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Synthesis of 3′-*O*-phosphonomethyl nucleosides with an adenine base moiety

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Abstract—A synthetic scheme has been developed for the synthesis of 2′-deoxythreose phosphonate nucleosides from β-hydroxy-γ-butyrolactone and of 2′-azido erythrose phosphonate nucleosides from dihydroxydihydrofuran-1-one. In addition several α-L-arabinofuranose phosphonate nucleosides were synthesized starting from protected α-D-galactofuranose. Unfortunately, none of the synthesized compounds show activity in an HIV-1 assay. One of these compounds, a locked phosphonate nucleoside, was evaluated (as diphosphate) for its potential to be incorporated into DNA using HIV-1 reverse transcriptase.

1. Introduction

One of the advantages of the antiviral phosphonate nucleosides that are currently on the market for the treatment of HIV infections is that they need to be given only once daily. Limitations of the currently used phosphonate drug (Tenofovir®), that need to be surpassed, are the long-term side effects, the emergence of resistance, and the presence of drug–drug interactions.[1] This research has led to the discovery of three types of phosphonate nucleosides with an adenine base moiety. The D-d4AP nucleoside was described by Kim et al. in 1991.[2] PMDTA (Fig. 1) was described as a selective anti-HIV-1/HIV-2 phosphonate nucleoside.[3] Both compounds demonstrated an anti-HIV activity (EC_{50}) of 2.0–3.0 μM against HIV-1. 2′Fd4AP[1] is a somewhat lesser active compound (EC_{50}: 12 μM) but with an excellent resistance profile (Fig. 1). In order to investigate the importance of the stereochemistry in the 1′- and 3′-position of PMDTA, we have now synthesized the isomeric nucleosides **1a** and **1b** (Fig. 2). PMDTA has a phosphonomethyl moiety at the 3′-position of the furanose ring and no substituent at the 4′-position.[3] The absence of a 4′-hydroxymethyl group avoids problems of steric hindrance during phosphorylation reactions by kinases. To further study the influence of different substituents on the anti-HIV activity, we have synthesized 2′- and 4′-modified analogs of PMDTA (**2–4**) as well as a locked phosphonate nucleoside with a 4′,2′-*O*-α-methylene bridge (**5**). The reason for synthesizing these compounds is to map the substitution landscape (especially in the 2′- and 4′-position) as a function of antiviral activity. The triphosphate of the locked phosphonate nucleoside was synthesized and tested as potential substrate for HIV-1 reverse transcriptase of PMDTA.

2. Results and discussion

The nucleosides **1a** and **1b** were synthesized starting from (*S*)-3-hydroxy-γ-butyrolactone (Scheme 1). The hydroxyl group in 3-position of (*S*)-3-hydroxy-γ-butyrolactone is protected by benzoylation[4] and the lactone function is reduced using Dibal-H in THF.[5] The anomeric hydroxyl group of compound **7** is protected with acetic anhydride in pyridine. The nucleobase (N^6-benzoyladenine) is introduced using $SnCl_4$ as Lewis catalyst,[6] giving a mixture of compounds **9a** and **9b** with the base moiety in β- and α-configuration, respectively. The benzoyl protecting groups of **9a** and **9b** are removed using satd ammonia in methanol. Finally, the phosphonate function is introduced on compounds **10a** and **10b**[7] using the triflate of diisopropylphosphonomethyl alcohol and NaH in THF. Hydrolysis of the phosphonate ester function of **11a** and **11b** is carried out with TMSI at 0 °C.[8] After purification by silica gel chromatography, Sephadex-DEAE A-25 resin and Dowex-sodium ion exchange resin, nucleoside phosphonates **1a** and **1b** were obtained.

Nucleoside **2** was synthesized starting from (*R*,*R*)-2,3-dihydroxydihydrofuran-1-one (Scheme 2). Compound **13**, with

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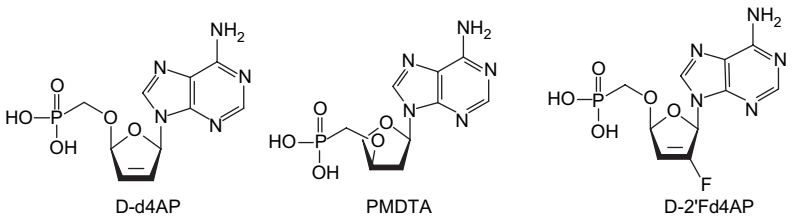


Figure 1. Structure of representative anti-HIV phosphonate nucleosides.

a TBDMS group at the 3-position, is a minor compound when selective protection of the 2-position of **12** is carried out with tertbutyldimethylsilyl chloride.[3] We realize that this multistep synthesis uses a compound, obtained in low yield, as starting material. However, no attempts were made to improve this yield, at this moment. The hydroxyl group in 2-position of compound **13** is protected with benzoyl chloride in pyridine. The lactone function is reduced to hemiketal using Dibal-H in THF. The anomeric hydroxyl group of compound **15** is protected with acetic anhydride in pyridine. The nucleobase (adenine) is introduced using $SnCl_4$ as Lewis catalyst. The presence of a 2-*O*-benzoyl group of compound **15** allows selective introduction of the base moiety in the α-configuration. Then the benzoyl protecting group is removed using satd ammonia in methanol. The introduction of the azido function into the 2′-position of compound **17** is performed through a nucleophilic substitution of the triflate-activated 2′-hydroxyl group with lithium azide.[9] The silyl protecting group is removed and the phosphonate function is then introduced in this position using the triflate of diisopropylphosphonomethyl alcohol and NaH in THF. Hydrolysis of the phosphonate ester function of compound **20** is carried out with TMSI at 0 °C. After purification by silica gel chromatography, Sephadex-DEAE A-25 resin and Dowex-sodium ion exchange resin, nucleoside phosphonate **2** was obtained.

The nucleosides **3**–**5** (Scheme 3) were synthesized starting from 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose **21**, which was obtained according to the procedure described previously.[10] The phosphonate function is introduced on

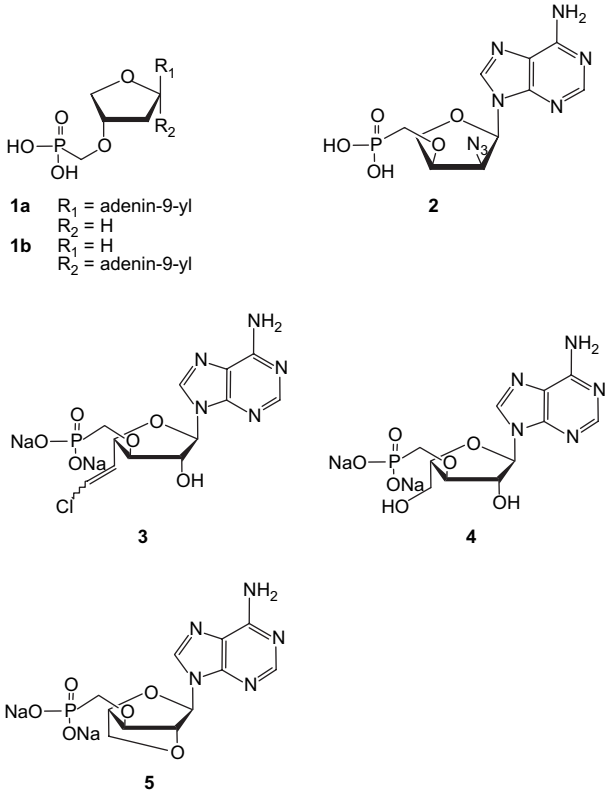
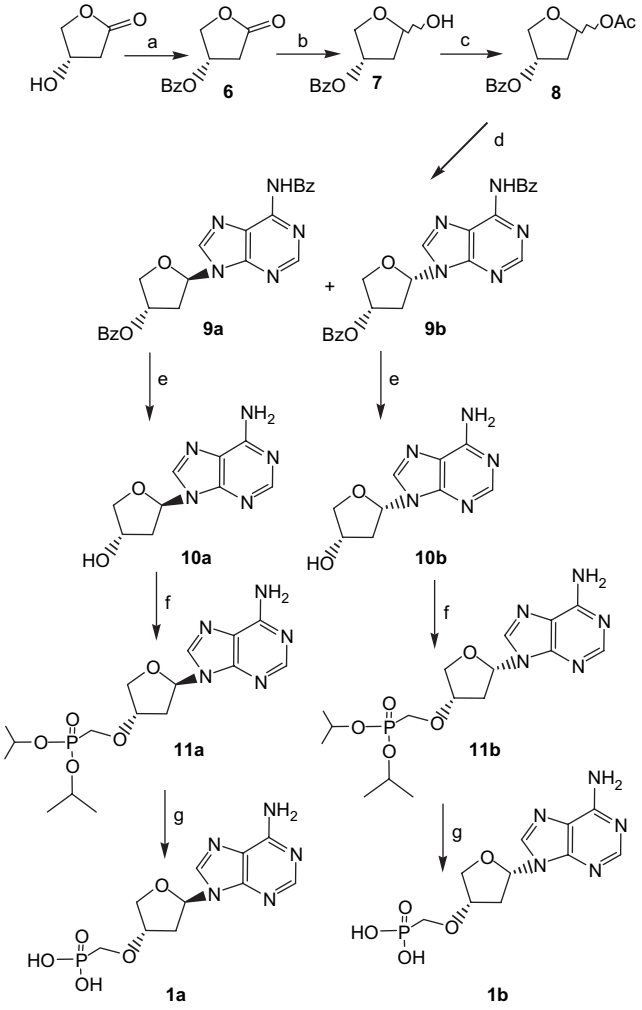
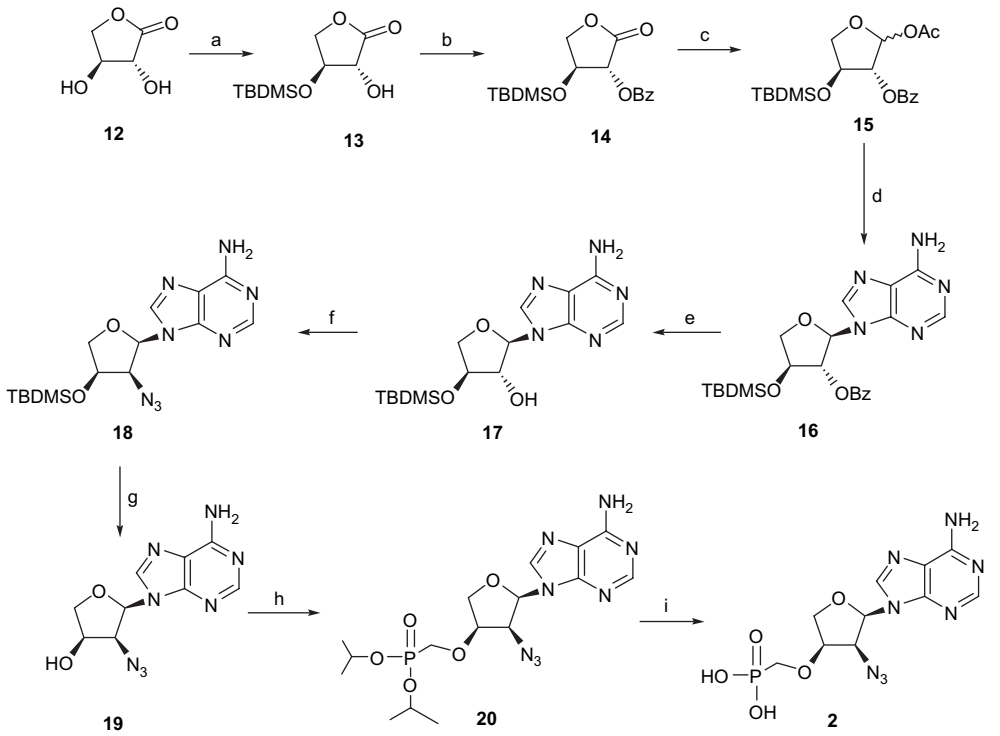


Figure 2. Structure of the target nucleosides.



Scheme 1. Synthesis of two stereoisomers of PMDTA. Reagents and conditions: (a) BzCl, pyridine, 89%; (b) Dibal-H, THF, 75%; (c) Ac_2O, Et_3N, DMAP, 83%; (d) $SnCl_4$, MeCN, N^6-benzoyladenine, **9a**: 19%, **9b**: 33%; (e) NH_3/MeOH, 86%; (f) trifluoromethanesulfonate of diisopropylphosphonomethanol, NaH, THF, 52%; (g) (1) TMSI, DCM; (2) Sephadex-DEAE, Dowex-Na^+, 45%.



Scheme 2. Synthesis of the 2′-deoxy-2′-azidophosphonate nucleoside. Reagents and conditions: (a) TBDMSCl, imidazole, MeCN, 10%; (b) BzCl, pyridine, 85%; (c) (1) Dibal-H, THF, 94%; (2) Ac_2O, Et_3N, 58%; (d) $SnCl_4$, MeCN, adenine, 39%; (e) NH_3/MeOH, 99%; (f) (1) trifluoromethanesulfonyl chloride, CH_2Cl_2; (2) LiN_3/DMF, 37%; (g) TBAF/THF, 86%; (h) trifluoromethanesulfonate of diisopropylphosphonomethanol, NaH, THF, 74%; (i) (1) TMSI, DCM; (2) Sephadex-DEAE, Dowex-Na^+, 41%.

21 using the triflate of diisopropylphosphonomethyl alcohol and NaH in THF,[3] followed by oxidative degradation of compound **22** to the aldehyde derivative **23** using H_5IO_6 and $NaIO_4$.[11] Wittig olefination of compound **23** with (chloromethyl)triphenylphosphonium chloride affords the chlorovinyl derivative **24**.[12] The isopropylidene protecting group of **24** is removed and replaced by benzoyl protecting groups. N^6-Benzoyladenine is introduced using $SnCl_4$ as Lewis catalyst. Removal of the benzoyl protecting groups with ammonia in methanol affords compound **27**.

After reduction of the aldehyde group of compound **23** to the free hydroxyl group with $NaBH_4$,[13] the free hydroxyl group of **28** was protected with a benzoyl group. The isopropylidene protection group of **29** is removed and replaced by benzoyl protecting groups. The presence of a 2-*O*-benzoyl group allows selective introduction of the base moiety N^6-benzoyladenine using $SnCl_4$ as Lewis catalyst in the α-configuration. Removal of the benzoyl protecting groups with ammonia in methanol afforded **32**. After introduction of a mesyl group on **32**, locked nucleoside **34** is obtained by intramolecular substitution using NaH.[14]

The diisopropyl protected nucleoside phosphonates (**27**, **32**, and **34**) were hydrolyzed with TMSI. After purification by silica gel chromatography, Sephadex-DEAE A-25 resin and Dowex-sodium ion exchange resin, nucleoside phosphonates as sodium salts (**3**–**5**) were obtained. We synthesized the diphosphate **35** of compound **5** (Fig. 3) to evaluate the potential of a locked nucleoside to function as chain terminator in a polymerase-catalyzed reaction. This diphosphate was synthesized using carbonyldiimidazole as coupling agent.

2.1. Biology

Compounds **1a**, **1b**, and **2**–**5** were evaluated as inhibitor of HIV-1 replication in MT-2 cells. None of the compounds shows activity at the highest concentration tested (200 μM).

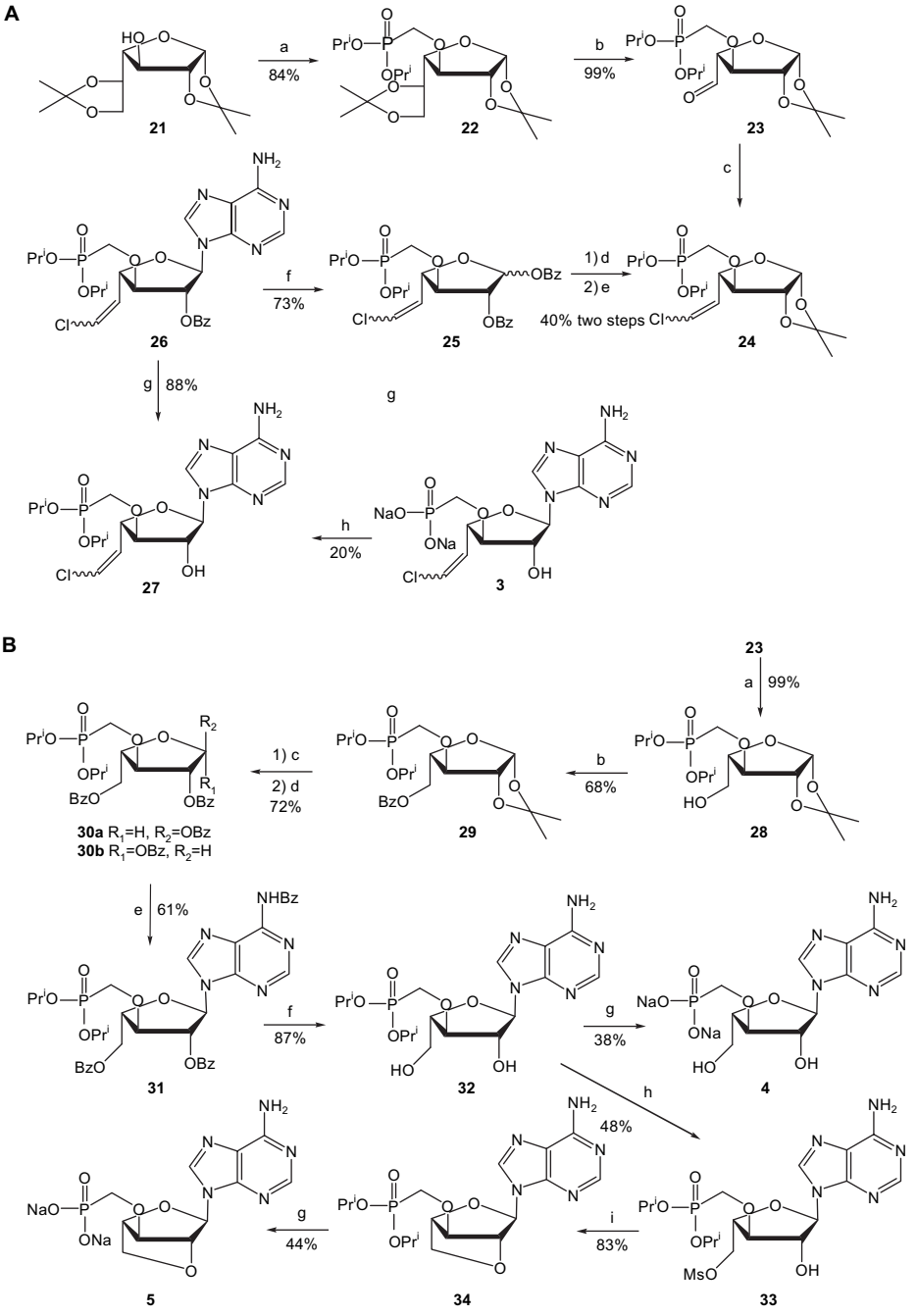
Because the phosphonate with the locked conformation lacks a hydroxyl group on the 2′-position, it is important to check whether this phosphonate nucleoside **35** can possibly act as a chain terminator. In order to behave as a chain terminator, the nucleotide analog should be incorporated into a growing DNA or RNA strand and prevent further elongation.

The ability of the phosphonate nucleoside **35** to terminate DNA synthesis was investigated by a single nucleotide incorporation assay using its diphosphate as potential substrate.

Even with a high concentration of diphosphorylphosphonate nucleoside (400 μM) and an enzyme (HIV reverse transcription) concentration of 1.44 U/μL, only weak incorporation (18% of the intact primer was elongated) could be seen. Therefore, only in extreme circumstances the phosphonate nucleoside will be able to act as a chain terminator.

3. Conclusion

The influence of stereochemistry on the anti-HIV activity of tetrahydrofuran nucleosides with a phosphonomethoxy substituent in the 3′-position has been investigated. Likewise, a L-erythrose nucleoside with a 2′-azido substituent and three



Scheme 3. Synthesis of the 4′-branched phosphonate nucleosides. (A) Reagents and conditions: (a) trifluoromethanesulfonate of diisopropylphosphonomethanol, NaH, THF, −78 °C, room temperature; (b) $NaIO_4$, H_5IO_6, EtOAc, room temperature; (c) $ClCH_2P(C_6H_5)_3Cl$, *n*-BuLi, THF, −78 °C; (d) TFA/H_2O, room temperature; (e) BzCl, pyridine, 0 °C, room temperature; (f) $SnCl_4$, MeCN, 0 °C, room temperature; (g) satd NH_3 in MeOH, room temperature; (h) (1) TMSI, DCM, 0 °C; (2) Sephadex-DEAE; (3) Dowex-Na^+. (B) Reagents and conditions: (a) $NaBH_4$, MeOH, room temperature; (b) $ClCH_2P(C_6H_5)_3Cl$, *n*-BuLi, THF, −78 °C; (c) TFA/H_2O, room temperature; (d) BzCl, pyridine, 0 °C, room temperature; (e) $SnCl_4$, MeCN, 0 °C, room temperature; (f) satd NH_3 in MeOH, room temperature; (g) (1) TMSI, DCM, 0 °C; (2) Sephadex-DEAE; (3) Dowex-Na^+; (h) MsCl, pyridine, 0 °C, room temperature; (i) NaH, THF, −78 °C, room temperature.

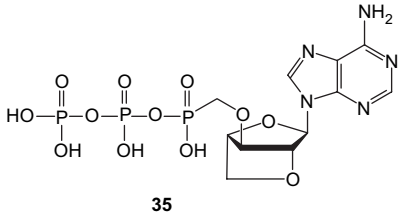


Figure 3. Structure of the diphosphate of the locked phosphonate nucleoside **5**.

examples of 4′-branched phosphonate nucleosides have been synthesized, but none of them show activity against HIV-1. The diphosphate analog **35** was synthesized to test its ability to function as chain terminator. Because of the flexibility of the phosphonomethoxy moiety connected at the 3′-position of the sugar moiety, hypothesis about the conformational preference of the sugar moiety for incorporation into DNA (to predict the potential outcome of this incorporation assay) was not taken into consideration. The phosphonate nucleoside **35** could act as a chain terminator, only in

extreme conditions. Given its low potency, it is unlikely that its parent phosphonate nucleoside **5** will be able to prevent the virus to replicate under physiological circumstances (even if sufficient amount of the diphosphate **35** could be generated into the cell by the action of kinases).

4. Experimental section

4.1. General

For all reactions, analytical grade solvents were used. All moisture sensitive reactions were carried out in oven-dried glassware (135 °C) under a nitrogen atmosphere. Anhydrous THF was refluxed over sodium/benzophenone and distilled. A Varian Unity 500 MHz spectrometer and a 200 MHz Varian Gemini apparatus were used for ^{1}H NMR and ^{13}C NMR. Exact mass measurements were performed on a quadrupole time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) equipped with a standard electrospray-ionization (ESI) interface; samples were infused in *i*-PrOH/H_2O 1:1 at 3 μL/min. Precoated aluminum sheets (Fluka Silica gel/TLC-cards, 254 nm) were used for TLC; the spots were examined with UV light. Column chromatography was performed on ICN silica gel 63-200. NMR signals of sugar protons and carbons are indicated with a prime, signals of base protons and carbons are given without a prime.

4.1.1. (*S*)-3-Benzoyloxy-γ-butyrolactone (6). To a solution of (*S*)-3-hydroxy-γ-butyrolactone (1.0 g, 9.8 mmol) in 25 mL pyridine was added dropwise BzCl (1.4 mL, 12.2 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was concentrated and coevaporated with toluene two times in vacuo. The residue was partitioned between H_2O (15 mL) and EtOAc (40 mL). The organic layer was washed with water and brine, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (*n*-hexane/EtOAc=8:1) to afford **6** (1.78 g, 8.6 mmol) as a white solid in 89% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 2.76–3.08 (m, 2H, C(2′)H_2), 4.58–4.70 (m, 2H, C(4′)H_2), 5.70–5.75 (m, 1H, C(3′)H), 7.49–8.07 (m, 5H, Ar-H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 33.21 (C-2′), 68.88 (C-4′), 71.73 (C-3′), 127.24 (Ar-C), 128.43 (C-Ar), 132.43 (C-Ar), 169.05 (Bz-*C*O), C-1′ was obscured in noise peak; Exact mass calcd for $C_{11}H_{10}O_4Na$ $[M+Na]^+$ 229.0477, found 229.0435.

4.1.2. (*S*)-3-Benzoyl-4-hydroxybutanal (lactol) (7). To a solution of **6** (0.780 g, 3.8 mmol) in 13 mL dry THF was slowly dropwise added 1.0 M diisopropyl aluminum hydride (4.7 mL, 4.7 mmol) in toluene at −78 °C. The reaction mixture was stirred at −78 °C, and as soon as the starting material was completely consumed (TLC, 1.5–2 h), methanol (2 mL) was slowly added to quench the reaction. The cooling bath was removed, 15 mL of a satd aq sodium potassium tartrate solution and 25 mL of EtOAc were added, and the mixture stirred vigorously for 3 h. The organic layer was washed with water and brine, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (*n*-hexane/EtOAc=8:2) to afford **7** (590 mg, 2.8 mmol) as a colorless oil in 75% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 2.30–2.40 (m, 2H, C(2′)H_2), 4.05–4.36 (m, 2H, C(4′)H_2), 5.30–5.82 (m, 2H, C(1′)H+C(3′)H), 7.40–7.62 (m, 3H, Ar-H), 8.00–8.07 (m, 2H, Ar-H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 39.67, 40.30 (C-2′)71.38, 72.63 (C-3′ or -4′), 73.94, 74.97 (C-4′ or -3′), 98.37, 98.43 (C-1′), 128.44, 128.53 (Ar-C), 129.69 (Ar-C), 133.27, 133.39 (Ar-C), 166.44 (Bz-*C*O); Exact mass calcd for $C_{11}H_{12}O_4Na$ $[M+Na]^+$ 231.0633, found 231.0629.

4.1.3. (*S*)-1-α,β-*O*-Acetyl-3-benzoyloxy-4-hydroxybutanal (lactol) (8). To a solution of **7** (150 mg, 0.72 mmol) in 6.5 mL Et_3N were added dropwise $(CH_3CO)_2O$ (0.34 mL) and DMAP (8.8 mg, 0.072 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 3 h, then concentrated in vacuo, and the residue was purified by chromatography on a silica gel column (*n*-hexane/EtOAc=9:1) to give compounds **8a** (117 mg, 0.47 mmol) as colorless oil in 65% yield and **8b** (32 mg, 0.13 mmol) as colorless oil in 18% yield. It was not identified at this stage which compound represents which isomer (α or β), but the mixture of **8a** and **8b** was used in the condensation reaction with the protected nucleobase.

Compound **8a**: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 2.07 (s, 3H, CH_3), 2.48–2.52 (m, 2H, C(2′)H_2), 4.14 (dd, J_1=10.6 Hz, J_2=2.2 Hz, 1H, C(4′)H_b), 4.26 (dd, J_1=10.6 Hz, J_2=4.2 Hz, 1H, C(4′)H_a), 5.61–5.65 (m, 1H, C(3′)H), 6.49 (t, *J*=4.0 Hz, 1H, C(1′)H), 7.40–8.05 (m, 5H, Ar-H).

Compound **8b**: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 2.07 (s, 3H, CH_3), 2.41–2.58 (m, 2H, C(2′)H_2), 4.22 (dd, J_1=10.6 Hz, J_2=2.6 Hz, 1H, C(4′)H_b), 4.36 (dd, J_1=10.6 Hz, J_2=5.2 Hz, 1H, C(4′)H_a), 5.61–5.65 (m, 1H, C(3′)H), 6.40 (d, *J*=4.4 Hz, 1H, C(1′)H), 7.43–7.60 (m, 3H, Ar-H), 8.05–8.09 (m, 2H, Ar-H).

4.1.4. 1-β-(N^6-Benzoyladenin-9-yl)-3-*O*-benzoyl-D-*threo*-tetrafuranose (9a) and 1-α-(N^6-benzoyladenin-9-yl)-3-*O*-benzoyl-D-*erythro*-tetrafuranose (9b). To a mixture of **8** (210 mg, 0.84 mmol) and N^6-benzoyladenine (405 mg, 1.62 mmol) in dry MeCN (30 mL) was dropwise added $SnCl_4$ (0.3 mL, 2.5 mmol) under N_2 at 0 °C. The reaction mixture was warmed to room temperature and stirred for 1.5 h. Then the reaction was quenched with satd $NaHCO_3$ and concentrated. The residue was partitioned between H_2O (20 mL) and EtOAc (100 mL). The organic layer was washed with water and brine, and concentrated under vacuum. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH=40:0.5) to afford a mixture of β/α isomers (187 mg, 0.44 mmol) in 52% yield, which were separated using preparative TLC and using CH_2Cl_2/MeOH/40:1 (three times), as eluent.

Compound **9a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.84–2.89 (m, 1H, C(2′)H_b), 3.23–3.28 (m, 1H, C(2′)H_a), 4.29 (d, *J*=10.6 Hz, 1H, C(4′)H_b), 4.63 (dd, J_1=4.3 Hz, J_2=10.6 Hz, 1H, C(4′)H_a), 5.92 (m, 1H, C(3′)H), 6.50 (t, *J*=6.5 Hz, 1H, C(1′)H), 7.45–7.60 (m, 6H, Ar-H), 8.02–8.08 (m, 4H, Ar-H), 8.15 (s, 1H, C(8 or 2)H), 8.78 (s, 1H, C(2 or 8)H), 9.32 (br s, 1H, NH); ^{13}C NMR (500 MHz,

$CDCl_3$) δ_C 38.59 (C-2′), 73.43 (C-4′), 74.34 (C-3′), 85.15 (C-1′), 123.88 (C-5), 127.88 (Ar-C), 128.45 (Ar-C), 128.73 (Ar-C), 129.64 (Ar-C), 132.69 (Ar-C), 133.40 (Ar-C), 141.80 (C-8), 149.69 (C-6), 151.45 (C-4), 152.52 (C-2), 164.74 (Bz-CO), 166.00 (Bz-CO); Exact mass calcd for $C_{23}H_{19}N_5O_4Na$ $[M+Na]^+$ 452.1335, found 452.1339.

Compound **9b**: ^1H NMR (500 MHz, $CDCl_3$) δ_H 2.90–2.96 (m, 1H, C(2′)H_b), 3.05 (d, J=15.4 Hz, 1H, C(2′)H_a), 4.40 (dd, J_1=4.4 Hz, J_2=10.0 Hz, 1H, C(4′)H_b), 4.46 (d, J=10.0 Hz, 1H, C(4′)H_a), 5.74 (m, 1H, C(3′)H), 6.56 (d, J=7.1 Hz, 1H, C(1′)H), 7.38–7.61 (m, 6H, Ar-H), 7.74 (d, J=8.3 Hz, 2H, Ar-H), 8.03 (d, J=7.6 Hz, 2H, Ar-H), 8.35 (s, 1H, C(8 or 2)H), 8.74 (s, 1H, C(2 or 8)H), 9.25 (br s, 1H, NH); ^{13}C NMR (500 MHz, $CDCl_3$) δ_C 38.63 (C-2′), 73.45 (C-3′), 74.80 (C-4′), 85.19 (C-1′), 123.44 (C-5), 127.90 (Ar-C), 128.59 (Ar-C), 128.76 (Ar-C), 129.36 (Ar-C), 132.69 (Ar-C), 133.54 (Ar-C), 140.92 (C-8), 149.49 (C-6), 151.43 (C-4), 152.52 (C-2), 164.67 (Bz-CO), 165.79 (Bz-CO); Exact mass calcd for $C_{23}H_{19}N_5O_4Na$ $[M+Na]^+$ 452.1335, found 452.1334.

4.1.5. 1-β-(Adenin-9-yl)-3-hydroxy-D-*threo*-tetrafuranose (10a) and 1-α-(adenin-9-yl)-3-hydroxy-D-*erythro*-tetrafuranose (10b). A solution of **9a** (90 mg, 0.21 mmol) in methanol satd with ammonium (26 mL) was stirred at room temperature overnight. The solution was concentrated under vacuum and the residue was purified by column chromatography (CH_2Cl_2/MeOH=9:1) to give compound **10a** (40 mg, 0.18 mmol) as a white solid in 86% yield.

Compound **10b** was prepared as described for **10a** using **9b** (110 mg, 0.26 mmol) as starting material. Compound **10b** (48 mg, 0.22 mmol) was obtained as a white solid in 86% yield.

Compound **10a**: ^1H NMR (200 MHz, DMSO-d_6) δ_H 2.27–2.34 (m, 1H, C(2′)H_a), 2.78–2.85 (m, 1H, C(2′)H_b), 3.76 (d, J=8.8 Hz, 1H, C(4′)H_a), 4.21 (dd, J_1=3.6 Hz, J_2=8.8 Hz, 1H, C(4′)H_b), 4.62 (br s, 1H, C(3′)H), 5.20 (d, J=3.6 Hz, 1H, OH), 6.37 (t, J=8.6 Hz, 1H, C(1′)H), 7.26 (br s, 2H, NH_2), 8.14 (s, 1H, C(8 or 2)), 8.30 (s, 1H, C(2 or 8)H); ^{13}C NMR (200 MHz, DMSO-d_6) δ_C 38.38 (C-2′), 70.56 (C-3′), 76.08 (C-4′), 84.22 (C-1′), 139.97 (A-C(8)), 152.78 (A-C(2)), 156.30 (A-C(6)), C-5 and C-4 were obscured in noise; Exact mass calcd for $C_9H_{11}N_5O_2Na$ $[M+Na]^+$ 244.0810, found 244.1191.

Compound **10b**: ^1H NMR (200 MHz, DMSO-d_6) δ_H 2.23–2.30 (m, 1H, C(2′)H_b), 2.63–2.71 (m, 1H, C(2′)H_a), 3.90–3.93 (m, 2H, C(4′)H_2), 4.45 (br s, 1H, C(3′)H), 5.80 (d, J=4.4 Hz, 1H, OH), 6.26 (dd, J_1=2.2 Hz, J_2=8.0 Hz, 1H, C(1′)H), 7.28 (s, 2H, NH_2), 8.13 (s, 1H, C(8 or 2)H), 8.35 (s, 1H, C(2 or 8)H); ^{13}C NMR (200 MHz, DMSO-d_6) δ_C 38.49 (C-2′), 66.99 (C-3′), 74.04 (C-4′), 80.90 (C-1′), 118.01 (A-C(5)), 140.02 (A-C(8)), 152.52 (A-C(2)), 155.76 (A-C(6)), 160.97 (A-C(4)); Exact mass calcd for $C_9H_{11}N_5O_2Na$ $[M+Na]^+$ 244.0810, found 244.1192.

4.1.6. 1-β-(Adenin-9-yl)-3-*O*-(diisopropylphosphonomethyl)-D-*threo*-tetrafuranose (11a) and 1-α-(adenin-9-yl)-3-*O*-(diisopropylphosphonomethyl)-D-*erythro*-tetrafuranose (11b). To the solution of **10a** (50 mg, 0.23 mmol) in 5 mL THF, which was cooled using dry-ice and acetone, was added sodium hydride 80% (11.4 mg, 0.46 mmol). The mixture was stirred for 10 min and the solution of the triflate of the phosphonate (136 mg, 0.46 mmol) in THF was slowly dropped into the reaction flask. Then the mixture was slowly warmed up to room temperature. The reaction was quenched with satd $NaHCO_3$ and concentrated. The residue was partitioned between H_2O and CH_2Cl_2. The organic layer was washed with water and brine, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH=98:2) to afford **11a** (25 mg, 0.06 mmol) as a colorless oil in 52% yield.

Compound **11b** was prepared as described for **11a** using **10b** (60 mg, 0.27 mmol) as starting material. Compound **11b** (30 mg, 0.07 mmol) was obtained as a colorless oil in 52% yield.

Compound **11a**: ^1H NMR (200 MHz, $CDCl_3$) δ_H 1.35–1.38 (m, 12H, CH_3), 2.62–2.74 (m, 1H, C(2′)H_a), 2.93–3.02 (m, 1H, C(2′)H_b), 3.78 (d, J=8.6 Hz, 2H, PCH_2), 4.17 (d, J=10 Hz, C(4′)H_b), 4.37 (dd, 1H, J_1=4.2 Hz, J_2=9.8 Hz, C(4′)H_a), 4.64 (br s, 1H, C(3′)H), 4.75–4.85 (m, 2H, $OCH(CH_3)_2$), 5.91 (br s, 2H, NH_2), 6.32 (t, 1H, J=6.6 Hz, C(1′)H), 7.89 (s, 1H, C(8 or 2)H), 8.33 (s, 1H, C(2 or 8)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.97 ($CH(CH_3)_3$), 37.63 (C-2′), 62.18 (PCH_2), 65.55 ($CH(CH_3)_3$), 71.32 ($CH(CH_3)_3$), 73.35 (C-4′), 81.73 (C-3′), 85.68 (C-1′), 139.49 (A-C(8)), 153.03 (A-C(2)), 155.61 (A-C(4)), C-5 and C-6 were obscured in noise; Exact mass calcd for $C_{16}H_{26}N_5O_5P_1Na$ $[M+Na]^+$ 422.1569, found 422.1572. Elem. and calcd for $C_{16}H_{16}N_5O_5P$ (MW, 399.1671) C: 48.10, H: 6.56, N: 17.54. Found C: 47.91, H: 6.24, N: 17.48.

Compound **11b**: ^1H NMR (200 MHz, $CDCl_3$) δ_H 1.25–1.35 (m, 12H, CH_3), 2.57–2.62 (m, 2H, C(2′)H_2), 3.70 (dd, J_1=1.4 Hz, J_2=8.8 Hz, 2H, PCH_2), 4.04 (dd, J_1=4.2 Hz, J_2=10.4 Hz, C(4′)H_a), 4.37 (d, J=10.4 Hz, C(4′)H_b), 4.47 (br s, 1H, C(3′)H), 4.71–4.78 (m, 2H, $OCH(CH_3)_2$), 5.75 (m, 2H, NH_2), 6.47 (dd, J_1=2.7 Hz, J_2=7.0 Hz, C(1′)H), 8.29 (s, 1H, C(8 or 2)), 8.34 (s, 1H, C(2 or 8)); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.87 ($CH(CH_3)_3$), 38.56 (C-2′), 62.41 (PCH_2), 67.95 ($CH(CH_3)_3$), 71.46 ($CH(CH_3)_3$), 73.80 (C-4′), 80.56 (C-3′), 83.63 (C-1′, 140.02 (A-C(8)), 153.03 (A-C(6)), 155.61 (A-C(2)); Exact mass calcd for $C_{16}H_{26}N_5O_5P_1Na_1$ $[M+Na]^+$ 422.1569, found 422.1573. Elem. and calcd for $C_{16}H_{16}N_5O_5P$ (MW, 399.1671) C: 48.10, H: 6.56, N: 17.54. Found C: 48.03, H: 6.32, N: 17.23.

4.1.7. 1-β-(Adenin-9-yl)-3-*O*-(phosphonomethyl)-D-*threo*-tetrafuranose sodium salt (1a) and 1-α-(adenin-9-yl)-3-*O*-(phosphonomethyl)-D-*erythro*-tetrafuranose sodium salt (1b). To the solution of **11a** (90 mg, 0.23 mmol) and Et_3N (0.33 mL) in DCM (9 mL) was added iodotrimethylsilane (0.26 mL, 1.84 mmol) at 0 °C. The reaction mixture was stirred for 2 h. Then, the reaction was quenched with 1.0 M TEAB solution. The mixture was concentrated and the residue was purified by chromatography column (CH_2Cl_2/MeOH=1:2) to give the crude title compound. Purification using Sephadex-DEAE A-25 with gradient TEAB solution from 0.01 to 0.8 M and ion exchange by Dowex-Na^+ resin afforded **1a** as a white solid (37 mg, 0.11 mmol) in 45% yield.

Compound **1b** was prepared as described for **1a** using **11b** (50 mg, 0.12 mmol) as starting material. Compound **1b** was obtained as a white solid (20 mg, 0.06 mmol) in 44% yield.

Compound **1a**: ^{1}H NMR (500 MHz, D_2O) δ_H 2.81 (br s, 2H, C(2′)H_2), 3.60–3.62 (m, 2H, PCH_2), 4.20–4.25 (m, 2H, C(4′)H_2), 4.64 (br s, 1H, C(3′)H), 4.47 (t, J=6.0 Hz, 1H, C(1′)H), 8.20 (s, 1H, A-C(8 or 2)H), 8.26 (s, 1H, C(2 or 8)H); ^{13}C NMR (500 MHz, D_2O) δ_C 39.40 (C-2′), 68.76 (d, J=61.1 Hz, PCH_2), 75.76 (C-4′), 83.39 (d, $J_{P,C}$=11.7 Hz, C-3′), 87.42 (C-1′), 121.73 (A-C(5)), 142.99 (A-C(8)), 151.31 (A-C(6)), 155.35 (A-C(2)), 158.24 (A-C(4)); ^{31}P NMR (500 MHz, D_2O) δ_P 14.74; Exact mass calcd for $C_{10}H_{15}N_5O_5P$ [M+H]$^+$ 316.0811, found 316.0805.

Compound **1b**: ^{1}H NMR (500 MHz, D_2O) δ_H 2.67 (d, J=14.9 Hz, 1H, C(2′)H_a), 2.79 (m, 1H, C(2′)H_b), 3.49–3.57 (m, 2H, PH_2), 4.09 (dd, J_1=10.2 Hz, J_2=4.1 Hz, 1H, C(4′)H_a), 4.34 (d, J=10.2 Hz, 1H, C(4′)H_b), 4.53–4.55 (m, 1H, C(3′)H), 6.40 (dd, J_1=8.0 Hz, J_2=2.2 Hz, 1H, C(1′)H), 8.22 (s, 1H, C(2 or 8)H), 8.58 (s, 1H, C(8 or 2)H); ^{13}C NMR (500 MHz, D_2O) δ_C 39.73 (C-2′), 69.54 (d, J=61.5 Hz, PCH_2), 76.49 (C-4′), 82.27 (d, $J_{P,C}$=11.7 Hz, C-3′), 86.17 (C-1′), 121.73 (A-C(5)), 144.17 (A-C(8)), 151.49 (A-C(6)), 155.34 (A-C(2)), 158.30 (A-C(4)); ^{31}P NMR (500 MHz, D_2O) δ_P 14.02, Exact mass calcd for $C_{10}H_{15}N_5O_5P$ [M+H]$^+$ 316.0811, found 316.0810.

4.1.8. 1-*O*-Acetyl-2-*O*-benzoyl-3-*O*-tertbutyldimethylsilyl-L-threose (15). To the solution of **14** (1.0 g, 2.97 mmol) in 10 mL THF was slowly dropwise added 1.0 M diisopropyl aluminum hydride (6 mL, 6 mmol) in toluene at −78 °C, and as soon as the starting material was completely consumed (4 h) methanol (8 mL) was added over a period of 5 min to quench the reaction. The cooling bath was removed, 50 mL of satd aq sodium potassium tartrate solution and 100 mL of EtOAc were added, and the mixture stirred vigorously for 3 h. The organic layer was washed with water and brine, and concentrated in vacuo. The residue (950 mg, 2.80 mmol) was dissolved in 25 mL of Et_3N and acetic anhydride was dropwise added (1.3 mL) followed by DMAP (0.1 equiv, 34 mg) at 0 °C. The reaction mixture was warmed to room temperature for 3 h and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (*n*-hexane/EtOAc=95:5, 9:1) to afford a mixture of α/β isomers of **15** (880 mg, 2.3 mmol) in 78% global yield for the two reactions. NMR showed that there were 66% of α isomer and 34% of β isomer in the mixture of **15**.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 0.12 (d, J=2.2 Hz, 6H, $SiCH_3$ (α isomer)), 0.17 (s, 6H, $SiCH_3$ (β isomer)), 0.92 (t, J=3.0 Hz, 18 H, C-CH_3, α+β isomer), 2.00 (s, 3H, CH_3, α isomer), 2.14 (s, 3H, CH_3, β isomer), 3.80 (dd, J_1=4.4 Hz, J_2=9.2 Hz, 1H, C(4′)H_a, α isomer), 4.01 (dd, J_1=4.0 Hz, J_2=9.2 Hz, 1H, C(4′)H_a, β isomer), 4.26 (m, 2H, C(4′)H_b, α+β isomer), 4.46 (m, 1H, C(3′)H, β isomer), 4.68 (m, 1H, C(3′)H, α isomer), 5.27 (m, 2H, C(2′)H, α+β isomer), 6.22 (s, 1H, C(1′)H, β isomer), 6.48 (d, J=4.0 Hz, C(1′)H, α isomer), 7.26–7.60 (m, 6H, Ar-H), 8.00–8.06 (m, 4H, Ar-H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C −4.87 ($SiCH_3$), −5.08 ($SiCH_3$), 17.86 ($C(CH_3)_3$), 20.84, 21.00 (CO-CH_3), 25.54 ($C(CH_3)_3$), 72.53 (C-3′, 1′α isomer), 73.38 (C-4′, 1′α isomer), 74.83 (C-3′, 1′β isomer), 75.38 (C-4′, 1′β isomer), 78.99 (C-2′, 1′α isomer), 83.21 (C-2′, 1′β isomer), 94.65 (C-1′, 1′α isomer), 99.90 (C-1′, 1′β isomer), 128.56 (Ar-C), 129.22 (Ar-C), 129.71 (Ar-C), 133.47 (Ar-C), 165.55 (Bz-*C*O), 169.70 (CH_3CO); Exact mass calcd for $C_{19}H_{28}O_6$-SiNa [M+Na]$^+$ 403.1553, found 403.1554.

4.1.9. 1-α-(Adenin-9-yl)-2-*O*-benzoyl-3-*O*-tertbutyldimethylsilyl-L-threose (16). To a mixture of **15** (1.0 g, 2.6 mmol) and N^6-benzoyladenine (0.7 g, 5.2 mmol) in dry MeCN (98 mL) was dropwise added $SnCl_4$ (0.98 mL, 7.8 mmol) under N_2 at 0 °C. The reaction mixture was stirred at room temperature for 1.5 h.

Then the reaction was quenched with satd $NaHCO_3$ and concentrated. The residue was partitioned between H_2O (50 mL) and EtOAc (200 mL). The organic layer was washed with water and brine, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH=98:2) to afford **16** (487 mg, 1.02 mmol) as a white solid in 39% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 0.14 (d, J=13.2 Hz, 6H, $SiCH_3$), 0.91 (s, 9H, CH_3), 4.33–4.36 (m, 2H, C(4′)H_2), 4.53–4.55 (m, 1H, C(3′)H), 5.62 (s, 1H, C(2′)H), 5.92 (br s, 2H, NH_2), 6.48 (s, 1H, C(1′)H), 7.47–7.65 (m, 3H, Ar-H), 8.08–8.12 (m, 2H, Ar-H), 8.13 (s, 1H, C(2 or 8)H), 8.38 (s, 1H, C(8 or 2)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C −5.27 ($SiCH_3$), −4.84 ($SiCH_3$), 17.95 ($C(CH_3)_3$), 25.63 ($C(CH_3)_3$), 75.31 (C-4′), 76.68 (C-3′), 82.87 (C-2′), 87.99 (C-1′), 119.81 (A-C(5)), 128.73 (Ar-C), 128.75 (Ar-C), 130.07 (Ar-C), 133.95 (Ar-C), 139.69 (A-C(8)), 149.83 (A-C(4)), 153.32 (A-C(2)), 155.59 (A-C(4)), 165.24 (OBz(CO)); Exact mass calcd for $C_{22}H_{29}N_5O_4SiNa$ [M+Na]$^+$ 478.1887, found 478.1911.

4.1.10. 1-α-(Adenin-9-yl)-3-*O*-tertbutyldimethylsilyl-L-threose (17). A solution of **16** (480 mg, 1.05 mmol) in MeOH satd with ammonia (79 mL) was stirred at room temperature overnight. The mixture was concentrated and the residue was purified by chromatography column (CH_2Cl_2/MeOH=92:8) to give compound **17** (370 mg, 1.04 mmol) in 99% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 0.10 (s, 6H, $SiCH_3$), 0.82 (s, 9H, CH_3), 2.47 (br s, 1H, OH), 4.20–4.23 (m, 1H, C(4′)H_a), 4.39–4.46 (m, 3H, C(4′)H_b, C(2′)H, C(3′)H), 1.82 (br s, 2H, NH_2), 6.10 (d, J=2.4 Hz, 1H, C(1′)H), 8.14 (s, 1H, C(2 or 8)H), 8.35 (s, 1H, C(8 or 2)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C −5.39 ($SiCH_3$), 17.41 ($C(CH_3)_3$), 25.24 ($C(CH_3)_3$), 76.11 (C-4′), 76.32 (C-3′), 81.36 (C-2′), 91.62 (C-1′), 119.38 (A-C(5)), 138.96 (A-C(8)), 148.62 (A-C(6)), 152.32 (A-C(2)), 155.26 (A-C(4)); Exact mass calcd for $C_{15}H_{25}N_5O_3SiNa$ [M+Na]$^+$ 374.1624, found 374.1624.

4.1.11. 1-α-(Adenin-9-yl)-2-azido-2-deoxy-3-*O*-tertbutyldimethylsilyl-L-erythrose (18). Compound **17** (150 mg, 0.43 mmol) was dissolved in anhydrous DCM (2 mL) and DMAP (130 mg) was added. The solution was cooled to 0 °C and CF_3SO_2Cl (90 μL, 0.86 mmol) was added dropwise. After being stirred at 0 °C for 2 h, the suspension was taken up in AcOH/H_2O(1:99)/CH_2Cl_2 and extracted twice. The organic layers were washed with satd $NaHCO_3$

solution and brine, dried over Na_2SO_4, evaporated and dried under vacuum resulting in a white solid. The crude reaction mixture was taken up in DMF (4 mL) and added to solid lithium azide (90 mg, 2.15 mmol) under N_2. The solution was stirred at room temperature overnight, concentrated and redissolved in CH_2Cl_2, and washed with $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and evaporated.

Purification by preparative TLC using as eluent CH_2Cl_2/MeOH=9:1 afforded **18** as a white solid (60 mg, 0.16 mmol) in 37% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 0.19 (d, J=1.8 Hz, 6H, $SiCH_3$), 0.96 (s, 9H, CH_3), 4.15 (d, J=5.2 Hz, 2H, C(4′)H_2), 4.29 (m, 1H, C(2′)H), 4.72 (m, 1H, C(3′)H), 5.71 (br s, 2H, NH_2), 6.48 (d, J=5.4 Hz, 1H, C(1′)H), 8.22 (s, 1H, C(2 or 8)H), 8.38 (s, 1H, C(8 or 2)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C −6.52 ($SiCH_3$), 18.45 ($C(CH_3)_3$), 24.23 (C(CH_3)$_3$), 62.56 (C-2′), 71.06 (C-4′), 71.79 (C-3′), 81.38 (C-1′), 120.25 (C(5)), 139.08 (A-C(8)), 146.40 (C(6)), 151.74 (C(2)), 153.95 (C(4)); Exact mass calcd for $C_{15}H_{24}N_8O_2SiNa$ $[M+Na]^+$ 399.1869, found 399.1703.

4.1.12. 1-α-(Adenin-9-yl)-2-azido-2-deoxy-L-erythrose (19). To the solution of **18** (120 mg, 0.32 mmol) in 3 mL of THF, was added at room temperature TBAF (64 μL, 0.64 mmol). The solution was stirred at room temperature for 2 h. The mixture was concentrated and the residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH=9:1) to afford **19** (70 mg, 0.27 mmol) as a white solid in 84% yield.

^{1}H NMR (200 MHz, DMSO-d_6) δ_H 4.04–4.06 (m, 2H, C(4′)H_2), 4.54–4.63 (m, 2H, C(2′)H, C(3′)H), 6.31 (d, J=4.6 Hz, 1H, OH), 6.37 (d, J=6.2 Hz, 1H, C(1′)H), 7.32 (br s, 2H, NH_2), 8.16 (s, 1H, A-C(2 or 8)H), 8.29 (s, 1H, A-C(8 or 2)H); ^{13}C NMR (200 MHz, DMSO-d_6) δ_C 63.63 (C-2′), 70.52 (C-4′), 74.25 (C-3′), 82.84 (C-1′), 119.02 (A-C(5)), 140.97 (A-C(8)), 150.89 (A-C(6)), 153.35 (A-C(2)), 156.75 (A-C(4)); Exact mass calcd for $C_9H_{10}N_8O_2Na_1$ $[M+Na]^+$ 285.0825, found 285.0839. Elem. and calcd for $C_9H_{10}N_8O_2$ (MW, 262.0927) C: 41.21, H: 3.85, N: 42.74. Found C: 41.54, H: 3.71, N: 42.48.

4.1.13. 1-α-(Adenin-9-yl)-2-azido-2-deoxy-3-*O*-(diisopropylphosphonomethyl)-L-erythrose (20). To the solution of **19** (30 mg, 0.11 mmol) in 3 mL of THF, which was cooled using dry-ice in acetone, was added sodium hydride 80% (7 mg, 0.22 mmol). The mixture was stirred for 10 min and the solution of the trifluoromethanesulfonate of the phosphonate reagent (65 mg, 0.22 mmol) in THF was slowly dropped into the reaction flask. Then the mixture was slowly warmed up to room temperature. The reaction was quenched with satd $NaHCO_3$ and concentrated. The residue was partitioned between H_2O and CH_2Cl_2. The organic layer was washed with water and brine, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH=94:6) to afford **20** (37 mg, 0.08 mmol) in 74% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.33–1.39 (m, 12H, CH_3), 3.80–3.99 (m, 2H, PCH_2), 4.16 (dd, J_1=4.8 Hz, J_2=10.2 Hz, 1H, C(4′)H_a), 4.32–4.51 (m, 2H, C(2′)H, C(4′)H_b), 4.57 (dd, J_1=4.4 Hz, J_2=8 Hz, 1H, C(3′)H), 4.74–4.85 (m, 2H, OCH(CH_3)$_2$), 5.87 (br s, 2H, NH_2), 6.47 (d, J=6.4 Hz, 1H, C(1′)H), 8.23 (s, 1H, C(2 or 8)H), 8.35 (s, 1H, C(8 or 2)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 24.05 (CH(CH_3)$_3$), 63.14 (C-2′), 65.70 (PCH_2), 70.97 (C-4′), 71.64 (CH(CH_3)$_3$), 80.25 (d, $J_{P,C}$=30.4 Hz, C-3′), 82.81 (C-1′), 118.79 (A-C(5)), 140.54 (A-C(8)), 150.19 (A-C(6)), 153.29 (A-C(2)), 155.56 (A-C(4)); Exact mass calcd for $C_{16}H_{25}N_8O_5PNa$ $[M+Na]^+$ 463.1583, found 463.1621. Elem. and calcd for $C_{10}H_{25}N_8O_5P$ (MW, 440.1685) C: 43.62, H: 5.72, N: 25.45. Found C: 43.93, H: 5.41, N: 25.07.

4.1.14. 1-α-(Adenin-9-yl)-2-azido-2-deoxy-3-*O*-(phosphonomethyl)-L-erythrose sodium salt (2). To a solution of **20** (80 mg, 0.18 mmol) and Et_3N (0.26 mL) in DCM (7.2 mL) was added iodotrimethylsilane (0.20 mL) at 0 °C. The reaction mixture was stirred for 4 h at room temperature. The reaction was quenched with 1.0 M TEAB solution. The mixture was concentrated and the residue was purified by column chromatography (CH_2Cl_2/MeOH=8:2, 5:5, 2:8) to give crude title compound. Purification using Sephadex-DEAE A-25 with gradient TEAB solution from 0.01 to 1.0 M and ion exchanges by Dowex-Na^+ resin afforded **2** (30 mg, 0.07 mmol) as a white solid in 41% yield.

^{1}H NMR (500 MHz, D_2O) δ_H 3.70 (d, J=10.8 Hz, 2H, CH_2-P), 4.19 (dd, J_1=10.2 Hz, J_2=4.2 Hz, 1H, C(4′)H_b), 4.52 (dd, J_1=10.2 Hz, J_2=2.3 Hz, 1H, C(4′)H_a), 4.59 (m, 1H, C(3′)H), 4.78 (dd, J_1=5.1 Hz, J_2=6.3 Hz, 1H, C(2′)H), 6.47 (d, J=6.3 Hz, 1H, C(1′)H), 8.24 (s, 1H, A-C(8)H), 8.53 (s, 1H, A-C(2)H); ^{13}C NMR (500 MHz, D_2O) δ_C 66.02 (C-2′), 70.77 (d, PCH_2), 74.24 (C-4′), 81.86 (C-3′), 85.92 (C-1′), 120.29 (A-C(5)), 144.72 (A-C(8)), 151.55 (A-C(6)), 155.54 (A-C(2)), 158.29 (A-C(4)); ^{31}P NMR (500 MHz, D_2O) δ_P 13.46; Exact mass calcd for $C_{10}H_{14}N_8O_5P$ $[M+H]^+$ 357.0825, found 357.0829.

4.1.15. 1,2:5,6-Di-*O*-isopropylidene-3-*O*-(diisopropylphosphonomethyl)-α-D-galactofuranose (22). To a solution of 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose **21** (1.16 g, 4.46 mmol) in dried THF (25 mL) was added sodium hydride (80% dispersion in mineral oil, 268 mg, 8.92 mmol) at −78 °C. Then the solution of the triflate of diisopropylphosphonomethanol[3] (2.64 g, 8.92 mmol) in dried THF (5 mL) was dropwise added, and the reaction mixture was slowly warmed to room temperature. The reaction was quenched with satd $NaHCO_3$ and concentrated. The residue was partitioned between H_2O and EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (DCM/MeOH=60:1) to afford **22** (1.64 g, 3.75 mmol, 84%) as colorless oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.29–1.51 (m, 24H, CH_3), 3.67–3.89 (m, 5H, PCH_2, C(4′)H, C(6′)H_2), 4.42 (dd, J_1=4.2 Hz, J_2=6.6 Hz, C(3′)H), 4.58 (d, J=4.0 Hz, C(2′)H), 4.22–4.31 (m, 1H, C(5′)H), 4.64–4.81 (m, 2H, CH(CH_3)$_2$), 5.79 (d, J=4.0 Hz, C(1′)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.91 (CH_3), 25.22 (CH_3), 26.71 (CH_3), 27.38 (CH_3), 64.89 (d, $J_{P,C}$=170.9 Hz, PCH_2), 65.59 (C-6′), 71.17 (CH(CH_3)$_2$), 71.29 (CH(CH_3)$_2$), 75.48 (C-5′), 83.44 (C-2′), 84.86 (C-4′), 84.52 (d, $J_{P,C}$=12.2 Hz), 104.92 (C-1′), 109.8

($C(O)_2$), 113.88 ($C(O)_2$); Exact mass (EI) calcd for $C_{16}H_{29}O_8P$ [M−C_3H_6O] 380.1600, found 380.1598.

4.1.16. 1,2-*O*-Isopropylidene-3-*O*-(diisopropylphosphonomethyl)-5-oxo-β-L-arabinofuranose (23). To a solution of **22** (5.00 g, 10.3 mmol) in 5 mL of ethyl acetate was added a well-stirred suspension of $NaIO_4$ (2.21 g, 10.3 mmol) and H_5IO_6 (1.17 g, 5.16 mmol) in 100 mL of ethyl acetate. After stirring for 5 h at room temperature, the mixture was filtered. The filtrate was concentrated to dry, affording **23** (3.74 g, 10.2 mmol) as colorless oil in 99% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.28–1.42 (m, 18H, CH_3), 3.77 (s, 1H, PCH_a), 3.82 (s, 1H, PCH_b), 4.28 (s, 1H, C(4′)H), 4.50 (s, 1H, C(3′)H), 4.63 (d, J=3.5 Hz, 1H, C(2′)H), 4.60–4.90 (m, 2H, $CH(CH_3)_2$), 6.02 (d, J=3.5 Hz, 1H, C(1′)H), 9.75 (s, 1H, C(5′)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 22.41 (CH_3), 25.66 (CH_3), 26.21 (CH_3), 64.55 (d, $J_{P,C}$=169.4 Hz, PCH_2), 71.62 ($CH(CH_3)_2$), 71.29 ($CH(CH_3)_2$), 82.51 (C-2′), 87.50 (d, $J_{P,C}$=10.7 Hz, (C-3′)), 88.58 (C-4′), 106.7 (C-1′), 112.67 ($C(O)_2$), 201.49 (C-5′).

4.1.17. 1,2-*O*-Isopropylidene-3-*O*-(diisopropylphosphonomethyl)-5-deoxy-5-chloromethylene-β-L-arabinofuranose (24). A suspension of (chloromethyl)triphenylphosphonium chloride (3.82 g, 11 mmol) in anhydrous THF (50 mL) was cooled to −78 °C and treated with a solution of *n*-BuLi in hexane (1.6 M, 6.87 mL, 11 mmol). After 1 h of stirring, a solution of aldehyde **23** (1.04 g, 2.75 mmol) in dry THF (50 mL) was added. The temperature of the bath was raised to 0 °C and stirring continued for 3 h. Following cautious addition of an aq satd solution of NH_4Cl, the reaction mixture was extracted with EtOAc (2×150 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (DCM/MeOH=98:2) to afford **24** contaminated with triphenylphosphine. A sample for NMR analysis was prepared by preparative TLC.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.32–1.52 (m, 15H, CH_3), 1.72 (s, 3H, CH_3), 3.62–4.04 (m, 3H, PCH_2, C(4′)H), 4.52–5.12 (m, 4H, C(3′)H, C(2′)H, $CH(CH_3)_2$), 5.93–5.95 (m, 1H, C(1′)H), 6.04–6.16 (m, 1H, C(5′)H), 6.31–6.38 (m, 1H, C(6′)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.94 (CH_3), 26.16 (CH_3), 26.65 (CH_3), 64.70 (d, $J_{P,C}$=170.9 Hz, PCH_2), 71.32 ($CH(CH_3)_2$), 83.01 (C-2′), 84.01 (C-4′), 88.40 (d, $J_{P,C}$=10.5 Hz, C-3′), 105.83 (C-1′), 113.15 ($C(O)_2$), 121.80 (C-5′), 133.61 (C-6′); Exact mass calcd for $C_{16}H_{28}Cl_1O_7PNa$ $[M+Na]^+$ 421.1159, found 421.1165.

4.1.18. 1,2-Di-*O*-benzoyl-3-*O*-(diisopropylphosphonomethyl)-5-deoxy-5-chloromethylene-α,β-L-arabinofuranose (25). A solution of crude product **24** in TFA/H_2O (3:1, 20 mL) was allowed to stand at room temperature for 2 h. The reaction mixture was neutralized with satd $NaHCO_3$ solution. The mixture was partitioned between the DCM (400 mL) and water (20 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by chromatography on silica gel (DCM/MeOH=20:1) to give the debenzoylated compound (460 g, 1.28 mmol) as a colorless amorphous solid in 46% of yield calculated from compound **23**.

To the solution of 3-*O*-(diisopropylphosphonomethyl)-5-deoxy-5-chloromethylene-α,β-L-arabinofuranose (450 mg, 1.25 mmol) in 100 mL pyridine was added dropwise BzCl (0.31 mL, 2.75 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was concentrated and coevaporated with 20 mL of toluene two times in vacuo. The residue was partitioned between H_2O (30 mL) and EtOAc (200 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (*n*-hexane/EtOAc=2:1) to afford **25** (610 mg, 1.07 mmol) as colorless oil in 86% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.30–1.34 (m, 12H, CH_3), 3.89–4.21 (m, 3H, PCH_2, C(4′)H), 4.54–4.86 (m, 3H, C(3′)H, $CH(CH_3)_2$), 5.56–5.62 (m, 1H, C(2′)H), 5.94–6.29 (m, 1H, C(5′)H), 6.39–6.51 (m, 1H, C(6′)H, (1′)H), 6.61–6.75 (m, 1H, C(6′)H), 7.35–7.68 (m, 9H, Ar-H), 7.92–8.18 (m, 6H, Ar-H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.95 (CH_3), 65.41 (d, $J_{P,C}$=167.9 Hz, PCH_2), 71.42 ($CH(CH_3)_2$), 80.37, 80.55 (C-4′), 83.28 (C-2′), 89.03 (d, $J_{P,C}$=10.7 Hz, (C-3′)), 94.60, 99.82 (C-1′), 122.89 (C-5′), 128.51, 128.69, 129.78, 129.90, 130.15 (Ar-C), 131.63 (C-6′), 133.67, 133.88 (Ar-C), 165.14 (BzCO); Exact mass calcd for $C_{27}H_{32}Cl_1O_9PNa$ $[M+Na]^+$ 589.1370, found 589.1375.

4.1.19. 1-(N^6-Benzoyladenin-9-yl)-2-*O*-benzoyl-3-*O*-(diisopropylphosphonomethyl)-5-deoxy-5-chloromethylene-α-L-arabinofuranose (26). To a mixture of **25** (270 mg, 0.48 mmol) and silylated N^6-benzoyladenine (225 mg, 0.96 mmol) in dry MeCN (30 mL) was dropwise added $SnCl_4$ (0.11 mL, 0.94 mmol) under N_2 at room temperature. The reaction mixture was stirred at room temperature for 4–5 h. Then the reaction was quenched with satd $NaHCO_3$ and concentrated. The residue was partitioned between H_2O (20 mL) and EtOAc (100 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (DCM/MeOH=40:1) to afford **26** (239 mg, 0.35 mmol) as a colorless amorphous solid in 73% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.30–1.37 (m, 12H, CH_3), 3.89–4.14 (m, 2H, PCH_2), 4.38–4.40 (m, 1H, C(4′)H), 4.56–4.84 (m, 2H, $CH(CH_3)_2$), 4.85–4.92 (m, 0.8H, C(3′)H), 5.60–5.62 (m, 0.2H, C(3′)H), 5.94–6.75 (m, 4H, C(2′)H, C(1′)H, C(5′)H, C(6′)H), 7.40–7.70 (m, 6H, Ar-H), 8.03–8.07 (m, 4H, Ar-H), 8.42 (s, 0.8H, C(8)H), 8.54 (s, 0.2H, C(8)H), 8.84 (s, 1H, C(2)H), 9.12 (s, 1H, NH); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.94 (CH_3), 65.65 (d, $J_{P,C}$=181.5 Hz, PCH_2), 71.51, 71.63 ($CH(CH_3)_2$), 80.28 (C-4′), 83.98 (C-2′), 87.90 (C-3′), 88.11 (C-1′), 122.00 (C-5), 123.20 (C-5′), 127.90, 128.81, 128.93, 129.37, 129.99 (Ar-C), 132.85 (C-6′), 134.18, (Ar-C), 141.95 (C-8), 149.72 (C-6), 153.09 (C-2), 165.32 (CO), C-4 was obscured in noise peak; Exact mass calcd for $C_{32}H_{35}Cl_1N_5O_8PNa$ $[M+Na]^+$ 706.1809, found 706.1806.

4.1.20. 1-(Adenin-9-yl)-3-*O*-(diisopropylphosphonomethyl)-5-deoxy-5-chloromethylene-α-L-arabinofuranose (27). A solution of **26** (328 mg, 0.48 mmol) in MeOH satd with ammonia (100 mL) was stirred at room

temperature overnight. The mixture was concentrated and the residue was purified by column chromatography (CH_2Cl_2/MeOH=20:1) to give compound **27** (210 mg, 0.42 mmol) as a white powder in 88% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.25–1.31 (m, 12H, CH_3), 3.84–4.08 (m, 3H, PCH_2, C(4′)H), 4.57–4.80 (m, 2H, C*H*(CH_3)$_2$), 4.85–4.92 (m, 1H, C(3′)H), 5.04 (dd, J_1= 5.1 Hz, J_2=4.7 Hz, 0.86H, C(2′)H), 5.42–5.47 (m, 0.16H, C(2′)H), 5.99–6.43 (m, 3H, C(1′)H, C(5′)H, C(6′)H), 6.59 (br s, 1.68H, NH), 6.45 (br s, 0.28H, NH), 7.97 (s, 0.86H, C(8)H), 8.07 (s, 0.24H, C(8)H), 8.16 (s, 1H, C(2)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.82 (CH_3), 65.59 (d, $J_{P,C}$= 181.5 Hz, PCH_2), 71.57, 71.72, 71.87, 71.99 (*C*H(CH_3)$_2$), 78.76 (C-4′), 81.46 (C-2′), 89.74 (C-3′, C-1′), 119.68 (C-5), 122.98 (C-5′), 129.78, 130.45 (C-6′), 139.34 (C-8), 149.12 (C-6), 152.76 (C-2), 155.64 (C-4); Exact mass calcd for $C_{18}H_{28}Cl_1N_5O_6P$ $[M+H]^+$ 476.1466, found 476.1465.

4.1.21. 1-(Adenin-9-yl)-3-*O*-(phosphonomethyl)-5-deoxy-5-chloromethylene-α-L-arabinofuranose disodium salt (3). To a solution of **27** (140 mg, 0.30 mmol) and Et_3N (1 mL) in DCM (25 mL) was added iodotrimethylsilane (0.43 mL, 3.0 mmol) at 0 °C. The reaction mixture was stirred for 2 h. The reaction was quenched with 1.0 M TEAB solution. The mixture was concentrated and the residue was purified by column chromatography (CH_2Cl_2/MeOH=2:1, 1:1, 1:2) to give crude 1-(adenin-9-yl)-3-*O*-(phosphonomethyl)-5-deoxy-5-chloromethylene-α-L-arabinofuranose triethylammonium salt. Purification using Sephadex-DEAE A-25 with gradient TEAB solution from 0.01 to 0.5 M and ion exchanges by the Dowex-Na^+ resin afforded **3** (25 mg, 0.06 mmol) as a colorless solid after precipitation from diethylether in 20% yield.

^{1}H NMR (200 MHz, D_2O) δ_H 3.23–3.54 (m, 2H, PCH_2), 3.76–3.87 (m, 1H, C(4′)H), 4.55–4.61 (m, 1.84H, C(3′)H, C(2′)H), 5.06–5.13 (m, 0.16H, C(2′)H), 5.76–5.96 (m, 2H, C(1′)H, C(5′)H), 6.13 (d, J=9.3 Hz, 0.16H, C(6′)H), 4.25 (d, J=13.2 Hz, 0.84H, C(6′)H), 7.77 (s, 1H, C(8)H), 8.01 (s, 0.84H, C(2)H), 8.04 (s, 0.84H, C(2)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 70.62 (d, $J_{P,C}$=151.1 Hz, PCH_2), 80.04, 81.29 (C-4′), 84.20 (C-2′), 90.06, 90.97 (C-1′), 91.06 (d, $J_{P,C}$=9.2 Hz, C-3′), 120.63 (C-5), 125.27, 125.61 (C-5′), 130.86, 132.56 (C-6′), 142.82 (C-8), 150.74 (C-6), 154.84 (C-2), 157.51 (C-4); Exact mass calcd for $C_{12}H_{16}N_5O_6P$ $[M+H]^+$ 392.0527, found 392.0521.

4.1.22. 1,2-*O*-Isopropylidene-3-*O*-(diisopropylphosphonomethyl)-β-L-arabinofuranose (28). To a solution of **23** (3.70 g, 10.1 mmol) in MeOH (50 mL) was added $NaBH_4$ (1.15 g, 30.9 mmol) at room temperature. After the mixture was stirred at room temperature for 2 h, AcOH (10%) was added to neutralize the reaction mixture. Then the mixture was concentrated and partitioned between EtOAc (200 mL) and H_2O (50 mL). The organic phase was washed with brine, dried with Na_2SO_4, and concentrated to dryness. The residue was purified by chromatography on a silica gel column (DCM/MeOH=95:1) to afford **28** (3.40 g, 10.0 mmol) as colorless oil in 99% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.33–1.36 (m, 15H, CH_3), 1.53 (s, 1H, CH_3), 3.71–3.94 (m, 4H, PCH_2, C(3′)H, C(4′)H), 4.08–4.17 (m, 2H, C(5′)H_2), 4.64 (d, J=4.0 Hz, 1H, C(2′)H), 4.65–4.92 (m, 2H, CH(CH_3)$_2$), 5.87 (d, J=4.0 Hz, 1H, C(1′)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.88 (CH_3), 26.28 (CH_3), 27.04 (CH_3), 62.40 (C-5′), 64.54 (d, $J_{P,C}$=169.4 Hz, PCH_2), 71.32 (*C*H(CH_3)$_2$), 71.45 (*C*H(CH_3)$_2$), 84.86 (C-2′), 84.98 (C-4′), 85.79 (d, $J_{P,C}$= 10.6 Hz, C-3′), 105.3 (C-1′), 113.03 ($C(O)_2$); Exact mass calcd for $C_{15}H_{29}O_8PNa$ $[M+Na]^+$ 391.1498, found 391.1492.

4.1.23. 1,2-*O*-Isopropylidene-3-*O*-(diisopropylphosphonomethyl)-5-*O*-benzoyl-β-L-arabinofuranose (29). To a solution of **28** (3.20 g, 8.7 mmol), DMAP (106 mg, 0.87 mmol) and Et_3N (3.7 mL, 26.1 mmol) in 50 mL of DCM was added dropwise BzCl (1.3 mL, mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was concentrated in vacuo. The residue was partitioned between H_2O (50 mL) and EtOAc (250 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (DCM/MeOH=98:2) to afford **29** (2.81 g, 5.9 mmol) as colorless oil in 68% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.31–1.36 (m, 15H, CH_3), 1.56 (s, 1H, CH_3), 3.80 (s, 1H, PCH_a), 3.85 (s, 1H, PCH_b), 4.11 (d, J=2.6 Hz, 1H, C(4′)H), 4.34–4.42 (m, 1H, C(5′)H_a), 4.50 (s, 1H, C(3′)H), 4.53 (d, J=1.5 Hz, 1H, C(5′)H_b), 4.68 (d, J=3.7 Hz, 1H, C(2′)H), 4.67–4.84 (m, 2H, C*H*(CH_3)$_2$), 5.92 (d, J=3.7 Hz, 1H, C(1′)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.94 (CH_3), 26.13 (CH_3), 27.04 (CH_3), 64.31 (C-5′), 64.68 (d, $J_{P,C}$=170.9 Hz, PCH_2), 71.36 (*C*H(CH_3)$_2$), 82.19 (C-4′), 84.16 (C-2′), 85.80 (d, $J_{P,C}$=12.2 Hz, C-3′), 105.80 (C-1′), 113.24 ($C(O)_2$), 128.45 (Ar-C), 128.78 (Ar-C), 133.24 (Ar-C), 166.23 (BzCO); Exact mass (EI) calcd for $C_{19}H_{27}O_8P$ $[M-C_3H_6O]$ 414.1444, found 414.1437.

4.1.24. 1,2,5-Tri-*O*-benzoyl-3-*O*-(diisopropylphosphonomethyl)-α-L-arabinofuranose (30a) and 1,2,5-tri-*O*-benzoyl-3-*O*-(diisopropylphosphonomethyl)-β-L-arabinofuranose (30b). A solution of **29** (2.71 g, 5.7 mmol) in TFA/H_2O (3:1, 20 mL) was allowed to stand at room temperature for 2 h. The reaction mixture was neutralized with satd $NaHCO_3$ solution. Then the mixture was partitioned between DCM (400 mL) and water (20 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by chromatography on silica gel (DCM/MeOH=30:1) to give 3-*O*-(diisopropylphosphonomethyl)-α,β-L-arabinofuranose (2.21 g, 5.1 mmol) as colorless oil in 89% yield.

To the solution of 3-*O*-(diisopropylphosphonomethyl)-α,β-L-arabinofuranose (2.11 g, 5.1 mmol) in 100 mL pyridine was added dropwise BzCl (14.0 mL, 12.2 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was concentrated and coevaporated with 20 mL toluene two times in vacuo. The residue was partitioned between H_2O (20 mL) and EtOAc (150 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (DCM/MeOH=95:5) to afford **30a** and **30b** (2.64 g, 4.1 mmol) as colorless oil in 81% yield. The H-1′

proton of **30a** appeared as a singlet while the H-1′ proton of **30b** is doublet (J=4.7 Hz).

Compound **30a**: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.26–1.39 (m, 12H, CH_3), 4.06–4.38 (m, 3H, PCH_2, C(4′)H), 4.57 (dd, J_1=12.8 Hz, J_2=5.5 Hz, 1H, C(5′)H_a), 4.68–4.91 (m, 4H, C(3′)H, C(5′)H_b, CH(CH_3)$_2$), 5.62 (s, 1H, C(2′)H), 6.72 (s, 1H, C(1′)H), 7.12–7.65 (m, 9H, Ar-H), 7.96–8.16 (m, 6H, Ar-H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.79 (CH_3), 63.22 (C-5′), 65.24 (d, $J_{P,C}$=169.4 Hz, PCH_2), 71.48 (*C*H(CH_3)$_2$), 71.60 (*C*H(CH_3)$_2$), 80.61 (C-4′), 83.31 (C-2′), 85.98 (d, $J_{P,C}$=12.2 Hz, C-3′), 100.04 (C-1′), 128.23–129.36 (Ar-C), 129.63–130.06 (Ar-C), 133.06–133.67 (Ar-C), 164.87, 165.32, 166.14 (BzCO); Exact mass (EI) calcd for $C_{33}H_{37}O_{11}P$ [M] 640.2073, found 640.2072.

Compound **30b**: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.26–1.36 (m, 12H, CH_3), 3.96–4.12 (m, 3H, PCH_2, C(4′)H), 4.48–4.82 (m, 4H, C(5′)H_2, C(3′)H, CH(CH_3)$_2$), 5.63 (dd, J_1=6.9 Hz, J_2=4.7 Hz, 1H, C(2′)H), 6.77 (d, J=4.7 Hz, 1H, C(1′)H), 7.23–7.61 (m, 9H, Ar-H), 7.86–8.12 (m, 6H, Ar-H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.82 (CH_3), 64.28 (C-5′), 65.12 (d, $J_{P,C}$=169.3 Hz, PCH_2), 71.38 (*C*H(CH_3)$_2$), 71.51 (*C*H(CH_3)$_2$), 77.52 (C-2′), 79.46 (C-4′), 82.52 (d, $J_{P,C}$=12.1 Hz, C-3′), 94.45 (C-1′), 128.39, 128.51, 129.24, 129.69, 132.76, 133.15, 133.39, 133.63 (Ar-C), 164.81, 165.32, 166.23 (BzCO); Exact mass (EI) calcd for $C_{33}H_{37}O_{11}P$ [M] 640.2073, found 640.2076.

4.1.25. 1-(N^6-Benzoyladenin-9-yl)-2,5-di-*O*-benzoyl-3-*O*-(diisopropylphosphonomethyl)-α-L-arabinofuranose (31). To a mixture of **30a** and **30b** (720 mg, 1.12 mmol) and N^6-benzoyladenine (537 mg, 2.24 mmol) in dry MeCN (50 mL) was added dropwise $SnCl_4$ (522 μL, 4.48 mmol) under N_2 at room temperature. The reaction mixture was stirred at room temperature for 24 h. Then the reaction was quenched with satd $NaHCO_3$ and concentrated. The residue was partitioned between H_2O (20 mL) and EtOAc (100 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (DCM/MeOH=100:1, 40:1) to afford **31** (520 mg, 0.69 mmol) as a colorless amorphous solid in 61% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 1.29–1.32 (m, 12H, CH_3), 4.05 (dd, J_1=9.5 Hz, J_2=13.9 Hz, 1H, PCH_a), 4.12 (dd, J_1=8.1 Hz, J_2=13.9 Hz, 1H, PCH_b), 4.58 (m, 6H, C(3′)H, C(4′)H, C(5′)H_2, CH(CH_3)$_2$), 6.11 (s, 1H, C(2′)H), 6.67 (s, 1H, C(1′)H), 7.30–7.67 (m, 9H, Ar-H), 8.02–8.06 (m, 6H, Ar-H), 8.50 (s, 1H, C(8)H), 8.79 (s, 1H, C(2)H), 9.55 (s, 1H, NH); ^{13}C NMR (500 MHz, $CDCl_3$) δ_C 23.79 (CH_3), 64.17 (C-5′), 65.05 (d, $J_{P,C}$=168 Hz, PCH_2), 71.32 (d, $J_{P,C}$=3.9 Hz, *C*H(CH_3)$_2$), 71.36 (d, $J_{P,C}$=3.9 Hz, *C*H(CH_3)$_2$), 71.51 (*C*H(CH_3)$_2$), 80.54 (C-2′), 83.42 (C-4′), 85.38 (d, $J_{P,C}$=9.8 Hz, C-3′), 88.00 (C-1′), 122.79 (C-5), 128.14, 128.22, 128.52, 129.14, 129.52, 129.71, 132.44, 133.13, 133.55, 133.83 (Ar-C), 141.57 (C-8), 149.62 (C-6), 151.53 (C-4), 152.70 (C-2), 164.67 (NHC=O), 165.12 (C(2′)OC=O), 165.90 (C(5′)OC=O); Exact mass calcd for $C_{38}H_{41}N_5O_{10}P$ $[M+H]^+$ 758.2591, found 758.2583.

4.1.26. 1-(Adenin-9-yl)-3-*O*-(diisopropylphosphonomethyl)-α-L-arabinofuranose (32). A solution of **31** (383 mg, 0.50 mmol) in MeOH satd with ammonia (100 mL) was stirred at room temperature overnight. The mixture was concentrated and the residue was purified by column chromatography (CH_2Cl_2/MeOH=20:1) to give compound **32** (196 mg, 0.44 mmol) as a colorless amorphous solid in 87% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.27–1.32 (m, 12H, CH_3), 3.80–3.98 (m, 4H, PCH_2, C(5′)H_2), 4.30 (t, J=5.1 Hz, C(4′)H), 4.60–4.80 (m, 2H, CH(CH_3)$_2$), 4.90 (dd, J_1=4.4 Hz, J_2=4.5 Hz, 1H, C(2′)H), 6.04 (d, J=4.4 Hz, 1H, C(1′)H), 6.32 (br s, 2H, NH), 8.04 (s, 1H, C(8)H), 8.16 (s, 1H, C(2)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.88 (CH_3), 61.58 (C-5′), 65.40 (d, $J_{P,C}$=167.8 Hz, PCH_2), 71.75 (m, *C*H(CH_3)$_2$), 79.27 (C-2′), 83.98 (C-4′), 85.73 (d, $J_{P,C}$=9.8 Hz, C-3′), 89.96 (C-1′), 119.43 (C-5), 139.25 (C-8), 149.12 (C-6), 152.73 (C-2), 155.49 (C-4); Exact mass calcd for $C_{17}H_{29}N_5O_7P$ $[M+H]^+$ 446.1805, found 446.1808.

4.1.27. 1-(Adenin-9-yl)-3-*O*-(phosphonomethyl)-α-L-arabinofuranose disodium salt (4). To a solution of **32** (60 mg, 0.13 mmol) and Et_3N (1 mL) in DCM (25 mL) was added iodotrimethylsilane (0.17 mL, 1.30 mmol) at 0 °C. After stirring for 2 h, the reaction mixture was quenched with 1.0 M TEAB solution. The mixture was concentrated and the residue was purified by column chromatography (CH_2Cl_2/MeOH=2:1, 1:1, 1:2) to give crude **4** as triethylammonium salt. Purification using Sephadex-DEAE A-25 with gradient TEAB solution from 0.01 to 0.5 M and ion exchanges by the Dowex-Na^+ resin afforded **4** (20 mg, 0.05 mmol) as a colorless solid after precipitation from diethylether in 38% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 3.73 (dd, J_1=9.0 Hz, J_2=12.9 Hz, 1H, PCH_a), 3.76 (dd, J_1=9.0 Hz, J_2=12.9 Hz, 1H, PCH_b), 3.86 (dd, J_1=12.7 Hz, J_2=5.1 Hz, 1H, C(5′)H_a), 3.89 (dd, J_1=12.7 Hz, J_2=3.9 Hz, 1H, C(5′)H_b), 4.18 (dd, J_1=3.9 Hz, J_2=5.1 Hz, 1H, C(4′)H), 4.47–4.50 (m, 1H, C(3′)H), 4.98 (dd, J_1=4.4 Hz, J_2=4.2 Hz, 1H, C(2′)H), 6.13 (d, J_1=4.4 Hz, 1H, C(1′)H), 8.21 (s, 1H, C(2)), 8.40 (s, 1H, C(8)); ^{13}C NMR (500 MHz, $CDCl_3$) δ_C 64.07 (C-5′), 70.40 (d, $J_{P,C}$=154.3 Hz, PCH_2), 80.82 (C-4′), 86.76 (C-2′), 88.24 (d, $J_{P,C}$=10.8 Hz, C-3′), 91.03 (C-1′), 121.40 (C-5), 143.37 (C-8), 151.49 (C-6), 155.49 (C-2), 158.26 (C-4); ^{31}P NMR (500 MHz, D_2O) δ_P 14.52; Exact mass calcd for $C_{11}H_{16}N_5O_7PNa$ $[M+Na]^+$ 384.0685, found 384.0685.

4.1.28. 1-(Adenin-9-yl)-3-*O*-(diisopropylphosphonomethyl)-5-*O*-methylsulfonyl-α-L-arabinofuranose (33). To a solution of **32** (128 mg, 0.29 mmol) in 200 mL of pyridine was added dropwise MsCl (0.35 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was concentrated and coevaporated with 5 mL toluene two times in vacuo. The residue was partitioned between H_2O (20 mL) and DCM (3×50 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (DCM/MeOH=15:1) to afford **33** (70 mg, 0.14 mmol) as a colorless amorphous solid in 48% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.31–1.37 (m, 12H, $C(CH_3)_2$), 3.10 (s, 3H, CH_3), 3.91 (dd, J_1=14.4 Hz, J_2=5.6 Hz, 1H, PCH_a), 4.06–4.21 (m, 2H, PCH_b, C(4′)H), 4.43 (dd, J_1=11.7 Hz, J_2=4.0 Hz, 1H, C(5′)H_a), 4.50 (dd, J_1=11.7 Hz, J_2=2.9 Hz, 1H, C(5′)H_b), 4.64–4.83 (m, 3H, C*H*(CH_3)$_2$, C(3′)H), 5.16 (dd, J_1=5.1 Hz, J_2=5.9 Hz, 1H, C(2′)H), 5.91–5.94 (m, 2H, NH_2, C(1′)H), 7.93 (s, 1H, C(8)H), 8.27 (s, 1H, C(2)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.85 (CH_3), 23.94 (CH_3), 37.72 (SCH_3), 66.41 (d, $J_{P,C}$=167.9 Hz, PCH_2), 67.53 (C-5′), 71.62 (d, $J_{P,C}$=6.1 Hz, POCH), 72.12 (d, $J_{P,C}$=6.1 Hz, POCH), 78.82 (C-4′), 79.97 (C-2′), 86.51 (d, $J_{P,C}$=4.6 Hz, C-3′), 89.72 (C-1′), 120.25 (C-5), 139.71 (C-8), 149.51 (C-6), 152.91 (C-2), 155.58 (C-4); Exact mass calcd for $C_{18}H_{31}N_5O_9PS$ $[M+H]^+$ 524.1580, found 524.1573.

4.1.29. 1-(Adenin-9-yl)-3-*O*-(diisopropylphosphonomethyl)-2,5-anhydro-α-L-arabinofuranose (34). To a solution of **33** (65 mg, 0.12 mmol) in dried THF (25 mL) was added sodium hydride (80% dispersion in mineral oil, 5 mg, 0.16 mmol) at −78 °C. Then the reaction mixture was slowly warmed to room temperature. The reaction was quenched with satd $NaHCO_3$ and concentrated. The residue was partitioned between H_2O (5 mL) and EtOAc (50 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (DCM/MeOH=15:1) to afford **34** (44 mg, 0.10 mmol, 83%) as colorless oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.28–1.32 (m, 12H, CH_3), 3.61 (dd, 1H, J_1=14.1 Hz, J_2=8.0 Hz, PCH_a), 3.65 (dd, 1H, J_1=14.1 Hz, J_2=8.0 Hz, PCH_b), 4.02 (d, J=8.8 Hz, 1H, C(5′)H_a), 4.05 (d, J=8.8 Hz, 1H, C(5′)H_b), 4.47 (br s, 1H, C(4′)H), 4.65–4.73 (m, 3H, POCH, C(3′)H), 4.84 (s, 1H, C(2′)H), 6.18 (s, 1H, C(1′)H), 8.20 (s, 1H, C(8)), 8.35 (s, 1H, C(2)); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.88 (CH_3), 64.42 (d, $J_{P,C}$=167.5 Hz, PCH_2), 71.50 (POCH), 72.05 (C-5′), 76.22 (C-4′), 77.92 (C-2′), 81.83 (C-3′), 86.67 (C-1′), 119.00 (C-5), 140.06 (C-8), 149.51 (C-4), 152.97 (C-2), 155.19 (C-6); Exact mass calcd for $C_{17}H_{27}N_5O_6PNa$ $[M+Na]^+$ 450.1518, found 450.1526.

4.1.30. 1-(Adenin-9-yl)-3-*O*-(phosphonomethyl)-2,5-anhydro-α-L-arabinofuranose disodium salt (5). To a solution of **34** (40 mg, 0.09 mmol) and Et_3N (1 mL) in DCM (25 mL) was added iodotrimethylsilane (0.12 mL, 0.90 mmol) at 0 °C. The reaction mixture was stirred for 2 h. The reaction was quenched with 1.0 M TEAB solution. The mixture was concentrated and the residue was purified by column chromatography (CH_2Cl_2/MeOH=2:1, 1:1, 1:2) to give crude **5** as triethylammonium salt. Purification using Sephadex-DEAE A-25 with gradient TEAB solution from 0.01 to 0.5 M and ion exchanges by the Dowex-Na^+ resin afforded **5** (15 mg, 0.04 mmol) as a colorless solid after precipitation from diethylether in 44% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 3.42–3.46 (m, 1H, C(5′)H_a), 3.56–3.60 (m, 1H, C(5′)H_b), 4.06 (d, J=8.9 Hz, 1H, PCH_a), 4.14 (d, J=8.9 Hz, 1H, PCH_b), 4.56 (s, 1H, C(4′)H), 4.83 (s, 1H, C(3′)H), 5.05 (s, 1H, C(2′)H), 6.05 (s, 1H, C(1′)H), 8.12 (s, 1H, C(8)H), 8.36 (s, 1H, C(2)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 69.62 (d, $J_{P,C}$=153.4 Hz, PCH_2), 74.87 (C-5′), 78.68 (C-4′), 81.10 (C-2′), 83.55 (C-3′), 88.69 (C-1′), 120.62 (C-5), 144.21 (C-8), 150.85 (C-6), 155.07 (C-2), 157.86 (C-4); ^{31}P NMR (500 MHz, D_2O) δ_P 13.88; Exact mass calcd for $C_{11}H_{15}N_5O_6P$ $[M+H]^+$ 344.0760, found 344.0753.

4.1.31. 1-(Adenin-9-yl)-3-*O*-(diphosphorylphosphonomethyl)-2,5-anhydro-α-L-arabinofuranose triethylammonium salt (35). After conversion of the sodium salt of compound **5** into the triethylammonium form on a Sephadex-DEAE ion exchange column (from 0 to 0.5 M TEAB in 45 min) the phosphonate nucleoside was lyophilized overnight. To the dried product (10 mg, 22 μmol), dry DMF (2 mL) and *N*,*N*′-carbonyldiimidazole (36 mg, 220 μmol) were added under anhydrous conditions. The reaction mixture was stirred under nitrogen for 4 h and followed up by TLC (*i*-PrOH/NH_4OH concd/H_2O=6:3:1; 0.1 M NH_4HCO_3/*i*-PrOH=2:8). After quenching the reaction with methanol (198 μmol, 8 μL) for 30 min to cleave the excess of *N*,*N*′-carbonyldiimidazole, tris(tetra-*n*-butylammonium) hydrogen pyrophosphate (0.4 M solution in dry DMF, 270 μmol, 675 μL) was added. The solution was stirred for 36 h at room temperature after which it was quenched by evaporation of the solvent under reduced pressure. Analytical ion exchange chromatography on a mono-Q column (1 M TEAB from 0 to 50% in 30 min) showed a double peak at the diphosphorylphosphonate nucleoside position. The dried reaction mixture was redissolved in 1 M TEAB (1 mL) and 1 N NaOH (44 μmol, 44 μL) was added. The reaction mixture was stirred for 30 min after the addition of NaOH and subsequently concentrated to dryness under reduced pressure. Purification of the diphosphate analog of the phosphonate nucleoside was performed on an ion exchange mono-Q column (1 M TEAB from 0 to 50% in 30 min). Compound **35** was obtained with a yield of 10% (2 μmol, 1.2 mg).

4.2. Incorporation of phosphonate nucleoside 5 by HIV reverse transcriptase in a DNA duplex

The incorporation of the modified nucleotide by HIV reverse transcriptase was probed by addition of the building block to a mixture containing a ^{32}P labeled primer/template complex (P1T1, Fig. 4) and enzyme. Incorporation was investigated by taking aliquots at specific time points and analysis of the samples by PAGE, autoradiography, and phosphor imaging. Reactions were run for 60 min at 37 °C. Aliquots were taken after 2, 4, 6, 8, 10, 15, 30, and 60 min and quenched by addition of a double volume of a stopmix (90% v/v formamide, 0.05% w/v bromophenol blue, 0.05% w/v xylene cyanol blue, 50 mM EDTA) and heating of this mixture at 90 °C for 5 min. The samples were analyzed by electrophoresis on a 16% denaturing polyacrylamide gel with TBE (89 mM Tris-borate, 2 mM EDTA, pH 8.3) as a buffer. The amount of the elongated primer was quantified using Optiquant image analysis software (Packard).

The experiments were carried out so that the primer/template complex concentration was kept constant at

P1 5′ – CAGGAAACAGCTATGAC- 3′

T1 5′ – **CCCCT**GTCATAGCTGTTTCCTG- 3′

Figure 4. Primer and template sequences used in single nucleotide incorporation assay for adenine base nucleotides.

250 nM while the diphosphorylphosphonate nucleoside and the enzyme concentrations were varied.

4.3. Antiviral activity and cytotoxicity

Appropriate compound dilutions were prepared in triplicate in 96-well plates. MT-2 cells were infected in bulk with HIV-1 IIIb for 3 h at 37 °C and added to plates at a density of 20,000/well in a final volume of 200 μL. After a 5-day incubation at 37 °C, the virus-induced cytopathic effect was determined using a cell viability assay. Media of 100 μL was removed from each well and replaced with 100 μL of phosphate-buffered saline containing 1.7 μg/mL XTT [2,3-bis(methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide] and 5 μg/mL phenazine methosulfate. Following a 1-h incubation at 37 °C, 20 μL/well of 2% Triton X-100 was added and absorbance was read at 450 nm. The data were plotted as cell death versus drug concentration. Cell death was expressed as a percentage of the signal from samples with fully suppressed virus replication following the subtraction of signal from untreated infected control. The concentration of each drug that inhibited the virus-induced cytopathic effect by 50% (EC50) was calculated using GraphPad Prizm program. Cytotoxicity in MT-2 cells was determined under identical conditions except that cells were uninfected and higher drug concentrations were tested.

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