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Synthesis and Properties of 2′-*O*,4′-*C*-Ethylene-Bridged Nucleic Acids (ENA) as Effective Antisense Oligonucleotides

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Abstract—Novel bicyclo nucleosides, 2′-*O*,4′-*C*-ethylene nucleosides and 2′-*O*,4′-*C*-propylene nucleosides, were synthesized as building blocks for antisense oligonucleotides to further optimize the 2′-*O*,4′-*C*-methylene-linkage of bridged nucleic acids (2′,4′-BNA) or locked nucleic acids (LNA). Both the 2′-*O*,4′-*C*-ethylene- and propylene-linkage within these nucleosides restrict the sugar puckering to the *N*-conformation of RNA as do 2′,4′-BNA/LNA. Furthermore, ethylene-bridged nucleic acids (ENA) having 2′-*O*,4′-*C*-ethylene nucleosides had considerably increased the affinity to complementary RNA, and were as high as that of 2′,4′-BNA/LNA ($\Delta T_m = +3 \sim 5\,^{\circ}C$ per modification). On the other hand, addition of 2′-*O*,4′-*C*-propylene modifications in oligonucleotides led to a decrease in the affinity to complementary RNA. As for the stability against nucleases, incorporation of one 2′-*O*,4′-*C*-ethylene or one 2′-*O*,4′-*C*-propylene nucleoside into oligonucleotides considerably increased their resistance against exonucleases to an extent greater than 2′,4′-BNA/LNA. These results indicate that ENA is more suitable as an antisense oligonucleotide and is expected to have better antisense activity than 2′,4′-BNA/LNA.

Introduction

Antisense oligonucleotides are now in increasing demand for their use as a gene-target validation tool in genomic-based drug discovery[1,2] and their potential to be developed as a new class of drugs for the treatment of inveterate diseases such as cancer, inflammation, and viral diseases.[3] Phosphorothioate oligodeoxynucleotides (PS ODN), currently the standard choice for most antisense designs, have favorable properties such as high nuclease resistance, and are able to be recognized by RNase H, which leads to the degradative inactivation of the target mRNA. However, they also have limitations in their use, such as low affinity to RNA derived from mixtures of 2^n (n = chain length -1) different diastereoisomers and nonsequence-specific protein binding, which can be the cause of significant side effects, such as inhibition of the blood clotting cascade, activation of the complement cascade and severe hypotension in vivo.[3,4] PS modification involves a dilemma where using PS ODN of longer size to offset their low affinity to RNA inevitably leads to an increase in unspecific binding and toxic effects. To overcome their limitations, considerable effort has been focused on the development of other types of modified oligonucleotides or oligonucleotide analogues as second-generation oligonucleotides that combine high nuclease resistance with superior RNA binding affinity and decrease the likelihood of eliciting unspecific biological responses that are not related to target mRNA binding.[5] Among numerous modified nucleic acids, several types of nucleic acid analogues are now under clinical development as antisense drugs. 2′-*O*-Alkyl modifications are known to be of value in enhancing the binding affinity to target RNA and nuclease resistance.[5–8] With 2′-*O*-modifications, nuclease resistance increases due to the bulkiness of 2′-*O*-chain.[6] In particular, a 2′-*O*-(2-methoxy)ethyl modification showed high RNA affinity (average $+2\,^{\circ}C$ per modification) with extraordinary nuclease stability.[7,8] Other backbone-modified nucleic

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acids such as PNA,[9] morpholino[10] and phosphoramidate (3′-NP DNA)[11,12] are capable of hybridizing to mRNA with increased thermal stabilities (PNA: +1∼2 °C per modification, morpholino: not more than 1 °C per modification and 3′-NP DNA: 2.2–2.6 °C per modification) due to the elimination of anionic repulsion between the phosphodiesters, and they also exhibit complete nuclease resistance. They have a completely different backbone from endogenous DNA and work as non-RNase H-mediated antisense molecules.

Recently, our group and Wengel's group have independently reported the synthesis of novel 2′-*O*,4′-*C*-methylene nucleosides whose sugar puckering is fixed in the *N*-conformation as in RNA, and that oligonucleotides containing these bridged nucleosides (2′,4′-BNA/LNA) showed an unprecedented level of affinity (+4∼8 °C per modification) toward their complementary RNA, higher than any other modified oligonucleotide (**b** in Fig. 1).[13–15] We have also carried out several studies on 2′,4′-BNA/LNA derivatives such as 3′,4′-BNA and 3′-amino-2′,4′-BNA.[16,17] Wengel's group reported the synthesis of 2′,4′-BNA/LNA derivatives such as 2′-thio-LNA,[18] 2′-amino-LNA[18] and an α-L-ribo isomer of LNA (α-L-LNA),[19] which all have a five-membered ring with 2′-*O*,4′-*C*-methylene linkage. Some of these LNA derivatives hybridize favorably to their target mRNA but to a lesser extent than 2′,4′-BNA/LNA. Wang et al. have reported the synthesis of 2′,4′-*C*-bridged 2′-deoxynucleosides and their corresponding oligonucleotides (**c** in Fig. 1). These show a moderate affinity to RNA (+1.9∼3.3 °C per modification), compared with 2′,4′-BNA/LNA.[20,21] Other bridged nucleosides, in which the oxygen at the 2′-position is replaced with a methylene group, have been introduced as less effective analogues (**d** in Fig. 1).[5] Recently, we have reported the synthesis of novel 2′-*O*,4′-*C*-ethylene thymidine, which has a less strained six-membered ring than the five-membered ring of 2′-*O*,4′-*C*-methylene thymidine (**e** in Fig. 1).[22] The corresponding oligonucleotides with 2′-*O*,4′-*C*-ethylene thymidine retain a binding affinity as high as 2′,4′-BNA/LNA and exhibit much more nuclease-resistance than 2′,4′-BNA/LNA.[22]

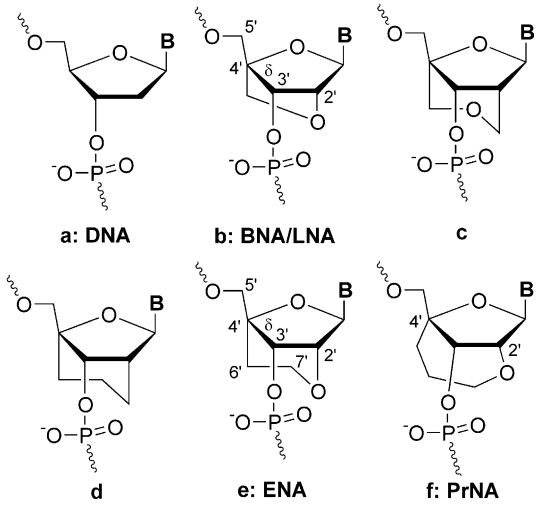


Figure 1. Structures of DNA and 2′,4′-bridged nucleic acid derivatives. 2′,4′-BNA/LNA: 2′-*O*,4′-*C*-methylene nucleic acids. ENA: 2′-*O*,4′-*C*-ethylene nucleic acids. PrNA:2′-*O*,4′-*C*-propylene nucleic acids.

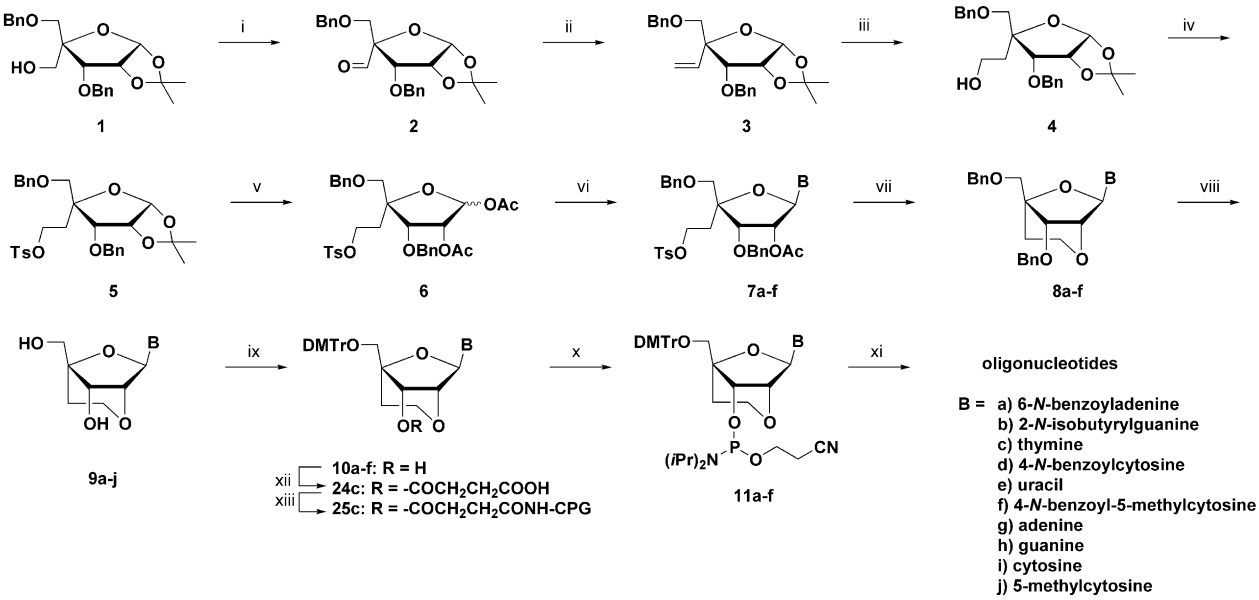
Here, we report the synthesis of 2′-*O*,4′-*C*-ethylene nucleosides and 2′-*O*,4′-*C*-propylene thymidine (**f** in Fig. 1) which is one carbon longer than ethylene-bridged nucleosides, and evaluated the basic properties of their oligonucleotides by circular dichroism (CD), UV melting analysis and nuclease resistance assay, to determine whether oligonucleotides with ENA residues were more suitable than those with propylene nucleic acid (PrNA) residues for use as antisense oligonucleotides.

Results and Discussion

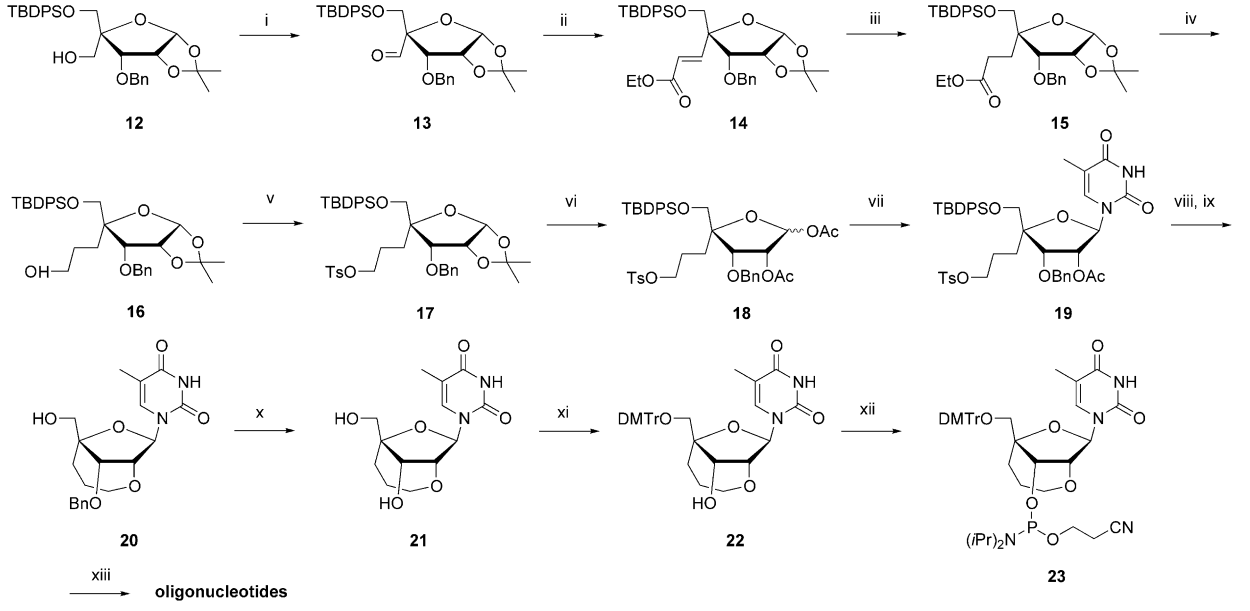
Synthesis of 2′-*O*,4′-*C*-ethylene nucleosides and 2′-*O*,4′-*C*-propylene thymidine

Novel nucleosides containing all possible natural nucleobases (adenine, guanine, thymine, cytosine, uracil and 5-methylcytosine) were synthesized by a procedure shown in Scheme 1. The hydroxymethyl group at the 4-position of the known pentofuranose derivative **1**[23] was converted to a hydroxyethyl group via Swern oxidation, Wittig reaction and hydroboration, followed by oxidation to give compound **4**. Tosylation and acetolysis of **4** afforded compound **6**, which is a useful common intermediate for coupling reactions with a variety of silylated nucleobases. Stereoselective coupling with 6-*N*-benzoyladenine, 2-*N*-isobutyrylguanine, thymine, 4-*N*-benzoylcytosine, uracil or 4-*N*-benzoyl-5-methylcytosine by Vorbrüggen's method[24] and the following base-induced ring closure by the treatment with 1 M NaOH/pyridine–H_2O afforded 2′-*O*,4′-*C*-ethylene-bridged nucleosides **8a–f**. Unfortunately, the yield of **7b** was quite low most likely due to a side reaction of debenzylation at the 3′- or 5′-position and the following ring-closure reaction of the 4′-*p*-toluenesulfonyloxyethyl group with the debenzylated 3′- or 5′-hydroxyl group. Furthermore, an undesired *N*-7 regioisomer that should have been separated by silica gel chromatography after the ring-closure reaction, was obtained. The stereochemistry of each isomer was determined based on the UV spectra of unprotected 2′-*O*,4′-*C*-ethylene-bridged guanosine **9h** which were analyzed under acidic, neutral and basic conditions. The deprotected bicyclonucleosides **9a–f** were obtained by debenzylation of **8b**, **8c** and **8e** using 10% $Pd(OH)_2$/C and of **8a**, **8d** and **8f** using BCl_3. By subsequent 5′-*O*-DMTr-protection and phosphitylation, the 2′-*O*,4′-*C*-ethylene-bridged nucleoside phosphoramidites **11a–f** were synthesized.

The synthesis of 2′-*O*,4′-*C*-propylene thymidine and its corresponding phosphoramidite was performed as shown in Scheme 2. Swern oxidation and chain extension by Horner–Wadsworth–Emmons olefination of the known 4′-*C*-hydroxymethyl pentofuranose derivative **12**[25] furnished the (*E*)-enoate **14**. $Pd(OH)_2$-catalyzed reduction for 6 h at 3.5 psi afforded olefin-selective hydrogenation of **14**, and the following reduction using L-selectride led to 4-hydroxypropyl furanose derivative **16**. Tosylation and acetolysis of **16** afforded **18**, the intermediate for the



Scheme 1. Synthesis of 2′-*O*,4′-*C*-ethylene nucleosides-3′-*O*-phosphoramidite and oligonucleotides. Reagents and conditions: (i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, 91%; (ii) $Ph_3P^+CH_3Br^-$, NaH, DMSO, 80%; (iii) 9-BBN, THF then H_2O_2, NaOH, 93%; (iv) TsCl, Et_3N, CH_2Cl_2 94%; (v) Ac_2O, H_2SO_4, AcOH, 89% (α/β = 1:5); (vi) silylated nucleobase, TMSOTf, ClC_2H_4Cl, reflux; (vii) 1 M NaOH, pyridine–H_2O; (viii) H_2, $Pd(OH)_2$, MeOH or BCl_3, CH_2Cl_2, −78 °C; (ix) DMTrCl, pyridine, CH_2Cl_2; (x) $((iPr)_2N)_2P(OC_2H_4CN)$, *N,N*-diisopropylammonium tetrazolide or $(iPr)_2NP(Cl)(OC_2H_4CN)$, diisopropylethylamine; (xi) DNA/RNA synthesizer; (xii) succinic anhydride, DMAP, CH_2Cl_2; (xiii) 2,3,4,5,6-pentachlorophenol, DCC, DMF; long-chain alkylamino-CPG, triethylamine, DMF.



Scheme 2. Synthesis of 2′-*O*,4′-*C*-propylene thymidine-3′-*O*-phosphoramidite and oligonucleotides. Reagents and conditions: (i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, 99% (ii) $(EtO)_2P(O)CH_2COOEt$, NaH, THF, 86%; (iii) $Pd(OH)_2$, H_2 (3.5 psi), MeOH, 71%; (iv) L-selectride, THF, 85%; (v) TsCl, Et_3N, CH_2Cl_2 77%; (vi) Ac_2O, H_2SO_4, AcOH, 79% (mainly β-anomer); (vii) silylated thymine, TMSOTf, ClC_2H_4Cl, reflux, 88%; (viii) 1 M NaOH, pyridine–H_2O; (ix) TBAF, THF, 82% (two steps); (x) H_2, $Pd(OH)_2$, MeOH, 59%; (xi) DMTrCl, pyridine, CH_2Cl_2, quant; (xii) $((iPr)_2N)_2P(OC_2H_4CN)$, *N,N*-diisopropylammonium tetrazolide, quant; (xiii) DNA/RNA synthesizer.

coupling reaction with silylated nucleobases. The reaction of **18** with *O*,*O*′-bis(trimethylsilyl)thymine under Vorbrüggen's conditions[24] afforded the β-anomer of thymidine derivative **19**, which was converted to the desired 2′-*O*,4′-*C*-propylene nucleoside **20** by treatment with 1 M NaOH/pyridine–H_2O. The debenzylation of **20** and the following 5′-*O*-DMTr-protection and phosphitylation gave 2′-*O*,4′-*C*-propylene thymidine phosphoramidite **23**, a building block for oligonucleotide synthesis.

Conformational analysis of 2′-*O*,4′-*C*-ethylene nucleosides and 2′-*O*,4′-*C*-propylene nucleosides

The conformational analysis of the bridged nucleosides obtained was carried out by means of ^1H NMR. In all of the bicyclic nucleoside analogues (**8**–**11**, **20**–**23**), the coupling constant ($J_{H1'-H2'}$) was 0 Hz, which was identical to that of 2′-*O*,4′-*C*-methylene nucleosides.[13] These results indicate that the furanose pucker of 2′-*O*,4′-*C*-ethylene

nucleosides and of 2′-*O*,4′-*C*-propylene thymidine were all fixed in the *N*-conformation, and C3′-*endo* conformation. Similar results were obtained by X-ray crystal structure analysis of the 2′-*O*,4′-*C*-ethylene-2-*N*-isobutyrylguanosine (**9b**), 2′-*O*,4′-*C*-ethylene adenosine (**9g**), 2′-*O*,4′-*C*-methylene adenosine and 2′-*O*,4′-*C*-methylene thymidine (the cell unit of 2′-*O*,4′-*C*-methylene thymidine had two conformers), which had the pseudorotation phase angle *P* of 12.1, 15.1, 20.2, 16.8 and 14.7°, respectively (Fig. 2). These *P* values corresponded to typical C3′-*endo* conformations.[26] On the basis of these data and the previously reported data of 2′-*O*,4′-*C*-methylene uridine (*P* angle = 17.4°),[13] the sugar puckering of 2′-*O*,4′-*C*-methylene nucleosides was found to adopt an envelope or near-envelope form, ^{3}E, and that of 2′-*O*,4′-*C*-ethylene nucleosides, an unsymmetrical form, $^{3}T_{2}$.[26] These differences appear clearly in the torsion angle δ of C5′–C4′–C3′–O3′ (see Fig. 1e) which is one of the defining angles of ribose conformation.[26] The δ angles of nucleosides described above were 78.9, 75.7, 62.5, 68.5 and 66.9°, respectively. Furthermore the mean δ angles of 2′-*O*,4′-*C*-ethylene nucleosides and 2′-*O*,4′-*C*-methylene nucleosides were 77 and 66°, respectively, and the mean δ angle of 2′-*O*,4′-*C*-ethylene nucleosides was approximately 11° larger than that of 2′-*O*,4′-*C*-methylene nucleosides. From the view of oligonucleotide structure, this difference in each nucleoside unit might result in a large difference overall which might, in turn, influence the duplex formation of oligonucleotides with complementary DNA and RNA (see section of CD spectra). Compared with 2′-*C*,4′-*C*-bridged nucleosides with the six-membered ring (**c** in Fig. 1), whose wide range in *p* values indicates flexibility (11.1–19.3°),[21] the range in *p* values of 2′-*O*,4′-*C*-ethylene nucleosides is relatively narrow. The torsion angles of C3′–C2′–O2′–C7′ of 2′-*O*,4′-*C*-ethylene nucleosides, **9b** and **9g**, were 62.3 and 61.6°, respectively, and those of C3′–C4′–C6′–C7′ of the same nucleosides were 60.7 and 59.9°, respectively. These results show that the six-membered rings of 2′-*O*,4′-*C*-ethylene nucleosides have a typical chair conformation. The torsion angles χ of the glycoside bonds were almost identical (from 157 to 169°) in all nucleosides, which suggests that these nucleosides adopt an *anti* orientation.

Synthesis of oligonucleotides containing 2′-*O*,4′-*C*-ethylene nucleosides or 2′-*O*,4′-*C*-propylene nucleosides

Oligonucleotides including the bridged nucleosides, 2′-*O*,4′-*C*-ethylene nucleosides, were successfully synthesized using the standard phosphoramidite approach on a DNA/RNA synthesizer. Coupling yields of 2′-*O*,4′-*C*-ethylene nucleoside units were more than 95%. However, the 2′-*O*,4′-*C*-propylene thymidine-3′-*O*-phosphoramidite unit did not give good yield (approximately 50–60%), even with the use of 5-ethylthio-1*H*-tetrazole,[27] which is well-known as an effective activator. This is probably due to steric hindrance of the propylene bridge of the 2′-*O*,4′-*C*-propylene thymidine unit. Two 2′-*O*,4′-*C*-propylene thymidine units were able to be incorporated into the oligonucleotide chain in moderate overall yield. On the other hand, to prepare oligonucleotides with 2′-*O*,4′-*C*-ethylene thymidine at their 3′ end, 2′-*O*,4′-*C*-ethylene thymidine-bound CPG **25c** was synthesized from **10c** by standard methods (Scheme 1). The oligonucleotides modified with ENA or PrNA residues were purified by reverse-phase HPLC. The structures of oligonucleotides were confirmed by negative ion ESI mass spectroscopy.

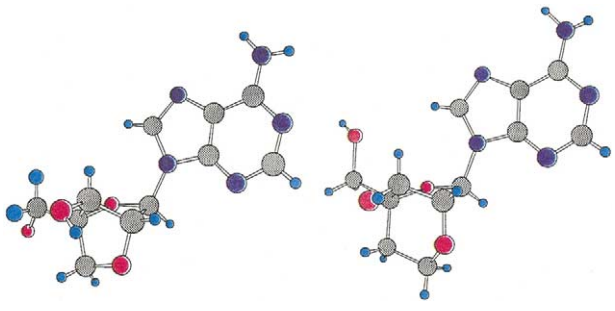


Figure 2. X-ray crystal structures of 2′-*O*,4′-*C*-methylene adenosine (left) and 2′-*O*,4′-*C*-ethylene adenosine (**9g**, right).

Circular dichroism (CD) analysis of the duplex of 2′-*O*,4′-*C*-bridged nucleic acids with complementary DNA or RNA

To investigate the properties of oligonucleotides containing novel 2′-*O*,4′-*C*-bridged nucleosides, the CD spectra of duplexes of oligonucleotides containing ENA or PrNA residues and their complementary DNA and RNA were evaluated. Cognate duplexes containing 2′-*O*,4′-*C*-methylene nucleosides show A-like conformation.[28] In Figure 3A, the spectrum of a duplex of an oligonucleotide containing six ENA residues and complementary DNA showed a positive cotton effect at 276 nm, and an A-like conformation similar to that in the spectrum of the duplex containing 2′-*O*,4′-*C*-methylene nucleosides. However, the intensity of the positive band of the duplex of the oligonucleotide containing ENA residues and complementary DNA was ca. 1.7-fold greater than that of the duplex with 2′-*O*,4′-*C*-methylene nucleosides and was similar to that of a duplex with six uridine residues (Fig. 3A). The results indicate that a duplex with some ENA residues form an A-like conformation. Their structures were closer to a natural DNA/RNA duplex than structures of duplexes with 2′,4′-BNA/LNA, maybe due to the difference of the δ angles between ENA and 2′,4′-BNA/LNA. In the case of complementary RNA, no remarkable difference between the CD spectra of a duplex with 2′-*O*,4′-*C*-methylene nucleosides and that with 2′-*O*,4′-*C*-ethylene nucleosides was observed (Fig. 3B). These data suggest that the oligonucleotide containing ENA residues can form a duplex with the complementary RNA strand and be cleaved by RNase H. This is also the case for the duplex of 2′,4′-BNA/LNA-DNA chimeric oligonucleotide with the complementary RNA.[29] Furthermore, the CD spectrum of a duplex of an oligonucleotide containing two PrNA residues with the complementary DNA was almost identical to that of an oligonucleotide containing ENA residues, even when bound to complementary RNA at room temperature (Fig. 3C and D). To investigate the difference in the properties of the two types of duplexes containing either ENA or PrNA

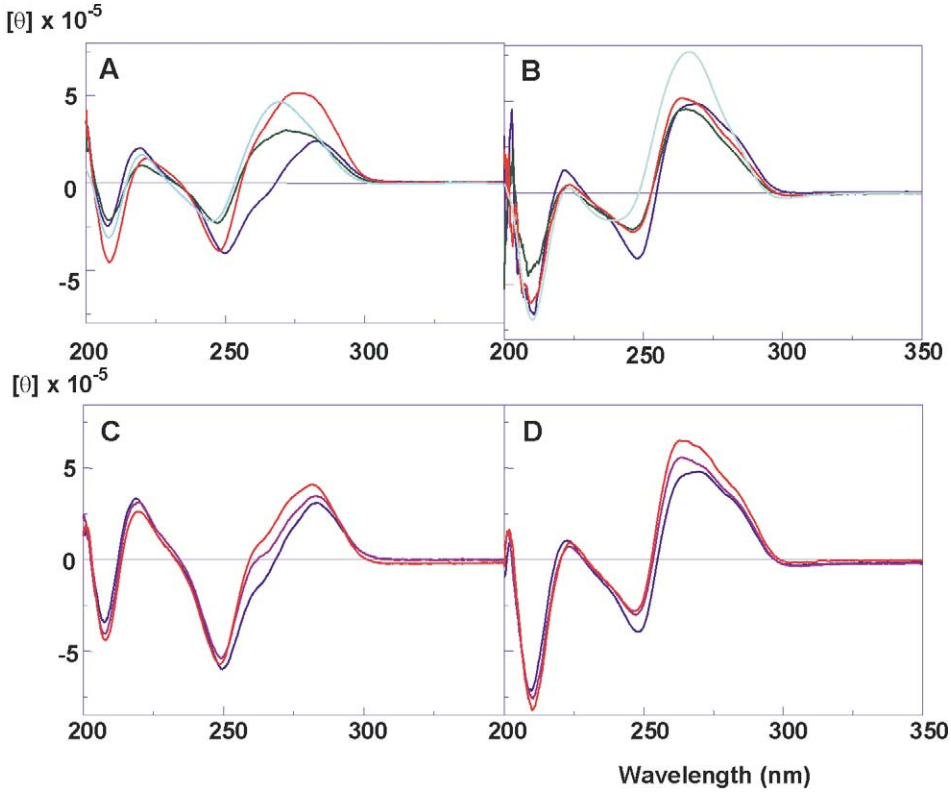


Figure 3. CD spectra of duplexes of 2′-*O*,4′-*C*-bridged nucleic acids and their complementary DNA or RNA: (A) duplex of modified oligonucleotides, 5′-d(GCGXXXXXXGCT)-3′ and DNA, 5′-d(AGCAAAAAACGC)-3′; (B) duplex of modified oligonucleotides, 5′-d(GCGXXXXXXGCT)-3′ and RNA 5′-r(AGCAAAAAACGC)-3′; (C) duplex of modified oligonucleotides, 5′-d(GCGTTXTTXGCT)-3′ and DNA, 5′-d(AGCAAAAAACGC)-3′. (D) duplex of modified oligonucleotides, 5′-d(GCGTTXTTXGCT)-3′ and RNA 5′-r(AGCAAAAAACGC)-3′. Blue: X = thymidine, green: X = 2′-*O*,4′-*C*-methylene thymidine, red: X = 2′-*O*,4′-*C*-ethylene thymidine, purple: 2′-*O*,4′-*C*-propylene thymidine, sky blue: duplex with 5′-r(GCGUUUUUUGCU)-3′, (U = uridine). Duplex concentration: 4 μM. Buffer: 100 mM NaCl, 10 mM sodium phosphate buffer (pH 7.2).

residues, the thermodynamic stability of these duplexes was evaluated by UV melting temperature analysis.

UV melting temperature analysis

It has been reported that the duplexes of the oligonucleotide containing six ENA residues and complementary RNA or DNA have a higher UV melting temperature (T_m) than a natural DNA/DNA and DNA/RNA duplex, which is also the case for duplexes containing 2′,4′-BNA/LNA residues.[22] We measured the T_m of the duplexes in a buffer of 100 mM NaCl and 10 mM sodium phosphate (pH 7.2). The T_m values of the duplex of the oligonucleotide containing two ENA residues with complementary RNA or DNA were increased by ca. 3.5 °C or 0.5 °C per modification, respectively (Table 1). These data confirmed our previous result that the incorporation of ENA residues into oligonucleotides increases the stability of the cognate duplexes.[22] On the other hand, a decrease in the T_m values of the duplexes of the oligonucleotide containing two PrNA residues (−0.5 or −2.0 °C per modification) was observed, and it was found that propylene-bridging of the nucleosides between the 2′- and the 4′-position was not suitable for the stabilization of the duplex and that bridging by a methylene or ethylene linkage between the 2′- and the 4′-position gave the best results in stabilizing the duplex formation.

Nuclease resistance of oligonucleotides modified with 2′-*O*,4′-*C*-bridged nucleosides

We have shown that oligonucleotides modified with an ENA residue have greater stability against exo- and endonucleases than those modified with a 2′,4′-BNA/LNA residue.[22] To compare the stability of oligonucleotides modified with 2′-*O*,4′-*C*-bridged nucleosides, such as 2′,4′-BNA/LNA, ENA and PrNA, or a PS linkage, a well known method to increase nuclease-resistance, a relatively high concentration of 3′-exonuclease, snake venom phosphodiesterase (SVPD, 7.14 μg/mL), was used, since it was previously found that

Table 1. T_m values (°C) of the modified oligonucleotides towards complementary RNA and DNA

Oligonucleotide 5′-d(GCGTTX-TTXGCT)-3′	Complementary RNA 5′-r(AGCAAAAAAC GC)-3′		Complementary DNA 5′-d(AGCAAAAAAC GC)-3′	
	T_m (°C)	ΔT_m (°C)/ modification	T_m (°C)	ΔT_m (°C)/ modification
DNA (X = T)	48		51	
ENA (X = eT)	55	3.5	52	0.5
PrNA (X = prT)	47	−0.5	47	−2.0

Duplex concentration: 4 μM. Buffer: 100 mM NaCl, 10 mM sodium phosphate buffer (pH 7.2). eT: 2′-*O*,4′-*C*-ethylene thymidine; prT: 2′-*O*,4′-*C*-propylene thymidine.

hydrolysis of the oligonucleotide with the ENA residue at 0.3 μg/mL of SVPD was very slow.[22] Under these conditions, natural DNA (oligo T_{12}) and an oligonucleotide containing a 2′,4′-BNA/LNA residue (5′-TTTTTTTTTTXT-3′: X = 2′-*O*,4′-*C*-methylene thymidine) were degraded rapidly (Fig. 4). On the other hand, the stability of an oligonucleotide modified with the ENA residue (5′-TTTTTTTTTTXT-3′: X = 2′-*O*,4′-*C*-ethylene thymidine) was identical to that of an oligonucleotide with a PS *R*p diastereomer. Later, we designed and synthesized an oligonucleotide containing two ENA residues (5′-TTTTTTTTTTXX-3′: X = 2′-*O*,4′-*C*-ethylene thymidine) to evaluate the total resistance of contiguous ENA residues, since we have previously reported that incorporating an ENA residue in oligonucleotides increased not only the nuclease resistance of the phosphodiester bond of the 3′-side of the ENA residue but also that of the 5′-side.[22] This oligonucleotide was more stable than an oligonucleotide with a PS *S*p diastereomer which is known to be a stable isomer.[30] These data indicate that oligonucleotides composed of contiguous ENA residues without PS modification could show more nuclease stability than PS ODN. Surprisingly, an oligonucleotide with a PrNA residue was the most stable in all tested compounds and ca. 70% of which remained even after incubation for 90 min. These data suggest that the incorporation of a longer alkylene linkage in the nucleoside between the 2′- and the 4′-position gives greater nuclease resistance, probably due to the blockage of the phosphodiester bond of the 3′-side of the nucleosides against nuclease attack, by the additional linkage on the sugar. In addition, dual-modification of incorporating an ENA residue and a PS bond into an oligonucleotide was successfully carried out. This oligonucleotide showed a high stability identical to that of the oligonucleotide with a PrNA residue (Fig. 4). Although it has been reported that 2′,4′-BNA/LNA oligonucleotides are more stable than natural DNA,[22,29,31] oligonucleotides with the ENA or the PrNA residues were much more resistant against exonucleases than 2′,4′-BNA/LNA residues. As well, we found that ENA oligonucleotides were stable in plasma, suggesting a potential for their use as antisense therapeutics in vivo (data not shown).

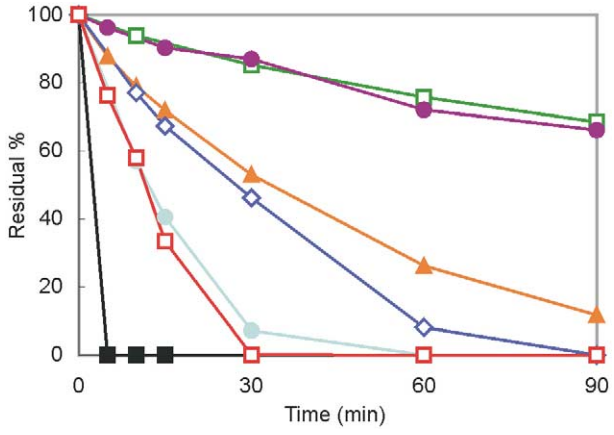


Figure 4. Stability of oligonucleotides with a phosphorothioate linkage, ENA or LNA, against snake venom phosphodiesterase. Oligo concentration: 26 μg/mL, enzyme concentration: 7.14 μg/mL; buffer: 50 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, at 37 °C. Open squares (green): 5′-TTT TTT TTT TXT-3′ (X = 2′-*O*,4′-*C*-propylene thymidine), closed circles (purple): 5′-TTT TTT TTT TXT-3′ (X = 2′-*O*,4′-*C*-ethylene thymidine-3′-phosphorothioate), closed triangles (orange): 5′-TTT TTT TTT TXX-3′ (X = 2′-*O*,4′-*C*-ethylene thymidine), open diamonds (blue): 5′-TTT TTT TTT TXT-3′ (X = thymidine-3′-phosphorothioate (*S*p)), closed circles (sky blue): 5′-TTT TTT TTT TXT-3′ [X = thymidine-3′-phosphorothioate (*R*p)], open squares (red): 5′-TTT TTT TTT TXT-3′ (X = 2′-*O*,4′-*C*-ethylene thymidine), closed squares (black): 5′-TTT TTT TTT TXT-3′ (X = 2′-*O*,4′-*C*-methylene thymidine).

Conclusion

We synthesized 2′-*O*,4′-*C*-bridged nucleosides, such as 2′-*O*,4′-*C*-methylene, -ethylene and -propylene derivatives, and their corresponding oligonucleotides, such as 2′,4′-BNA/LNA, ENA and PrNA. As well, we evaluated the basic properties and the possibility of these oligonucleotides to be used as antisense drugs. Regarding the coupling yields of the synthesized oligonucleotides and their binding affinity to complementary RNA, 2′,4′-BNA/LNA and ENA were superior to PrNA, owing to differences in their steric hindrance. Another important factor to consider in creating antisense oligonucleotides as therapeutic agents is nuclease resistance. Oligonucleotides lending more steric hindrance, such as ENA and PrNA, are more effective in increasing nuclease resistance than 2′,4′-BNA/LNA. Moreover, oligonucleotides with contiguous ENA modification or dual-modification by incorporation of an ENA residue and PS bond lead to the formation of much more stable duplexes than those with PS ODN. ENA modification permits designing antisense oligonucleotides with or without a PS bond. From these results, we consider that the ENA modification is most suitable for 2′-*O*,4′-*C*-alkylene-bridged nucleosides and that oligonucleotides with ENA residues have a potential to be used as antisense therapeutics. Application of ENA oligonucleotides as antisense oligonucleotides will be reported elsewhere in the near future.

Experimental

General methods

^1H and ^{13}C NMR were recorded on Varian Mercury 400 (400 MHz) and JEOL JNM-ECP500 (125 MHz), respectively. IR spectra were recorded on a JASCO FT/IR-610 spectrometer. All reactions were monitored by thin-layer chromatography performed on Merck silica gel plates (Merck Art 5715 silica gel 60 F_{254} plate) with UV detection. For column chromatography, Merck Art 9385 silica gel 60 (230–400 mesh) or Kanto Chemicals silica gel 60, spherical particles (40–100 μm) were used.

3,5-Di-*O*-benzyl-4-*C*-formyl-1,2-*O*-isopropylidene-α-D-erythropentofuranose (2). Oxalyl chloride (6.02 mL, 69.0 mmol) was added to CH_2Cl_2 (200 mL) cooled at −78 °C. A solution of DMSO (7.87 mL, 110 mmol) in anhydrous CH_2Cl_2 (100 mL) was added dropwise to this solution. After stirring for 20 min a solution of **1**[23] (9.21 g, 23.02 mmol) in anhydrous CH_2Cl_2 (100 mL) was added dropwise to this mixture and the mixture was stirred for 30 min. Triethylamine (28 mL, 200 mmol)

was added to this reaction mixture and the mixture was slowly warmed to room temperature. The reaction mixture was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with H_2O and brine, dried over $MgSO_4$ and then concentrated in vacuo. The residue was purified by chromatography on silica gel (hexane/EtOAc = 5:1) to give **2** as a colorless oil (8.31 g, 20.88 mmol, 91%). ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.09, 26.51, 69.18, 72.80, 73.78, 178.36, 79.69, 89.76, 104, 86, 114.14, 127.70, 127.80, 127.96, 128.11, 128.40, 128.48, 137.04, 137.57, 199.94. ^1H NMR (400 MHz, $CDCl_3$) δ 1.35 (3H, s), 1.60 (3H, s), 3.61 (1H, d, 11 Hz), 3.68 (1H, d, 11 Hz), 4.37 (1H, d, 4.4 Hz), 4.46 (1H, d, 12 Hz), 4.52 (1H, d, 12 Hz), 4.59 (1H, d, 12 Hz), 4.59 (1H, dd, 3.4, 4.4 Hz), 4.71 (1H, d, 12 Hz), 5.84 (1H, d, 3.4 Hz), 7.3 (10H, m), 9.91 (1H, s). IR (film) ν_{max} 3064, 3032, 2987, 2939, 2865, 1731, 1101, 1022 cm^{-1}. FAB-MS (mNBA): 397 $(M-H)^+$, 421 $(M+Na)^+$. ESI-HRMS (positive): calcd for $C_{23}H_{26}O_6Na$ $[M+Na]^+$ 421.1627, found 421.1631. $[\alpha]_D$ +27.4° (0.51, MeOH).

3,5-Di-*O*-benzyl-4-*C*-vinyl-1,2-*O*-isopropylidene-α-D-erythropentofuranose (3). Sodium hydride (60% in mineral, 3.7 g, ca. 93.6 mmol) was added to DMSO (35 mL) under a nitrogen atmosphere and the mixture was stirred for 45 min at 80 °C. After the mixture was cooled down to room temperature, a solution of methyltriphenylphosphonium bromide (33.4 g, 93.6 mmol) in anhydrous DMSO (72 mL) was added dropwise to the mixture at 0 °C and the mixture was stirred for 20 min at room temperature. A solution of **2** (28.7 g, 71.98 mmol) in anhydrous DMSO (50 mL) was added dropwise and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was poured into H_2O in an ice-bath and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and then evaporated in vacuo. The residue was purified by chromatography on silica gel (hexane/EtOAc = 18:1) to give **3** as a colorless oil (22.7 g, 57.3 mmol, 80%). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.63, 26.10, 72.49, 72.76, 73.44, 76.79, 77.34, 78.34, 86.46, 103.91, 113.36, 116.30, 127.57, 127.58, 127.86, 127.93, 128.08, 128.13, 128.31, 128.36, 128.48, 135.49, 137.89, 138.10. ^1H NMR (400 MHz, $CDCl_3$) δ 1.28 (3H, s), 1.52 (3H, s), 3.31 (1H, d, 11 Hz), 3.34 (1H, d, 11 Hz), 4.25 (1H, d, 4.9 Hz), 4.40 (1H, d, 12 Hz), 4.52 (1H, d, 12 Hz), 4.57 (1H, dd, 3.9, 4.9 Hz), 4.59 (1H, d, 12 Hz), 4.76 (1H, d, 12 Hz), 5.25 (1H, dd, 1.8, 11 Hz), 5.52 (1H, dd, 1.8, 18 Hz), 5.76 (1H, d, 3.9 Hz), 6.20 (1H, dd, 11, 18 Hz), 7.3 (10H, m). IR (film) ν_{max} 3357, 3065, 3032, 2985, 2939, 2865, 1725, 1104, 1027 cm^{-1}. FAB-MS (mNBA): 419 $(M+Na)^+$. ESI-HRMS (positive): calcd for $C_{24}H_{28}O_5Na$ $[M+Na]^+$ 419.1834, found 419.1829.

3,5-Di-*O*-benzyl-4-*C*-hydroxyethyl-1,2-*O*-isopropylidene-α-D-erythropentofuranose (4). A 0.5 M THF solution of 9-BBN (9-borabicyclo[3.3.1]nonane) (80 mL, 40 mmol) was added dropwise to a solution of **3** (5.50 g, 13.89 mmol) in anhydrous THF (200 mL) under a nitrogen atmosphere and the mixture was stirred at room temperature overnight. H_2O was added to the reaction mixture until evolution of gas ceased, 3 N NaOH solution (30 mL) was added and then slowly 30% aqueous hydrogen peroxide solution was added while keeping the temperature between 30 and 50 °C. This mixture was stirred for 30 min and partitioned between H_2O and EtOAc. The organic layer was washed with neutral phosphate buffer solution and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by chromatography on silica gel (hexane/EtOAc = 2:1) to give **4** as a colorless oil (5.37 g, 12.97 mmol, 93%). ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.03, 26.38, 33.87, 58.77, 72.43, 73.23, 73.55, 78.52, 79.12, 87.21, 104.23, 113.60, 127.63, 127.71, 127.83, 127.88, 128.38, 137.73, 137.83. ^1H NMR (400 MHz, $CDCl_3$) δ 1.33 (3H, s), 1.66 (3H, s), 1.78 (1H, ddd, 4.0, 8.5, 15 Hz), 2.51 (1H, ddd, 3.4, 6.4, 15 Hz), 3.31 (1H, d, 10 Hz), 3.54 (1H, d, 10 Hz), 3.80 (2H, m), 4.13 (1H, d, 5.3 Hz), 4.43 (1H, d, 12 Hz), 4.52 (1H, d, 12 Hz), 4.55 (1H, d, 12 Hz), 4.65 (1H, dd, 4.0, 5.3 Hz), 4.77 (1H, d, 12 Hz), 5.77 (1H, d, 4.0 Hz), 7.3 (10H, m). IR (film) ν_{max} 3507, 2937, 2870, 1104, 1026, cm^{-1}. FAB-MS (mNBA): 415 $(M+H)^+$. FAB-HRMS (positive): calcd for $C_{24}H_{30}O_6Na$ $[M+Na]^+$ 437.1940, found 437.1945. $[\alpha]_D$ +57.4° (0.91, MeOH).

3,5-Di-*O*-benzyl-4-*C*-(*p*-toluenesulfonyloxyethyl)-1,2-*O*-isopropylidene-α-D-erythropentofuranose (5). Triethylamine (1.8 mL, 13 mmol), *N,N*-dimethylaminopyridine (DMAP, 30 mg, 0.25 mmol), and *p*-toluenesulfonyl chloride (858 mg, 4.5 mmol) were added to a solution of **4** (1.03 g, 2.5 mmol) in anhydrous CH_2Cl_2 (35 mL) under a nitrogen atmosphere at 0 °C and the mixture was stirred at room temperature overnight. The reaction mixture was partitioned between the CH_2Cl_2 and saturated $NaHCO_3$ solution. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by chromatography on silica gel (hexane/EtOAc = 3:1) to give **5** as a colorless oil (1.34 g, 2.6 mmol, 94%). ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.60, 25.93, 26.43, 31.26, 67.55, 72.35, 73.30, 73.52, 78.50, 78.80, 85.34, 104.26, 113.09, 127.61, 127.67, 127.75, 127.86, 127.88, 128. 38, 129.74, 133.24, 137.70, 137. 84, 144.47. ^1H NMR (400 MHz, $CDCl_3$) δ 1.33 (3H, s), 1.49 (3H, s), 1.99 (1H, dt, 7.6 and 15 Hz), 2.47 (3H, s), 2.60 (1H, ddd, 5.7, 7.6, 15 Hz), 3.28 (1H, d, 10 Hz), 3.45 (1H, d, 10 Hz), 4.11 (1H, d, 5.3 Hz), 4.32 (2H, m), 4.42 (1H, d, 12 Hz), 4.50 (1H, d, 12 Hz), 4.54 (1H, d, 12 Hz), 4.62 (1H, dd, 4.0, 5.2 Hz), 4.76 (1H, d, 12 Hz), 5.74 (1H, d, 4.0 Hz), 7.3 (12H, m), 7.78 (2H, d, 8.3 Hz). IR (film) ν_{max} 2940, 2868, 1359, 1176, 1099, 1024, 958, cm^{-1}. FAB-MS (mNBA): 569 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{31}H_{36}O_8SNa$ $[M+Na]^+$ 591.2029, found 591.2050.

3,5-Di-*O*-benzyl-4-*C*-(*p*-toluenesulfonyloxyethyl)-1,2-di-*O*-acetyl-D-erythropentofuranose (6). Acetic anhydride (1.88 mL, 20 mmol) and concentrated H_2SO_4 (0.01 mL) were added to a solution of **5** (1.34 g, 2.36 mmol) in acetic acid (15 mL) and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into H_2O (60 mL) in an ice-bath, stirred for 30 min and then partitioned between brine and EtOAc. The organic layer was washed with neutral phosphate buffer solution, saturated $NaHCO_3$ solution, brine and dried over

$MgSO_4$ and then concentrated. The residue was purified by chromatography on silica gel (hexane/EtOAc = 2:1) to give **6** as a colorless oil (1.29 g, 2.11 mmol, 89%, α:β = 1:5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.65, 20.90, 21.59, 32.24, 67.24, 73.37, 74.16, 74.27, 78.96, 85.64, 97.50, 127.61, 127.74, 127.85, 127.94, 128.38, 128.42, 129.77, 133.27, 137.37, 137.74, 144.54, 169.05, 169.49. ^1H NMR (400 MHz, $CDCl_3$) δ (β derivative) 1.86 (3H, s), 2.05 (3H, s), 2.08 (1H, m), 2.18 (1H, m), 2.42 (3H, s), 3.30 (1H, d, 10 Hz), 3.33 (1H, d, 10 Hz), 4.23 (1H, d, 5.1 Hz), 4.24 (2H, m), 4.42 (2H, s), 4.45 (1H, d, 12 Hz), 4.55 (1H, d, 12 Hz), 5.28 (1H, d, 5.1 Hz), 6.01 (1H, s), 7.3 (12H, m), 7.73 (2H, d, 8.3 Hz). IR (film) ν_{max} 3032, 2868, 1749, 1361, 1218, 1099, 952, cm^{-1}. FAB-MS (mNBA): 613 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{32}H_{36}O_{10}SNa$ $[M+Na]^+$ 635.1927, found 635.1938.

3′,5′-Di-*O*-benzyl-4′-*C*-(*p*-toluenesulfonyloxyethyl)-2′-*O*-acetyl-6-*N*-benzoyladenosine (7a). Trimethylsilylated 6-*N*-benzoyladenosine (500 mg, about 2.0 mmol), which was prepared according to Vorbrüggen's method,[24] was added to a solution of **6** (600 mg, 0.98 mmol) in anhydrous 1,2-dichloroethane (15 mL) at room temperature under a nitrogen atmosphere. After dropwise addition of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.36 mL, 2 mmol) to the mixture, the mixture was stirred at 50 °C for 4 h. Saturated $NaHCO_3$ solution and CH_2Cl_2 were added to the reaction mixture and the mixture was partitioned between these two layers. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and then concentrated in vacuo. The residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH = 50:1) to give **7a** as a colorless amorphous solid (405 mg, 0.51 mmol, 52%). ^1H NMR (400 MHz, $CDCl_3$) δ 2.0 (1H, m), 2.06 (3H, s), 2.32 (1H, dt, 6.0 and 15 Hz), 2.40 (3H, s), 3.36 (1H, d, 10 Hz), 3.58 (1H, d, 10 Hz), 4.22 (2H, m), 4.39 (1H, d, 12 Hz), 4.45 (1H, d, 12 Hz), 4.47 (1H, d, 12 Hz), 4.59 (1H, d, 12 Hz), 4.62 (1H, d, 5.6 Hz), 5.94 (1H, dd, 4.5 and 5.6 Hz), 6.21 (1H, d, 4.5 Hz), 7.2-7.3 (12H, m), 7.54 (2H, m), 7.62 (1H, dt, 1.2 and 6.2 Hz), 7.72 (2H, d, 8.3 Hz), 8.02 (2H, m), 8.21 (1H, s), 8.75 (1H, s), 8.97 (1H, brs). FAB-MS (mNBA): 792 $(M+H)^+$.

3′,5′-Di-*O*-benzyl-2′-*O*,4′-*C*-ethylene-6-*N*-benzoyladenosine (8a). Of a 2 N NaOH mixture solution, comprising pyridine/MeOH/H_2O = 65:30:5, 5 mL was added to **7a** (238 mg, 0.30 mmol) in pyridine/MeOH/H_2O = 65:30:5 (5 mL) at 0 °C and the mixture was stirred at room temperature for 15 min. The reaction mixture was neutralized with 1 N HCl solution and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and then concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 50:1) to afford **8a** as a colorless amorphous solid (133 mg, 0.23 mmol, 78%). ^1H NMR (400 MHz, $CDCl_3$) δ 1.44 (1H, d, 13 Hz), 2.31 (1H, dd, 13 and 19 Hz), 3.56 (1H, d, 11 Hz), 3.70 (1H, d, 11 Hz), 4.10 (2H, m), 4.24 (1H, s), 4.45 (1H, d, 12 Hz), 4.53–4.67 (4H, m), 6.52 (1H, s), 7.3 (10H, m), 7.53 (2H, m), 7.62 (1H, m), 8.03 (2H, d, 7.6 Hz), 8.66 (1H, s), 8.78 (1H, s), 9.00 (1H, brs). FAB-MS (mNBA): 578 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{33}H_{32}N_7O_7$ $[M+H]^+$ 578.2404, found 578.2407.

2′-*O*,4′-*C*-Ethylene-6-*N*-benzoyladenosine (9a). A 1 M boron trichloride solution (1.5 mL, 1.5 mmol) in CH_2Cl_2 was slowly added dropwise to a solution of **8a** (116 mg, 0.20 mmol) in anhydrous CH_2Cl_2 (5 mL) at −78 °C and the mixture was stirred at −78 °C for 3 h. To the reaction mixture was added a 1 M boron trichloride solution (1.5 mL, 1.5 mmol) in CH_2Cl_2 and the mixture was stirred for 2 h. The mixture was slowly warmed to room temperature and then quickly cooled to −78 °C. Then, MeOH (5 mL) was added to the mixture. The reaction mixture was slowly warmed to room temperature and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 9:1) to afford **9a** as a white powder (49 mg, 0.17 mmol, 84%). ^1H NMR (400 MHz, CD_3OD) δ 1.45 (1H, dd, 4.3 and 13 Hz), 2.12 (1H, m), 3.72 (1H, d, 12 Hz), 3.79 (1H, d, 12 Hz), 4.04 (1H, dd, 7.3 and 12 Hz), 4.15 (1H, dt, 4.3 and 9.4 Hz), 4.36 (1H, d, 3.2 Hz), 4.43 (1H, d, 3.2 Hz), 6.57 (1H, s), 7.57 (2H, m), 7.66 (1H, m), 8.09 (2H, d, 8.0 Hz), 8.72 (1H, s), 8.85 (1H, s). FAB-MS (mNBA): 398 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{19}H_{20}N_5O_5$ $[M+H]^+$ 398.1464, found 398.1460.

2′-*O*,4′-C-Ethyleneadenosine (9g). A solution of **9a** (14 mg, 0.035 mmol) in MeOH saturated with ammonia (1 mL) was left standing overnight. The mixture was concentrated and the residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 10:1) to afford **9g** as a white powder (10 mg, 0.034 mmol, 98%). ^1H NMR (400 MHz, CD_3OD) δ 1.32 (1H, dd, 4 and 13 Hz), 2.04 (1H, dt, 7.4 and 12 Hz), 3.53 (1H, dd, 5 and 12 Hz), 3.61 (1H, dd, 5.2 and 12 Hz), 3.90 (1H, dd, 7.4 and 12 Hz), 3.97 (1H, dt, 4 and 12 Hz), 4.15 (1H, d, 3.1 Hz), 4.21 (1H, d, 3.1 Hz), 5.27 (1H, t, 5.2 Hz), 5.39 (1H, d, 3.1 Hz), 6.33 (1H, s), 7.29 (2H, s), 7.66 (1H, m), 8.14 (1H, s), 8.42 (1H, s). FAB-MS (mNBA): 294 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{12}H_{16}N_5O_4$ $[M+H]^+$ 294.1202, found 294.1196. UV (λ_{max}): 260 (pH 7), 260 (pH 1), 258 (pH 13).

5′-*O*-Dimethoxytrityl-2′-*O*,4′-*C*-ethylene-6-*N*-benzoyladenosine (10a). To a solution of **9a** (14 mg, 0.035 mmol) in anhydrous pyridine (1 mL) 4,4′-dimethoxytritylchloride (18 mg, 0.053 mmol) was added and the mixture was stirred at 40 °C for 5 h. A small amount of MeOH was added to the reaction mixture and then the solvent was evaporated in vacuo. The residue was partitioned between H_2O and $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$ solution and brine and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 100:5) to afford **10a** (18 mg, 0.026 mmol, 73%) as a colorless amorphous solid. ^1H NMR (400 MHz, $CDCl_3$) δ 1.63 (1H, m), 2.14 (1H, 7.5, 12, and 13 Hz), 3.37 (1H, d, 11 Hz), 3.41 (1H, d, 11 Hz), 3.79 (6H, s), 4.10 (2H, m), 4.48 (1H, d, 3.3 Hz), 4.59 (1H, d, 3.3 Hz), 6.54 (1H, s), 6.85 (4H, m), 7.2-7.6 (12H, m), 8.02 (2H, m), 8.45 (1H, s), 8.82 (1H, s), 9.02 (1H, brs). FAB-MS

(mNBA): 700 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{40}H_{38}N_5O_7$ $[M+H]^+$ 700.2771, found 700.2747.

5′-*O*-(4,4′-Dimethoxytrityl-2′-*O*,4′-*C*-ethylene-6-*N*-benzoyladenosine-3′-*O*-(2-cyanoethyl *N*,*N*-diisopropyl)phosphoramidite (11a). To a solution of **10a** (16 mg, 0.023 mmol) in anhydrous CH_2Cl_2 (0.5 mL), *N*,*N*-diisopropylammonium tetrazolide (10 mg) was added and then 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite (20 μL) was added dropwise in an ice bath. The mixture was stirred at room temperature overnight. The reaction mixture was washed with saturated $NaHCO_3$ solution and brine and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/EtOAc = 2:1) to afford **11a** (20 mg, 0.022 mmol, 97%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.0–1.2 (12H, m), 1.54 (1H, m), 2.15 (1H, m), 2.33 (2H, m), 3.3–3.6 (6H, m), 3.80 (6H, s), 4.08 (2H, m), 4.65 (1H, m), 4.75 (1H, m), 6.53 (1H, s), 6.84 (4H, m), 7.2–7.6 (12H, m), 8.01 (2H, m), 8.53 (1H, s), 8.83 (1H, s), 9.01 (1H, brs). FAB-MS (mNBA): 900 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{49}H_{55}N_7O_8P$ $[M+H]^+$ 900.3850, found 900.3860.

3′,5′-Di-*O*-benzyl-4′-*C*-(*p*-toluenesulfonyloxyethyl)-2′-*O*-acetyl-2-*N*-isobutyrylguanosine (7b). Trimethylsilylated 2-*N*-isobutyrylguanosine (650 mg, about 1.5 mmol), which was prepared according to Vorbrüggen's method,[24] was added to a solution of **6** (400 mg, 0.65 mmol) in anhydrous 1,2-dichloroethane (10 mL) at room temperature under a nitrogen atmosphere. After addition of TMSOTf (0.2 mL, 1.2 mmol) to the mixture, the mixture was stirred at 50 °C for 4 h. Saturated $NaHCO_3$ solution was added to the reaction mixture and the organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and then concentrated in vacuo to give a product **7b** which was used in the next reaction without further purification.

3′,5′-Di-*O*-benzyl-2′-*O*,4′-*C*-ethylene-2-*N*-isobutyrylguanosine (8b). An aqueous solution of 1 N NaOH (2 mL) was added to a solution of **7b** (about 200 mg) in pyridine (2 mL) and the mixture was stirred at room temperature for 15 min. The reaction mixture was neutralized with 1 N HCl solution and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and then concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2:MeOH = 50:1) to afford **8b** as a colorless amorphous solid (20 mg, 0.036 mmol, 6%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (3H, s), 1.29 (3H, s), 1.43 (1H, dd, 3 and 13 Hz), 2.28 (1H, m), 2.59 (1H, qui, 6.9 Hz), 3.54 (1H, d, 11 Hz), 3.68 (1H, d, 11 Hz), 4.03 (2H, m), 4.15 (1H, d, 3.0 Hz), 4.31 (1H, d, 3.0 Hz), 4.45 (1H, d, 12 Hz), 4.56 (1H, d, 12 Hz), 4.61 (1H, d, 12 Hz), 4.63 (1H, d, 12 Hz), 6.18 (1H, s), 7.2–7.4 (10H, m), 8.19 (1H, s), 11.93 (1H, brs). FAB-MS (mNBA): 560 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{30}H_{34}N_5O_6$ $[M+H]^+$ 560.2509, found 560.2503.

2′-*O*,4′-*C*-Ethylene-2-*N*-isobutyrylguanosine (9b). To a solution of **8b** (10 mg, 0.018 mmol) in MeOH (2 mL) was added 20% palladium hydroxide on carbon (20 mg). The mixture was stirred under a hydrogen atmosphere at atmospheric pressure for 5 h. The reaction mixture was filtered in order to remove the catalyst and the filtrate was concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 10:2) to afford **9b** as a colorless oil (5 mg, 0.013 mmol, 72%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.21 (3H, s), 1.22 (3H, s), 1.41 (1H, dd, 4 and 13 Hz), 2.18 (1H, m), 2.69 (1H, qui, 6.9 Hz), 3.69 (1H, d, 12 Hz), 3.76 (1H, d, 12 Hz), 4.0 (2H, m), 4.26 (1H, d, 3.2 Hz), 4.30 (1H, d, 3.2 Hz), 6.30 (1H, s), 8.40 (1H, s). FAB-MS (mNBA): 380 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{16}H_{22}N_5O_6$ $[M+H]^+$ 380.1570, found 380.1555.

2′-*O*,4′-*C*-Ethyleneguanosine (9h). A solution of **9b** (0.5 mg) in MeOH saturated with ammonia (0.5 mL) was allowed to stand at 60 °C for 5 h. The mixture was concentrated to afford **9h** as a white powder (0.4 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.24 (1H, m), 2.01 (1H, m), 3.50 (1H, dd, 5.1 and 12 Hz), 3.58 (1H, dd, 5.1 and 12 Hz), 3.87 (2H, m), 4.08 (2H, m), 5.17 (1H, t, 5.1 Hz), 5.32 (1H, d, 5.1 Hz), 6.08 (1H, s), 6.55 (2H, brs), 7.14 (1H, brs), 8.01 (1H, s). FAB-MS (mNBA): 310 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{12}H_{16}N_5O_5$ $[M+H]^+$ 310.1152, found 310.1160. UV (λ_{max}): 255 (pH 7), 256 (pH 1), 258–266 (pH 13).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,4′-*C*-ethylene-2-*N*-isobutyrylguanosine (10b). The preparation of **10b** was carried out according to the same procedure used for **10a** to give **10b** (94%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): 1.26 (3H, d, 1.4 Hz), 1.28 (3H, d, 1.4 Hz), 1.66 (1H, m), 2.15 (1H, m), 2.59(1H, qui, 6.9 Hz), 3.65 (1H, m), 3.78 (1H, m), 4.06 (2H, m), 4.35 (1H, m), 4.38 (1H, d, 3.2 Hz), 6.23 (1H, s), 6.8 (4H, m), 7.2–7.5 (9H, m), 8.01 (1H, s), 8.19 (1H, brs). FAB-MS (mNBA): 682 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{37}H_{40}N_5O_8$ $[M+H]^+$ 682.2877, found 682.2891.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,4′-*C*-ethylene-2-*N*-isobutyrylguanosine-3′-*O*-(2-cyanoethyl *N*,*N*-diisopropyl)-phosphoramidite (11b). The preparation of **11b** was carried out according to the same procedure used for **11a** to give **11b** (73%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.1–1.4 (19H, m), 2.1(1H, m), 2.4 (2H, m), 2.6 (1H, m), 3.3–3.6 (6H, m), 3.8 (6H, s), 4.0–4.6 (4H, m), 6.2 (1H, s), 6.8 (4H, m), 7.2–7.5 (9H, m), 8.1 (1H, s). FAB-MS (mNBA): 882$(M+H)^+$. ESI-HRMS (positive): calcd for $C_{46}H_{57}N_7O_9P$ $[M+H]^+$ 882.3955, found 882.3948.

3′,5′-Di-*O*-benzyl-4′-C-(*p*-toluenesulfonyloxyethyl)- 2′-*O*-acetyl-5-methyluridine (7c). Trimethylsilylated thymine (500 mg, about 2 mmol), which was prepared according to Vorbrüggen's method was added to a solution of **6** (650 mg, 1.06 mmol) in anhydrous 1,2-dichloroethane (15 mL) at room temperature under nitrogen atmosphere. TMSOTf (0.36 mL, 2 mmol) was added dropwise to the mixture and the mixture was stirred at 50 °C for 1 h. Saturated $NaHCO_3$ solution was added to the reaction mixture and the mixture was filtered through Celite. CH_2Cl_2 was added to the filtrate. The organic

layer was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and then concentrated in vacuo. The residue was purified by chromatography on silica gel (using hexane/EtOAc = 1.2:1) to give **7c** as a colorless amorphous solid (432 mg, 0.64 mmol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (3H, d, 0.9 Hz), 1.94 (1H, dt, 7.5 and 15 Hz), 2.06 (3H, s), 2.23 (1H, dt, 6.0 and 15 Hz), 2.42 (3H, s), 3.38 (1H, d, 10 Hz), 3.67 (1H, d, 10 Hz), 4.17 (2H, m), 4.36 (1H, d, 6.0 Hz), 4.41 (1H, d, 12 Hz), 4.44 (1H, d, 12 Hz), 4.48 (1H, d, 12 Hz), 4.58 (1H, d, 12 Hz), 5.39 (1H, dd, 5.1 and 6.0 Hz), 6.04 (1H, d, 5.1 Hz), 7.3 (12H, m), 7.73 (2H, dt, 1.8 and 8.3 Hz), 8.18 (1H, s). FAB-MS (mNBA): 679 $(M+H)^+$.

3′,5′-Di-*O*-benzyl-2′-*O*,4′-*C*-ethylene-5-methyluridine (8c). Of 2 N NaOH mixture solution, comprising pyridine/MeOH/H_2O = 65:30:5, 5 mL was added to **7c** (418 mg, 0.62 mmol) in pyridine/MeOH/H_2O = 65:30:5 (5 mL) at 0 °C and the mixture was stirred at room temperature for 15 min. The reaction mixture was neutralized with 1 N HCl solution and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and then concentrated in vacuo. The residue was purified by chromatography on a silica gel column (hexane/EtOAc = 1:1) to afford **8c** as a colorless amorphous solid (228 mg, 0.49 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (1H, d, 13 Hz), 1.41 (3H, s), 2.28 (1H, dt, 9.4 and 13 Hz), 3.60 (1H, d, 11 Hz), 3.76 (1H, d, 11 Hz), 3.94 (1H, d, 3.0 Hz), 4.10 (1H, d, 7.0 Hz), 4.14 (1H, d, 7.0 Hz), 4.31 (1H, d, 3.0 Hz), 4.51 (1H, d, 12 Hz), 4.54 (1H, d, 12 Hz), 4.58 (1H, d, 12 Hz), 4.75 (1H, d, 12 Hz), 6.06 (1H, s), 7.3 (10H, m), 7.91 (1H, s), 8.42 (1H, brs). FAB-MS (mNBA): 465$(M+H)^+$. ESI-HRMS (positive): calcd for $C_{26}H_{28}N_2O_6$ $[M+H]^+$ 465.2025, found 465.2018.

2′-*O*,4′-*C*-Ethylene-5-methyluridine (9c). To a solution of **8c** (195 mg, 0.42 mmol) in MeOH (10 mL) was added 20% palladium hydroxide on carbon (100 mg). The mixture was stirred under hydrogen atmosphere at atmospheric pressure for 5 h. The reaction mixture was filtered in order to remove the catalyst and the filtrate was concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 10:1) to afford **9c** as a colorless powder (76 mg, 0.268 mmol, 64%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.33 (1H, dd, 3.8 and 13 Hz), 1.86 (3H, d, 0.9 Hz), 1.94 (1H, ddd, 7.5, 11.7 and 13 Hz), 3.68 (1H, d, 12 Hz), 3.75 (1H, d, 12 Hz), 3.9–4.0 (2H, m), 4.05 (1H, d, 3.2 Hz), 4.09 (1H, d, 3.2 Hz), 6.00 (1H, s), 8.28 (1H, d, 1.1 Hz). FAB-MS (mNBA): 285 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{12}H_{17}N_2O_6$ $[M+H]^+$ 285.1086, found 285.1074.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,4′-*C*-ethylene-5-methyluridine (10c). The preparation of **10c** was carried out according to the same procedure used for **10a** to give **10c** (81%) as a colorless amorphous solid. ^{1}H NMR (270 MHz, DMSO-d_6) δ 11.36 (1H, s), 7.68 (1H, s), 6.90–7.44 (13H, m), 5.89 (1H, s), 5.55 (1H, d), 4.09 (1H, m), 4.04 (1H, d), 3.82 (2H, m), 3.74 (6H, s), 3.19 (2H, m), 1.99 (1H, m), 1.36 (1H, m), 1.17 (3H, s). FAB-MS (mNBA): 587 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{33}H_{34}N_2O_8Na$ $[M+Na]^+$ 609.2213, found 609.2213.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,4′-*C*-ethylene-5-methyluridine-3′-*O*-(2-cyanoethyl *N*,*N*-diisopropyl)phosphoramidite (11c). The preparation of **11c** was carried out according to the same procedure used for **11a** to give **11c** (89%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.1–1.2 (15H, m), 1.4 (1H, m), 2.08 (1H, m), 2.4 (2H, m), 3.2–4.0 (14H, m), 4.38 (2H, m), 4.47 (1H, m), 6.06 (1H, s), 6.8–6.9 (4H, m), 7.2–7.5 (9H, m), 7.91 (1H, m). FAB-MS (mNBA): 787 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{42}H_{51}N_4O_9PNa$ $[M+Na]^+$ 809.3291, found 809.3275.

3′,5′-Di-*O*-benzyl-4′-*C*-(*p*-toluenesulfonyloxyethyl)-2′-*O*-acetyl-4-*N*-benzoylcytidine (7d). Trimethylsilylated 4-*N*-benzoylcytosine (300 mg, about 1.0 mmol), which was prepared according to Vorbrüggen's method,[24] was added to a solution of **6** (383 mg, 0.626 mmol) in anhydrous 1,2-dichloroethane (4 mL). TMSOTf (0.18 mL, 0.995 mmol) at 0 °C was added to the mixture and the mixture was stirred at 50 °C for 1 h. Saturated $NaHCO_3$ solution and CH_2Cl_2 was added to the mixture and the mixture was stirred. The resulting white precipitates were removed by filtrating through Celite. The organic layer of the filtrate was washed with brine, dried over $MgSO_4$ and then concentrated in vacuo to give **7d** as a colorless amorphous solid (397 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (1H, br), 8.18 (1H, d, 7.4 Hz), 7.87 (2H, d, 7.5 Hz), 7.72 (2H, d, 8.3 Hz), 7.61–7.57 (1H, m), 7.51–7.48 (2H, m), 7.43–7.21 (13H,m), 6.02 (1H, d, 2.9 Hz), 5.40 (1H, dd, 5.8, 2.9 Hz), 4.57 (1H, d, 11 Hz), 4.39 (1H, d, 11 Hz), 4.32–4.28 (3H, m), 4.19–4.16 (2H, m), 3.69 (1H, d, 11 Hz), 3.31 (1H, d, 11 Hz), 2.40 (3H, s), 2.30–2.23 (1H, m), 2.06 (3H, s), 1.95–1.89 (1H, m). FAB-MS (mNBA): 768 $(M+H)^+$.

3′,5′-Di-*O*-benzyl-2′-*O*,4′-*C*-ethylene-4-*N*-benzoylcytidine (8d). Of a 2 N NaOH solution, 68 mL was added to a solution of **7d** (6.80 g, 8.86 mmol) in pyridine (136 mL) at 0 °C and the mixture was stirred at room temperature for 1 h. The reaction mixture was neutralized by dropwise addition of aqueous 20% acetic acid and extracted with chloroform. The organic layer was washed with brine and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 100:3) to afford **8d** (3.3 g, 6.02 mmol, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (2H, brs), 7.89 (2H, d, 7.6 Hz), 7.64–7.60 (1H, m), 7.54–7.51 (2H, m), 7.48–7.37 (3H, m), 7.36–7.26 (8H, m), 6.18 (1H,s), 4.70 (1H, d, 11 Hz), 4.60 (1H, d, 11 Hz), 4.55 (1H, d, 11 Hz), 4.46 (1H, d, 2.9 Hz), 4.42 (1H, d, 11 Hz), 4.10–4.02 (2H,m), 3.89 (1H, d, 2.9 Hz), 3.75 (1H, d, 11 Hz), 3.62 (1H, d, 11 Hz), 2.34–2.26 (1H, m), 1.39–1.36 (1H, m). FAB-MS (mNBA): 554 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{32}H_{32}N_3O_6$ $[M+H]^+$ 554.2291, found 554.2305.

2′-*O*,4′-*C*-Ethylene-4-*N*-benzoylcytidine (9d). A solution (31.7 mL) of 1.0 M trichloroborane in CH_2Cl_2 was added dropwise to a solution of **8d** (2.06 g, 3.72 mmol) in anhydrous CH_2Cl_2 (317 mL) at −78 °C and the mix-

ture was stirred at −78 °C for 1 h. The reaction mixture was slowly warmed to −20 °C and stirred at between 20 °C and −10 °C for 2 h. MeOH (12 mL) was slowly added to the mixture and the mixture was stirred for 10 min. The pH of the reaction mixture was adjusted to 7–8 by dropwise addition of saturated $NaHCO_3$ solution. The mixture was warmed to room temperature and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 100:5) to afford **9d** (1.21 g, 3.24 mmol, 87%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.23 (1H, brs), 8.70 (1H, d, 7.2 Hz), 8.00 (2H, d, 7.5 Hz), 7.3-6 (4H, m), 5.97 (1H, s), 5.35 (1H, dd, 5 and 10 Hz), 4.10 (1H, dd, 5 and 10 Hz), 4.03 (1H, d, 3.2 Hz), 3.95–3.85 (2H, m) 3.83 (1H, d, 3.2 Hz), 3.65–3.51 (2H, m), 2.06–1.98 (1H, m), 1.26 (1H, m). FAB-MS (mNBA): 374 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{18}H_{20}N_3O_6$ $[M+H]^+$ 374.1352, found 374.1341.

2′-*O*,4′-*C*-Ethylenecytidine (9i). A solution of **9d** (0.1 g, 0.268 mmol) in MeOH saturated with ammonia (12 mL) was allowed to stand overnight. The mixture was concentrated in vacuo to afford **9i** (54 mg, 75%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.18 (1H, d, 7.4 Hz), 7.10 (2H, br), 5.84 (1H, s), 5.69 (1H, d, 7.6 Hz), 5.27–5.24 (2H, m), 3.86 (1H, d, 3.2 Hz), 3.90–3.78 (2H, m), 3.76 (1H, d, 3.2 Hz), 3.56 (1H, dd, 5.5 and 12 Hz), 3.49 (1H, dd, 5.5 and 12 Hz), 2.01–1.93 (1H,dt, 7.5 and 12 Hz), 1.22 (1H, dd, 3.6 and 13 Hz). FAB-MS (mNBA): 270 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{11}H_{16}N_3O_6$ $[M+H]^+$ 270.1090, found 270.1092.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,4′-*C*-ethylene-4-*N*-benzoylcytidine (10d). The preparation of **10d** was carried out according to the same procedure used for **10a** to give **10d** (90%) as a colorless amorphous solid. ^{1}H NMR (270 MHz, DMSO-d_6) δ 11.27 (1H, brs), 8.59 (1H, m), 6.92–8.01 (19H, m), 6.03 (1H, s), 5.56 (1H, m), 4.17 (1H, m), 4.08 (1H, m), 3.86 (2H, m), 3.77 (6H, s), 3.24 (2H, m), 1.98 (1H, m), 1.24 (1H, m). FAB-MS (mNBA): 676 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{39}H_{38}N_3O_8$ $[M+H]^+$ 676.2659, found 676.2648.

5′-*O*-(4,4′-Dimethoxytrityl-2′-*O*,4′-*C*-ethylene-4-*N*-benzoylcytidine-3′-*O*-(2-cyanoethyl *N*,*N*-diisopropyl)phosphoramidite (11d). The preparation of **11b** was carried out according to the same procedure used for **11a** to give **11d** (84%) as a colorless compound. ^{1} H NMR (400 MHz, $CDCl_3$) δ 1.1–1.2 (12H, m), 1.35 (1H,m), 2.11 (1H, m), 2.3 (2H, m), 3.35–3.7 (6H, m), 3.8 (6H, m), 3.9–4.1 (2H, m), 4.33 (1H, m), 4.45 (1H, m), 6.23 (1H, s), 6.9 (4H, m), 7.3–7.9 (15H, m), 8.7–8.8 (1H, m). FAB-MS (mNBA): 876 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{48}H_{55}N_5O_9P$ $[M+H]^+$ 876.3738, found 876.3744.

3′,5′-Di-*O*-benzyl-2′-*O*-acetyl-4′-*C*-(*p*-toluenesulfonyloxyethyl)uridine (7e). Trimethylsilylated uracil (200 mg, about 0.8 mmol), which was prepared according to Vorbrüggen's method, was added to a solution of **6** (200 mg, 0.327 mmol) in anhydrous 1,2-dichloroethane (8 mL) at room temperature under a nitrogen atmosphere. After dropwise addition of TMSOTf (0.145 mL, 0.8 mmol) to the mixture, the mixture was stirred at 70 °C for 1 h. Saturated $NaHCO_3$ solution was added to the reaction mixture. Then, the mixture was filtered through Celite and CH_2Cl_2 was added to the filtrate. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and then concentrated in vacuo. The residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH = 100:2) to give **7e** as a colorless oil (199 mg, 0.299 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94 (1H, dt, 7.4 and 15 Hz), 2.07 (3H, s), 2.23 (1H,dt,5.9 and 15 Hz), 2.43 (3H, s), 3.36 (1H, d, 10 Hz), 3.65 (1H, d, 10 Hz), 4.17 (2H, dd, 6 and 7 Hz), 4.31 (1H, d, 5.9 Hz), 4.38 (1H, d, 11 Hz), 4.39 (1H, d, 11 Hz), 4.40 (1H, d, 11 Hz), 4.58 (1H, d, 11 Hz), 5.29 (1H, dd, 2.4 and 8.2 Hz), 5.33 (1H, dd, 4.5 and 6 Hz), 6.00 (1H, d, 4.5 Hz), 7.2–7.4 (12H, m), 7.61 (1H, d, 8.2 Hz), 7.74 (1H, d, 8.3 Hz), 8.14 (1H, brs). FAB-MS (mNBA): 665 $(M+H)^+$.

3′,5′-Di-*O*-benzyl-2′-*O*,4′-*C*-ethyleneuridine (8e). Of 1 N NaOH solution, 2 mL was added to a solution of **7e** (194 mg, 0.292 mmol) in pyridine (3 mL) at 0 °C and the mixture was stirred at room temperature for 30 min. The reaction mixture was neutralized with 1 N HCl solution and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and then concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 100:3) to afford **8e** as a colorless oil (105 mg, 0.233 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (1H, m), 2.29 (1H, m), 3.63 (1H, d, 11 Hz), 3.74 (1H, d, 11 Hz), 3.87 (1H, d, 2.9 Hz), 4.03 (2H, m), 4.29 (1H, d, 2.9 Hz), 4.49 (1H, d, 12 Hz), 4.50 (1H, d, 11 Hz), 4.53 (1H, d, 11 Hz), 4.73 (1H, d, 12 Hz), 5.20 (1H, dd, 2 and 8 Hz), 6.04 (1H, s), 7.2–7.4 (10H, m), 8.13 (1H, d, 8.2 Hz), 8.57 (1H, brs). FAB-MS (mNBA): 451 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{25}H_{26}N_2O_6Na$ $[M+Na]^+$ 473.1688, found 473.1695.

2′-*O*,4′-*C*-Ethyleneuridine (9e). To a solution of **8e** (100 mg, 0.222 mmol) in MeOH (4 mL) was added 20% palladium hydroxide on carbon (90 mg). The mixture was stirred under a hydrogen atmosphere at atmospheric pressure for 5 h. The reaction mixture was filtered in order to remove the catalyst and the filtrate was concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 10:1) to afford **9e** as a colorless oil (45 mg, 0.167 mmol, 75%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.35 (1H, dd, 4 and 13 Hz), 2.13 (1H, ddd, 7, 11 and 13 Hz), 3.66 (1H, d, 12 Hz), 3.73 (1H, d, 12 Hz), 3.91–4.08 (2H,m), 4.01 (1H, d, 3.2 Hz), 4.12 (1H, d, 3.2 Hz), 5.66 (1H, d, 8.2 Hz), 6.00 (1H, s), 8.37 (1H, d, 8.2 Hz). FAB-MS (mNBA): 271 $(M+H)^+$. FAB-HRMS (positive): calcd for $C_{11}H_{15}N_2O_6$ $[M+H]^+$ 271.0930, found 271.0924.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,4′-*C*-ethyleneuridine (10e). The preparation of **10e** was carried out according to the same procedure used for **10a** to give **10e** (42%) as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 1.35 (1H, dd, 3 and 14 Hz), 2.03 (1H, ddd, 8, 11 and

14 Hz), 2.46 (1H, d, 8 > Hz), 3.36 (1H, d, 11 Hz), 3.41 (1H, d, 11 Hz), 3.80 (3H, s), 3.81 (3H, s), 3.97 (2H, m), 4.21 (1H, d, 3.2 Hz), 4.33 (1H, brm), 5.31 (1H, m), 6.10 (1H, s), 6.86 (4H, m), 7.2–7.5 (9H, m), 8.27 (1H, d, 8.2 Hz), 8.43 (1H, brs). FAB-MS (mNBA): 573 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{32}H_{32}N_2O_8Na$ $[M+Na]^+$ 595.2056, found 595.2039.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,4′-*C*-ethyleneuridine-3′-*O*-(2-cyanoethyl *N*,*N*-diisopropyl)phosphoramidite (11e). The preparation of **11e** was carried out according to the same procedure used for **11a** to give **11e** (quant.) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.1–1.2 (13H, m), 2.09 (1H, m), 2.4 (2H, m), 3.3–3.6 (6H, m), 3.81 (6H, m), 3.94 (2H, m), 4.35 (1H, m), 4.47 (1H, m), 5.18 (1H, d, 8.2 Hz), 6.08 (1H, s), 6.86 (4H, m), 7.2–7.4 (9H, m), 8.31 (1H, d, 8.2 Hz). FAB-MS (mNBA): 773 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{41}H_{49}N_4O_9PNa$ $[M+Na]^+$ 795.3135, found 795.3167.

2′-*O*-Acetyl-3′,5′-di-*O*-benzyl-4′-*C*-(*p*-toluenesulfonyloxyethyl)-4-*N*-benzoyl-5-methylcytidine (7f). Trimethylsilylated *N*-4-benzoyl 5-methylcytosine (400 mg, about 1.2 mmol), which was prepared according to Vorbrüggen's method[24] was added to a solution of **6** (400 mg, 0.653 mmol) in anhydrous 1,2-dichloroethane (6 mL). After addition of TMSOTf (0.180 μL, 1.0 mmol) to the mixture at 0 °C, the mixture was stirred at 50 °C for 1 h. The reaction mixture was warmed to room temperature. Saturated $NaHCO_3$ solution and CH_2Cl_2 were added to the reaction mixture and the mixture was stirred. The mixture was filtered through Celite in order to remove the white precipitate. The organic layer of the filtrate was washed with brine, dried over $MgSO_4$ and then concentrated in vacuo to give **7f** as a colorless amorphous solid (320 mg, 0.409 mmol, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68 (3H, s), 1.95 (1H, dt, 7.3 and 15 Hz), 2.07 (3H, s), 2.25 (1H, dt, 6 and 15 Hz), 2.43 (3H, s), 3.40 (1H, d, 10 Hz), 3.71(1H, d, 10 Hz), 4.18 (2H, m), 4.37 (1H, d, 5.8 Hz), 4.42 (1H, d, 12 Hz), 4.46 (1H, d, 12 Hz), 4.51 (1H, d, 12 Hz), 4.61 (1H, d, 12 Hz), 5.42 (1H, dd, 4.9 and 5.8 Hz), 6.07 (1H, d, 4.9 Hz), 7.2–7.6 (17H, m), 7.74 (2H, d, 8.3 Hz), 8.28 (2H, d, 7.0 Hz). FAB-MS (mNBA): 782 $(M+H)^+$.

3′,5′-Di-*O*-benzyl-2′-*O*,4′-*C*-ethylene-4-*N*-benzoyl-5-methylcytidine (8f). Of 1 N NaOH solution, 5 mL was added to a solution of **7f** (310 mg, 0.396 mmol) in pyridine (5 mL) at 0 °C and the mixture was stirred at room temperature for 20 min. The reaction mixture was neutralized by dropwise addition of aqueous 20% acetic acid and extracted with CH_2Cl_2. The organic layer was washed with brine and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 100:2) to afford **8f** (190 mg, 0.334 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (1H, m), 1.58 (3H, s), 2.30 (1H, dt, 10 and 13 Hz), 3.64 (1H, d, 11 Hz), 3.79 (1H, d, 11 Hz), 3.95 (1H, d, 3.0 Hz), 4.04 (2H, dd, 2.3 and 10 Hz), 4.37 (1H, d, 3.0 Hz), 4.50 (1H, d, 12 Hz), 4.56 (1H, d, 11 Hz), 4.61 (1H, d, 11 Hz), 4.76 (1H, d, 12 Hz), 6.11 (1H, s), 7.2–7.5 (13H, m), 8.09 (1H, s), 8.29 (2H, m). FAB-MS (mNBA): 568 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{33}H_{34}N_3O_6$ $[M+H]^+$ 568.2448, found 568.2455.

2′-*O*,4′-*C*-Ethylene-4-*N*-benzoyl-5-methylcytidine (9f). A 1.0 M boron trichloride solution (1.6 mL) in CH_2Cl_2 was added dropwise to a solution of **8f** (120 mg, 0.211 mmol) in anhydrous CH_2Cl_2 (5 mL) at −78 °C and the mixture was stirred at −78 °C for 4 h. MeOH (1 mL) was slowly added dropwise to the mixture and the mixture was stirred for 10 min. The pH of the reaction mixture was adjusted to 7–8 by dropwise addition of saturated $NaHCO_3$ solution. The reaction mixture was warmed to room temperature and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 100:6) to afford **9f** (29 mg, 0.075 mmol, 36%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.24 (1H, m), 2.01 (3H, s), 2.0 (1H, m), 3.54 (1H, dd, 5.4 and 12 Hz), 3.64 (1H, dd, 5.4 and 12 Hz), 3.88 (3H, m), 4.10 (1H, m), 5.36 (1H, d, 5.4 Hz), 5.49 (1H, t, 5.0 Hz), 5.95 (1H, s), 7.4–7.6 (3H, m), 8.21 (2H, m), 8.49 (1H, s), 13.17 (1H, brs). FAB-MS (mNBA): 388 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{19}H_{21}N_3O_6Na$ $[M+Na]^+$ 410.1328, found 410.1336.

2′-*O*,4′-*C*-Ethylene-5-methylcytidine (9j). A solution of **9f** (11.6 mg, 0.030 mmol) in MeOH saturated with ammonia (2 mL) was allowed to stand overnight. The mixture was concentrated to afford **9j** as a white solid (8.5 mg, 0.030 mmol, quant.). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 (1H, m), 1.82 (3H, s), 1.97 (1H, m), 3.49 (1H, dd, 5 and 12 Hz), 3.58 (1H, dd, 5 and 12 Hz), 3.85 (2H, m), 5.23 (1H, d, 5 Hz), 5.32 (1H, t, 5 Hz), 5.84 (1H, s), 6.7 (1H, brs), 7.2 (1H, brs), 8.08 (1H, s). FAB-MS (mNBA): 284 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{12}H_{18}N_3O_5$ $[M+H]^+$ 284.1246, found 284.1238. UV (λ_{max}): 279 (pH7), 289 (pH1), 279 (pH13).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,4′-*C*-ethylene-4-*N*-benzoyl-5-methylcytidine (10f). The preparation of **10f** was carried out according to the same procedure used for **10a** to give **10f** (93%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (1H, m), 1.49 (3H, s), 2.06 (1H, m), 2.59 (1H, d, 8.6 Hz), 3.36 (1H, d, 11 Hz), 3.39 (1H, d, 11 Hz), 3.80 (3H, s), 3.81 (3H, s), 3.99 (2H, m), 4.30 (1H, d, 3.3 Hz), 4.39 (1H, m), 6.12 (1H, s), 6.85 (4H, m), 7.2–7.5 (12H, m), 8.03 (1H, s), 8.28 (2H, m). FAB-MS (mNBA): 690 $(M+H)^+$. FAB-HRMS (positive): calcd for $C_{40}H_{41}N_3O_8$ $[M+H]^+$ 690.2815, found 690.2812.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,4′-*C*-ethylene-4-*N*-benzoyl-5-methylcytidine-3′-*O*-(2-cyanoethyl *N*,*N*-diisopropyl)phosphoramidite (11f). The preparation of **11b** was carried out according to the same procedure used for **11a** to give **11f** (89%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) d 1.1–1.2 (12H, m), 1.36 (3H, s), 1.37 (1H, m), 2.10 (1H, m), 2.36 (2H, m), 3.3–3.6 (6H, m), 3.81 (6H, m), 3.98 (2H, m), 4.42 (1H, m), 4.49 (1H, m), 6.11 (1H, s), 6.88 (4H, m), 7.2–7.5 (12H, m), 8.14 (1H, s), 8.28 (2H, m). FAB-MS (mNBA): 890 $(M+H)^+$. ESI-HRMS (positive): calcd for $C_{49}H_{57}N_5O_9P$ $[M+H]^+$ 890.3894, found 890.3884.

5-*O*-(*tert*-Butyldiphenylsilyl)-4-*C*-formyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythropentofuranose (13). Oxalyl chloride (8.3 mL, 95 mmol) was added to anhydrous

CH_2Cl_2 (200 mL) at −78 °C. A solution of DMSO (11.4 mL, 160 mmol) in anhydrous CH_2Cl_2 (100 mL) was added dropwise to this solution. After stirring for 20 min, a solution of **12** (26.3 g, 47.9 mmol) in anhydrous CH_2Cl_2 (100 mL) was added dropwise to this mixture and the mixture was stirred for 30 min. Triethylamine (42 mL, 300 mmol) was added to this reaction mixture and this was slowly warmed to room temperature. The reaction was quenched with H_2O. The organic layer was washed with H_2O and brine, dried over $MgSO_4$ and then evaporated in vacuo. The residue was purified by chromatography on silica gel (hexane/EtOAc = 7:1) to give **13** as a colorless oil (26.1 g, 47.8 mmol, 99%). ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.21, 26.13, 26.66, 26.76, 63.05, 72.73, 78.57, 79.08, 90.62, 104.91, 114.15, 127.76, 127.79, 127.83, 128.07, 128.51, 129.81, 129.86, 132.51, 132.82, 135.49, 135.57, 136.99, 200.17. ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (9H, s), 1.37 (3H, s), 1.62 (3H, s), 3.79 (1H, d, 11 Hz), 3.88 (1H, d, 11 Hz), 4.54 (1H, d, 4.4 Hz), 4.62 (1H, d, 12 Hz), 4.65 (1H, dd, 3.7 and 4.4 Hz), 4.74 (1H, d, 12 Hz), 5.87 (1H, d, 3.7 Hz), 7.3–7.6 (15H), 9.89 (1H, s). IR (film) ν_{max} 2933, 2858, 1732, 1113, 1022, 702 cm^{-1}. ESI-HRMS (positive): calcd for $C_{32}H_{38}O_6SiNa$ $[M+Na]^+$ 569.2335, found 569.2354.

5-*O*-(*tert*-Butyldiphenylsilyl)-4-*C*-(2-ethoxycarbonyl-(*E*)-vinyl)-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythropentofuranose (14). Sodium hydride (60% in mineral, 2.3 g, about 57.4 mmol) was added to diethylphosphonoacetic acid ethyl ester (11.5 mL, 57.4 mmol) in anhydrous THF (200 mL) at 0 °C. After stirring for 10 min, a solution of **13** (26.1 g, 47.8 mmol) in anhydrous THF (200 mL) was added dropwise to this solution and the mixture was stirred for 30 min at room temperature. The reaction was quenched with H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and then evaporated in vacuo. The residue was purified by chromatography on silica gel (hexane/EtOAc = 7:1) to give **14** as a colorless oil (25.4 g, 41.2 mmol, 86%). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.23, 19.25, 25.66, 26.07, 26.77, 60.21, 65.60, 72.53, 77.21, 78.08, 86.68, 103.99, 113.48, 122.66, 127.68, 127.73, 127.80, 127.96, 128.49, 129.73, 129.78, 132.71, 133.12, 135.49, 135.64, 137.56, 145.51, 166.21. ^1H NMR (400 MHz, $CDCl_3$) δ 1.04 (9H, s), 1.30 (3H, t, 7.3 Hz), 1.45 (3H, s), 1.62 (3H, s), 3.54 (1H, d, 10 Hz), 3,58 (1H, d, 10 Hz), 4.2 (2H, m), 4.39 (1H, d, 6.3 Hz), 4.59 (1H, d, 10 Hz), 4.58 (1H, dd, 4.4 and 6.3 Hz), 4.77 (1H, d, 10 Hz), 5.90(1H, d, 4.4 Hz), 6.24 (1H, d, 16 Hz), 7.3–7.7 (15H, m). IR (KBr) ν_{max} 2929, 2857, 1717, 1302, 1139, 1106, 1034, 704 cm^{-1}. FAB-MS (mNBA): 639 $[M+Na]^+$. ESI-HRMS (positive): calcd for $C_{36}H_{44}O_7SiNa$ $[M+Na]^+$ 639.2754, found 639.2755.

5-*O*-(*tert*-Butyldiphenylsilyl)-4-*C*-(2-ethoxycarbonylethyl)-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythropentofuranose (15). To a solution of **14** (26.0 g, 42.14 mmol) in EtOAc (100 mL) was added 20% palladium hydroxide on carbon (8.0 g). The mixture was stirred under a hydrogen atmosphere at an atmospheric pressure of 3.5 psi for 10 h. The reaction mixture was filtered and the filtrate was evaporated in vacuo. The residue was purified by chromatography on silica gel (hexane/EtOAc = 7:1) to give **15** as a colorless oil (18.5 g, 29.9 mmol, 71%) and the debenzylated compound (6.0 g, 11.34 mmol, 27%). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.16, 19.20, 26.19, 26.59, 26.80, 27.00, 29.02, 60.12, 66.29, 72.35, 77.97, 79.16, 86.88, 104.17, 113.27, 127.67, 127.73, 127.76, 128.40, 129.68, 129.76, 132.89, 133.17, 135.53, 135.61, 137.92, 173.88. ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (9H, s), 1.18 (3H, t, 7.0 Hz), 1.35 (3H, s), 1.64 (3H, s), 1.82 (1H, m), 2.16 (1H, m), 2.52 (2H, m), 3.40 (1H, d, 11 Hz), 3.61 (1H, d, 11 Hz), 4.04 (2H, dd, 7.0 and 14 Hz), 4.30 (1H, d, 5.1 Hz), 4.58 (1H, d, 12 Hz), 4.67 (1H, dd, 4.4 and 5.1 Hz), 4.80 (1H, d, 12 Hz), 5.78 (1H, d, 4.4 Hz), 7.3–7.7 (15H, m). IR (KBr) ν_{max} 2933, 2858, 1733, 1111, 1025, 703 cm^{-1}. FAB-MS (mNBA): 617 [M-H], 641 $[M+Na]^+$. ESI-HRMS (positive): calcd for $C_{36}H_{46}O_7SiNa$ $[M+Na]^+$ 641.2911, found 641.2910.

5-*O*-(*tert*-Butyldiphenylsilyl)-4-*C*-hydroxypropyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythropentofuranose (16). To a solution of **15** (1.95 g, 3.15 mmol) in anhydrous THF (40 mL) was added 1.0 M of lithium tri-*s*-butylborohydride in THF (L-selectride, 10 mL, 10 mmol) and the reaction mixture was stirred for 30 min at room temperature. The reaction was quenched with aqueous 20% acetic acid and filtered through Celite. The filtrate was extracted with EtOAc and the organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and then evaporated in vacuo. The residue was purified by chromatography on silica gel (hexane/EtOAc = 4:1) to give **16** as a colorless oil (1.55 g, 2.69 mmol, 85%). IR (film) ν_{max} 3487, 2934, 2858, 1112, 1025, 703 cm^{-1}. ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.21, 26.06, 26.58, 26.77, 26.80, 27.51, 62.57, 65.97, 72.42, 77.83, 79.15, 87.95, 104.13, 113.14, 127.68, 127.73, 127.82, 128.42, 129.70, 129.75, 132.92, 133.29, 135.53, 135.65, 137.95. ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (9H, s), 1.35 (3H, s), 1.63 (3H, s), 1.54–1.70 (2H, m), 1.97 (1H, t, 6.1 Hz), 2.17 (1H, m), 3.44 (1H, d, 11 Hz), 3.56 (2H, m), 3.74 (1H, 11 Hz), 4.35 (1H, d, 5.1 Hz), 4.59 (1H, d, 12 Hz), 4.68 (1H, dd, 4.4 and 5.1 Hz), 4.80 (1H, d, 12 Hz), 5.79 (1H, d, 4.4 Hz), 7.3–7.7 (15H, m). FAB-HRMS (mNBA): calcd for $C_{34}H_{44}O_6SiNa$ $[M+Na]^+$ 599.2805, found 599.2819.

5-*O*-(*tert*-Butyldiphenylsilyl)-4-*C*-(*p*-toluenesulfonyloxypropyl)-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythropentofuranose (17). Pyridine (0.24 mL, 3.0 mmol) and *p*-toluenesulfonyl chloride (286 mg, 1.5 mmol) were added to a solution of **16** (610 mg, 1.06 mmol) in anhydrous CH_2Cl_2 (10 mL) under a nitrogen atmosphere at 0 °C and the mixture was stirred at room temperature overnight. The reaction mixture was partitioned between CH_2Cl_2 and saturated $NaHCO_3$. The organic layer was washed with brine, dried over $MgSO_4$ and then evaporated in vacuo. The residue was purified by chromatography on silica gel (hexane/EtOAc = 7:1) to give **17** as a colorless oil (600 mg, 0.82 mmol, 77%). IR (film) ν_{max} 2933, 2858, 1360, 1176, 1112, 704 cm^{-1}. ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.17, 21.56, 23.20, 26.17, 26.73, 26.77, 27.80, 66.33, 71.09, 72.37, 78.04, 79.25,

87.20, 104.16, 113.13, 127.70, 127.76, 127.81, 127.87, 128.42, 129.70, 129.79, 132.85, 133.11, 133.13, 135.51, 135.58, 137.89, 144.44. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (9H, s), 1.32 (3H, s), 1.50 (3H, s), 1.42–1.56 (2H, m), 1.82 (1H, m), 2.05 (1H, m), 2.39 (3H, s), 3.34 (1H, d, 11 Hz), 3.56 (1H, d, 11 Hz), 3.90–4.00 (2H, m), 4.25 (1H, d, 5.1 Hz), 4.54 (1H, d, 12 Hz), 4.63 (1H, dd, 3.7 and 5.1 Hz), 4.77 (1H, d, 12 Hz), 5.74 (1H, d, 3.7 Hz), 7.3–7.8 (19H, m). ESI-HRMS: calcd for $C_{41}H_{50}O_8SiSNa$ $[M+Na]^+$ 753.2893, found 753.2878.

5-*O*-(*tert*-Butyldiphenylsilyl)-4-*C*-(*p*-toluenesulfonyloxypropyl)-3-*O*-benzyl-1,2-di-*O*-acetyl-D-erythropentofuranose (18). Acetic anhydride (1.0 mL, 10 mmol) and concentrated H_2SO_4 (0.01 mL) were added to a solution of **17** (600 mg, 0.82 mmol) in acetic acid (5 mL) and the mixture was stirred at room temperature for 30 min. The reaction mixture was poured into H_2O (10 mL) in an ice-bath, stirred for 30 min and then extracted with EtOAc. The organic layer was washed with neutral phosphate buffer, saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and then evaporated in vacuo. The residue was purified by chromatography on silica gel (hexane/EtOAc = 5:1) to give mainly a β-anomer of **18** as a colorless oil (500 mg, 0.65 mmol, 79%). IR (film) ν_{max} 1748, 1362, 1220, 1176, 1112, 704 cm^{-1}. ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.32, 20.75, 20.96, 21.58, 22.85, 26.90, 28.23, 67.30, 71.15, 73.50, 74.75, 79.07, 87.79, 97.70, 127.51, 127.77, 127.84, 128.43, 129.74, 129.82, 129.94, 132.71, 133.05, 133.24, 135.49, 135.59, 137.59, 144.50, 169.20, 169.71. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (9H, s), 1.60–1.90 (4H, m), 1.80 (3H, s), 2.06 (3H, s), 2.40 (3H, s), 3.53 (2H, s), 3.98 (2H, m), 4.34 (1H, d, 5.2 Hz), 4.50 (1H, d, 11 Hz), 4.57 (1H, d, 11 Hz), 5.33 (1H, d, 5.2 Hz), 6.08 (1H, s), 7.3–7.8 (19H, m). ESI-HRMS: calcd for $C_{42}H_{50}O_{10}SiSNa$ $[M+Na]^+$ 797.2792, found 797.2798.

5′-*O*-(*tert*-Butyldiphenylsilyl)-4′-*C*-(*p*-toluenesulfonyloxypropyl)-3′-*O*-benzyl-2′-*O*-acetyl-5-methyluridine (19). Trimethylsilylated thymine (500 mg, about 2 mmol), which was prepared according to Vorbrüggen's method,[24] was added to a solution of **18** (490 mg, 0.64 mmol) in anhydrous 1,2-dichloroethane (15 mL) at room temperature under a nitrogen atmosphere. TMSOTf (0.2 mL, 1.1 mmol) was added dropwise to the mixture and the mixture was stirred at 50 °C for 3 h. The reaction mixture was quenched with saturated $NaHCO_3$ solution and filtered through Celite. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and then evaporated in vacuo. The residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH = 100:3) to give **19** as a colorless amorphous solid (475 mg, 0.56 mmol, 88%). IR (KBr) ν_{max} 2931, 1748, 1693, 1361, 1228, 1176, 1111, 703 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10 (9H, s), 1.40–1.52 (2H, m), 1.55 (3H, s), 1.72–1.82 (2H, m), 2.08 (3H, s), 2.40 (3H, s), 3.49 (1H, d, 12 Hz), 3.75 (1H, d, 12 Hz), 3.92 (2H, m), 4.37 (1H, d, 6.6 Hz), 4.02 (1H, d, 12 Hz), 4.57 (1H, d, 12 Hz), 5.36 (1H, t, 6.6 Hz), 6.12 (1H, d, 6.6 Hz), 7.3–7.7 (19H, m), 7.71 (1H, d, 2.2 Hz), 8.08 (1H, s). ESI-HRMS: calcd for $C_{45}H_{52}N_2O_{10}SiSNa$ $[M+Na]^+$ 863.3009, found 863.2991.

3′-*O*-Benzyl-2′-*O*,4′-*C*-propylene-5-methyluridine (20). Of 1.0 N NaOH solution, 3 mL was added to a solution of **19** (475 mg, 0.56 mmol) in pyridine (5 mL) at 0 °C and the mixture was stirred at room temperature for 20 min. The reaction mixture was neutralized by dropwise addition of aqueous 20% acetic acid and extracted with CH_2Cl_2. The organic layer was washed with neutral phosphate buffer and brine and evaporated in vacuo. The obtained crude compound was diluted with THF (5 mL) and 1.0 M of tetrabutylammonium fluoride in THF solution was added to this solution. The reaction mixture was stirred for 6 h at room temperature and then partitioned between H_2O and CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$ and then evaporated in vacuo. The residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH = 40:1) to give **20** as a colorless amorphous solid (180 mg, 0.46 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50–1.90 (4H, m), 1.87 (3H, s), 3.70 (1H, d, 12 Hz), 3.80 (1H, d, 12 Hz), 4.05 (1H, m), 4.30 (1H, dt, 1.5 and 11 Hz), 4.37 (1H, d, 5.9 Hz), 4.42 (1H, d, 5.9 Hz), 4.56 (1H, d, 11 Hz), 4.82 (1H, d, 11 Hz), 6.02 (1H, s), 7.3–7.4 (5H, m), 7.72 (1H, d, 1.5 Hz), 8.25 (1H, brs). IR (KBr) ν_{max} 3393, 2956, 1468, 1118 cm^{-1}. FAB-MS (mNBA): 389 $[M+H]^+$. ESI-HRMS (positive): calcd for $C_{20}H_{25}N_2O_6$ $[M+H]^+$ 389.1713, found 389.1714.

2′-*O*,4′-*C*-Propylene-5-methyluridine (21). To a solution of **20** (175 mg, 0.45 mmol) in MeOH (5 mL) was added 20% palladium hydroxide on carbon (105 mg). The mixture was stirred under a hydrogen atmosphere at atmospheric pressure overnight. The reaction mixture was filtered and the filtrate was evaporated in vacuo. The residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH = 10:1) to give **21** as a colorless amorphous solid (81 mg, 0.27 mmol, 59%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.58 (1H, m), 1.71 (1H, m), 1.84 (2H, m), 1.85 (3H, s), 3.36 (1H, s), 3.66 (1H, d, 12 Hz), 3.74 (1H, d, 12 Hz), 3.94 (1H, dt, 3.7 and 11 Hz), 4.11 (1H, d, 5.9 Hz), 4.28 (1H, m), 4.50 (1H, 5.9 Hz), 5.95 (1H, s), 8.28 (1H, s). FAB-MS (mNBA): 299 $[M+H]^+$. ESI-HRMS (positive): calcd for $C_{13}H_{19}N_2O_6$ $[M+H]^+$ 299.1243, found 299.1237.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,4′-*C*-propylene-5-methyluridine (22). The preparation of **22** was carried out according to the same procedure used for **10a** to give **22** as a colorless amorphous solid (quant.). ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.83, 25.03, 31.46, 55.26, 66.10, 68.18, 70.64, 80.89, 86.94, 89.38, 91.37, 110.14, 113.36, 123.81, 127.18, 128.09, 128.13, 130.10, 135.17, 135.19, 135.37, 136.13, 144.22, 149.68, 149.99, 158.72, 158.74, 163.80. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (3H, s), 1.67 (1H, m), 1.76 (1H, m), 1.83 (2H, m), 2.73 (1H, brs), 3.37 (2H, s), 3.79 (6H, s), 4.06 (1H, m), 4.20 (1H, m), 4.30 (1H, d, 6.6 Hz), 4.72 (1H, d, 6.6 Hz), 5.99 (1H, s), 6.84 (4H, m), 7.3–7.7 (9H, m), 8.44 (1H, s), 8.61 (1H, m). IR (KBr) ν_{max} 3403, 2951, 1688, 1509, 1252 cm^{-1}. FAB-MS (mNBA): 601 $[M+H]^+$. ESI-HRMS (positive): calcd for $C_{34}H_{36}N_2O_8Na$ $[M+H]^+$ 623.2369, found 623.2365.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,4′-*C*-propylene-5-methyluridine-3′-*O*-(2-cyanoethyl *N*,*N*-diisopropyl)phosphoramidite (23). The preparation of **23** was carried out

according to the same procedure used for **11a** to give **23** as a colorless amorphous solid (quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (3H, s), 1.22 (3H, s), 1.29 (3H, s), 1.31 (3H, s), 1.78 (3H, s), 1.5-2.4 (6H, m), 2.6-2.8 (2H, m), 3.6–3.8 (4H, m), 3.80 (6H, s), 4.03 (1H, m), 4.13 (1H, m), 4.36 (1H, d, 6.6 Hz), 4.98 (1H, d, 6.6 Hz), 6.04 (1H, s), 6.8–6.9 (4H, m), 7.3-7.5 (9H), 7.91 (1H, d, 1.5 Hz), 8.25 (1H, brs). FAB-MS (mNBA): 801 $[M+H]^+$. ESI-HRMS (positive): calcd for $C_{43}H_{54}N_4O_9P$ $[M+H]^+$ 801.3628, found 801.3648.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-succinyl-2′-*O*,4′-*C*-ethylene thymidine (24c). To a stirred solution of **10c** (580 mg, 0.988 mmol) and DMAP (217 mg, 1.78 mmol) in 10 mL of pyridine was added succinic anhydride (178 mg, 1.78 mmol). After stirring overnight, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (8% MeOH in CH_2Cl_2) to afford **24c** (580 mg, 85%). IR (KBr) ν_{max} 3179, 3064, 2931, 1694, 1509, 1252, 1176 cm^{-1}. ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.78, 28.36, 29.08, 29.11, 55.24, 60.48, 62.87, 66.89, 76.30, 83.42, 85.94, 86,81, 110.27, 113.31, 113.32, 124.11, 125.29, 127.19, 128.06, 128.21, 128.24, 129.02, 130.17, 134.87, 135.04, 135.45, 137.04, 137.84, 143.77, 148.51, 149.69, 158.72, 158.74, 165.15, 171.34, 175.81. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (3H, s), 1.45 (1H, d, 12 Hz), 2.07 (1H, dd, 8.3 and 12 Hz), 2.54 (1H, m), 2.64 (1H, m), 2.75 (2H, m), 3.17 (1H, d, 11 Hz), 3.43 (1H, d, 11 Hz), 3.79 (6H, s), 4.02 (2H, m), 4.55 (1H, d, 3.0 Hz), 5.40 (1H, d, 3.0 Hz), 6.11 (1H, s), 7.16 (4H, m), 7.1–7.4 (9H, m), 7.93 (1H, s), 8.61 (1H, brs). FAB-MS (mNBA): 686 [M–H]. ESI-HRMS (positive): calcd for $C_{37}H_{38}N_2O_{11}Na$ $[M+Na]^+$ 709.2373, found 709.2372.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,4′-*C*-ethylene thymidine-bound controlled pore glass (CPG, 25c). To a solution of **24** (515 mg, 0.75 mmol) in 6 mL of DMF were added 2,3,4,5,6-pentachlorophenol (330 mg, 1.12 mmol) and 1,3-dicyclohexylcarbodiimide (DCC, 231 mg, 1.12 mmol). After stirring for 24 h, insoluble material was removed by filtration. The filtrate was concentrated in vacuo, and benzene was added to the residue. Any insoluble material was removed by repeated-filtration to give the desired ester. Long-chain alkylamino CPG (CPG Inc., 6.0 g, 170 μmol amino group/gram) was suspended in 15 mL of DMF containing 280 μL (2 mmol) of triethylamine, and to this was added pentachlorophenyl ester. After the mixture was left standing for 8 h at 60 °C and for 24 h at room temperature, the CPG was filtered, washed with CH_2Cl_2 and MeOH, and dried in vacuo to afford CPG **25c**. The loaded nucleoside amount, as determined by acid treatment and measurement of the trityl cation (A_{500}; ε = 71,700), was 62 μmol/g. The residual amino groups were capped with acetic anhydride in the presence of 1-methylimidazole in pyridine-THF.

X-Ray crystallographic data

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 195962-195965. Copies of the data can be obtained, free of charge, upon application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK, (fax: +44- (0)1223-336033 or e-mail: deposit @ccdc.cam.ac.uk).

Synthesis of modified oligonucleotides containing 2′-*O*,4′-*C*-ethylene nucleosides or 2′-*O*,4′-*C*-propylene nucleosides. Modified oligonucleotides containing 2′-*O*,4′-*C*-ethylene nucleosides were prepared by solid-phase phosphoramidite chemistry using Applied Biosystems DNA/RNA synthesizer 392. Reagent solutions were purchased from Applied Biosystems. The coupling of 2′-*O*,4′-*C*-ethylene nucleoside-3′-phosphoramidite was performed according to standard synthesis cycles except for an elongation of the coupling time (15 min). After synthesis, the CPGs were treated with concentrated aqueous ammonia at 60 °C for 5 h. The crude products were purified by C18 silica gel column chromatography with a gradient of CH_3CN (Cosmosil 75 C18-OPN, Nacalai Tesque, Japan, 8 × 100 mm, 50 mM triethylammonium bicarbonate (pH 7.5)), treated with 80% acetic acid in H_2O for 20 min, and then purified by reverse phase HPLC with a gradient of CH_3CN (Wakosil DNA, Wako Pure Chemical Industries, Ltd, Japan, 10 × 250 mm, 0.1 M triethylammonium acetate (pH 7.0)). The structures of modified oligonucleotides were determined by negative ion ESI mass spectroscopy. 5′-d(GCGXXXXXXGCT)-3′, X = mT: 2′-*O*,4′-*C*-methylene thymidine, calcd: 3801.47, found: 3801.04, 5′-d(GCGXXXXXXGCT)-3′, X = eT: 2′-*O*,4′-*C*-ethylene thymidine, calcd: 3885.63, found: 3885.26, 5′-d(GCGTTXTTXGCT)-3′, X = eT, calcd: 3717.45, found: 3717.02, 5′-d(GCGTTXTTXGCT)-3′, X = prT: X = 2′-*O*,4′-*C*-propylene thymidine, calcd: 3745.50, found: 3745.05, 5′-d(TTTTTTTTTTXT)-3′, X = mT, calcd: 3616.40, found: 3616.04, 5′ - d(TTTTTTTTTTXT) - 3′, X = eT, calcd: 3630.43, found: 3630.06, 5′ - d(TTTTTTTTTTXT) - 3′, X = prT, calcd: 3644.43, found: 3643.84, 5′-d(TTTTTTTTTTXT)-3′, X = Ts(*S*p): thymidine 3′-phosphorothioate (*S*p isomer), calcd: 3604.43, found: 3603.94, 5′ - d(TTTTTTTTTTXT)-3′, X = Ts(*R*p), thymidine 3′-phos phorothioate (*R*p isomer), calcd: 3604.43, found: 3604.52, 5′ - d(TTTTTTTTTTXT) - 3′, eTs: 2′ - *O*,4′ - *C*-ethylene thymidine 3′-phosphorothioate, calcd: 3646.46, found, 3646.05, 5′-d(TTTTTTTTTTXX)-3′, X = eT, calcd: 3672.43, found: 3672.28.

UV melting analysis

Each modified oligonucleotide and their corresponding RNA or DNA were mixed (final concentration, 4 μM) and dissolved into a buffer containing 10 mM phosphate (pH 7.2) and 100 mM NaCl, heated at 95 °C for 10 min, and then cooled down to room temperature. The melting temperature (T_m) of the complexes was measured with UV spectrometer UV-3100PC (Shimadzu, Japan) equipped with a temperature controller TCC-controller (Shimadzu, Japan).

Measurement of CD spectra of duplexes. The CD spectra of the complexes described above were measured with a JASCO J-500C spectropolarimeter.

Nuclease stability of oligonucleotides

To each solution of 40 μg of oligonucleotides in 1.425 mL of reaction buffer (50 mM Tris–HCl (pH8.0) and 10 mM $MgCl_2$) was added 10.7 μg of snake venom phosphodiesterase (Worthington) in 75 μL of H_2O at 37 °C. Of the reaction mixture, 200 μL was taken at each sampling time point and immediately heated at 90 °C for 4 min to inactivate the enzyme. The reaction mixture was analyzed by reverse phase HPLC. The residual ratio of oligonucleotides to the initial amount was determined from their peak areas ratios.

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