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SYNTHESIS AND IN VITRO ACTIVITY OF 4′ AND 5′-MODIFIED ANALOGUES OF APIOSYL NUCLEOSIDES AS POTENT ANTI-HCV AGENTS

Hua Li, Wonjae Lee, and Joon Hee Hong

BK21-Project Team, College of Pharmacy, Chosun University, Kwangju, Republic of Korea

□ *Novel doubly branched apio dideoxynucleosides were synthesized starting from 1,3-dihydroxyacetone using an ozonolysis and Grignard addition as key steps, and evaluated for anti-hepatitis C virus (HCV) activity. The adenine derivative* ***24*** *showed significant anti-HCV activity, indicating that the branches at the 4′,5′-position of the apiosyl ring led to favorable interaction with HCV polymerase.*

Keywords anti-HCV agent; ozonolysis; Grignard addition

INTRODUCTION

Apiosyl nucleosides[1,2] such as 3TC that belong to a novel class of nucleosides, which have the oxygen of the furanose and C2-methylene transposed, show anti-HIV activity and resistance to enzymatic deamination. Similarly, adenine analogues such as apio-ddA, **1**[3] and aminoapio-ddA, **2**[4] (Figure 1) show comparable anti-HIV and anti-HBV activity to the parent 2′,3′-dideoxy adenosine. Apio-ddA is more resistant to adenosine deaminase (ADA) and shows enhanced stability of the glycosidic bond under acidic and enzymatic conditions compared to natural 2′,3′-dideoxynucleosides (ddNs) nucleosides.[5] Apio nucleoside phosphonates can be assembled from natural precursor molecules and form duplexes with DNA and RNA with thermal stability, similar to that of the natural nucleic acid association.[6] Diphosphoryl phosphonate of apio nucleoside **3**, a substrate of several polymerases, can be enzymatically incorporated into DNA.[7,8] These nucleosides can substitute for ribonucleosides in the catalytic site of

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Address correspondence to Joon Hee Hong, College of Pharmacy, Chosun University, Kwangju 501-759, Republic of Korea. E-mail: hongjh@chosun.ac.kr

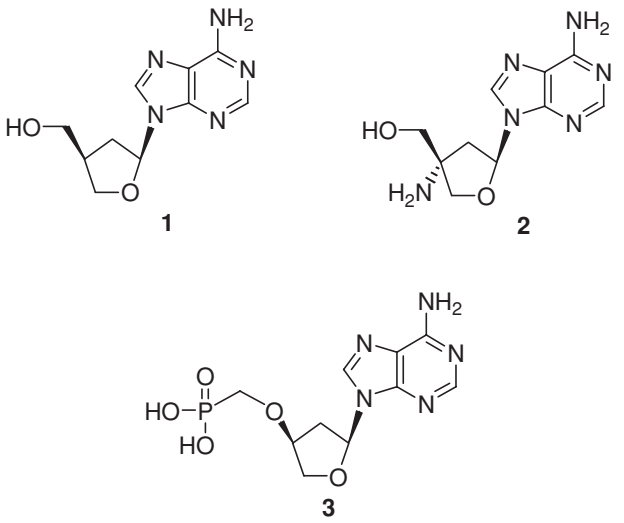


FIGURE 1 Examples of apiosyl nucleoside analogues as potent antiviral agents.

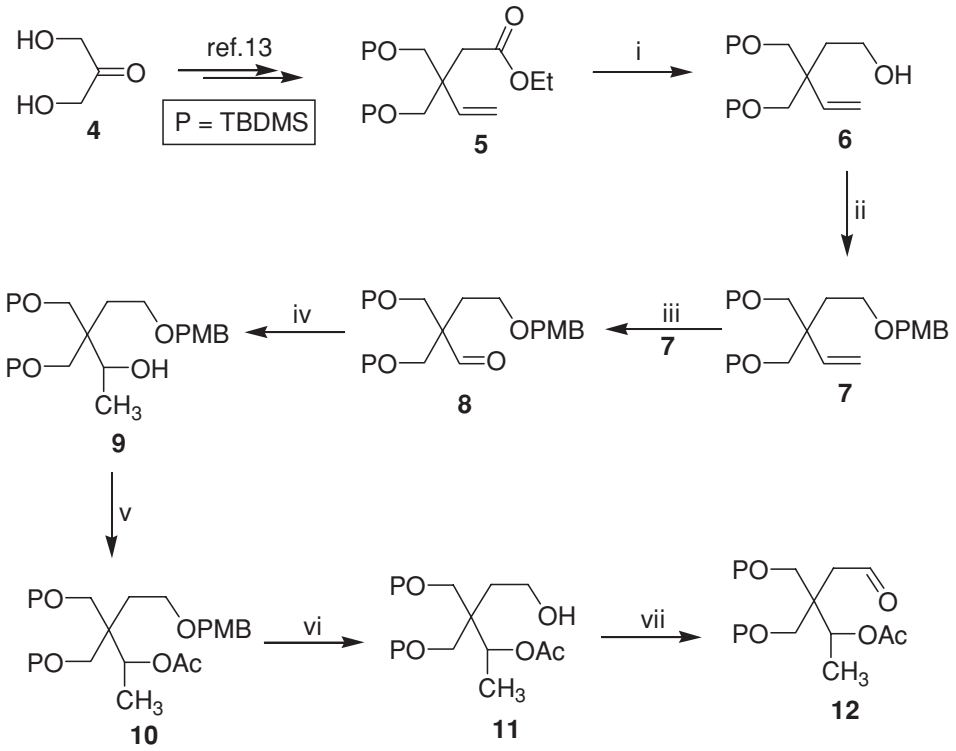
a hammerhead ribozyme, although the catalytic efficiency of the ribozyme is significantly reduced.[9]

Because branched nucleoside derivatives are a drug of choice in curing viral infections, including hepatitis C virus (HCV), a number of nucleoside derivatives have been synthesized and evaluated for anti-HCV activity. For example, 2′-C-methylcytidine,[10] 2′-C-methyladenosine,[11] and 2′-C-hydroxymethyladenosine[12] are potent and selective anti-HCV agents. These nucleosides are converted into their triphosphates and incorporated into proviral RNA, resulting in viral RNA chain termination, because subsequent incorporation of the substrate, nucleoside triphosphate, is sterically hindered by the 2′-methyl group. On the basis of potent anti-HCV activity of furanosyl nucleosides, it is known that the hydroxyl functional group of 3′-position and 2′ (β)-methyl group were essential for nucleosides to show anti-HCV activity. Therefore, we applied similar structural environments to the design of novel doubly branched apiosyl nucleosides. We introduced not only hydroxylmethy funtional group at 4′-position but also methyl group at 5′-position for the purpose of causing the favorable interaction of 4′-*C*-hydroxymethyl with NS5b RNA dependent RNA polymerase and the steric repulsion like the 5′-methyl group.

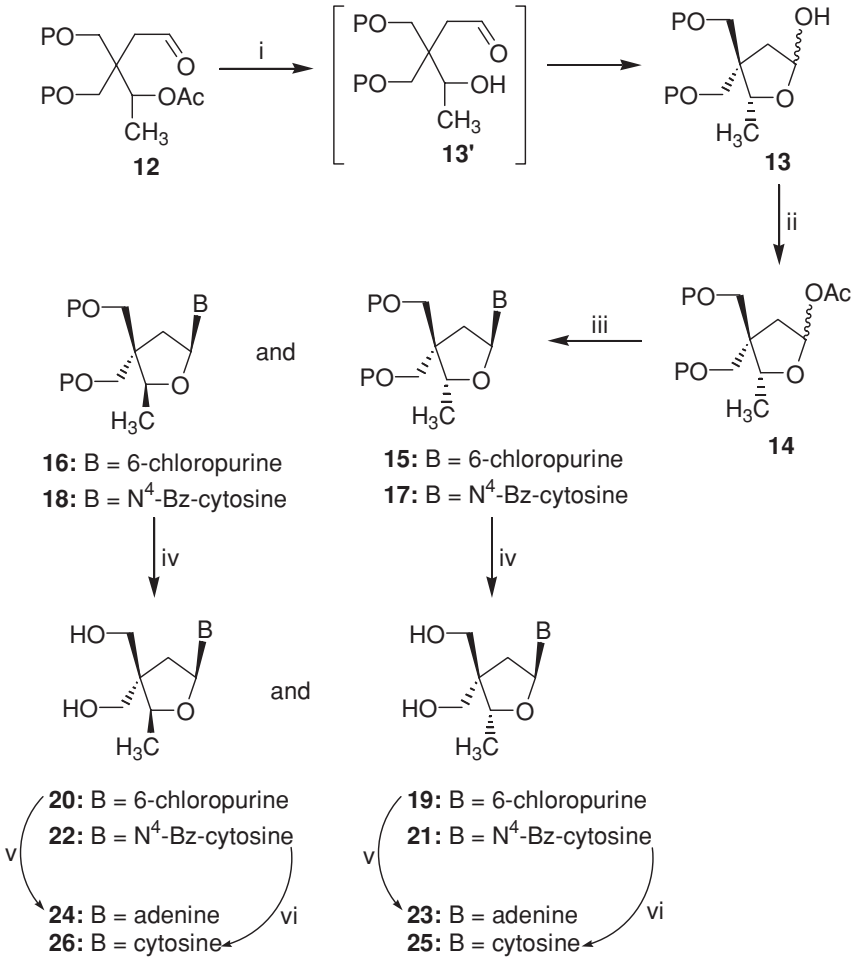
RESULTS AND DISCUSSION

To synthesize the target branched apiosyl nucleosides, we utilized the unsaturated ester intermediate **5** as a starting material, which was readily prepared by previously reported procedure from 1,3-dihydroxyacetone

4.[13] Ester **5** was subjected to diisobutylaluminum hydride (DIBALH) reduction to give the alcohol **6** in high yield. The primary hydroxyl group of **6** was protected as a temporary *p*-methoxybenzyl ether (PMB) by reaction[14] with PMBCl and NaH in DMF to afford the protected olefin **7** in a yield of 95%. The olefin of **7** was treated with ozone in methylene chloride at –78°C, followed by the decomposition of the ozonide by dimethylsulfide (DMS) to give the aldehyde **8**. Compound **8** was subjected to carbonyl addition with methylmagnesium bromide to provide the secondary alcohol derivative **9**, which was acetylated with acetic anhydride in pyridine to give the ester **10**. Oxidative deprotection of the PMB ether of **10** was effected with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[15] in CH_2Cl_2 with a small amount of water to give the alcohol **11**, which was subjected to Swern oxidation [DMSO,$(COCl)_2$, TEA][16] to provide aldehyde **12** in a quantitative yield. Deacetylation of **12** using NaOMe/MeOH provided the lactol derivative **13**, and subsequent acetylation with acetic anhydride in pyridine yielded a glycosyl donor **14**, which is ready for the condensation with bases Scheme 1.



SCHEME 1 Synthesis of aldehyde intermediate **12**. Reagents: i) BIBAL-H, CH_2Cl_2; ii) PMBCl, naH, THF; iii) O_3/DMS, CH_2Cl_2; iv) CH_3MgBr, THF; v) Ac_2O, pyridine; vi) DDQ, CH_2Cl_2-H_2O; vii) $(COCl)_2$, DMSO, CH_2Cl_2, −78°C.



SCHEME 2 Synthesis of target nucleosides. Reagents: i) NaOMe, MeOH; ii) Ac2O, DMAP, pyridine; iii) persilylated bases, TMSOTf, $ClCH_2CH_2Cl$; iv) TBAF, THF/CH_3CN; v) NH_3/MeOH, steel bomb, 90°C; vi) NH_3/MeOH, room temperature.

Synthesis of the desired nucleosides is depicted in Scheme 2. The glycosyl donor **14** was condensed with silylated 6-chloropurine in the presence of trimethylsilyl trifluoromethansulfonate (TMSOTf) and separated by silica gel column chromatography to provide the protected nucleosides **15** and **16**. As shown in Figure 2, the stereochemistries of **15** and **16** were unambiguously determined on the basis of the NOE correlations. On irradiation of C_2-H, a relatively weak nuclear Overhauser effect (NOE) was observed at C_5-H of **15** (0.3%), but not at C_5-H of **16** (0.8%).

Desilylation of **15** and **16** with *n*-tetrabutylammonium fluoride (TBAF) afforded the nucleoside analogues **19** and **20**, respectively. Chloropurine derivatives **19** and **20** were individually transformed to **23** and **24** by treating with methanolic ammonia in a steel bomb at 90°C.

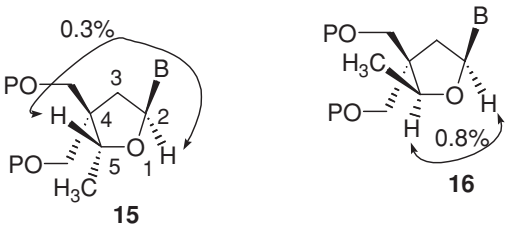


FIGURE 2 NOE comparisons of compounds **15** and **16**.

For the synthesis of cytosine analogues, the glycosyl donor **14** was condensed with silylated N^4-benzoyl cytosine in the presence of TMSOTf and separated by silica gel column chromatography to give the protected nucleosides **17** and **18**. The stereochemistries of **17** and **18** were as described for **15** and **16** on the basis of the NOE correlations. Desilylation of each anomer was performed by TBAF in the THF-CH_3CN co-solvent system followed by debenzolyation with methanolic ammonia to afford the final cytosine nucleosides **25** and **26**, respectively.

All the synthesized compounds were tested for anti-HCV activity using an in vitro assay that is suitable for monitoring anti-HCV activities of compounds. This system is composed of a human hepatocarcinoma cell line (Huh-7) supporting multiplication of an HCV replicon named NK-R2AN. Compound **24** significantly inhibited the replication of the replicon NK-R2AN in Huh-7 cells by 50% at 19 μM. Therefore, two branches at the 4′,5′-position make the apiosyl ring conformation favorable for phosphorylation at the 4′-hydroxyl group and subsequent incorporation into the growing RNA chain by the polymerase.

EXPERIMENTAL

Melting points were determined on a Mel-temp II laboratory device and are uncorrected. NMR spectra were recorded on a JEOL 300 Fourier transform spectrometer (JEOL, Tokyo, Japan); chemical shifts are reported in parts per million (δ) and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and dd (doublet of doublets). UV spectra were obtained on a Beckman DU-7 spectrophotometer (Beckman, South Pasadena, CA, USA). The elemental analyses were performed using a Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA). Thin layer chromatography (TLC) was performed on Uniplates (silica gel) purchased from Analtech Co. (7558, Newark, DE, USA). All reactions were performed under an atmosphere of nitrogen unless otherwise specified. Dry dichloromethane, benzene, and pyridine were obtained by distillation from CaH_2. Dry THF was obtained by distillation from Na and benzophenone immediately prior to use.

3,3-Bis-(tert-butyl-dimethyl-silanyloxymethyl)-pent-4-en-1-ol (6): To a solution of **5** (5.5 g, 13.19 mmol) in CH_2Cl_2 (100 mL), DIBAL-H (27.71 mL, 1.0 M solution in hexane) was added slowly at −20°C, and the mixture was stirred for 2 hours at the same temperature. To the mixture, methanol (28 mL) was added. The mixture was stirred at room temperature for 2 hours, and the resulting solid was filtered through a Celite pad. The filtrate was concentrated under vacuum and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:7) to give alcohol **6** (4.55 g, 92%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.70–5.63 (m, 1H), 4.91–4.98 (m, 2H), 3.71–3.64 (m, 4H), 3.52 (m, 2H), 1.45–1.39 (m, 2H), 0.81 (m, 18H), 0.01 (m, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 149.6, 107.9, 70.5, 59.1, 38.7, 34.1, 25.3, 18.7, −5.4; Anal. Calc. for $C_{19}H_{42}O_3Si_2$: C, 60.90; H, 11.30. Found: C, 60.94; H, 11.26.

1-[3,3-Bis-(tert-butyl-dimethyl-silanyloxymethyl)-pent-4-enyloxymethyl]-4-methoxy-benzene (7): Compound **6** (5.2 g, 13.87 mmol) was dissolved in dry DMF (50 mL). After cooling the solution to 0°C, NaH (0.4 g, 60% in mineral oil, 16.6 mmol) was added. The solution was stirred at 0 °C for 30 minutes and then 4-methoxybenzyl chloride (2.6 g, 16.6 mmol) was slowly added. After warming the solution to room temperature, it was stirred for 3 hours. The solvent was removed under reduced pressure and the residue was quenched with H_2O followed by extraction with EtOAc (2 × 60 mL). The organic layers were combined, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **7** (5.83 g, 85%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.25 (d, J = 8.2 Hz, 2H), 6.91 (d, J = 8.2 Hz, 2H), 5.82–5.05 (m, 2H), 4.65 (s, 2H), 3.73–3.63 (m, 7H), 3.38 (t, J = 6.8 Hz, 2H), 1.43–1.36 (m, 2H), 0.82 (m, 18H), 0.02 (m, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.7, 148.5, 130.4, 129.7, 113.6, 109.3, 76.2, 70.6, 65.6, 57.4, 39.8, 31.8, 25.5, 18.4, −5.5; Anal. Calc. for $C_{27}H_{50}O_4Si_2$: C, 65.53; H, 10.18. Found: C, 65.59; H, 10.21.

2,2-Bis-(tert-butyl-dimethyl-silanyloxymethyl)-4-(4-methoxy-benzyloxy)-butyraldehyde (8): A solution of compound **7** (4.5 g, 9.1 mmol) in anhydrous CH_2Cl_2 (60 mL) was cooled down to −78°C, and ozone gas was then bubbled into the reaction mixture until a blue color persisted for an additional 5 minutes. The reaction mixture was degassed with nitrogen, and dimethyl sulfide (2.79 mL, 38 mmol) was slowly added at –78°C. The mixture was stirred for 1 hour at room temperature under argon gas and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:30) to give compound **8** (1.79 g, 80%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.78 (s, 1H), 7.26 (d, J = 8.2 Hz, 2H), 6.97 (d, J = 8.2 Hz, 2H), 4.62 (s, 2H), 4.02–3.94 (m, 4H), 3.75 (s, 3H), 3.36 (dd, J = 7.0, 1.2 Hz, 2H), 1.75–1.68 (m, 2H), 0.81

(m, 18H), 0.02 (m, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 203.2, 160.2, 130.1, 129.2, 114.1, 75.7, 65.3, 62.3, 56.8, 55.8, 25.6, 24.5, 18.3, −5.6.

3,3-Bis-(tert-butyl-dimethyl-silanyloxymethyl)-5-(4-methoxy-benzyloxy)-pentan-2-ol (9): To a solution of **8** (2.1 g, 4.22 mmol) in dry THF (30 mL) was slowly added CH_3MgBr (5.0 mL, 1.0 M solution in THF) at −78°C. After 5 hours, saturated NH_4Cl solution (5 mL) and water (50 mL) were sequentially added, and the reaction mixture was slowly warmed to room temperature. The mixture was extracted with EtOAc (60 mL) two times. The combined organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **9** (1.86 g, 86%) as colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22 (d, $J = 8.0$ Hz, 2H), 6.90 (d, $J = 8.1$ Hz, 2H), 4.65 (s, 2H), 3.72–3.63 (m, 7H), 3.36–3.30 (m, 3H), 1.37–1.29 (m, 2H), 1.20 (d, $J = 6.8$ Hz, 3H), 0.82 (m, 18H), 0.02 (m, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.1, 131.2, 128.4, 112.9, 75.8, 68.5, 65.6, 62.8, 56.5, 45.5, 25.7, 24.8, 18.4, 17.2, −5.4; Anal. Calc. for $C_{27}H_{52}O_5Si_2$: C, 63.23; H, 10.22. Found: C, 63.26; H, 10.19.

2,2-Bis-(tert-butyl-dimethyl-silanyloxymethyl)-4-(4-methoxy-benzyloxy)-1-methyl-butyl acetate (10): To a solution of compound **9** (2.5 g, 4.87 mmol) in anhydrous pyridine (30 mL), Ac_2O (497 mg, 1.53 mmol) was slowly added, and the mixture was stirred overnight under nitrogen. The pyridine was evaporated under reduced pressure and co-evaporated with toluene. The residue was poured into water (50 mL) and extracted with EtOAc (50 mL) two times. The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:25) to give compound **10** (2.46 g, 91%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.26 (d, $J = 8.1$ Hz, 2H), 6.89 (d, $J = 8.1$ Hz, 2H), 4.65 (s, 2H), 4.13 (q, $J = 6.8$ Hz, 1H), 3.75–3.67 (m, 7H), 3.34 (dd, $J = 6.2$, 1.2 Hz, 2H), 2.03 (s, 3H), 1.39–1.31 (m, 5H), 0.83 (s, 18H), 0.02 (m, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.1, 159.9, 130.7, 129.1, 113.6, 76.5, 72.5, 66.3, 64.2, 56.8, 43.1, 25.7, 24.9, 18.4, 17.4, 14.3, −5.6; Anal. Calc. for $C_{29}H_{54}O_6Si_2$: C, 62.77; H, 9.81. Found: C, 62.81; H, 9.78.

2,2-Bis-(tert-butyl-dimethyl-silanyloxymethyl)-4-hydroxy-1-methyl-butyl acetate (11): To a solution of compound **10** (2.2 g, 3.96 mmol) in CH_2Cl_2/H_2O mixture (119 mol CH_2Cl_2, 5.94 mol H_2O) was added DDQ (1.08 g, 4.75 mmol) and the mixture was stirred for 2 hour at room temperature. Saturated $NaHCO_3$ (20 mL) was added to quench the reaction. The organic layer was separated, washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give compound **11** (2.46 g, 83%) as a colorless

oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.13 (m, 1H), 3.73–3.65 (m, 4H), 3.51 (t, J = 6.6 Hz, 2H), 2.02 (s, 3H), 1.42–1.36 (m, 5H), 0.81 (m, 18H), 0.01 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.2, 71.6, 64.2, 58.7, 41.8, 27.9, 25.4, 18.4, 17.5, 15.6, −5.5; Anal. Calc. for $C_{21}H_{46}O_5Si_2$: C, 58.01; H, 10.66. Found: C, 58.09; H, 10.70.

2,2-Bis-(tert-butyl-dimethyl-silanyloxymethyl)-1-methyl-4-oxo-butyl acetate (12): To a stirred solution of oxalyl chloride (0.06 mL, 0.68 mmol) in CH_2Cl_2 (6 mL) was added a solution of DMSO (0.06 mL, 0.9 mmol) in CH_2Cl_2 (0.34 mL) dropwise at –78°C. The resulting solution was stirred at –78°C for 5 minutes, and a solution of alcohol **11** (197 mg, 0.454 mmol) in CH_2Cl_2 (3 mL) was added dropwise. The mixture was stirred at –78°C for 20 minutes and triethylamine (0.32 mL, 2.26 mmol) was added. The resulting mixture was warmed to 0 °C and stirred for 30 minutes. H_2O (6 mL) was added, and the solution was stirred at room temperature for 20 minutes. The mixture was poured into water (40 mL), extracted with EtOAc (40 mL) two times. The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:30) to give compound **12** (193 mg, 99%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.75 (s, 1H), 4.12 (m, 1H), 3.74–3.67 (m, 4H), 2.34 (dd, J = 8.4, 6.2 Hz, 1H), 2.03 (s, 3H), 0.81 (s, 18H), 0.01 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 201.5, 171.5, 71.8, 65.0, 58.7, 38.4, 37.3, 25.5, 18.6, 15.7, −5.4.

(*rel*)-(2′*S*,5′*R* and 5′*S*)-4,4-Bis-(tert-butyl-dimethyl-silanyloxymethyl)-5-methyl-tetrahydro-furan-2-ol (13): To a solution of **12** (2.3 g, 5.31 mmol) in methanol (10 mL) was added NaOMe (1.0 mmol, 1.0 M in MeOH). The mixture was stirred for 4 hours at room temperture and neutralized with acetic acid (0.1 mL). The mixture was concentrated under reduced pressure. The residue was poured into water (60 mL) and extracted with EtOAc (60 mL) two times. The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:25) to give compound **13** (1.74 g, 84%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.67 (m, 1H), 3.91 (m, 1H), 3.72–3.63 (m, 4H), 1.94–1.89 (m, 1H), 1.82 (m, 1H), 1.22 (d, J = 6.2 Hz, 3H), 0.81 (m, 18H), 0.01 (m, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 93.1, 92.9, 68.0, 67.8, 63.8, 63.7, 47.3, 38.4, 34.3, 34.2, 25.5, 25.4, 18.6, −5.7, −5.6.

(*rel*)-(2′*S*,5′*R* and 5′*S*)-Acetic acid 4,4-bis-(tert-butyl-dimethyl-silanyloxymethyl)-5-methyl-tetrahydro-furan-2-yl ester (14): To a solution of compound **13** (3.1 g, 7.93 mmol) in anhydrous pyridine (30 mL), Ac_2O (1.21 g, 11.9 mmol) and DMAP (36 mg, 0.3 mmol) were slowly added, and the

mixture was stirred overnight under nitrogen. The pyridine was evaporated under reduced pressure and co-evaporated with toluene. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:25) to give diastereomeric compound **14** (2.95 g, 86%) as a syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.13 (dd, J = 5.4, 1.2 Hz, 1H), 3.93 (m, 1H), 3.74–3.65 (m, 4H), 2.09–2.01 (m, 4H), 1.83–1.76 (m, 1H), 1.22 (dd, J = 6.6, 4.2 Hz, 3H), 0.81 (m, 18H), 0.01 (m, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.9, 170.8, 97.3, 68.1, 64.2, 64.1, 47.0, 46.9, 31.3, 25.5, 18.4, 17.5, 15.2, −5.7.

(*rel*)-(2′*S*,5′*R*)-6-Chloro-9-[4,4-Bis-(tert-butyl-dimethyl-silanyloxymethyl)-5-methyl-tetrahydro-furan-2-yl] purine (15) and (*rel*)-(2′*S*,5′*S*)-6-chloro-9-[4,4-bis-(tert-butyl-dimethyl-silanyloxymethyl)-5-methyl-tetrahydro-furan-2-yl] purine (16): 6-Chloropurine (541 mg, 3.5 mmol), anhydrous HMDS (20 mL), and a catalytic amount of ammonium sulfate (20 mg) were refluxed to a clear solution (overnight), and the solvent was distilled under anhydrous conditions. The residue was dissolved in anhydrous 1,2-dichloroethane (DCE, 10 mL). To this mixture, a solution of **14** (757 mg, 1.75 mmol) in dry DCE (10 mL) and TMSOTf (778 mg, 3.5 mmol) was added, and the resulting mixture was stirred at room temperature for 2 hours. The reaction mixture was quenched with 20 mL of saturated $NaHCO_3$ and stirred for 20 minutes. The resulting solid was filtered through a Celite pad, and the filtrate was extracted twice with CH_2Cl_2. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane, 2:1) to give compound **15**(286 mg, 31%) and **16** (304 mg, 33%) as white solids, respectively: compound for **15**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.43 (s, 1H), 8.11 (s, 1H), 6.14 (br s, 2H), 6.01 (dd, J = 5.4, 1.4 Hz, 1H), 3.92 (m, 1H), 3.72–3.64 (m, 4H), 2.25 (dd, J = 12.6, 6.6 Hz, 1H), 1.87 (dd, J = 12.6, 8.6 Hz, 1H), 1.19 (d, J = 6.8 Hz, 3H), 0.82 (m, 18H), 0.01 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.7, 152.6, 148.4, 147.8, 129.5, 82.5, 68.4, 64.2, 48.1, 31.9, 25.6, 18.7, 14.7, −5.5; Anal. Calc. for $C_{24}H_{43}ClN_4O_3Si_2$: C, 54.67; H, 8.22; N, 10.63. Found: C, 54.71; H, 8.19; N, 10.69; compound for **16**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.51 (s, 1H), 8.29 (s, 1H), 6.11 (br s, 2H), 6.00 (d, J = 5.8 Hz, 1H), 3.87 (q, J = 6.2 Hz, 1H), 3.78–3.69 (m, 4H), 2.31 (dd, J = 12.8, 7.2 Hz, 1H), 1.90 (dd, J = 12.7, 6.8 Hz, 1H), 1.16 (d, J = 6.6 Hz, 3H), 0.81 (m, 18H), 0.01 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.2, 151.7, 147.4, 145.8, 128.1, 83.3, 67.9, 63.6, 49.7, 32.2, 25.4, 18.3, 14.9, −5.6; Anal. Calc. for $C_{24}H_{43}ClN_4O_3Si_2$: C, 54.67; H, 8.22; N, 10.63. Found: C, 54.65; H, 8.25; N, 10.59.

(*rel*)-(2′*S*,5′*R*)-1-[4,4-Bis-(tert-butyl-dimethyl-silanyloxymethyl)-5-methyl-tetrahydro-furan-2-yl] N^4-benzoylcytosine (17) and (*rel*)-(2′*S*,5′*S*)-1-[4,4-bis-(tert-butyl-dimethyl-silanyloxymethyl)-5-methyl-tetrahydro-furan-2-yl] N^4-benzoylcytosine (18): The glycosyl donor **14** (389 mg, 0.9 mmol) was condensed with N^4-benzoylcytosine (387 mg, 1.8 mmol) by the same

procedure as described for the preparation of **15** and **16** to give **17** (148mg, 28%) and **18** (142 mg, 27%) as white solids, respectively: compound for **17**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.96–7.91 (m, 2H), 7.51–7.42 (m, 4H), 5.89 (d, J = 5.6, 1.8 Hz, 1H), 5.77 (d, J = 6.8 Hz, 1H), 3.89 (q, J = 6.8 Hz, 1H), 3.70–3.62 (m, 4H), 2.21 (dd, J = 12.8, 6.8 Hz, 1H), 1.88 (dd, J = 12.7, 8.4 Hz, 1H), 1.18 (d, J = 6.8 Hz, 3H), 0.81 (m, 18H), 0.01 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.3, 164.9, 159.7, 134.7, 133.4, 131.2, 129.5, 127.4, 99.3, 79.2, 68.9, 63.6, 63.1, 49.3, 30.5, 25.5, 18.7, 15.1, −5.6; Anal. Calc. for $C_{30}H_{49}N_3O_5Si_2$: C, 61.29; H, 8.40; N, 7.15. Found: C, 61.34; H, 8.37; N, 7.19; compound for **18**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.12 (d, J = 6.2 Hz, 7.90 (m, 1H), 7.49–7.40 (m, 4H), 5.93 (t, J = 5.4, Hz, 1H), 5.71 (d, J = 7.0 Hz, 1H), 3.91 (q, J = 6.7 Hz, 1H), 3.73–3.61 (m, 4H), 2.19 (dd, J = 13.0, 8.2 Hz, 1H), 1.91 (dd, J = 12.9, 6.2 Hz, 1H), 1.16 (d, J = 6.4 Hz, 3H), 0.83 (m, 18H), 0.02 (m, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.2, 164.5, 158.2, 133.8, 132.6, 131.4, 128.1, 127.4, 124.5, 98.6,80.1, 67.4, 63.7, 63.3, 48.8, 30.7, 25.6, 18.4, 14.9, −5.7; Anal. Calc. for $C_{30}H_{49}N_3O_5Si_2$: C, 61.29; H, 8.40; N, 7.15. Found: C, 61.26; H, 8.44; N, 7.11.

(*rel*)-(2′*S*,5′*R*)-6-Chloro-9-[4,4-Bis-(hydroxymethyl)-5-methyl-tetrahydrofuran-2-yl] purine (19): To a solution of **15** (322 mg, 0.61 mmol) in tetrahydrofurane/acetonitrile (1/1 co-mixture) (12 mL), tetrabutylammonium fluoride (1.83 mL, 1.0 M solution in THF) was added at 0°C. The mixture was stirred overnight at room temperature, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:7) to give compound **19** (144 mg, 79%) as a white solid: UV (MeOH) λ_{max} 264.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.96 (s, 1H), 8.67 (s, 1H), 5.96 (dd, J = 5.6, 1.8 Hz, 1H), 3.92 (m, 1H), 3.46–3.35 (m 4H), 2.21 (dd, J = 12.8, 6.8 Hz, 1H), 1.89 (dd, J = 12.8, 9.2 Hz, 1H), 1.20 (d, J = 6.7 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.9, 152.1, 147.2, 127.6, 81.4, 69.1, 61.7, 61.3, 47.1, 31.5, 14.5; Anal. Calc. for $C_{12}H_{15}ClN_4O_3$: C, 48.25; H, 5.06; N, 18.76. Found: C, 48.32; H, 5.12; N, 18.69.

(*rel*)-(2′*S*,5′*S*)-6-Chloro-9-[4,4-Bis-(hydroxymethyl)-5-methyl-tetrahydrofuran-2-yl] purine (20): Purine derivative **20** was synthesized from **16** by the same procedure described for **19**: yield 75%; UV (MeOH) λ_{max} 264.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.85 (s, 1H), 8.36 (s, 1H), 5.99 (d, J = 5.4 Hz, 1H), 3.92 (m, 1H), 3.45–3.33 (m 4H), 2.24 (dd, J = 12.6, 8.2 Hz, 1H), 1.91 (dd, J = 12.6, 6.2 Hz, 1H), 1.19 (d, J = 6.6 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.3, 153.2, 146.4, 128.1, 81.8, 68.9, 61.2, 60.8, 47.5, 30.8, 14.6; Anal. Calc. for $C_{12}H_{15}ClN_4O_3$: C, 48.25; H, 5.06; N, 18.76. Found: C, 48.18; H, 4.96; N, 18.82.

(*rel*)-(2′*S*,5′*R*)-1-[4,4-Bis-(hydroxymethyl)-5-methyl-tetrahydro-furan-2-yl]*N*^{4}-benzoylcytosine (21): Compound **21** was synthesized from **17** by the same procedure described for **19**: yield 79%; UV (MeOH) λ_{max} 259.0 nm;

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.24 (d, J = 6.7 Hz, 1H), 7.87 (m, 2H), 7.49–7.41 (m, 3H), 5.85 (m, 1H), 5.62 (d, J = 7.0 Hz, 1H), 3.86 (q, J = 6.7 Hz, 1H), 3.51–3.43 (m, 4H), 2.19 (dd, J = 13.2, 8.8 Hz, 1H), 1.84 (dd, J = 13.1, 7.4 Hz, 1H), 1.16 (d, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.7, 164.3, 158.5, 135.2, 132.4, 130.3, 127.5, 124.4, 100.4, 78.7, 67.3, 61.7, 61.3, 46.1, 30.1, 14.3; Anal. Calc. for $C_{18}H_{21}N_3O_5$: C, 60.16; H, 5.89; N, 11.69. Found: C, 60.09; H, 5.78; N, 11.77.

(*rel*)-(2′*S*,5′*S*)-1-[4,4-Bis-(hydroxymethyl)-5-methyl-tetrahydro-furan-2-yl]N^4-benzoylcytosine (22): Compound **22** was synthesized from **18** by the same procedure described for **19**: yield 79%; UV (MeOH) λ_{max} 259.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.19 (d, J = 6.6 Hz, 1H), 7.89 (d, J = 5.2 Hz, 1H), 7.47–7.42 (m, 4H), 5.93 (dd, J = 5.2, 1.2 Hz, 1H), 5.48 (d, J = 6.8 Hz, 1H), 3.86 (q, J = 6.8 Hz, 1H), 3.46–3.40 (m, 4H), 2.24 (dd, J = 12.8, 8.2 Hz, 1H), 1.89 (m, 1H), 1.14 (d, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.1, 165.1, 157.7, 134.5, 131.4, 129.1, 126.9, 123.4, 98.9, 79.1, 68.2, 63.3, 63.0, 47.2, 30.6, 15.7. Anal. Calc. for $C_{18}H_{21}N_3O_5$: C, 60.16; H, 5.89; N, 11.69. Found: C, 60.22; H, 5.91; N, 11.65.

(*rel*)-(2′*S*,5′*R*)-9-[4,4-Bis-(hydroxymethyl)-5-methyl-tetrahydro-furan-2-yl] adenine (23): A solution of **19** (180 mg, 0.6 mmol) in saturated methanolic ammonia (15 mL) was stirred in a steel bomb at 90°C overnight and the mixture was concentrated. The residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$, 1:4) to give compound **23** (144 mg, 86%) as a white solid: m.p. 199∼201°C; UV (MeOH) λ_{max} 261.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.29 (s, 1H), 8.15 (s, 1H), 5.99 (d, J = 6.2 Hz, 1H), 4.93 (t, J = 5.2 Hz, 1H, D_2O exchangeable), 4.87 (t, J = 5.4 Hz, 1H, D_2O exchangeable), 3.93 (q, J = 6.8 Hz, 1H), 3.48–3.39 (m 4H), 2.18 (dd, J = 12.6, 6.6 Hz, 1H), 1.86 (dd, J = 12.7, 8.8 Hz, 1H), 1.19 (d, J = 6.8 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.7, 152.5, 149.8, 141.3, 119.4, 80.8, 68.3, 61.2, 60.9, 46.3, 30.1, 15.0; Anal. Calc. for $C_{11}H_{17}N_3O_4$ (+0.5 MeOH): C, 50.83; H, 6.48; N, 23.71. Found: C, 50.91; H, 6.36; N, 23.77.

(*rel*)-(2′*S*,5′*S*)-9-[4,4-Bis-(hydroxymethyl)-5-methyl-tetrahydro-furan-2-yl] adenine (24): Compound **24** was synthesized from **20** by the same procedure described for **23**: yield 82%: m.p. 199 ∼ 201°C; UV (MeOH) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.31 (s, 1H), 8.22 (s, 1H), 5.96 (t, J = 5.8 Hz, 1H), 4.91 (t, J = 5.3 Hz, 1H, D_2O exchangeable), 4.85 (t, J = 5.4 Hz, 1H, D_2O exchangeable), 3.89 (m, 1H), 3.46–3.33 (m 4H), 2.15 (m, 1H), 1.85 (dd, J = 12.8, 8.2 Hz, 1H), 1.21 (d, J = 6.9 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.6, 152.8, 148.4, 142.0, 118.5, 79.2, 68.6, 60.9, 60.4, 46.5, 30.6, 14.6; Anal. Calc. for $C_{12}H_{17}N_5O_3$ (+1.0 H_2O): C, 48.47; H, 6.44; N, 23.55. Found: C, 48.53; H, 6.50; N, 23.51.

(*rel*)-(2′*S*,5′*R*)-1-[4,4-Bis-(hydroxymethyl)-5-methyl-tetrahydro-furan-2-yl] cytosine (25): A solution of **21** (123 mg, 0.34 mmol) in saturated methanolic ammonia (10 mL) was stirred overnight at room temperature and the mixture was concentrated. The residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$, 1:4) to give compound **25** (73 mg, 84%) as a white solid: m.p. 202∼204°C; UV (MeOH) λ_{max} 271.0 nm; ^1H NMR (DMSO-d_6, 300 MHz) δ 7.86 (d, J = 7.4 Hz, 1H), 5.87 (dd, J = 5.8, 1.8 Hz, 1H), 5.68 (d, J = 7.5 Hz, 1H), 4.89 (t, J = 5.4 Hz, 1H, D_2O exchangeable), 4.79 (t, J = 5.2 Hz, 1H, D_2O exchangeable), 3.89 (m, 1H), 3.47–3.38 (m, 4H), 2.20 (dd, J = 13.0, 6.2 Hz, 1H), 1.89 (dd, J = 12.9, 8.7 Hz, 1H), 1.24 (d, J = 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 165.2, 156.3, 142.3, 95.5, 79.0, 68.2, 60.7, 60.3, 47.2, 30.1; Anal. Calc. for $C_{11}H_{17}N_3O_4$ (+1.0 MeOH): C, 50.16; H, 7.37; N, 14.62. Found: C, 50.09; H, 7.33; N, 14.71.

(*rel*)-(2′*S*,5′*S*)-1-[4,4-Bis-(hydroxymethyl)-5-methyl-tetrahydro-furan-2-yl] cytosine (26): Compound **26** was synthesized from **22** by the same procedure described for **25**: yield 87%: m.p. 198∼201°C; UV (MeOH) λ_{max} 271.5 nm; ^1H NMR (DMSO-d_6, 300 MHz) δ 7.84 (d, J = 7.6 Hz, 1H), 5.92 (d, J = 6.0 8 Hz, 1H), 5.62 (d, J = 7.7 Hz, 1H), 4.87 (t, J = 5.2 Hz, 1H, D_2O exchangeable), 4.77 (t, J = 5.2 Hz, 1H, D_2O exchangeable), 3.92 (q, J = 7.0 Hz, 1H), 3.49–3.39 (m, 4H), 2.18 (dd, J = 12.6, 7.2 Hz, 1H), 1.86 (dd, J = 12.7, 8.8 Hz, 1H), 1.22 (d, J = 6.9 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 165.4, 156.5, 143.0, 96.1, 79.6, 69.6, 61.8, 61.5, 47.8, 29.9; Anal. Calc. for $C_{11}H_{17}N_3O_4$ (+1.0 H_2O): C, 48.34; H, 7.00; N, 15.37. Found: C, 48.26; H, 7.03; N, 15.42.

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