* = 5.7, H−C(2′)-d4T); 5.06 (*s*, 2 CH_2N); 4.37 (*m*, CH_2(3′)-AZT); 3.99–3.79 (*m*, 2 CH_2(5′), 2 H−C(4)-AZT); 2.53–2.38 (*m*, CH_2(2′)-AZT); 1.90 (*s*, Me−C(5)); 1.87 (*s*, Me−C(5)). ^{13}C-NMR ($CDCl_3$): 163.0, 150.6 (C=O); 135.8 (C(6)); 134.5 (C(6)); 129.0 (C(2′)); 110.3 (C(5)); 87.1 (C(1′)); 84.2 (C(4′)); 61.7 (C(5′)); 59.6 (C(3′)); 43.9 (CH_2N); 37.1 (C(2′)-AZT); 13.1 (*Me*−C(5)). ESI-HR-MS: 659.2297 ($[M+Na]^+$, $C_{28}H_{32}N_{10}NaO_8^+$; calc. 659.2302).

General Procedure for Preparation of [AZT]N³-(CH₂)₃-N³[AZT] (**10**). To a soln. of the nucleoside AZT (267 mg, 1 mmol) in acetone (50 ml) was added K_2CO_3 (690 mg, 5 mmol) and $(CH_2)_3Br_2$ (110 mg, 0.5 mmol). The mixture was refluxed for 72 h. After evaporation of the solvent, the residue was dissolved in AcOEt (100 ml), washed with H_2O (3 × 20 ml), dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by SiO_2 CC using a mixture of $CHCl_3$/MeOH 20 : 1 as the eluent to afford **10** (200 mg, 70%). White syrup. ^{1}H-NMR ($CDCl_3$): 7.49 (*s*, CH_2(6)); 6.06 (*t*, *J* = 6.2, CH_2(1′)); 4.38–4.32 (*m*, CH_2(3′)); 3.95–3.74 (*m*, 2 CH_2(5′), 2 H−C(4), 2 CH_2N); 2.50–2.33 (*m*, 2 CH_2(2′)); 1.92 (*m*, CH_2); 1.84 (*s*, 2 Me−C(5)). ^{13}C-NMR ($CDCl_3$): 163.6 (C=O); 151.0 (C=O); 135.3 (C(6)); 110.5 (C(5)); 87.8 (C(1′)); 84.8(C(4′)); 62.0 (C(5′)); 60.0 (C(3′)); 39.2 (CH_2N); 37.4 (C(2′)); 26.3 (CH_2); 13.5 (*Me*−C(5)). ESI-HR-MS: 597.2105 ($[M+Na]^+$, $C_{23}H_{30}N_{10}NaO_8^+$; calc. 597.2146).

General Procedure for Preparation of **13** *and* **14**. Under N_2, to a suspension of bis[benzimidazole] in MeCN was added *N*,*O*-bis(trimethylsilyl)acetamide (2.4 equiv.) and heated under reflux for 1 h. After the mixture was cooled to r.t., 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (2 equiv.) in MeCN and trimethylsilyl trifluoromethanesulfonate (2.5 equiv.) were added and heated under reflux for 4 h. The mixture was concentrated under reduced pressure, the residue was purified by SiO_2 CC using a mixture of $CHCl_3$/MeOH 20 : 1 as the eluent to afford the compounds **13** and **14**.

*2,2′-(Methanediyl)bis[1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-benzimidazole]* (**13**). Yield: 306 mg (40%). Yellow solid. ^{1}H-NMR ($CDCl_3$): 7.75 (*d*, *J* = 7.5, 2 arom. H); 7.57 (*d*, *J* = 7.5, 2 arom. H); 7.27 (*m*, 4 arom. H); 6.76 (*d*, *J* = 7.2, CH_2(1′)); 5.56 (*m*, CH_2(2′)); 5.44 (*m*, CH_2(3′)); 4.87 (*s*, CH_2); 4.41 (*m*, 2 CH_2(5′), 2 H−C(4)); 2.20 (*s*, 2 MeCO); 2.18 (*s*, 2 MeCO); 0.81 (*s*, 2 MeCO). ^{13}C-NMR ($CDCl_3$): 170.1, 169.6, 169.1 (C=O); 149.1 (C(2)); 142.9, 132.9, 123.5, 123.1, 120.0, 112.0 (arom. C); 85.8 (C(1′)); 79.2 (C(4′)); 70.0 (C(2′)); 69.2 (C(3′)); 63.0 (C(5′)); 29.9 (CH_2); 20.7, 20.5, 18.1 (Me). ESI-HR-MS: 765.2609 ($[M+H]^+$, $C_{37}H_{41}O_{14}N_4^+$; calc. 765.2614).

*2,2′-(Propane-1,3-diyl)bis[1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-benzimidazole]* (**14**). Yield: 243 mg (31%). Yellow solid. ^{1}H-NMR ($CDCl_3$): 7.73 (*d*, *J* = 7.5, 2 arom. H); 7.60 (*d*, *J* = 7.8, 2 arom. H); 7.25 (*m*, 4 arom. H); 6.12 (*d*, *J* = 6.9, CH_2(1′)); 5.58 (*t*, *J* = 6.9, CH_2(2′)); 5.46 (*dd*, *J* = 4.2, 6.6, CH_2(3′)); 4.43 (*d*, *J* = 3.0, 2 CH_2(5′)); 4.23 (*m*, 2 H−C(4)); 3.15 (*m*, 2 CH_2); 2.59 (*m*, CH_2); 2.22 (*s*, 2 MeCO); 2.15 (*s*, 2 MeCO); 1.86 (*s*, 2 MeCO). ^{13}C-NMR ($CDCl_3$): 170.2, 169.5, 169.2 (C=O); 154.0 (C(2)); 142.9, 132.6, 122.7, 119.4, 111.5 (arom. C); 86.3 (C(1′)); 79.8 (C(4′)); 70.9 (C(2′)); 69.3 (C(3′)); 62.9 (C(5′)); 26.4, 24.7 (CH_2); 20.7, 20.5, 19.9 (Me). ESI-HR-MS: 793.2930 ($[M+H]^+$, $C_{39}H_{45}O_{14}N_4^+$; calc. 793.2927).

General Procedure for the Preparation of Compounds **2** *and* **3**. Compounds **13** and **14** were dissolved in dry MeOH to which was added MeONa (20 equiv.). The soln. was stirred at r.t. for 48 h, and the solvent was then removed under vacuum. The residue was suspended in H_2O, and the suspension was extracted with AcOEt. The combined org. extracts were dried over Na_2SO_4, filtered, and evaporated to yield a white solid. The solid was purified by SiO_2 CC using $CHCl_3$/MeOH 8 : 1 as the eluent to afford **2** and **3**.

*2,2′-Bis[1-(β-D-ribofuranosyl)-1*H*-benzimidazole]* (**2**). Yield: 78 mg (80%). White powder. ^{1}H-NMR ($CDCl_3$/(D_6)DMSO): 7.50 (*d*, *J* = 5.1, 2 arom. H); 7.18 (*d*, *J* = 5.1, 2 arom. H); 6.84 (*m*, 4 arom. H); 5.84 (*d*, *J* = 6.0, CH_2(1′)); 4.51 (*s*, CH_2); 4.17 (*t*, *J* = 6.8, CH_2(2′)); 3.98 (*m*, CH_2(3′)); 3.71 (*m*, 2 H−C(4)); 3.53 (*m*, 2 CH_2(5′)). ^{13}C-NMR ((D_6)DMSO): 150.8 (C(2)); 142.8, 134.0, 123.1, 122.8, 119.3, 114.0 (arom. C); 89.4 (C(1′)); 86.5 (C(4′)); 73.0 (C(2′)); 70.1 (C(3′)); 61.9 (C(5′)); 28.2 (CH_2). ESI-HR-MS: 513.1981 ($[M+H]^+$, $C_{25}H_{29}O_8N_4^+$; calc. 513.1981).

*2,2′-(Propane-1,3-diyl)bis[1-(β-D-ribofuranosyl)-1*H*-benzimidazole]* (**3**). Yield: 100 mg (88%). White powder. ^{1}H-NMR ($CDCl_3$/(D_6)DMSO): 7.68 (*m*, 2 arom. H); 6.82 (*m*, 4 arom. H); 6.69 (*m*, 2 arom. H); 6.00 (*d*, *J* = 6.0, CH_2(1′)); 4.89 (br. *s*, 2 OH); 4.55 (*m*, CH_2(2′)); 4.26 (*m*, CH_2(3′)); 3.96 (*m*, 2 CH_2); 3.76 (*m*, 2 H−C(4)); 3.20 (*m*, 2 CH_2(5′)); 2.69 (*m*, CH_2). ^{13}C-NMR ((D_6)DMSO): 160.2 (C(2)); 148.0, 138.5, 127.3, 123.9, 118.5 (arom. C); 93.7 (C(1′)); 91.2 (C(4′)); 77.0 (C(2′)); 75.1 (C(3′)); 66.8 (C(5′)); 32.1, 30.5 (CH_2). ESI-HR-MS: 541.2298 ($[M+H]^+$, $C_{27}H_{33}O_8N_4^+$; calc. 541.2293).

Cells. HEK293T Cell lines were cultured in *Dulbecco* modified Eagle's medium supplemented with 10% fetal calf serum, 100 U of penicillin, and 100 μg of streptomycin per ml.

Plasmids. The plasmid pNL4-3.Luc.R-E- (NIH) was a non-infectious HIV-1 recombinant clone, into which the firefly luciferase gene was inserted into the pNL4–3 nef and two frameshifts (5′ Env and Vpr aa 26) rendered this clone Env- and Vpr- and allowed only a single cycle of replication. The plasmid pGL4.73 [hRluc/SV40] (*Promega*) was intended for use as internal control reporters to co-transfect HEK 293T cells with pNL4-3.Luc.R-E-.

Cells Transfection. Plate 2×10^5 cells per well in 500 μl *Dulbecco* modified Eagle's medium without antibiotics or serum in 24-well, sterile micro titer plates. The cells of each well were transfected with 0.8 μg of pNL4-3.Luc.R-E- and 0.1 μg phRL (*Promega*), and incubated with or without different compounds for 48 h.

Luciferase and Renilla Luciferase Activity Assay. After 48 h, the growth medium was removed from the cultured cells and gently applied a sufficient volume of PBS (500 ml) to rinse the cells on the bottom of the culture platel. 100 μl 1X luciferase assay lysis buffer were added into each well and the cells were harvested immediately. Fifty μl of luciferase assay reagent and 20 μl of cell lysate were added to the luminometer tube. The mixture was stirred and the tube placed in a luminometer for luciferase activity measurement. Fifty μl of renilla luciferase assay reagent and 20 μl of the same lysate sample were added to the luminometer tube for renilla luciferase activity measurement.

Expressions of p24 Detection. HEK 293T Cells were transfected with plasmids and incubated with or without different chemical compounds for 48 h as aforementioned. Then the cells were rinsed with PBS and lysed with 1% *Triton-100*/PBS. Expressions of p24 were detected by enzyme-linked immunosorbent assay using HIV-p24 diagnostic kit. Briefly, 50 μl of the cell lysats were incubated with each well of 96-well plate coated with p24 MAb for 40 min at 37°. The plate was then washed five times with wash buffer and incubated with the polyclonal HRP-anti-p24 for 30 min at 37°. After incubation, the plates were washed again five times with wash buffer, and 100 μl of a 1:1 soln. of HRP substrate/H_2O_2 was added. The substrate was incubated for 10 min at 37° before adding the stop solution, and the reading was done at the absorbance 450 nm. Assays were performed in triplicate.

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REFERENCES

[1] F. Barré-Sinoussi, J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, L. Montagnier, *Science* **1983**, *220*, 868.
[2] C. Flexner, *Nat. Rev. Drug Discovery* **2007**, *6*, 959.
[3] H. Mitsuya, K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. N. Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, S. Broder, *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7096.
[4] Y. Hamamoto, H. Nakashima, T. Matsui, A. Matsuda, T. Ueda, T. Yamamoto, *Antimicrob. Agents Chemother.* **1987**, *31*, 907.
[5] FDA, http://www.fda.gov/oashi/aids/virals.html, Drugs used in the Treatment of HIV Infection.
[6] E. J. Arts, M. A. Wainberg, *Antimicrob. Agents Chemother.* **1996**, *40*, 527.
[7] S. A. Riddler, R. E. Anderson, J. W. Mellors, *Antiviral Res.* **1995**, *27*, 189.
[8] J. Balzarini, *Biochem. Pharmacol.* **1999**, *58*, 1.
[9] S. Velázquez, R. Alvarez, A. San-Félix, M. L. Jimeno, E. De Clercq, J. Balzarini, M. J. Camarasa, *J. Med. Chem.* **1995**, *38*, 1641.
[10] M. Renoud-Grappin, C. Fossey, G. Fontaine, D. Ladurée, A. M. Aubertin, A. Kirn, *Antiviral Chem. Chemother.* **1998**, *9*, 205.

[11] S. Velázquez, V. Tuñón, M. L. Jimeno, C. Chamorro, E. De Clercq, J. Balzarini, M. J. Camarasa, *J. Med. Chem.* **1999**, *42*, 5188.
[12] L. B. Townsend, R. V. Devivar, S. R. Turk, M. R. Nassiri, J. C. Drach, *J. Med. Chem.* **1996**, *38*, 4098.
[13] M. T. Migawa, J.-L. Girardet, J. A. Walker II, G. W. Koszalka, S. D. Chamberlain, J. C. Drach, L. B. Townsend, *J. Med. Chem.* **1998**, *41*, 1242.
[14] K. S. Gudmundsson, G. A. Freeman, J. C. Drach, L. B.Townsend, *J. Med. Chem.* **2000**, *43*, 2473.
[15] J. Parsch, J. W. Engels, *J. Am. Chem. Soc.* **2002**, *124*, 5664.
[16] Z. Li, Y. Xiong, Y. Peng, J. Pan, Y. Chen, X. Wu, S. Hussain, P. Tien, D. Y. Guo, *FEBS Lett*. **2005**, *579*, 3100.
[17] H.-P. Hu, S.-C. Hsieh, C.-C. King, W.-K. Wang, *Virology* **2007**, *368*, 376.
[18] J. Neumann, J. Stitz, R. König, E. Seibold, S. Norley, E. Flory, K. Cichutek, *J. Biotechnol.* **2006**, *124*, 615.
[19] K. S. Sagar, C.-C. Chang, W.-K. Wang, J.-Y. Linc, S.-S. Lee, *Bioorg. Med. Chem.* **2004**, *12*, 4045.
[20] D. R. Adams, C. Perez, M. Maillard, J.-C. Florent, M. Evers, Y. Hénin, S. Litvak, L. Litvak, C. Monneret, D. S. Grierson, *J. Med. Chem.* **1997**, *40*, 1550.

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