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Synthesis and conformation of 3′,4′-BNA monomers, 3′-*O*,4′-*C*-methyleneribonucleosides

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Abstract—In order to develop novel 2′,5′-linked oligonucleotide analogues aimed for antivirus reagents and antisense/antigene oligonucleotides, novel nucleoside analogues, 3′-*O*,4′-*C*-methyleneribonucleosides (3′,4′-BNA monomers) were synthesized via two synthetic routes. The first route starting from uridine utilized a regioselective ring-closure reaction of the 4′-*C*-(*p*-toluenesulfonyl)oxymethyluridine derivative. The second route involved a coupling reaction of 1,2,3-tri-*O*-acetyl-4-*C*-(*p*-toluenesulfonyl)oxymethylribofuranose derivative with nucleobases followed by oxetan-ring formation to afford the 3′,4′-BNA monomers bearing all four nucleobases. By means of ^{1}H NMR, X-ray crystallography and computational analysis, the sugar puckering of the 3′,4′-BNA monomers was found to be restricted in S-conformation ($C_{1'}$-*exo*–$C_{2'}$-*endo* puckering mode).

1. Introduction

It is well known that nucleic acids (DNA and RNA) play an important role in storage and transmission of genetic information in a living cell. They are consisted of three components, a nucleobase, a ribofuranose sugar and a phosphodiester linkage. In most DNAs and RNAs, nucleoside units are connected via a 3′-5′ internucleoside phosphodiester linkage. On the other hand, there are some biologically active nucleic acids bearing a 2′-5′ internucleoside phosphodiester linkage. Studies on these '2′,5′-linked nucleic acids' have focused on both their structural uniqueness and characteristic biological activity (Fig. 1).[1,2] A 2′,5′-linked oligoadenylate (2–5A) is well known to enhance the activity of 2–5A-dependent RNaseL, which plays an important role in prevention of virus infection.[1] To develop an antiviral drug, various types of modified 2–5A analogues have been synthesized to date.[3] In addition, oligonucleotide analogues having 2′,5′-phosphodiester linkages were also synthesized by some groups,[4] and it was revealed that the 2′,5′-linked oligonucleotides have a tendency to hybridize with their RNA complements rather than DNA complements,[4,5] and that they also have resistance towards several types of nucleases.[6] All of these characters are quite favorable for antisense technology.

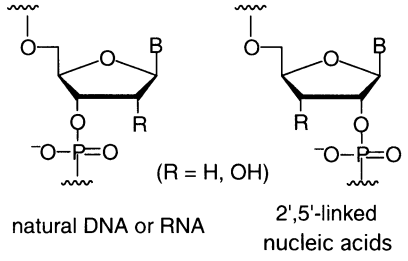

Figure 1. Structures of natural DNA, RNA and 2′,5′-linked nucleic acids.

For isolation and enhancement of specific biological activities, conformational restraint of the parent compounds by chemical modification is well known to be effective. In nucleosides, the ribofuranose ring is flexible and exists in an equilibrium of some major sugar conformations, such as N- and S-type conformations (Fig. 2).[7] If the conformation of the nucleoside for a 2′,5′-linked oligonucleotide is restrained in a suitable form, the hybridization process with ssRNA should profit from less negative entropy changes during duplex formation. Furthermore, it would contribute to the development of novel 2–5A analogues as an effective antiviral reagent.

We have already accomplished the synthesis of 2′-*O*,4′-*C*-methylene-bridged nucleic acids, 2′,4′-BNA[8,9] (LNA),[10] which have a strictly locked N-type sugar conformation

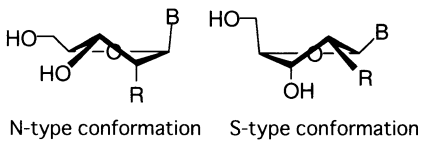

Figure 2. N- and S-type conformation of nucleosides.

Keywords: nucleic acid analogues; nucleosides; conformation; oxetanes.

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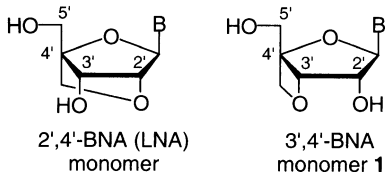


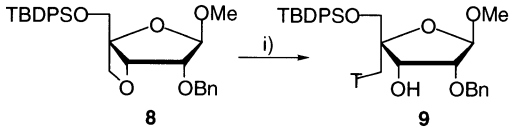
Figure 3. Structures of 2′,4′-BNA (LNA) and 3′,4′-BNA monomers.

by a methylene bridging between 2′-oxygene and 4′-carbon atoms in a ribonucleoside (Fig. 3). The effectiveness of restraining the sugar puckering for hybridization with ssDNA, ssRNA and dsDNA has also been demonstrated.[8–11] Here, we describe the synthesis of another type of BNA monomers, 3′-*O*,4′-*C*-methyleneribonucleosides **1** (3′,4′-BNA monomers), which have the ribofuranose moiety conformationally restrained by a fused oxetane ring.[12–14] The conformational analyses of these nucleoside analogues are also carried out.

2. Results and discussion

2.1. Synthesis of 3′,4′-BNA monomers, 3′-*O*,4′-*C*-methyleneuridine and -cytidine, from uridine

At first, we synthesized the 3′,4′-BNA monomers, 3′-*O*,4′-*C*-methyleneuridine and -cytidine **1a** and **1b**, from 4′-(*p*-toluenesulfonyl)oxymethyluridine **2**[9] which was prepared from uridine (Scheme 1). The primary hydroxy group in **2** was selectively protected by a dimethoxytrityl group to give **3** in 68% yield. Ring-closure reaction of **3** was effectively accomplished under alkaline conditions to yield the oxetane **4**. The oxetane structure of **4** was confirmed by observation of the low-field shifts of the oxetane-ring protons in its ^{1}H NMR spectrum. Three proton signals on the ring of **4** appeared at 4.52 and 4.83 ppm as an AB-type quartet (J=8 Hz) (for CH_2) and at 5.11 ppm as a doublet (J=4 Hz) (for 3′-H). The exclusive oxetane-ring formation would be contributable to the predominant S-type sugar puckering of the diol **3**. In the ^{1}H NMR of **3**, the $J_{1',2'}$ value was 8 Hz, which indicates that the diol **3** exists in S-type conformation.[15] In this conformation, only the 3′-hydroxy group in **3** seems to be located near the 4′-carbon center, while the 2′-hydroxy group is too far away to attack the 4′-methylene carbon. Finally, the 5′-dimethoxytrityl group of **4** was removed by 1% trichloroacetic acid in dichloroethane to give the desired 3′,4′-BNA monomer **1a** in 94% yield.

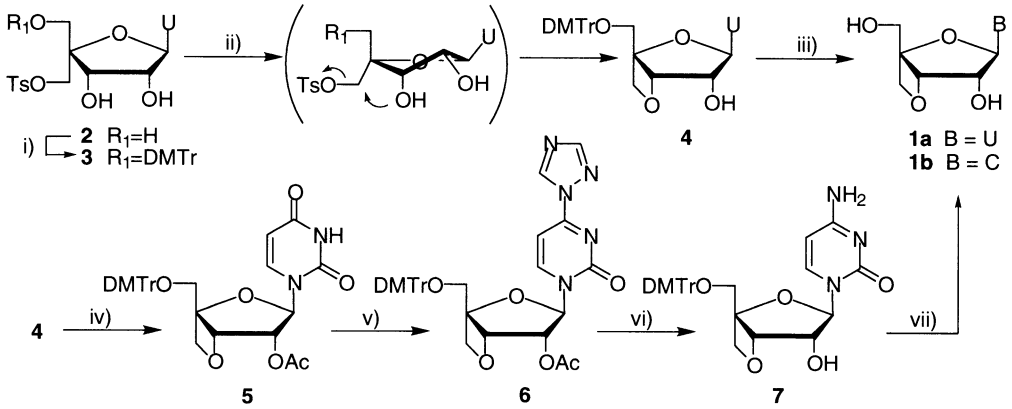


Scheme 2. *Reagents and conditions*: (i) 2TMS·T, TMSOTf, MeCN, rt, 88%.

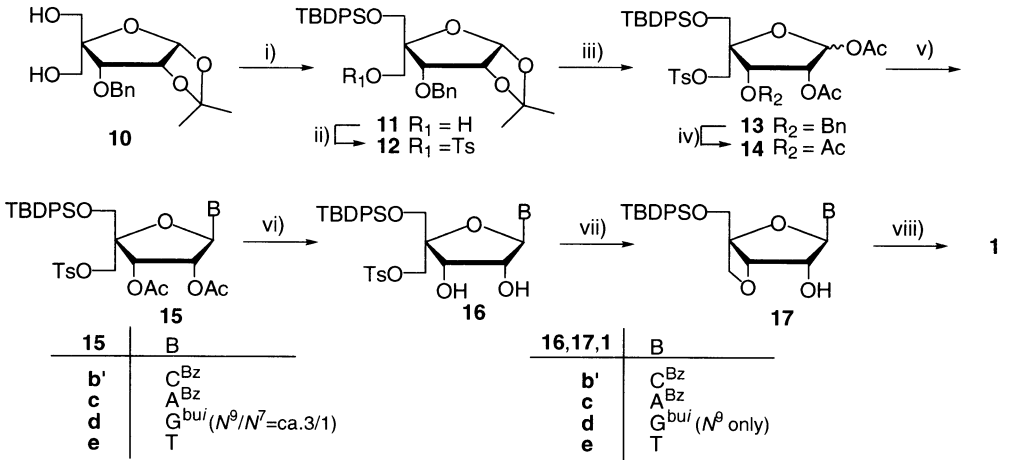
To synthesize the cytidine analogue of **1**, transformation of the nucleobase from uracil to cytosine was examined according to the literature.[16] Acetylation of the 2′-hydroxy group in **4** by acetic anhydride–pyridine gave **5**, quantitatively, and then **5** was treated with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine to give the 4-triazolopyrimidine derivative **6** in 64% yield. The cytidine analogue **7** was obtained by mild ammonolysis of **6** in 82% yield. Deprotection of the 5′-hydroxy group in **7** afforded the 3′,4′-BNA cytosine monomer **1b**.

2.2. Synthesis of 3′,4′-BNA monomers from D-glucose

To synthesize 3′,4′-BNA monomers containing all four natural nucleobases, cytosine, adenine, guanine and thymine, we investigated another synthetic route. Preliminary synthetic study for introduction of the thymine nucleobase into oxetane derivative **8** resulted in exclusive oxetane-ring opening, yielding only **9** (Scheme 2). Considering this finding, we examined the oxetane-ring formation after introduction of a nucleobase into 1,2,3-tri-*O*-acetyl-4-(*p*-toluenesulfonyl)oxymethylribofuranose derivative **14** (Scheme 3). The starting material **10** was prepared from D-glucose by a several-step sequence according to the literature.[17] Selective desymmetrization of **10** was performed by treatment with *tert*-butyldiphenylchlorosilane and triethylamine in dichloromethane to give the desired compound **11** (67%) along with a small amount of its isomer. *p*-Toluenesulfonyl group was introduced into **11** to give **12** in 97% yield. Acid-catalyzed removal of the isopropylidene group in **12** and following acetylation gave the diacetate **13** in 86% yield as an anomeric mixture (α/β=ca. 1:4).



Scheme 1. *Reagents and conditions*: (i) 4,4′-dimethoxytrityl chloride, pyridine, rt, 68%; (ii) NaHMDS, THF, rt, 63%; (iii) 1%TCA in $Cl_2CH_2CH_2Cl_2$, rt, 94%; (iv) Ac_2O, pyridine, rt, 100%; (v) 1,2,4-triazole, 4-chlorophenyl phosphorodichloridate, pyridine, rt, 64%; (vi) aqueous NH_3, 1,4-dioxane, rt, 82%; (vii) 1%TCA in $Cl_2CH_2CH_2Cl_2$, rt, 100%.



Scheme 3. *Reagents and conditions*: (i) TBDPSCl, Et_3N, CH_2Cl_2, rt, 67%; (ii) TsCl, Et_3N, DMAP, CH_2Cl_2, rt, 97%; (iii) AcOH, Ac_2O, c. H_2SO_4, rt, 86%; (iv) 10% Pd–C, H_2, AcOEt, $CHCl_3$, rt, then Ac_2O, pyridine, rt, 91%; (v) sililated base, TMSOTf, $ClCH_2CH_2Cl$, rt, 70–82%; (vi) K_2CO_3, MeOH, rt, 63–100%; (vii) NaHMDS, THF, rt, 82–100%; (viii) TBAF, THF, rt, 65–78%.

Palladium-catalyzed hydrogenolysis of **13** gave a debenzylated product, which was then treated with acetic anhydride in pyridine to give triacetate **14** in 91% yield.

Introduction of a nucleobase to triacetate **14** was accomplished under Vorbrüggen's conditions.[18] Silylated nucleobase (2TMS·C^{Bz}, 2TMS·A^{Bz}, 3TMS·G^{Bui} or 2TMS·T) was reacted with triacetate **14** in dichloroethane in the presence of trimethylsilyltriflate. In this reaction, the desired cytidine, adenosine or thymidine derivative **15b′**,**c** and **15e** was yielded in 70–82% as the sole isomer, while the guanosine analogue **15d** was obtained as a mixture of N^9- and N^7-regioisomer (72%, N^9/N^7=ca. 3:1). Deprotection of the acetyl group in **15** was performed with potassium carbonate in methanol to give the corresponding diols **16**, respectively, in 63–100% yields. After this deprotection step, each isomer of the guanosine derivative was easily separated by column chromatography. From an analysis of the ^1H and ^{13}C NMR spectra of these compounds, it was found that the major isomer was the desired N^9-isomer and the minor one was the N^7-isomer.[19] Oxetane derivatives **17** were prepared in 82–100% yields by treatment of the diols **16** with sodium hexamethyldisilazide. Finally, deprotection of the *tert*-butyldiphenylsilyl group in **17** gave 3′,4′-BNA monomers **1** in 65–78% yields.

2.3. Conformational analysis of 3′,4′-BNA monomers

We performed the conformational analysis of 3′,4′-BNA monomers **1** to clarify their ribofuranose sugar conformation. Generally, ^1H NMR measurements of nucleoside analogues give us useful conformational information. It is well known that the nucleosides and their analogues exist in an equilibrium between S- and N-type conformations based on their furanose-ring puckering. Altona and co-workers reported ^1H–^1H coupling constants $J_{1'2'}$, $J_{2'3'}$ and $J_{3'4'}$ had a correlationship with ribofuranose ring conformation, and several types of equations to predict the probability in S-type conformation (*S*%) of the ribofuranose ring were also proposed.[15] In 3′,4′-BNA monomers **1**, $J_{3'4'}$ is not obtainable because these compounds have no hydrogen atom at the C4′ position. Therefore, the simplest equation (Eq. (1)) was only available for 3′,4′-BNA monomers **1**. Observed coupling constant $J_{1'2'}$ values and calculated *S*% values are summarized in Table 1. In our ^1H NMR measurements, natural ribonucleosides, uridine, cytidine, adenosine and guanosine, showed various $J_{1'2'}$ values due to the diversity of their ribofuranose ring puckering, and the calculated *S*% values of these ribonucleosides were 52, 26, 80 and 71%, respectively. On the other hand, all 3′,4′-BNA monomers showed relatively large $J_{1'2'}$ values (7.3–7.6 Hz), regardless of the type of nucleobase. The *S*% values of **1** ranged from 91 to 96%. These results indicate that the furanose-ring conformation of 3′,4′-BNA monomers **1** was effectively restricted in S-type conformation by the 3′-*O*,4′-*C*-methylene bridge.

$$S(\%) = 100(J_{1'2'} - 1)/6.9 \quad (1)$$

Table 1. Coupling constants and *S* (%) values of nucleosides

Nucleosides	$J_{1'2'}$ (Hz)	*S* (%)
Uridine	4.6	52
Cytidine	2.8	26
Adenosine	6.5	80
Guanosine	5.9	71
1a	7.5	94
1b′	7.3	91
1c	7.5	94
1d	7.6	96
1e	7.5	94

All ^1H NMR data were measured in CD_3OD.

Table 2. Selected torsion angles, pseudorotational phase angle *P* and maximum torsion angle ν_{max} of **1e** determined from X-ray structure

ν_0 ($C_{4'}$–$O_{4'}$–$C_{1'}$–$C_{2'}$)	−29.6°
ν_1 ($O_{4'}$–$C_{1'}$–$C_{2'}$–$C_{3'}$)	32.5°
ν_2 ($C_{1'}$–$C_{2'}$–$C_{3'}$–$C_{4'}$)	−23.5°
ν_3 ($C_{2'}$–$C_{3'}$–$C_{4'}$–$O_{4'}$)	7.1°
ν_4 ($C_{3'}$–$C_{4'}$–$O_{4'}$–$C_{1'}$)	14.2°
δ ($C_{5'}$–$C_{4'}$–$C_{3'}$–$O_{3'}$)	126.2°
χ ($O_{4'}$–$C_{1'}$–$N_{1'}$–$C_{2'}$)	−108.0°
γ ($O_{5'}$–$C_{5'}$–$C_{4'}$–$C_{3'}$)	74.1°
P	136.3°
ν_{max}	32.5°

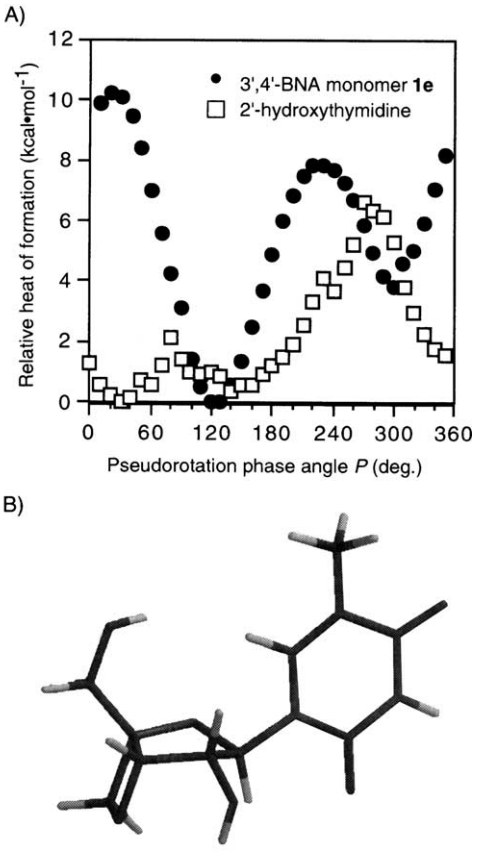


Figure 4. (A) Variation of heat of formation for **1e** and 2′-hydroxythymidine with pseudorotational phase angle *P*. (B) The most stable conformation of **1e** (*P*=130°).

Next, we carried out X-ray crystallographic analysis of 3′,4′-BNA monomer **1e**.[12b] The selected torsion angles of **1e** obtained from the X-ray crystallography are summarized in Table 2. The conformation of the furanose ring can be described by the pseudorotation phase angle (*P*) and the maximum torsion angle (ν_{max}) calculated from the endocyclic sugar torsion angles.[7] In the 3′,4′-BNA monomer, the calculated *P*-value (136.3°) showed that **1e** existed in $C_{1'}$-*exo*–$C_{2'}$-*endo* conformation, which was one of the S-type conformations. In addition, the maximum torsion angle ν_{max} of **1e** (32.5°) was similar to that of typical nucleosides. The dihedral angles ν_3 and ν_4 were smaller than those of natural nucleosides, owing to the restrained structure of the oxetane-fused furanose ring, whereas the value of other dihedral angles ν_0, ν_1, ν_2, bond lengths and bond angles of **1e** were comparable to those of natural nucleosides.[7]

Finally, a PM3 semi-empirical calculation was carried out to analyze the stable conformation of 3′,4′-BNA monomer **1**.[20] By using the X-ray structure of **1e**, various initial structures of **1e** were generated, where the ν_{max}-value was constrained at 32.3° and the *P*-value was varied by changing the dihedral angles ν_0–ν_4. The results of PM3 calculation are illustrated in Fig. 4(A). In the case of 2′-hydroxythymidine, only one large energy barrier was observed for the $O_{4'}$-*exo* puckering mode (*P*=ca. 270°), which came from the steric repulsion between the nucleobase and the 5′-carbon atom.[7,21] On the contrary, 3′,4′-BNA monomer **1e** had two large energy barriers, both of which were attributable to the strain of the oxetane-ring structure. These two energy barriers existed for $C_{3'}$-*endo* (*P*=ca. 20°) and $C_{4'}$-*endo* (*P*=ca. 230°) furanose puckering modes. The lowest energy state was observed for the $C_{1'}$-*exo*–$C_{2'}$-*endo* (*P*=ca. 130°) pucker (Fig. 4(B)), and this result was in good agreement with that from the X-ray crystallographic analysis. The energies for $C_{3'}$-*endo* and $C_{4'}$-*endo* states were about 10 and 8 kcal/mol above the energy calculated for $C_{1'}$-*exo*–$C_{2'}$-*endo* sugar puckering, respectively. Thus, it was clearly demonstrated that the sugar conformation of 3′,4′-BNA monomers 3′-*O*,4′-*C*-methyleneribonucleic acids, was effectively restricted at the $C_{1'}$-*exo*–$C_{2'}$-*endo* state.

3. Conclusion

The conformationally restricted nucleoside analogues, 3′-*O*,4′-*C*-methyleneribonucleosides (3′,4′-BNA monomers **1**) were effectively prepared by two synthetic approaches using uridine or D-glucose as the starting materials. These nucleoside analogues have a oxetane-fused ring consisting of a methylene bridging between 3′-oxygene and 4′-carbon atoms. From ^{1}H NMR analysis, it was demonstrated that the 3′,4′-BNA monomers **1** preferred S-type sugar puckering. Furthermore, the detailed conformation of **1** was studied by X-ray crystallography and PM3 calculation, which revealed that the sugar puckering of **1** was restricted in the $C_{1'}$-*exo*–$C_{2'}$-*endo* state.

4. Experimental

4.1. General considerations

All melting points were measured on a Yanagimoto micro-melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL EX-270 (^{1}H, 270 MHz; ^{13}C, 67.8 MHz) or JEOL GX-500 (^{1}H, 500 MHz) spectrometer. IR spectra were recorded on a JASCO FT/IR-200 spectrometer. Mass spectra were measured on JEOL JMS-D300 or JMS-600 mass spectrometer. Optical rotations were recorded on a JASCO DIP-370 instrument. For column chromatography, Merck Kieselgel 60 (70–200 mesh) or Fuji Silysia BW-127ZH (100–200 mesh) was used.

4.1.1. 5′-*O*-(4,4′-Dimethoxytrityl)-4′-*C*-(*p*-toluenesulfonyl)-oxymethyluridine (3). Under N_2 atmosphere, 4,4′-dimethoxytrityl chloride (242 mg, 0.71 mmol) was added to a stirred solution of **2**[9] (255 mg, 0.60 mmol) in pyridine (5 mL) at room temperature. After having been stirred for 14 h at the same temperature, the reaction mixture was partitioned with saturated $NaHCO_3$ and the mixture was extracted with CH_2Cl_2. Usual work-up and purification by SiO_2 column chromatography ($CHCl_3$/MeOH/Et_3N, 30:1:1) afforded **3** (297 mg, 0.41 mmol, 68%) as a white solid, mp 104–105°C (Et_2O–hexane). $[\alpha]_D^{20}$=−15.3 (*c* 1.1, acetone). IR (KBr): 3396, 2937, 2737, 2675, 2493, 1691, 1474, 1397, 1173, 1035 cm^{-1}. ^{1}H NMR (acetone-d_6) δ: 2.41 (3H, s), 3.22, 3.33 (2H, ABq, *J*=10 Hz), 3.79 (6H, s), 4.29 (1H, dd, *J*=6, 6 Hz), 4.34, 4.41 (2H, AB, *J*=11 Hz), 4.40 (1H, d, *J*=6 Hz), 5.35 (1H, d, *J*=8 Hz), 5.82 (1H, d, *J*=6 Hz), 6.89 (4H, d, *J*=9 Hz), 7.26–7.41 (7H, m), 7.43 (1H, d,

J=8 Hz), 7.70 (2H, d, J=8 Hz). ^{13}C NMR (acetone-d_6) δ: 21.6, 55.5, 64.6, 70.7, 72.7, 74.3, 85.8, 87.8, 88.9, 102.8, 114.0, 127.7, 128.7, 128.8, 130.7, 130.9, 131.0, 133.9, 141.1, 145.5, 151.4, 159.7, 163.3; MS (EI) m/z: 737 (M^+-OCH_3, 11.0), 583 (11.5), 304 (100). Anal. calcd for $C_{38}H_{38}N_2O_{11}S\cdot 1/6H_2O$: C, 62.19; H, 5.26; N, 3.81; S, 4.15. Found: C, 62.37; H, 5.26; N, 3.60; S, 4.15.

4.1.2. 5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*,4′-*C*-methyleneuridine (4). Under N_2 atmosphere, sodium bis(trimethylsilylamide) (8.96 mmol, 1.0 M solution in THF) was added to a stirred solution of **3** (735 mg, 0.90 mmol) in THF (11 mL) at room temperature. After having been stirred for 48 h at the same temperature, the reaction mixture was partitioned with saturated $NaHCO_3$ and the mixture was extracted with $CHCl_3$. Usual work-up and purification by SiO_2 column chromatography ($CHCl_3$/MeOH/Et_3N, 30:1:0, then 30:1:2) afforded **4** (311 mg, 0.56 mmol, 63%) as a white foam, mp 120–121°C (Et_2O–hexane). $[\alpha]_D^{21}=-37.7$ (c 1.1, acetone). IR (KBr): 3395, 3222, 3062, 2930, 1693, 1508, 1461, 1385, 1298, 1252, 1177, 1034 cm^{-1}. ^{1}H NMR (acetone-d_6) δ: 2.92 (1H, br), 3.47, 3.51 (2H, ABq, J=10 Hz), 3.85 (6H, s), 4.36 (1H, dd, J=8, 4 Hz), 4.52, 4.83 (2H, ABq, J=8 Hz), 5.11 (1H, d, J=4 Hz), 5.57 (1H, d, J=8 Hz), 6.51 (1H, d, J=8 Hz), 6.96 (4H, d, J=9 Hz), 7.39–7.41 (7H, m), 7.52 (2H, d, J=5 Hz), 7.71 (1H, d, J=9 Hz). ^{13}C NMR (acetone-d_6) δ: 55.4, 64.1, 75.5, 79.0, 85.5, 86.2, 87.1, 88.8, 103.3, 113.7, 113.9, 127.6, 128.4, 128.6, 128.8, 129.9, 130.9, 131.1, 136.3, 136.4, 141.2, 145.7, 151.6, 159.6, 163.4. MS (EI) m/z: 558 (M^+, 2.2), 303 (64.1), 227 (8.7), 114 (100). Anal. calcd for $C_{31}H_{30}N_2O_8$: C, 66.7; H, 5.41; N, 5.35. Found: C, 66.5; H, 5.43; N, 5.35.

4.1.3. 3′-*O*,4′-*C*-Methyleneuridine (1a). To a stirred solution of **4** (107 mg, 0.21 mmol) in dichloroethane (6.0 mL) was added 1% trichloroacetic acid in dichloroethane (1.0 mL) at room temperature. After having been stirred for 15 min at the same temperature, the reaction mixture was concentrated under reduced pressure. The remaining residue was chromatographed on silica gel ($CHCl_3$/MeOH, 10:1) to give **1a** (85 mg, 0.20 mmol, 94%) as a white solid, mp 215–216°C (Et_2O–hexane). $[\alpha]_D^{17}=-18.2$ (c 1.2, acetone). IR (KBr): 3639, 1924, 1714, 1464, 1134 cm^{-1}. ^{1}H NMR (acetone-d_6) δ: 3.74, 3.82 (2H, ABq, J=12 Hz), 4.21 (1H, dd, J=7, 5 Hz), 4.42, 4.82 (2H, ABq, J=8 Hz), 5.05 (1H, d, J=5 Hz), 5.66 (1H, d, J=9 Hz), 6.38 (1H, d, J=7 Hz), 7.67 (1H, d, J=9 Hz). ^{13}C NMR (acetone-d_6) δ: 62.3, 75.3, 78.3, 85.9, 86.9, 89.4, 103.4, 141.6, 151.7, 163.4. MS (EI) m/z: 256 (M^+, 2.1), 113 (100). Anal. calcd for $C_{10}H_{12}N_2O_6\cdot 1/2H_2O$: C, 45.29; H, 4.94; N, 10.56. Found: C, 45.07; H, 4.82; N, 10.15.

4.1.4. 2′-*O*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*,4′-*C*-methyleneuridine (5). Under N_2 atmosphere, acetic anhydride (41 mg, 0.40 mmol) was added to a stirred solution of **4** (188 mg, 0.34 mmol) in pyridine (3.5 mL) at room temperature. After having been stirred for 10 h at the same temperature, the reaction mixture was concentrated under reduced pressure. The remaining residue was chromatographed on silica gel (AcOEt) to give **5** (203 mg, 0.34 mmol, 100%) as a white solid, mp 120–121°C (AcOEt–hexane). $[\alpha]_D^{21}=-33.7$ (c 0.91, acetone). IR (KBr): 3471, 3200, 3062, 2953, 2836, 1697, 1610, 1508, 1456, 1380, 1250, 1178, 1040 cm^{-1}. ^{1}H NMR (acetone-d_6) δ: 2.04 (3H, s), 3.47, 3.61 (2H, AB, J=10 Hz), 3.79 (6H, s), 4.53, 4.79 (2H, AB, J=8 Hz), 5.19 (1H, dd, J=7, 5 Hz), 5.27 (1H, d, J=5 Hz), 5.54 (1H, d, J=8 Hz), 6.60 (1H, d, J=7 Hz), 6.90 (4H, d, J=9 Hz), 7.30–7.49 (9H, m), 7.70 (1H, d, J=8 Hz), 10.10 (1H, br). ^{13}C NMR (acetone-d_6) δ: 20.3, 55.5, 64.0, 76.4, 79.1, 84.6, 86.3, 87.3, 87.4, 103.5, 114.0, 127.7, 128.7, 128.9, 130.9, 136.3, 136.4, 141.1, 145.8, 151.4, 159.7, 170.4. MS (FAB) m/z: 601 (MH^+). Anal. calcd for $C_{33}H_{32}N_2O_9\cdot 1/2H_2O$: C, 65.02; H, 5.36; N, 4.65. Found: C, 65.02; H, 5.46; N, 4.60.

4.1.5. 1-[2-*O*-Acetyl-5-*O*-(4,4′-dimethoxytrityl)-3-*O*,4-*C*-methylene-β-D-ribofuranosyl]-4-(1,2,4-triazol-1-yl)pyrimidinone (6). Under N_2 atmosphere, dichlorophenyl phosphorodichloridate (221 mg, 0.90 mmol) was added to a stirred solution of **5** (118 mg, 0.20 mmol) in pyridine (2 mL) at room temperature. After having been stirred for 3 min, 1,2,4-triazole (180 mg, 2.61 mmol) was added to the reaction mixture at 0°C. Stirring was continued for 10 h at room temperature, and then the reaction mixture was concentrated under reduced pressure. The remaining residue was chromatographed on silica gel (AcOEt) to afford **6** (82 mg, 0.13 mmol, 64%) as a pale yellow solid, mp 78–80°C (CH_2Cl_2–hexane). $[\alpha]_D^{22}=-10.1$ (c 1.5, acetone). IR (KBr): 3860, 3122, 2048, 1746, 1682, 1548, 1509, 1281, 1249, 1062 cm^{-1}. ^{1}H NMR (acetone-d_6) δ: 2.05 (3H, s), 3.56, 3.70 (2H, ABq, J=10 Hz), 3.76 (6H, s), 4.59, 4.84 (2H, ABq, J=8 Hz), 5.31–5.36 (2H, m), 6.74 (1H, d, J=5 Hz), 6.89 (4H, d, J=9 Hz), 7.29–7.51 (9H, m), 8.24 (1H, s), 8.47 (1H, d, J=7 Hz), 9.27 (1H, s). ^{13}C NMR (acetone-d_6) δ: 20.3, 55.5, 63.9, 78.0, 79.1, 85.1, 87.2, 87.5, 87.6, 88.2, 90.4, 95.5, 114.0, 127.7, 128.7, 128.8, 130.9, 136.2, 136.4, 144.2, 145.7, 147.8, 149.2, 154.7, 154.9, 159.6, 160.3, 170.5. MS (FAB) m/z: 652 (MH^+).

4.1.6. 5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*,4′-*C*-methylenecytidine (7). To a stirred solution of **6** (150 mg, 0.23 mmol) in 1,4-dioxane (6.5 mL) was added NH_4OH (1 mL) at room temperature. After having been stirred for 10 h at the same temperature, the reaction mixture was concentrated under reduced pressure. The remaining residue was chromatographed on silica gel ($CHCl_3$/MeOH/Et_3N, 30:2:3) to afford **7** (107 mg, 0.19 mmol, 82%) as a white solid, mp 139–140°C (Et_2O–hexane). $[\alpha]_D^{22}=+0.60$ (c 0.34, MeOH). IR (KBr): 3345, 3199, 2946, 1651, 1604, 1503, 1373, 1294, 1250, 1179, 1037 cm^{-1}. ^{1}H NMR (CD_3OD): δ: 3.40, 3.50 (2H, ABq, J=10 Hz), 3.76 (6H, s), 4.14 (1H, dd, J=7, 4 Hz), 4.48, 4.72 (2H, ABq, J=7 Hz), 4.98 (1H, d, J=4 Hz), 5.79 (1H, d, J=7 Hz), 6.56 (1H, d, J=7 Hz), 6.85 (4H, d, J=9 Hz), 7.21–7.42 (9H, m), 7.67 (1H, d, J=7 Hz). ^{13}C NMR (CD_3OD) δ: 55.7, 64.5, 76.8, 79.8, 86.2, 87.3, 87.8, 90.1, 97.0, 114.2, 128.1, 128.9, 129.3, 131.3, 136.8, 142.8, 146.1, 160.3, 167.3. MS (FAB) m/z: 558 (MH^+). Anal. calcd for $C_{31}H_{31}N_3O_7\cdot 2H_2O$: C, 62.72; H, 5.94; N, 7.08. Found: C, 63.01; H, 5.56; N, 7.08.

4.1.7. 3′-*O*,4′-*C*-Methylenecytidine (1b). To a stirred solution of **7** (72 mg, 0.13 mmol) in dichloroethane (2 mL) was added 1% trichloroacetic acid in dichloroethane (1 mL) at room temperature. After having been stirred for 15 min at

the same temperature, the reaction mixture was concentrated under reduced pressure. The remaining residue was chromatographed on silica gel ($CHCl_3$/MeOH, 10:1) to afford **1b** (33 mg, 0.13 mmol, 100%) as a white solid, mp 224–224°C (*i*PrOH). $[\alpha]_D^{23}=+32.6$ (*c* 0.49, H_2O). IR (KBr): 3348, 1653, 1494, 1374, 1286, 1125, 1042 cm^{-1}. ^1H NMR (CD_3OD) δ: 3.73, 3.81 (2H, ABq, *J*=12 Hz), 4.16 (1H, dd, *J*=7, 5 Hz), 4.52, 4.81 (2H, ABq, *J*=8 Hz), 5.04 (1H, d, *J*=5 Hz), 5.94 (1H, d, *J*=7 Hz), 6.41 (1H, d, *J*=7 Hz), 7.68 (1H, d, *J*=7 Hz). ^{13}C NMR (D_2O) δ: 62.0, 74.6, 79.5, 86.39, 86.42, 89.3, 98.2, 142.8, 158.9, 167.1. Mass (EI) *m/z*: 255 (M^+, 1.9), 224 (0.9), 112 (100). Anal. calcd for $C_{10}H_{13}N_3O_5\cdot 1/3H_2O$: C, 45.98; H, 5.27; N, 16.09. Found: C, 45.98; H, 5.02; N, 15.82.

4.1.8. Methyl 2-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-3-*O*,4-*C*-methylene-β-D-ribofuranoside (8). Under N_2 atmosphere, a solution of methyl 5-*O*-(*tert*-butyldiphenylsilyl)-3-*O*,4-*C*-methylene-β-D-ribofuranoside[22] (143 mg, 0.34 mmol) in THF (2 mL) was added to a suspension of hexane-washed NaH (60% in mineral oil (w/w), 17 mg, 0.41 mmol) in THF (1 mL) at 0°C. After having been stirred for 30 min at the same temperature, benzyl bromide (65 mg, 0.38 mmol) was added to the reaction mixture at 0°C. Stirring was continued for 16 h at room temperature. After addition of water, the mixture was extracted with AcOEt. Usual work-up and purification by SiO_2 column chromatography (hexane/AcOEt, 8:1) afforded **8** (147 mg, 0.29 mmol, 85%) as a colorless oil. $[\alpha]_D^{22}=-49.8$ (*c* 1.65, $CHCl_3$). IR (KBr): 2934, 2860, 1111, 1010 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.01 (9H, s), 3.49 (3H, s), 3.66 (1H, dd, *J*=5, 5 Hz), 3.68, 3.74 (2H, AB, *J*=11 Hz), 4.45, 4.88 (2H, AB, *J*=7 Hz), 4.62, 4.68 (2H, AB, *J*=12 Hz), 5.01 (1H, d, *J*=5 Hz), 5.37 (1H, d, *J*=5 Hz), 7.30–7.44 (11H, m), 7.63–7.76 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 19.2, 26.8, 56.9, 63.5, 72.5, 78.4, 82.0, 83.9, 85.2, 108.9, 127.6, 127.7, 127.7, 127.9, 128.0, 128.4, 129.8, 132.9, 133.1, 135.6, 135.6, 137.5. MS (EI) *m/z*: 504 (M^+, 0.1), 91 (100). Anal. calcd for $C_{30}H_{36}O_5Si$: C, 71.39; H, 7.19. Found: C, 71.45; H, 7.12.

4.1.9. Methyl 2-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-4-*C*-(thymin-1-yl)methyl-β-D-ribofuranoside (9). Under N_2 atmosphere, silylated thymine (2TMS·T) (176 mg, 0.68 mmol) and trimethylsilyl trifluoromethanesulfonate (0.773 M in dichloroethane, 0.34 mL, 0.26 mmol) were added to a stirred solution of **8** (131 mg, 0.26 mmol) in MeCN (2.5 mL) at room temperature. After having been stirred for 41 h, the reaction mixture was partitioned with saturated $NaHCO_3$, and the mixture was extracted with AcOEt. Usual work-up and purification by SiO_2 column chromatography (hexane/AcOEt, 2:1) afforded **9** (146 mg, 0.23 mmol, 88%) as a white solid, mp 62–65°C. $[\alpha]_D^{23}=+64.8$ (*c* 0.80, $CHCl_3$). IR (KBr): 2934, 1681, 1470, 1112, 1036 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.10 (9H, s), 1.92 (3H, s), 3.17 (3H, s), 3.63, 3.73 (2H, AB, *J*=11 Hz), 3.67 (1H, d, *J*=5 Hz), 3.83, 4.28 (2H, AB, *J*=15 Hz), 4.06 (1H, dd, *J*=5, 12 Hz), 4.44, 4.50 (2H, AB, *J*=12 Hz), 4.58 (1H, s), 4.71 (1H, d, *J*=12 Hz), 7.11 (1H, s), 7.24 (2H, d, *J*=7 Hz), 7.35–7.46 (9H, m), 7.64 (2H, d, *J*=8 Hz), 7.68 (2H, d, *J*=8 Hz). ^{13}C NMR ($CDCl_3$) δ: 12.3, 19.4, 26.9, 46.8, 55.0, 69.3, 74.0, 86.5, 105.7, 127.8, 127.8, 128.2, 128.3, 128.4, 128.5, 129.9, 130.0, 132.8, 132.9, 135.6, 136.9, 142.7, 152.8, 163.5. MS (EI) *m/z*: 573 (M^+-*tert*-Bu, 50.1), 91 (100). Anal. calcd for $C_{35}H_{42}N_2O_7Si$: C, 66.64; H, 6.71; N, 4.44. Found: C, 66.63; H, 6.88; N, 4.41.

4.1.10. 3-*O*-Benzyl-5-*O*-*tert*-butyldiphenylsilyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (11). Under N_2 atmosphere, triethylamine (2.69 g, 26.6 mmol) and *tert*-butyldiphenylsilyl chloride (7.09 g, 25.8 mmol) were added to a stirred solution of **10**[17] (2.50 g, 8.1 mmol) in dichloromethane (50 mL) at room temperature. After having been stirred for 11 h at the same temperature, the reaction mixture was partitioned with saturated $NaHCO_3$ and the mixture was extracted with $CHCl_3$. Usual work-up and purification by SiO_2 column chromatography (hexane/AcOEt, 4:1 to 3:1) afforded **11** (2.97 g, 5.4 mmol, 67%) as a white solid, mp 98–99°C (hexane). $[\alpha]_D^{22}=+46.2$ (*c* 0.92, $CHCl_3$). IR (KBr): 3553, 2936, 1463, 1379, 1107 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.13 (9H, s), 1.50 (3H, s), 1.78 (3H, s), 2.56 (1H, t, *J*=7 Hz), 3.82, 3.92 (2H, ABq, *J*=11 Hz), 3.94 (2H, m), 4.57 (1H, d, *J*=5 Hz), 4.64, 4.95 (2H, ABq, *J*=12 Hz), 4.83 (1H, dd, *J*=4, 5 Hz), 5.95 (1H, d, *J*=4 Hz), 7.44–7.55 (11H, m), 7.72–7.78 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 19.2, 26.2, 26.5, 26.8, 63.2, 65.4, 72.5, 77.9, 79.1, 87.4, 104.4, 113.7, 127.6, 127.7, 128.0, 128.5, 129.5, 129.7, 132.9, 133.1, 134.7, 135.5, 137.2. Anal. calcd for $C_{32}H_{41}O_6Si$: C, 70.04; H, 7.35. Found: C, 70.19; H, 7.38.

4.1.11. 3-*O*-Benzyl-5-*O*-*tert*-butyldiphenylsilyl-1,2-*O*-isopropylidene-4-*C*-(*p*-toluenesulfonyl)oxymethyl-α-D-*erythro*-pentofuranose (12). Under N_2 atmosphere, triethylamine (286 mg, 2.83 mmol), *p*-toluenesulfonyl chloride (133 mg, 0.073 mmol) and 4,4′-dimethylaminopyridine (5.6 mg, 0.046 mmol) were added to a stirred solution of **11** (250 mg, 0.46 mmol) in dichloromethane (5 mL) at room temperature. After having been stirred for 16 h at the same temperature, the reaction mixture was partitioned with saturated $NaHCO_3$ and the mixture was extracted with $CHCl_3$. Usual work-up and purification by SiO_2 column chromatography (hexane/AcOEt, 1:6) afforded **12** (311 mg, 0.44 mmol, 97%) as a colorless oil. $[\alpha]_D^{23}=+0.47$ (*c* 1.76, $CHCl_3$). IR (KBr): 2935, 1595, 1462, 1363, 1174 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.08 (9H, s), 1.40 (3H, s), 1.46 (3H, s), 2.48 (3H, s), 3.68, 3.83 (2H, ABq, *J*=11 Hz), 4.45 (2H, dd, *J*=4, 5 Hz), 4.62 (2H, ABq, *J*=12 Hz), 4.64, 4.81 (2H, ABq, *J*=12 Hz), 4.68 (1H, dd, *J*=5 Hz), 5.81 (1H, d, *J*=4 Hz), 7.32 (2H, d, *J*=8 Hz), 7.42–7.72 (15H, m), 7.82 (2H, d, *J*=8 Hz), 7.66 (4H, m), 7.72 (2H, d, *J*=8 Hz). ^{13}C NMR ($CDCl_3$) δ: 19.1, 21.5, 26.1, 26.4, 26.7, 64.4, 70.0, 72.5, 78.1, 78.9, 85.4, 104.2, 113.6, 127.3, 127.7, 127.9, 128.0, 128.4, 129.6, 129.7, 129.8, 132.7, 132.8, 135.5, 137.2, 144.4. MS (EI) *m/z*: 646 (M^+-*tert*-Bu, 3.2), 91 (100). HRMS (EI) calcd for $C_{35}H_{37}O_8SSi$ (M^+-*tert*-Bu) 645.1978. Found: 645.1969.

4.1.12. 3-*O*-Benzyl-5-*O*-*tert*-butyldiphenylsilyl-1,2-di-*O*-acetyl-4-*C*-(*p*-toluenesulfonyl)oxymethyl-α- and -β-D-*erythro*-pentofuranose (13). To a stirred solution of **12** (3.70 g, 5.3 mmol) in acetic acid (56 mL) were added acetic anhydride (6 mL, 64 mmol) and concentrated sulfuric acid (56 μL, 1.1 μmol). The solution was stirred at room temperature for 2 h. Then the reaction mixture was poured into ice-water (300 mL) and stirred for 30 min. Saturated NH_4Cl was added, and the mixture was extracted with

$CHCl_3$. Usual work-up and purification by SiO_2 column chromatography (hexane/AcOEt, 1:2) afforded **13** (3.36 g, 4.5 mmol, 86%) as a pale yellow oil. IR (KBr): 2934, 2863, 1751, 1365, 1217, 1106 cm^{-1}. ^1H NMR ($CDCl_3$) (major, β-anomer) δ: 1.02 (9H, s), 1.77 (3H, s), 1.98 (3H, s), 2.39 (3H, s), 3.61, 3.76 (2H, ABq, J=11 Hz), 4.21–4.58 (5H, m), 5.26 (1H, d, J=5 Hz), 5.94 (1H, s), 7.15–7.59 (13H, m), 7.58–7.66 (4H, m), 7.72 (2H, d, J=8 Hz). (minor, α-anomer) δ: 1.02 (9H, s), 1.98 (3H, s), 2.36 (3H, s), 3.48, 3.58 (2H, ABq, J=11 Hz), 4.21–4.58 (5H, m), 5.12 (1H, dd, J=5, 6 Hz), 6.33 (1H, d, J=5 Hz), 7.15–7.59 (13H, m), 7.58–7.66 (4H, m), 7.72 (2H, d, J=8 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.2, 19.3, 20.5, 20.8, 21.6, 26.7, 26.8, 60.3, 64.8, 69.1, 73.6, 74.1, 78.6, 85.3, 97.4, 127.4, 127.6, 127.7, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 129.5, 129.6, 128.98, 129.9, 132.4, 132.8, 132.9, 135.4, 135.5, 135.6, 136.9, 144.5, 168.7, 169.4. HRMS (FAB) calcd for $C_{40}H_{46}N_2O_{10}SSiNa$ (MH^+-Na) 769.2479. Found: 769.2484.

4.1.13. 5-*O*-*tert*-Butyldiphenylsilyl-4-(*p*-toluenesulfonyl)-oxymethyl-1,2,3-tri-*O*-acetyl-α- and -β-D-*erythro*-pentofuranose (14). Under H_2 atmosphere, 10% Pd–C (1.0 g) was added to a stirred solution of **13** (1.85 g, 2.5 mmol) in AcOEt–$CHCl_3$ (10:1, v/v, 220 mL) at room temperature. After having been stirred for 16 h, the reaction mixture was filtered, and the filtrate was concentrated. The residue was dissolved in pyridine (10 mL), and acetic anhydride (0.68 g, 6.7 mmol) was added at room temperature. After having been stirred for 49 h, the reaction mixture was concentrated under reduced pressure. The remaining residue was chromatographed on silica gel (hexane/AcOEt, 2:1) to afford **14** (1.58 g, 2.26 mmol, 91%) as a pale yellow oil. IR (KBr): 2935, 1755, 1603, 1506, 1367, 1216 cm^{-1}. ^1H NMR ($CDCl_3$) (major, β-anomer) δ: 0.98 (9H, s), 1.79 (3H, s), 2.08 (6H, s), 2.41 (3H, s), 3.55, 3.78 (2H, ABq, J=11 Hz), 4.21, 4.40 (2H, ABq, J=10 Hz), 5.32 (1H, d, J=5 Hz), 5.51 (1H, d, J=5 Hz), 6.01 (1H, s), 7.25–7.26 (2H, m), 7.29–7.43 (6H, m), 7.60 (4H, d, J=7 Hz), 7.76 (2H, d, J=8 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.1, 19.0, 20.3, 20.7, 20.9, 26.6, 55.1, 60.3, 65.4, 67.7, 72.1, 74.3, 84.3, 97.2, 113.5, 127.8, 128.0, 129.5, 129.7, 129.8, 132.2, 132.5, 132.6, 135.4, 135.5, 144.8, 168.6, 169.1. HRMS (FAB) calcd for $C_{35}H_{42}O_{11}SSiNa$ (MH^++Na) 721.2115. Found: 721.2122.

4.1.14. N^4-Benzoyl-5′-*O*-*tert*-butyldiphenylsilyl-2′,3′-di-*O*-acetyl-4′-*C*-(*p*-toluenesulfonyl)oxymethylcytidine (15b′). Under N_2 atmosphere, silylated N^4-benzoylcytosine ($2TMS{\cdot}C^{Bz}$) (0.5 M solution in dichloroethane, 0.96 mL, 0.48 mmol) and trimethylsilyl trifluoromethanesulfonate (76 mg, 0.32 mmol) were added to a stirred solution of **14** (250 mg, 0.32 mmol) in dichloroethane (5 mL) at room temperature. After having been refluxed for 5 h, the reaction mixture was cooled and partitioned with saturated $NaHCO_3$, and the mixture was extracted with $CHCl_3$. Usual work-up and purification by SiO_2 column chromatography ($CHCl_3$/MeOH, 30:1) afforded **15b′** (225 mg, 0.26 mmol, 82%) as a white foam, mp 73–74°C. $[\alpha]_D^{23}$=+25.4 (c 0.88, $CHCl_3$). IR (KBr): 3067, 2934, 1753, 1674, 1482, 1240, 1105 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.19 (9H, s), 2.16 (3H, s), 2.20 (3H, s), 2.51 (3H, s), 3.87, 3.96 (2H, ABq, J=11 Hz), 4.24, 4.34 (2H, ABq, J=11 Hz), 5.63 (1H, dd, J=6, 6 Hz), 5.79 (1H, d, J=6 Hz), 6.22 (1H, d, J=6 Hz), 7.35–8.04 (22H, m). ^{13}C NMR ($CDCl_3$) δ: 19.1, 20.3, 21.6, 26.6, 26.8, 65.2, 67.5, 70.9, 73.9, 85.4, 87.7, 127.6, 127.8, 128.0, 128.1, 129.0, 129.8, 130.3, 131.5, 132.1, 132.3, 133.2, 135.4, 135.5, 135.6, 145.2, 168.9, 169.4. MS (FAB) m/z: 854 (MH^+). Anal. calcd for $C_{44}H_{47}N_3O_{11}SSi{\cdot}1/2H_2O$: C, 61.24; H, 5.61; N, 4.87; S, 3.72. Found: C, 61.01; H, 5.33; N, 4.62; S, 3.89.

4.1.15. N^6-Benzoyl-5′-*O*-*tert*-butyldiphenylsilyl-2′,3′-di-*O*-acetyl-4′-*C*-(*p*-toluenesulfonyl)oxymethyladenosine (15c). Under N_2 atmosphere, silylated N^6-benzoyladenine ($2TMS{\cdot}A^{Bz}$) (0.5 M solution in dichloroethane, 0.96 mL, 0.48 mmol) and trimethylsilyl trifluoromethanesulfonate (76 mg, 0.32 mmol) were added to a stirred solution of **14** (250 mg, 0.32 mmol) in dichloroethane (5 mL) at room temperature. After having been refluxed for 23 h, the reaction mixture was cooled and partitioned with saturated $NaHCO_3$, and the mixture was extracted with $CHCl_3$. Usual work-up and purification by SiO_2 column chromatography (hexane/AcOEt, 1:2 to 1:4) afforded **15c** (197 mg, 0.23 mmol, 70%) as a white foam, mp 84–86°C. $[\alpha]_D^{22}$=−12.5 (c 1.03, $CHCl_3$). IR (KBr): 2934, 1756, 1704, 1640, 1604, 1232, 1182, 1106 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.02 (9H, s), 2.04 (3H, s), 2.16 (3H, s), 2.40 (3H, s), 3.83, 3.89 (2H, ABq, J=9 Hz), 4.30, 4.38 (2H, ABq, J=10 Hz), 5.94 (1H, d, J=5 Hz), 6.06 (1H, dd, J=6, 5 Hz), 6.12 (1H, d, J=6 Hz), 7.24–7.57 (15H, m), 7.61 (2H, d, J=6 Hz), 7.73 (2H, d, J=8.0 Hz), 8.02 (1H, s), 8.04 (1H, s), 8.54 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 19.0, 20.2, 21.5, 26.6, 64.6, 67.3, 71.6, 72.8, 85.5, 86.1, 123.5, 127.2, 127.7, 127.8, 127.9, 128.4, 128.7, 129.7, 129.9, 130.0, 131.8, 132.0, 132.4, 132.7, 133.3, 135.35, 135.38, 141.6, 145.0, 149.6, 151.3, 152.6, 164.6, 169.0, 169.1. MS (FAB) m/z: 878 (MH^+). Anal. calcd for $C_{45}H_{47}N_5O_{10}SSi{\cdot}1/10H_2O$: C, 61.43; H, 5.41; N, 7.96; S, 3.63. Found: C, 61.54; H, 5.60; N, 7.81; S, 3.60.

4.1.16. 5′-*O*-*tert*-Butyldiphenylsilyl-2′,3′-di-*O*-acetyl-N^2-isobutyryl-4′-*C*-(*p*-toluenesulfonyl)oxymethylguanosine (15d). Under N_2 atmosphere, silylated N^2-isobutyrylguanine ($3TMS{\cdot}G^{Bui}$) (0.5 M solution in dichloroethane, 0.96 mL, 0.48 mmol) and trimethylsilyl trifluoromethanesulfonate (76 mg, 0.32 mmol) were added to a stirred solution of **14** (250 mg, 0.32 mmol) in dichloroethane (5 mL) at room temperature. After having been refluxed for 13 h, the reaction mixture was cooled and partitioned with saturated $NaHCO_3$, and the mixture was extracted with $CHCl_3$. Usual work-up and purification by SiO_2 column chromatography ($CHCl_3$/MeOH, 33:1) afforded **15d** (198 mg, 0.23 mmol, 72%, a mixture of N^9- and N^7-isomers, N^9/N^7=ca. 3:1) as a white foam, mp 83–84°C. IR (KBr): 2936, 1751, 1685, 1608, 1561, 1368, 1244 cm^{-1}. ^1H NMR ($CDCl_3$) (major, N^9-isomer) δ: 0.94 (9H, s), 1.14 (3H, d, J=7 Hz), 1.15 (3H, d, J=6 Hz), 2.05 (3H, s), 2.08 (3H, s), 2.40 (3H, s), 2.53 (1H, hep, J=7 Hz), 3.73, 3.76 (2H, ABq, J=6 Hz), 4.37 (2H, s), 5.93 (1H, d, J=5 Hz), 6.00 (2H, m), 7.21–7.74 (15H, m), 9.26 (1H, br), 12.20 (1H, br). ^{13}C NMR ($CDCl_3$) δ: 18.8, 20.2, 21.5, 26.5, 36.1, 64.7, 67.4, 71.2, 72.6, 85.1, 85.4, 85.7, 96.0, 121.5, 127.6, 127.7, 127.8, 129.8, 129.9, 130.0, 131.8, 132.1, 132.5, 135.2, 135.3, 135.4, 135.5, 137.8, 145.1, 147.5, 148.0, 155.7, 169.1, 169.3, 178.4. MS (FAB) m/z: 860 (MH^+).

4.1.17. 5′-*O*-*tert*-Butyldiphenylsilyl-2′,3′-di-*O*-acetyl-5-methyl-4′-*C*-(*p*-toluenesulfonyl)oxymethyluridine (15e). Under N_2 atmosphere, silylated thymine (2TMS·T) (0.5 M solution in dichloroethane, 0.96 mL, 0.48 mmol) and trimethylsilyl trifluoromethanesulfonate (76 mg, 0.32 mmol) were added to a stirred solution of **14** (250 mg, 0.32 mmol) in dichloroethane (5 mL) at room temperature. After having been refluxed for 5 h, the reaction mixture was cooled and partitioned with saturated $NaHCO_3$, and the mixture was extracted with $CHCl_3$. Usual work-up and purification by SiO_2 column chromatography (hexane/AcOEt, 1:2 to 1:4) afforded **15e** (164 mg, 0.22 mmol, 79%) as a white foam, mp 71–72°C. $[\alpha]_D^{24}$=+5.7 (*c* 0.65, $CHCl_3$). IR (KBr): 2934, 1753, 1695, 1465, 1369, 1236 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.09 (3H, s), 1.53 (3H, s), 2.05 (3H, s), 2.12 (3H, s), 2.40 (3H, s), 3.76 (2H, s), 4.02, 4.21 (2H, ABq, *J*=10 Hz), 5.58 (1H, dd, *J*=7, 6 Hz), 5.69 (1H, d, *J*=6 Hz), 6.10 (1H, d, *J*=7 Hz), 7.21–7.67 (15H, m), 9.28 (1H, br). ^{13}C NMR ($CDCl_3$) δ: 11.8, 19.3, 20.3, 21.6, 26.9, 65.8, 67.3, 71.0, 72.1, 84.8, 85.5, 112.1, 127.8, 128.0, 128.1, 129.8, 130.2, 130.3, 131.4, 132.3, 135.0, 135.2, 135.5, 145.1, 150.5, 163.3, 169.1, 169.4. MS (FAB) *m/z*: 765 (MH^+). Anal. calcd for $C_{38}H_{44}N_2O_{11}SSi\cdot 1/10H_2O$: C, 59.53; H, 5.81; N, 3.65; S, 4.17. Found: C, 59.74; H, 5.84; N, 3.37; S, 4.01.

4.1.18. N^4-Benzoyl-5′-*O*-*tert*-butyldiphenylsilyl-4′-*C*-(*p*-toluenesulfonyl)oxymethylcytidine (16b′). To a stirred solution of diacetate **15b′** (113 mg, 0.13 mmol) in MeOH (4 mL) was added K_2CO_3 (11 mg, 0.079 mmol). After having been stirred for 15 min, the reaction mixture was neutralized with concentrated HCl and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel ($CHCl_3$/MeOH, 25:1) to afford **16b′** (74 mg, 0.096 mmol, 73%) as a white solid, mp 97–98°C. $[\alpha]_D^{23}$=+49.8 (*c* 0.16, $CHCl_3$). IR (KBr): 3379, 1649, 1493, 1361 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.01 (9H, s), 2.38 (3H, s), 3.74 (2H, s), 4.24 (1H, dd, *J*=5, 5 Hz), 4.33, 4.47 (2H, ABq, *J*=11 Hz), 4.34 (1H, d, *J*=5 Hz), 5.72 (1H, d, *J*=5 Hz), 7.22–7.74 (19H, m), 7.92–7.96 (2H, m). ^{13}C NMR ($CDCl_3$) δ: 19.1, 21.6, 26.8, 64.2, 69.1, 72.4, 76.9, 88.8, 92.5, 97.2, 127.3, 127.8, 128.0, 128.5, 128.9, 129.7, 130.2, 131.8, 132.0, 132.5, 132.6, 133.3, 135.4, 135.5, 144.1, 144.8, 162.8. MS (FAB) *m/z*: 770 (MH^+). Anal. calcd for $C_{40}H_{43}N_3O_9SSi\cdot 1/4H_2O$: C, 62.04; H, 5.66; N, 5.43; S, 4.14. Found: C, 62.03; H, 6.01; N, 5.42; S, 3.99.

4.1.19. N^6-Benzoyl-5′-*O*-*tert*-butyldiphenylsilyl-4′-*C*-(*p*-toluenesulfonyl)oxymethyladenosine (16c). To a stirred solution of **15c** (54 mg, 0.062 mmol) in MeOH (3 mL) was added K_2CO_3 (3 mg, 0.019 mmol). After having been stirred for 15 min, the reaction mixture was neutralized with concentrated HCl and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel ($CHCl_3$/MeOH, 15:1) to give the desired product **16c** (44 mg, 0.056 mmol, 90%) as a white solid, mp 94–95°C. $[\alpha]_D^{22}$=+10.0 (*c* 1.2, $CHCl_3$). IR (KBr): 3332, 2932, 1701, 1612, 1180, 1105 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.95 (9H, s), 2.37 (3H, s), 3.72, 3.81 (2H, ABq, *J*=11 Hz), 4.45 (2H, s), 4.52 (1H, d, *J*=5 Hz), 4.86 (1H, dd, *J*=5, 7 Hz), 5.90 (1H, d, *J*=7 Hz), 7.21–7.60 (17H, m), 7.75 (2H, d, *J*=8 Hz), 7.95 (2H, d, *J*=7 Hz), 7.96 (1H, s), 8.46 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 19.1, 21.4, 26.7, 64.2, 68.7, 72.5, 74.2, 87.5, 89.0, 127.8, 127.9, 128.0, 128.8, 129.7, 130.0, 132.2, 132.4, 132.6, 133.0, 133.3, 135.4, 135.5, 141.9, 144.8, 149.2, 150.9, 152.0. MS (FAB) *m/z*: 794 (MH^+). Anal. calcd for $C_{41}H_{43}N_5O_8SSi\cdot 1/4H_2O$: C, 61.67; H, 5.49; N, 8.77; S, 4.01. Found: C, 61.70; H, 5.59; N, 8.57; S, 3.88.

4.1.20. 5′-*O*-*tert*-Butyldiphenylsilyl-N^2-isobutyryl-4′-*C*-(*p*-toluenesulfonyl)oxymethylguanosine (16d) and its N^7-isomer. To a stirred solution of **15d** (111 mg, 0.13 mmol) in MeOH (4 mL) was added K_2CO_3 (11 mg, 0.077 mmol). After having been stirred for 15 min, the reaction mixture was neutralized with concentrated HCl and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel ($CHCl_3$/MeOH, 25:1 to 20:1) to afford **16d** (63 mg, 0.081 mmol, 63%) and its N^7-isomer (18 mg, 0.023 mmol, 18%) **16d**: a white solid, mp 97–98°C. $[\alpha]_D^{22}$=+8.5 (*c* 1.0, $CHCl_3$). IR (KBr): 3164, 2933, 1678, 1606, 1564, 1363, 1182 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.92 (9H, s), 1.20 (6H, d, *J*=7 Hz), 2.35 (3H, s), 2.96 (1H, hep, *J*=7 Hz), 3.64, 3.80 (2H, ABq, *J*=11 Hz), 4.50 (2H, s), 4.64 (2H, m), 5.87 (1H, d, *J*=4 Hz), 7.20–7.75 (15H, m), 10.54 (1H, br), 12.36 (1H, br). ^{13}C NMR ($CDCl_3$) δ: 18.9, 19.1, 21.6, 26.7, 26.8, 29.7, 36.0, 64.4, 69.1, 72.3, 75.1, 87.5, 89.4, 96.1, 120.3, 127.7, 127.8, 127.9, 129.8, 129.9, 132.2, 132.7, 135.4, 135.5, 138.1, 144.8, 147.9, 148.3, 156.0, 180.5. MS (FAB) *m/z*: 776 (MH^+). Anal. calcd for $C_{38}H_{45}N_5O_9SSi\cdot H_2O$: C, 57.49; H, 5.97; N, 8.82; S, 4.13. Found: C, 57.59; H, 5.67; N, 8.41; S, 4.00. N^7-isomer: mp 87–88°C. $[\alpha]_D^{22}$=+26.0 (*c* 1.2, $CHCl_3$). IR (KBr): 3164, 2932, 1682, 1608, 1369, 1105 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.96 (9H, s), 1.28 (6H, d, *J*=6 Hz), 2.37 (3H, s), 2.84 (1H, hep, *J*=6 Hz), 3.68, 3.82 (2H, ABq, *J*=11 Hz), 4.37 (2H, m), 4.45 (2H, s), 6.03 (1H, d, *J*=5 Hz), 7.22–7.76 (14H, m), 7.98 (1H, s), 10.13 (1H, br), 12.54 (1H, br). ^{13}C NMR ($CDCl_3$) δ: 18.9, 21.4, 26.8, 29.7, 35.8, 36.2, 55.2, 64.2, 69.3, 72.5, 87.8, 91.7, 96.1, 111.0, 113.9, 127.9, 128.0, 129.7, 129.8, 129.9, 130.1, 132.0, 132.2, 132.5, 132.7, 135.5, 141.3, 144.8, 148.0, 180.3. MS (FAB) *m/z*: 776 (MH^+). Anal. calcd for $C_{38}H_{45}N_5O_9SSi\cdot H_2O$: C, 57.49; H, 5.97; N, 8.82; S, 4.13. Found: C, 57.77; H, 5.87; N, 8.92; S, 4.22.

4.1.21. 5′-*O*-*tert*-Butyldiphenylsilyl-5-methyl-4′-*C*-(*p*-toluenesulfonyl)oxymethyluridine (16e). To a stirred solution of **15e** (35 mg, 0.046 mmol) in MeOH (2 mL) was added K_2CO_3 (4 mg, 0.028 mmol). After having been stirred for 15 min, the reaction mixture was neutralized with concentrated HCl and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel (hexane/AcOEt, 1:2 to 1:4) to afford **16e** (31 mg, 0.046 mmol, 100%) as a white solid, mp 91–92°C. $[\alpha]_D^{22}$=+12.7 (*c* 0.84, $CHCl_3$). IR (KBr): 3379, 3067, 2934, 1695, 1468, 1363, 1107 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.04 (9H, s), 1.56 (3H, s), 2.38 (3H, s), 3.72, 3.75 (2H, ABq, *J*=8 Hz), 4.26 (2H, s), 4.36 (2H, m), 5.84 (1H, d, *J*=6 Hz), 7.21–7.71 (15H, m), 9.74 (1H, br). ^{13}C NMR ($CDCl_3$) δ: 12.0, 19.3, 21.5, 26.9, 65.3, 69.0, 72.0, 74.9, 86.9, 88.6, 96.1, 111.3, 128.0, 129.8, 130.2, 131.8, 132.4, 135.3, 135.5, 144.9, 151.5, 163.7. MS (FAB) *m/z*: 681 (MH^+). Anal. calcd for $C_{34}H_{40}N_2O_9SSi\cdot H_2O$: C, 58.43; H, 6.06; N, 4.01; S, 4.59. Found: C, 58.67; H, 6.26; N, 3.72; S, 4.59.

4.1.22. N^4-Benzoyl-5′-*O*-*tert*-butyldiphenylsilyl-3′-*O*,4′-*C*-methylenecytidine (17b′). Under N_2 atmosphere, sodium bis(trimethylsilylamide) (0.056 mL, 0.056 mmol, 1.0 M solution in THF) was added to a stirred solution of **16b′** (43 mg, 0.056 mmol) in THF (2 mL) at room temperature. After having been stirred for 1 h at the same temperature, the reaction mixture was partitioned with saturated $NaHCO_3$, and the mixture was extracted with $CHCl_3$. Usual work-up and purification by SiO_2 column chromatography ($CHCl_3$/MeOH, 15:1) afforded **17b′** (34 mg, 0.056 mmol, 100%) as a white solid, mp 78–79°C. $[\alpha]_D^{21}=+21.0$ (*c* 0.17, $CHCl_3$). IR (KBr): 3447, 1654 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.07 (9H, s), 3.96 (2H, s), 4.11 (1H, dd, *J*=6, 5 Hz), 4.52, 4.76 (2H, ABq, *J*=8 Hz), 5.14 (1H, d, *J*=5 Hz), 6.44 (1H, d, *J*=6 Hz), 7.38–7.96 (17H, m). ^{13}C NMR ($CDCl_3$) δ: 19.2, 26.8, 63.3, 76.5, 77.9, 78.0, 85.7, 87.5, 91.9, 96.1, 97.5, 125.5, 127.3, 127.9, 128.0, 128.5, 128.9, 130.2, 132.2, 132.5, 132.7, 133.3, 135.4, 135.5, 144.5, 162.6. MS (FAB) *m/z*: 598 (MH^+). Anal. calcd for $C_{33}H_{35}N_3O_6Si\cdot 1/4H_2O$: C, 65.81; H, 5.94; N, 6.98. Found: C, 65.83; H, 5.57; N, 7.06.

4.1.23. N^4-Benzoyl-5′-*O*-*tert*-butyldiphenylsilyl-3′-*O*,4′-*C*-methyleneadenosine (17c). Under N_2 atmosphere, sodium bis(trimethylsilylamide) (0.34 mL, 0.34 mmol, 1.0 M solution in THF) was added to a stirred solution of **16c** (111 mg, 0.14 mmol) in THF (3 mL) at room temperature. After having been stirred for 3 h at the same temperature, the reaction mixture was partitioned with saturated $NaHCO_3$, and the mixture was extracted with $CHCl_3$. Usual work-up and purification by SiO_2 column chromatography (AcOEt/EtOH, 20:1 to 10:1) afforded **17c** (76 mg, 0.13 mmol, 90%) as a white solid, mp 76–77°C. $[\alpha]_D^{22}=+5.6$ (*c* 1.0, $CHCl_3$). IR (KBr): 3283, 2932, 1702, 1613, 1458, 1250, 1109 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.07 (9H, s), 3.91 (2H, s), 4.64, 4.96 (2H, ABq, *J*=8 Hz), 4.70 (1H, dd, *J*=7, 5 Hz), 5.30 (1H, d, *J*=5 Hz), 6.46 (1H, d, *J*=7 Hz), 7.33–7.65 (13H, m), 8.03 (2H, d, *J*=7 Hz), 8.14 (1H, s), 8.70 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 19.2, 26.7, 63.0, 75.8, 78.1, 85.3, 86.5, 88.3, 122.5, 127.3, 127.8, 127.9, 128.5, 128.7, 130.0, 131.9, 132.4, 132.5, 132.8, 133.1, 135.4, 135.5, 141.4, 149.2, 151.7, 152.7, 169.6. MS (FAB) *m/z*: 622 (MH^+). Anal. calcd for $C_{34}H_{35}N_5O_5Si\cdot 3/4AcOEt$: C, 64.61; H, 6.01; N, 10.18. Found: C, 64.24; H, 5.70; N, 10.10.

4.1.24. 5′-*O*-*tert*-Butyldiphenylsilyl-N^2-isobutyryl-3′-*O*,4′-*C*-methyleneguanosine (17d). Under N_2 atmosphere, sodium bis(trimethylsilylamide) (0.041 mL, 0.041 mmol, 1.0 M solution in THF) was added to a stirred solution of **16d** (13 mg, 0.017 mmol) in THF (2 mL) at room temperature. After having been stirred for 1 h at the same temperature, the reaction mixture was partitioned with saturated $NaHCO_3$, and the mixture was extracted with $CHCl_3$. Usual work-up and purification by SiO_2 column chromatography ($CHCl_3$/MeOH, 15:1) afforded **17d** (8 mg, 0.014 mmol, 82%) as a white solid, mp 84–85°C. $[\alpha]_D^{25}=-14.6$ (*c* 0.97, $CHCl_3$). IR (KBr): 3395, 3159, 2933, 1682, 1609, 1564 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.04 (9H, s), 1.22 (6H, d, *J*=7 Hz), 2.76 (1H, hep, *J*=7 Hz), 3.86 (2H, s), 4.56, 4.83 (2H, ABq, *J*=8 Hz), 4.63 (1H, dd, *J*=7, 4 Hz), 5.23 (1H, d, *J*=4 Hz), 6.38 (1H, d, *J*=7 Hz), 7.32–7.41 (6H, m), 7.60–7.63 (4H, m), 7.90 (1H, s), 10.02 (1H, br), 12.21 (1H, br). ^{13}C NMR ($CDCl_3$) δ: 19.0, 19.3, 26.8, 29.7, 36.1, 63.4, 75.5, 78.1, 85.7, 86.0, 88.1, 96.1, 120.1, 127.9, 130.0, 132.5, 132.7, 135.4, 135.5, 138.0, 148.2, 155.4, 179.7. MS (EI) *m/z*: 546 (M^+-*tert*-Bu, 29.6), 199 (100). Anal. Calcd. for $C_{31}H_{37}N_5O_6Si$: C, 61.67; H, 6.18; N, 11.60. Found: C, 61.42; H, 6.22; N, 11.46.

4.1.25. 5′-*O*-*tert*-Butyldiphenylsilyl-5-methyl-3′-*O*,4′-*C*-methyleneuridine (17e). Under N_2 atmosphere, sodium bis(trimethylsilylamide) (0.18 mL, 0.18 mmol, 1.0 M solution in THF) was added to a stirred solution of **16e** (52 mg, 0.077 mmol) in THF (3 mL) at room temperature. After having been stirred for 1 h at the same temperature, the reaction mixture was partitioned with saturated $NaHCO_3$, and the mixture was extracted with $CHCl_3$. Usual work-up and purification by SiO_2 column chromatography ($CHCl_3$/MeOH, 15:1) afforded **17e** (38 mg, 0.074 mmol, 96%) as a white solid, mp 53–54°C. $[\alpha]_D^{25}=+8.1$ (*c* 0.98, $CHCl_3$). IR (KBr): 3195, 3066, 2932, 1695, 1466, 1108 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.09 (9H, s), 1.65 (3H, s), 3.91, 3.94 (2H, ABq, *J*=12 Hz), 4.08 (1H, m), 4.56, 4.76 (2H, ABq, *J*=9 Hz), 5.14 (1H, d, *J*=4 Hz), 6.45 (1H, d, *J*=7 Hz), 7.16 (1H, s), 7.37–7.46 (4H, m), 7.63–7.70 (6H, m), 9.48 (1H, br). ^{13}C NMR ($CDCl_3$) δ: 12.0, 19.3, 21.4, 26.7, 26.9, 29.7, 63.5, 75.4, 78.3, 85.1, 85.5, 88.1, 96.1, 112.1, 127.8, 128.0, 130.1, 130.2, 132.2, 132.7, 135.2, 135.5, 151.1, 163.6. MS (EI) *m/z*: 451 (M^+-*tert*-Bu, 100). Anal. calcd for $C_{27}H_{32}N_2O_6Si\cdot H_2O$: C, 61.58; H, 6.51; N, 5.32. Found: C, 61.81; H, 6.52; N, 5.42.

4.1.26. N^4-Benzoyl-3′-*O*,4′-*C*-methylenecytidine (1b′). To a stirred solution of **17b′** (24 mg, 0.040 mmol) in dry THF (2 mL) was added to tetrabutylammonium fluoride (0.048 mL, 0.048 mmol, 1.0 M solution in THF) at room temperature. After having been stirred for 15 min, the reaction mixture was concentrated under reduced pressure. The remaining residue was chromatographed on silica gel (AcOEt/EtOH, 20:1 to 10:1) to afford **1b′** (10 mg, 0.028 mmol, 70%) as a white solid, mp 150–151°C (AcOEt). $[\alpha]_D^{22}=-56.6$ (*c* 0.35, CH_3OH). IR (KBr): 3405, 1700 cm^{-1}. ^1H NMR (CD_3OD) δ: 3.79, 3.87 (2H, ABq, *J*=12 Hz), 4.24 (1H, dd, *J*=7, 5 Hz), 4.55, 4.85 (2H, ABq, *J*=8 Hz), 5.10 (1H, d, *J*=5 Hz), 6.56 (1H, d, *J*=7 Hz), 7.52–7.67 (4H, m), 7.98–8.02 (2H, m), 8.20 (1H, d, *J*=8 Hz). ^{13}C NMR (CD_3OD) δ: 62.5, 77.0, 79.1, 86.9, 88.0, 91.4, 99.4, 129.18, 129.24, 129.4, 129.8, 130.7, 133.8, 134.2, 145.6, 147.2, 165.1. MS (FAB) *m/z*: 360 (MH^+). Anal. calcd for $C_{17}H_{17}N_3O_6\cdot H_2O$: C, 54.11; H, 5.08; N, 11.14. Found: C, 54.22; H, 5.12; N, 11.31.

4.1.27. N^6-Benzoyl-3′-*O*,4′-*C*-methyleneadenosine (1c). To a stirred solution of **17c** (17 mg, 0.027 mmol) in THF (2 mL) was added tetrabutylammonium fluoride (0.032 mL, 0.032 mmol, 1.0 M solution in THF) at room temperature. After having been stirred for 15 min, the reaction mixture was concentrated under reduced pressure. The remaining residue was chromatographed on silica gel (AcOEt/EtOH, 20:1 to 10:1) to afford **1c** (7 mg, 0.018 mmol, 65%) as a white solid, mp 228–229°C (*i*PrOH). IR (KBr): 3329, 2923, 2856, 1705, 1613, 1459, 1250, 1107 cm^{-1}. ^1H NMR (CD_3OD) δ: 3.82, 3.86 (2H, ABq, *J*=13 Hz), 4.64, 4.94

(2H, ABq, J=8 Hz), 4.80 (1H, dd, J=8, 4 Hz), 5.22 (1H, d, J=4 Hz), 6.57 (1H, d, J=8 Hz), 7.56–7.59 (2H, m), 7.64–7.68 (1H, m), 8.08–8.10 (2H, m), 8.67 (1H, s), 8.73 (1H, s). ^{13}C NMR (DMSO-d_6) δ: 60.8, 73.6, 77.4, 84.9, 86.3, 86.9, 125.8, 128.7, 128.9, 132.9, 133.5, 143.7, 150.7, 152.2, 152.8. MS (FAB) m/z: 384 (MH^+). Anal. calcd for $C_{18}H_{18}N_5O_5 \cdot 1/3H_2O$: C, 55.53; H, 4.57; N, 17.99. Found: C, 55.77; H, 4.56; N, 17.74.

4.1.28. N^2-Isobutyryl-3′-*O*,4′-*C*-methyleneguanosine (1d). To a stirred solution of **17d** (32 mg, 0.042 mmol) in THF (3 mL) was added tetrabutylammonium fluoride (0.10 mL, 0.10 mmol, 1.0 M solution in THF) at room temperature. After having been stirred for 15 min, the reaction mixture was concentrated under reduced pressure. The remaining residue was chromatographed on silica gel (AcOEt/EtOH, 20:1 to 10:1) to afford **1d** (20 mg, 0.033 mmol, 78%) as a white solid, mp 234–235°C (*i*PrOH). $[\alpha]_D^{23}=-47.5$ (c 0.13, CH_3OH). IR (KBr): 3316, 1688, 1607 cm^{-1}. ^{1}H NMR (CD_3OD) δ: 1.23 (6H, d, J=7 Hz), 2.67–2.77 (1H, hep, J=7 Hz), 3.76, 3.83 (2H, ABq, J=12 Hz), 4.55, 4.65 (2H, ABq, J=8 Hz), 4.92 (1H, m), 5.15 (1H, d, J=5 Hz), 6.35 (1H, d, J=8 Hz), 8.23 (1H, s). ^{13}C NMR (CD_3OD) δ: 19.3, 37.0, 62.5, 75.9, 79.1, 86.8, 87.5, 88.4, 97.2, 121.4, 140.0, 150.0, 151.2, 181.7. MS (FAB) m/z: 366 (MH^+). Anal. calcd for $C_{15}H_{19}N_5O_6 \cdot 1/2H_2O$: C, 48.13; H, 5.38; N, 18.71. Found: C, 48.32; H, 5.15; N, 18.24.

4.1.29. 5-Methyl-3′-*O*,4′-*C*-methyleneuridine (1e). To a stirred solution of **17e** (32 mg, 0.062 mmol) in THF (2 mL) was added tetrabutylammonium fluoride (0.075 mL, 0.075 mmol, 1.0 M solution in THF) at room temperature. After having been stirred for 15 min, the reaction mixture was concentrated under reduced pressure. The remaining residue was chromatographed on silica gel (AcOEt/EtOH, 20:1 to 10:1) to afford **1e** (12 mg, 0.044 mmol, 71%) as a white solid, mp 119–120°C (AcOEt). $[\alpha]_D^{25}=-63.1$ (c 0.44, MeOH); IR (KBr): 4016, 3451, 1723 cm^{-1}. ^{1}H NMR (CD_3OD) δ: 1.89 (3H, s), 3.75, 3.83 (2H, ABq, J=12 Hz), 4.14 (1H, dd, J=8, 5 Hz), 4.51, 4.83 (2H, ABq, J=8 Hz), 5.05 (1H, d, J=5 Hz), 6.42 (1H, d, J=8 Hz), 7.52 (1H, s). ^{13}C NMR (CD_3OD) δ: 12.4, 62.5, 75.3, 79.1, 86.6, 86.9, 88.8, 112.4, 138.0, 152.8, 166.1. MS (FAB) m/z: 277 ($M+Li^+$). Anal. calcd for $C_{11}H_{14}N_2O_6 \cdot H_2O$: C, 45.83; H, 5.59; N, 9.72. Found: C, 45.81; H, 5.51; N, 9.71.

4.2. Computation

Semi-empirical MO calculations of **1e** and 2′-hydroxythymidine were carried out on an SGI O_2 workstation using Spartan version 5.1.[20] All initial structures used for the MO calculations were generated by reference to the X-ray structures of **1e**. Geometry optimization utilizing the PM3 Hamiltonian was carried out with constraints on torsion angles, ν_0–ν_4, χ and γ. The torsion angles χ and γ were fixed at −108.0° and 74.1° for **1e** and −163.9° and 51.8° for 2′-hydroxythymidine, respectively. The values of endocyclic sugar torsion angles ν_0–ν_4 were determined to vary the pseudorotation phase angle P every 10°, and the maximum torsion angles ν_{max} were fixed at 32.5° for **1e** and 38.6° for 2′-hydroxythymidine.

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