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Synthesis and Properties of *Trans*-3′,4′-Bridged Nucleic Acids Having Typical S-type Sugar Conformation

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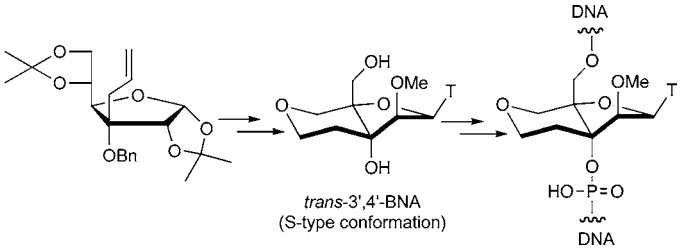

The synthesis of nucleoside analogues with a conformationally restricted sugar moiety is of great interest. The present research describes the synthesis of BNA (bridged nucleic acid) monomers **1** and **2** bearing a 4,7-dioxabicyclo[4.3.0]nonane skeleton and a methoxy group at the C2′ position. Conformational analysis showed that the sugar moiety of these monomers is restricted in a typical S-type conformation. It was difficult to synthesize the phosphoramidite derivative of the ribo-type monomer **1**, while the phosphoramidite of the arabino-type monomer **2** was successfully prepared and incorporated into oligodeoxynucleotides (ODNs). The hybridization ability of the obtained ODN derivatives containing **2** with complementary strands was evaluated by melting temperature (T_m) measurements. As a result, the ODN derivatives hybridized with DNA and RNA complements in a sequence-selective manner, though the stability of the duplexes was lower than that of the corresponding natural DNA/DNA or DNA/RNA duplex.

Introduction

Development of oligonucleotide (ON) analogues that tightly bind with single-stranded (ss) and/or double-stranded (ds) nucleic acid has attracted considerable attention in the fields of medical and molecular biology. Generally, the sugar moiety of nucleosides or ONs has relatively high flexibility and is in equilibrium between several metastable conformations (e.g., N- and S-types). However, the conformational flexibility is restricted in the hybridization process. The sugar moiety in dsRNA predominantly adopts N-type conformation, while that in dsDNA is rather flexible but mainly has S-type conformation (Figure 1a).[1] Conformational restriction of the ONs in a suitable form to hybridize with the target strands is one promising approach to enhance the binding affinity and selectivity of the ONs by means of an entropic gain in the hybridization process.[2,3]

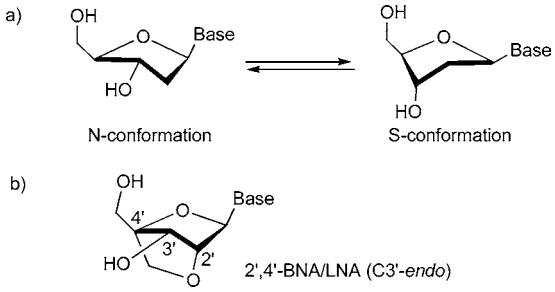

FIGURE 1. (a) Typical sugar puckering of nucleosides, N- and S-type conformation. (b) Structure of 2′,4′-BNA/LNA bearing a locked N-type sugar conformation.

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(1) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
(2) Herdewijn, P. *Liebigs Ann.* **1996**, 1337−1348.
(3) Kool, E. T. *Chem. Rev.* **1997**, *97*, 1473−1488.

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On the basis of this concept, many kinds of ON derivatives with a conformationally restricted sugar moiety have been prepared.[2−10] The 2′-*O*-substitution of RNA is one simple but effective ON modification, where the sugar conformation is induced to be in N-type by a gauche effect between O2′ and O4′ atoms.[6] Oligonucleotide N3′→P5′ phosphoramidates, in which the 3′-oxygen atom is replaced by the less electron-withdrawing nitrogen atom, also have an N-type sugar conformation, due to the reduced gauche effect between N3′ and O4′ atoms.[7] Both ON analogues increase the stability of duplexes formed with the complementary strand, especially with the complementary RNA. Recently, our group[11,12] and Wengel's group[13] independently achieved the synthesis of 2′,4′-BNA/LNA, of which the sugar conformation is exactly locked in N-type by an additional methylene bridge between O2′ and C4′ atoms (Figure 1b).[14] The ON derivatives containing 2′,4′-BNA/LNA monomers showed unprecedentedly high binding affinity with the RNA complement[5,8,10−13] and have been applied in an antisense strategy.[15]

On the other hand, studies on the ON analogues with a DNA-selective binding affinity are of great interest. Wengel and co-workers demonstrated the DNA-selective hybridization of the pyrene-conjugated ON analogue, where the pyrene unit was supposed to recognize the minor-groove structure of dsDNA.[16] Very recently, bipyridyl-functionalized 2′-amino-LNA was employed for high-affinity DNA targeting.[17] On the other hand, restriction of the sugar conformation of ONs within an S-type by an additional bridged structure would be another strategy for DNA-selective hybridization. Several nucleoside analogues having an S-type sugar moiety with a bridged structure have been synthesized and incorporated into ONs (Figure 2a).[18−23] These ON analogues with S-type sugar conformation, however, showed only moderate increase or considerable decrease in the duplex stability, probably due to inappropriate restriction of the sugar conformation or steric repulsion between the additional bridged structure and neighboring nucleotide residues. Recently, our group[24] and Nielsen's group[25] independently reported the synthesis of a novel S-type nucleoside analogue **1** (Figure 2b). Preliminary molecular modeling showed that the additional bridged structure between C3′ and C4′ atoms of **1** was located outside the B-type DNA duplex structure; therefore, it is expected that the bridged structure has no adverse contact with the neighboring residues.

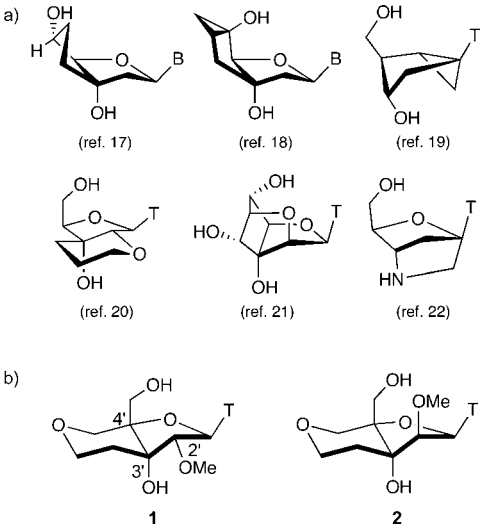


FIGURE 2. (a) Structure of selected bicyclic and tricyclic nucleoside analogues with S-type sugar conformation. (b) Structure of *trans*-3′,4′-BNAs **1** and **2**. B = nucleobases, T = thymin-1-yl.

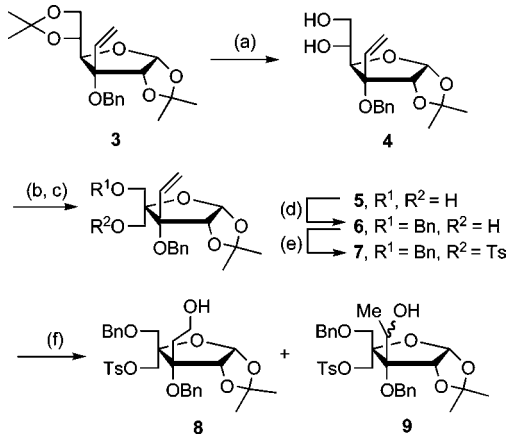
In this paper, we describe in detail the synthesis of nucleoside analogues **1** and **2** (*trans*-3′,4′-BNA-thymine monomers) which have a conformationally restricted S-type sugar moiety with a 4,7-dioxabicyclo[4.3.0]nonane skeleton (Figure 2b). The hybridization properties of the ONs containing **2** are also discussed.

Results and Discussion

For the synthesis of *trans*-3′,4′-BNA monomers **1** and **2**, we chose the known D-allofuranose derivative **3**[26] as the starting material. As shown in Scheme 1, the acetonide group of **3** was removed by treatment with 80% acetic acid at 40 °C to give the diol **4** in 90% yield. Oxidative cleavage of the diol **4** with sodium periodate gave the corresponding aldehyde, which was then employed for an aldol-Canizzaro reaction to afford **5** in

(4) Herdewijn, P. *Biochim. Biophys. Acta* **1999**, *1489*, 167−179.
(5) Imanishi, T.; Obika, S. *J. Synth. Org. Chem., Jpn.* **1999**, *57*, 969−980.
(6) Manoharan, M. *Biochim. Biophys. Acta* **1999**, *1489*, 117−130.
(7) Gryaznov, S. M. *Biochim. Biophys. Acta* **1999**, *1489*, 131−140.
(8) Meldgaard, M.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3539−3554.
(9) Leumann, C. J. *Bioorg. Med. Chem.* **2002**, *10*, 841−854.
(10) Imanishi, T.; Obika, S. *Chem. Commun.* **2002**, 1653−1659.
(11) Obika, S.; Nanbu, D.; Hari, Y.; Morio, K.; In, Y.; Ishida, T.; Imanishi, T. *Tetrahedron Lett.* **1997**, *38*, 8735−8738.
(12) Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.; Morio, K.; Doi, T.; Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401−5404.
(13) Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455−456. Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607−3630.
(14) The similar bridged nucleic acid analogues with N-type sugar conformation were reported by other groups. See: Wang, G.; Girardet, J.-L.; Gunic, E. *Tetrahedron* **1999**, *55*, 7707−7724. Wang, G.; Gunic, E.; Girardet, J.-L.; Stoisavljevic, V. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1147−1150. Morita, K.; Hasegawa, C.; Kaneko, M.; Tsutsumi, S.; Sone, J.; Ishikawa, T.; Imanishi, T.; Koizumi, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 73−76. Morita, K.; Takagi, M.; Hasegawa, C.; Kaneko, M.; Tsutsumi, S.; Sone, J.; Ishikawa, T.; Imanishi, T.; Koizumi, M. *Bioorg. Med. Chem.* **2003**, *11*, 2211−2226.
(15) Vester, B.; Wengel, J. *Biochemistry* **2004**, *43*, 13233−13241. Jepsen, J. S.; Sørensen, M. D.; Wengel, J. *Oligonucleotides* **2004**, *14*, 130−146.
(16) Bryld, T.; Højland, T.; Wengel, J. *Chem. Commun.* **2004**, 1064−1065.
(17) Babu, B. R.; Hrdlicka, P. J.; McKenzie, C. J.; Wengel, J. *Chem. Commun.* **2005**, 1705−1707.
(18) Tarköy, M.; Bolli, M.; Schweizer, B.; Leumann, C. *Helv. Chim. Acta* **1993**, *76*, 481−510. Bolli, M.; Trafelet, H. U.; Leumann, C. *Nucleic Acids Res.* **1996**, *24*, 4660−4667. Bolli, M.; Leumann, C. *Angew. Chem., Int. Ed. Engl.*, **1995**, *34*, 694−696. Bolli, M.; Litten, J. C.; Schütz, R.; Leumann, C. J. *Chem. Biol.* **1996**, *3*, 197−206.
(19) Steffens, R.; Leumann, C. J. *J. Am. Chem. Soc.* **1997**, *119*, 11548−11549. Steffens, R.; Leumann, C. J.; *J. Am. Chem. Soc.* **1999**, *121*, 3249−3255. Renneberg, D.; Leumann, C. J.; *J. Am. Chem. Soc.* **2002**, *124*, 5993−6002.
(20) Altmann, K.-H.; Imwinkelried, R.; Kesselring, R.; Rihs, G. *Tetrahedron Lett.* **1994**, *35*, 7625−7628. Maier, M. A.; Choi, Y.; Gaus, H.; Barchi Jr, J. J.; Marquez, V. E.; Manoharan, M. *Nucleic Acids Res.* **2004**, *32*, 3642−3650.
(21) Nielsen, P.; Pfundheller, H. M.; Olsen, C. E.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3423−3433.
(22) Ravn, J.; Thorup, N.; Nielsen, P. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1855−1861.
(23) Kværnø, L.; Wightman, R. H.; Wengel, J. *J. Org. Chem.* **2001**, *66*, 5106−5112.
(24) Obika, S.; Sekiguchi, M.; Osaki, T.; Shibata, N.; Masaki, M.; Hari, Y,; Imanishi, T. *Tetrahedron Lett.* **2002**, *43*, 4365−4368.
(25) Thomasen, H.; Meldgaard, M.; Freitag, M.; Petersen, M.; Wengel, J.; Nielsen, P. *Chem. Commun.* **2002**, 1888−1889.
(26) Prashad, M.; Fraser-Reid, B. *J. Org. Chem.* **1985**, *50*, 1564−1566.

SCHEME 1 [a]



[a] Reagents, conditions, and yields: (a) 80% AcOH aq, 40 °C, 3 h, 90%; (b) $NaIO_4$, THF−H_2O (1:8), 0 °C, 1 h; (c) 37% HCHO aq, 1 M NaOH aq, THF−H_2O (1:1), rt, 18 h, 82% over two steps; (d) NaH, BnBr, DMF, 0 °C, 1 h, 54%; (e) *p*-TsCl, Et_3N, DMAP, CH_2Cl_2, rt, 20 h, 92%; (f) see Table 1.

TABLE 1. Hydroboration of 7 with Several Borane Reagents[a]

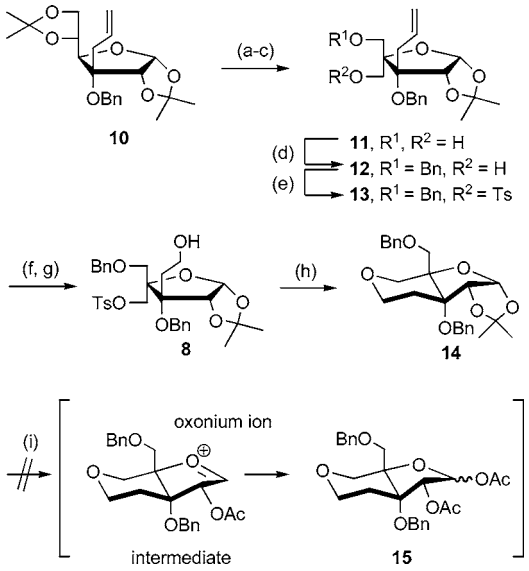
run	reagent	equiv	yield of **8**[b] (%)	yield of **9**[b] (%)
1	borane−THF	3.0	23	58
2	borane−1,4-oxathiane	3.0	25	64
3	thexylborane	1.5	33	
4	thexylborane with 2.0 mol % of $Rh(PPh_3)_3Cl$	1.5	56	16
5	9-BBN	1.5	no reaction	

[a] Every reaction was carried out at 0 °C. [b] Isolated yield.

82% yield. The pro-*R* hydroxymethyl group in **5** was selectively benzylated to give **6** in 54% yield, and tosylation of **6** gave **7** in 92% yield. Next, hydroboration of **7** was carried out with several borane reagents (Table 1). Treatment of **7** with borane−tetrahydrofuran or borane−1,4-oxathiane complex gave the desired terminal alcohol **8** in 23% and 25% yield, respectively (runs 1 and 2). However, unfortunately, the Markovnikov-type adduct **9** was obtained as a major product, probably due to undesired chelation between the borane reagents and oxygen atoms in **7**. To avoid formation of such chelate structure, more bulky borane reagents were used for further evaluation of the reaction (runs 3−5). Although the reaction with 9-borabicyclo-[3.3.1]nonane (9-BBN) did not proceed at all (run 5), the reaction with thexylborane[27] gave the desired compound **8** in moderate yield (run 3). Addition of a catalytic amount of Wilkinson's reagent[28] improved the yield up to 56% (run 4). However, the yield and reproducibility of this hydroboration/oxidation approach were still insufficient.

To overcome this problem, we selected an approach for the synthesis of compound **8** via oxidative cleavage of olefin and subsequent reduction of the corresponding allyl derivative (Scheme 2),[24] instead of the hydroboration/oxidation of the vinyl derivative **7** (Scheme 1). Consequently, the compound **8** was effectively obtained from the known allyl derivative **10**[29] as shown in Scheme 2. Ring-closure reaction of **8** under alkaline conditions successfully proceeded to give the tricyclic compound

SCHEME 2 [a]



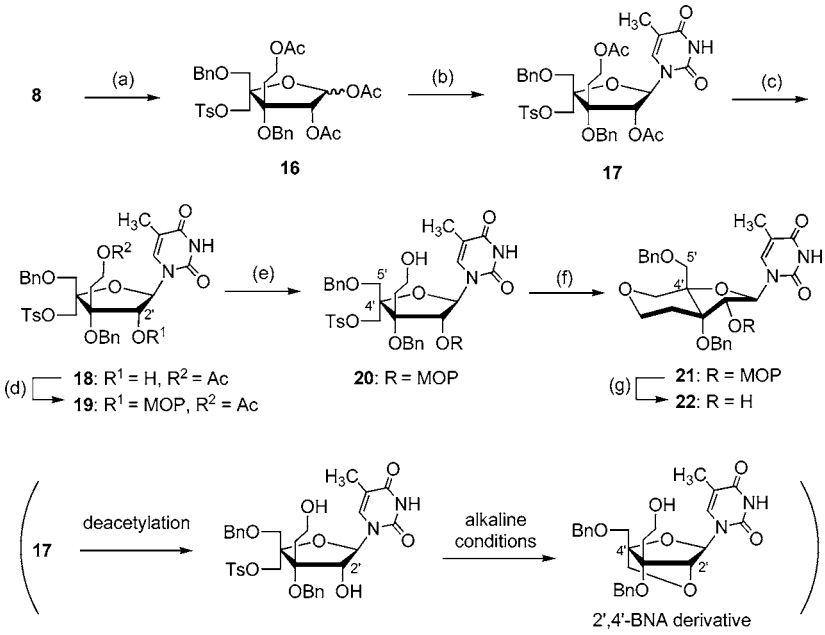
[a] Reagents, conditions, and yields: (a) 80% AcOH aq, 40 °C, 3 h; (b) $NaIO_4$, THF−H_2O (1:1), 0 °C, 45 min; (c) 37% HCHO aq, 1 M NaOH aq, THF−H_2O (1:1), rt, 18 h, 65% over three steps; (d) NaH, BnBr, DMF, 0 °C, 1.5 h, 65%; (e) *p*-TsCl, Et_3N, DMAP, CH_2Cl_2, rt, 19 h, 93%; (f) OsO_4, $NaIO_4$, THF−H_2O (1:1), 0 °C, rt, 2 h; (g) $NaBH_4$, THF−H_2O (1:1), 0 °C, 1 h 63% over two steps. (h) NaHMDS, THF, reflux, 12 h, 86%; (i) concd H_2SO_4 (cat.), AcOH, Ac_2O, rt.

14 in 86% yield. Next, we attempted conversion of **14** to the diacetate **15**, which would be a key intermediate for coupling with several nucleobases. However, reaction of **14** with acetic acid, acetic anhydride and a catalytic amount of sulfonic acid failed to give the desired diacetate **15**. From this result, it was supposed that the additional *trans*-fused six-membered ring severely restricted the conformational flexibility of the furanose ring and prevented the formation of the oxonium intermediate (Scheme 2).

As an alternative approach, we decided to introduce a nucleobase into the sugar moiety before formation of the *trans*-fused bicyclic ring structure (Scheme 3). Acetolysis of **8** gave the corresponding triacetate **16** in 98% yield, which was coupled with thymine nucleobase to afford the *β*-isomer **17** in 95% yield according to Vorbrüggen's procedure. After removal of both acetyl groups in **17**, the obtained diol was treated under alkaline conditions. As a result, the ring-closure reaction from the 2′-hydroxyl group proceeded exclusively and the 2,5-dioxabicyclo-[2.2.1]heptane structure (2′,4′-BNA skeleton) was formed, instead of the desired *trans*-fused 4,7-dioxabicyclo[4.3.0]nonane structure. Therefore, it is necessary to protect the 2′-hydroxyl group before the ring-closure reaction. Since it is known that a 2′-*O*-acyl group in fully acylated ribonucelosides showed high reactivity under controlled conditions,[30] we attempted several reaction conditions. Consequently, regioselective deprotection of the 2′-*O*-acetyl group in **17** was successfully accomplished by using aqueous methylamine to give **18** in 87% yield. Treatment of **18** with 2-methoxypropene gave **19** in 85% yield, and removal of the acetyl group of **19** under alkaline conditions afforded **20** in 87% yield. Ring-closure reaction of **20** by using

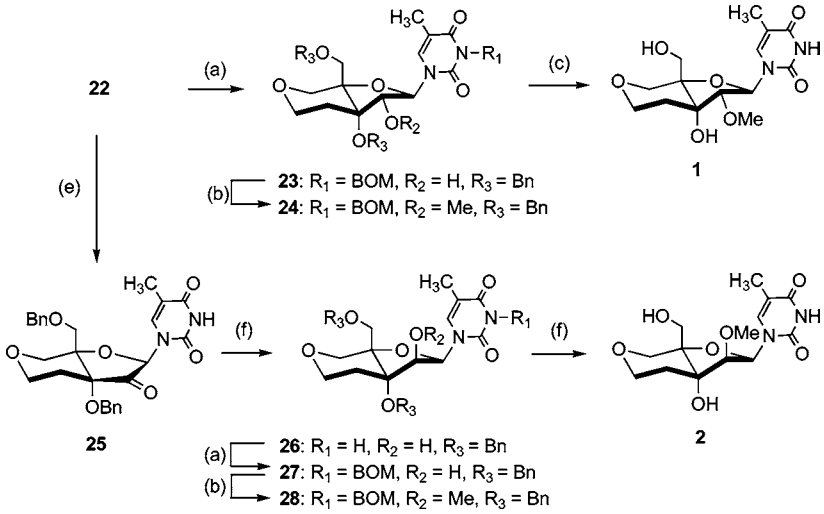
(27) Negishi, E.; Brown, H. C. *Synthesis* **1974**, 77−89.
(28) Evans, D. A.; Fu, G. C.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1992**, *114*, 6671−6679.
(29) Bar, N. C.; Roy, A.; Achari, B.; Mandal, S. B. *J. Org. Chem.* **1997**, *62*, 8948−8951.
(30) Nishino, S.; Takamura, H.; Ishido, Y. *Tetrahedron* **1986**, *42*, 1995−2004.

SCHEME 3 [a]



[a] Reagents, conditions, and yields: (a) Ac_2O, CH_3COOH, concd H_2SO_4 (cat.), rt, 6 h, 98%; (b) thymine, BSA, TMSOTf, $ClCH_2CH_2Cl$, reflux, 19 h, 95%; (c) 40% $MeNH_2$ aq, THF, 0 °C, 87%; (d) 2-methoxypropene, *p*-TsOH·H_2O, CH_2Cl_2, 0 °C, 1.5 h, 85%; (e) 2 M NaOH aq, THF−MeOH (3:1), rt, 1.5 h, 87%; (f) NaHMDS, THF, reflux, 6 h, 73%; (g) *p*-TsOH·H_2O, THF−MeOH (2:1), 0 °C, 1 h, 85%.

SCHEME 4 [a]

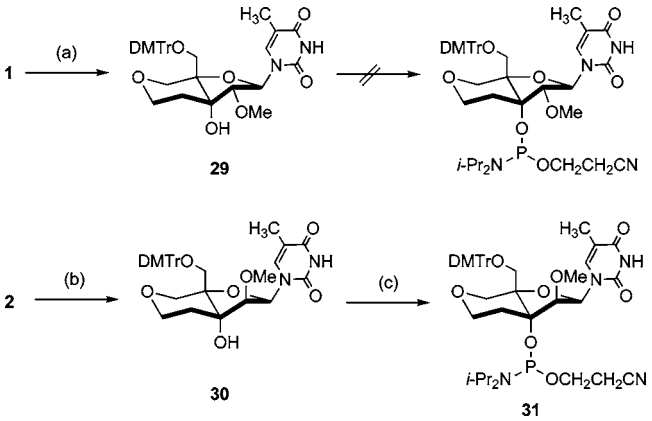


[a] Reagents, conditions, and yields: (a) BOMCl, DBU, DMF, 0 °C, 45 min, 90% for **23**; 3 h, 68% for **27**; (b) NaH, MeI, DMF, 0 °C, 30 min, 86% for **24**; 30 min, 93% for **28**; (c) 20% $Pd(OH)_2$/C, cyclohexene, MeOH, reflux, 7 h, 39%; (d) Dess−Martin periodinane, CH_2Cl_2, rt, 30 min, 97%; (e) DIBAL-H, THF, −78 °C, 5 h, 67%; (f) 20% $Pd(OH)_2$/C, ammonium formate, MeOH, reflux, 7.5 h, 63%.

sodium hexamethyldisilazide (NaHMDS) successfully proceeded to afford **21** in 73% yield. The methoxypropyl (MOP) group in **21** was removed by treatment with *p*-toluenesulfonic acid to give **22** in 85% yield. Thus, we achieved the synthesis of the desired *trans*-3′,4′-BNA skeleton. To incorporate the monomer into ONs, the 2′-hydroxyl group of **22** has to be removed or appropriately protected. At first, 2′-deoxygenation of **22** was attempted by the procedures previously reported.[31−33] However, unfortunately, the desired 2′-deoxy derivative was not obtained, probably due to steric hindrance around the 2′-hydroxyl group. Next, we examined the protection of the 2′-hydroxyl group. As a protecting group we selected a methyl group because of its small size. As shown in Scheme 4, thymine nucleobase of **22** was protected with a benzyloxymethyl (BOM) group to give **23** in 90% yield. The obtained **23** was then treated with sodium hydride and iodomethane in DMF to afford the 2′-*O*-methyl derivative **23** in 86% yield. Deprotection of the BOM group and both 3′- and 5′-*O*-benzyl groups by Pd-mediated hydrogenolysis afforded *trans*-3′,4′-BNA monomer **1** in 39% yield along with its 3′-*O*-benzyl derivative. The low reactivity of the

(31) Robins, M. J.; Wilson, J. S. *J. Am. Chem. Soc.* **1981**, *103*, 932−933.
(32) Pankiewicz, K.; Matsuda, A.; Watanabe, K. A. *J. Org. Chem.* **1982**, *47*, 485−488.
(33) Wang, Z.; Prudhomme, D. R.; Buck, J. R.; Park, M.; Rizzo, C. J. *J. Org. Chem.* **2000**, *65*, 5969−5985.

SCHEME 5 [a]



[a] Reagents, conditions, and yields: (a) DMTrCl, pyridine, 50 °C, 2.5 h, 77%; (b) DMTrOTf, CH_2Cl_2−pyridine, rt, 5 h, 100%; (c) $NC(CH_2)_2OP[N(Pr^i)_2]_2$, dicyanoimidazole, MeCN−THF, 50 °C, 15 h, 55%.

3′-benzyloxy group was probably due to the steric hindrance around the 3′-position that involves 1,3-diaxial interaction with the hydrogens on the *trans*-fused six-membered ring and gauche interaction with the 2′-methoxy group. ^{1}H NMR and X-ray crystallographic analysis revealed that **1** has a typical S-type (C3′-*exo*) conformation and the torsion angle δ (C5′−C4′−C3′−O3′) of 174.6°.[24] The torsion angle χ was −125.8°, indicating anti orientation of the nucleobase moiety.[24] Next, the arabino-type derivative **2** was prepared as shown in Scheme 4. The 2′-hydroxyl group of **22** was oxidized with Dess−Martin periodinane to give the 2′-oxo derivative **25** in 97% yield. Reduction of the obtained ketone **25** with sodium borohydride showed no significant stereoselectivety (data not shown). After several attempts with various reducing reagents, it was found that the reduction with diisobutyl aluminum hydride (DIBAL-H) gave the desired arabino-type product **26** stereoselectively. The stereochemistry at the C2′ position was confirmed by comparison of the coupling constants between H1′ and H2′ ($^3J_{H1'-H2'}$), which was changed from 7 Hz (for **22**) to 4 Hz (for **26**). Protection of the thymine nucleobase with the BOM group, methylation of the 2′-hydroxyl group with sodium hydride and iodomethane and Pd-mediated hydrogenolysis afforded the desired compund **2** in 40% yield over three steps. Reactivity of the 3′-benzyloxy group of the arabino-type compound **28** was improved compared with that of the ribo-type compound **24**.

To incorporate the *trans*-3′,4′-BNA monomers **1** and **2** into oligodeoxynucleotides (ODNs), we attempted to synthesize the corresponding phosphoramidite derivatives (Scheme 5). The 5′-*O*-dimethoxytrityl derivative **29** was obtained in 77% yield by treatment of **1** with dimethoxytrityl chloride in pyridine. However, the following phosphitylation of **29** did not proceed at all, even if the reaction temperature was elevated up to 60 °C. We tried some other phosphitylation conditions; however, the desired phosphoramidite derivative was not obtained at all. On the other hand, the *trans*-3′,4′-BNA arabino-type monomer **2** was successfully converted to the corresponding phosphoramidite compound. The dimethoxytritylation at the 5′-*C*-hydroxyl group of **2** was successfully accomplished by treating with 4,4′-dimethoxytrityl triflate (DMT-OTf)[34] to give **30** in quantitative yield. Phosphitylation of **30** proceeded at 50 °C to give the corresponding phosphoramidite **31** in 55% yield.

(34) Tarköy, M.; Bolli, M.; Leumann, C. *Helv. Chim. Acta* **1994**, *77*, 716−744.

TABLE 2. Melting Temperatures (°C) of the Duplexes between ODN1−ODN4 and Their Complementary Strands[a]

sequence of ODNs (5′→3′)	target strands: DNA complement	target strands: RNA complement
d(GCGTTTTTTGCT) (**ODN1**)	50	45
d(GCGTT**T**TTTGCT) (**ODN2**)	36	33
d(GCG**T**TTTTTGCT) (**ODN3**)	37	35
d(GCGTTTTT**T**GCT) (**ODN4**)	38	36

[a] The UV melting profiles were recorded in 10 mm sodium phosphate buffer (pH 7.2) containing 100 mm NaCl at a scan rate of 0.5 °C/min with detection at 260 nm. The final concentration of each ODN was 4.0 μM. The *trans*-3′,4′-BNA arabino-type monomer is shown in bold.

TABLE 3. Melting Temperatures (°C) of the Duplexes between ODN1 or ODN2 and Their Complementary DNA Strands with or without One Base Mismatch[a]

ODN	target sequence: 3′−d(CGCAA**N**AAACGA)-5′: **N** = A	G	C	T
ODN1	50	41 (−9)[b]	37 (−13)[b]	38 (−12)[b]
ODN2	36	25 (−11)[b]	20 (−16)[b]	23 (−13)[b]

[a] Conditions for melting temperature measurements are shown in footnote of Table 2 and in the Experimental Section. [b] The values in parentheses are differences in T_m values between mismatched and matched duplexes.

Incorporation of the phosphoramidite **31** into 12-mer ODNs **ODN2**−**ODN4** was attempted by the usual phosphoramidite protocol on an automated DNA synthesizer. However, the coupling efficiency of phosphoramidite **31** was quite low under standard coupling conditions. After several examinations of the coupling conditions, the ODNs containing the *trans*-3′,4′-BNA arabino-type monomer were synthesized with prolonged coupling time (24 h) and elevated coupling temperature (50 °C). The coupling efficiency, estimated from the absorption of the released trityl cation, was improved up to 40%. After deblocking and cleavage from the solid support by using aqueous ammonia at 55 °C, the resultant ODNs were purified by reversed-phase HPLC. The composition of ODNs (**ODN2**−**ODN4**) was confirmed by MALDI-TOF MS analysis.

The hybridization properties of **ODN2**−**ODN4** toward their complementary strands were evaluated by UV melting experiments (Table 2). The melting temperatures (T_m's) of the duplexes between natural ODN (**ODN1**) and its DNA and RNA complements were 50 and 45 °C, respectively. All three modified ODNs (**ODN2**−**ODN4**) exhibited a decrease in T_m value by −11 to −14 °C when they form duplexes with the DNA complement. A similar decrease in T_m value ($\Delta T_m = -9$ to −12 °C) was observed when targeting the RNA complement. Thus, incorporation of the *trans*-3′,4′-BNA arabino-type monomer into ODNs decreases the duplex stability, regardless of the position of the modification and the type of the target nucleic acid (DNA or RNA). To investigate in detail the duplex-forming properties of the *trans*-3′,4′-BNA-modified ODNs, we examined the sequence selectivity of **ODN1** and **ODN2** by means of T_m measurement with the target DNA sequences containing one base mismatch (Table 3). The T_m values of the mismatched duplexes containing natural ODN (**ODN1**) decreased by −9 °C to −13 °C, compared to that of the full-matched duplex. In the case of *trans*-3′,4′-BNA modified ODN (**ODN2**), the differences in T_m values between matched and mismatched duplexes are larger than those observed for the duplexes containing **ODN1**,

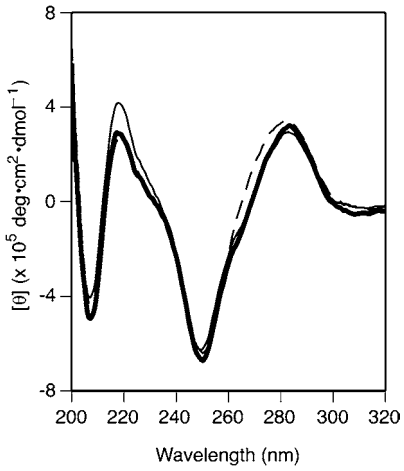


FIGURE 3. CD spectra of the DNA duplexes between **ODN1** and full-matched strand (thin line), **ODN1** and one base mismatched strand (dashed line), and **ODN2** and full-matched strand (thick line). The spectra were recorded in 10 mm sodium phosphate buffer (pH 7.2) containing 100 mm NaCl at 10 °C. Concentration of each strand was 4 μm.

indicating that the *trans*-3′,4′-BNA-T moiety in **ODN2** recognized the corresponding adenine nucleobase in the complementary strand. To investigate the helical structure of the duplex containing *trans*-3′,4′-BNA, we measured the CD spectrum of the DNA duplex containing **ODN2** at 10 °C. The spectrum is shown in Figure 3 along with those of the natural DNA duplexes between **ODN1** and its complementary sequences with or without one base mismatch. The CD spectrum of the duplex containing **ODN2** was quite similar to those of the matched or mismatched natural DNA duplex. All spectra showed a large positive Cotton band around 280 nm and large negative Cotton band around 250 nm, indicating a typical B-type helix formation. This result means that the modification of ODNs with one *trans*-3′,4′-BNA unit does not affect the overall helical structure of the DNA duplex, though the thermal stability of the duplexes decreased significantly compared with that of the natural DNA duplexes. The conformational change of the *trans*-3′,4′-BNA-modified duplex is limited to a local one as well as a mismatched DNA duplex.

The sugar puckering of the *trans*-3′,4′-BNA monomers was locked in C3′-exo conformation, which is predominantly observed in DNA duplexes with B-type helical structure, and the nucleobase orientation of *trans*-3′,4′-BNA monomers was found to be anti that is favorable to Watson−Crick base pair formation.[24] However, modification of ODNs with the *trans*-3′,4′-BNA arabino-type monomer **2** failed to stabilize the duplex. One possibility to account for the destabilization of duplexes by the *trans*-3′,4′-BNA modification would be the effect of the 2′-*O*-methyl group. It was previously reported that the ODNs containing 2′-*O*-methylarabinonucleoside exhibited a sharp decrease in thermal stability of the duplexes with their DNA complements (ΔT_m/modification = −4 °C to −6 °C).[35] In the case of *trans*-3′,4′-BNA modification, a larger decrease in the T_m value was observed (ΔT_m/modification = −12 °C to −14 °C). The sugar puckering of **2** was restricted in the C3′-exo conformation with the arabino-configured 2′-*O*-methyl group in an axial position. Therefore, the steric effect of the 2′-*O*-methyl group of **2** might be more significant than that of the conformationally unrestricted 2′-*O*-methylarabinonucleoside. Another possibility to explain the destabilization would be the large δ values (δ = 174.6°) of *trans*-3′,4′-BNA monomers,[24] which deviated from the mean δ value of one typical B-type DNA duplex, Dickerson's dodecamer ($\delta = 123 \pm 21°$).[36] This difference in δ value might cause an extension of the helical pitch and destabilization of the duplex structure. Alternatively, the pattern of hydration, stabilizing the DNA helical structure,[37] might be affected by the additional hydrophobic six-membered ring in the *trans*-3′,4′-BNA monomer. To investigate the origin of the decreased thermal stability in detail and develop a conformationlly restricted nucleic acids with enhanced binding affinity toward DNA complements, further chemical modification of the *trans*-3′,4′-BNA is now in progress in our laboratory.

Conclusion

In this study, we have developed the nucleoside analogues, *trans*-3′,4′-BNAs **1** and **2**, bearing a *trans*-fused additional six-membered ring for restriction of the sugar puckering into S-type conformation. Conformational analysis revealed that these nucleoside analogues have a C3′-exo sugar pucker, a typical S-type conformation. The arabino-type analogue **2** was successfully incorporated into ODNs. The corresponding ODN derivatives containing **2** showed sequence-selective binding with the complementary strands, though the binding affinity was lower than that of the natural ODNs. We believe that the present results will be useful for further development of the nucleic acid analogues with S-type sugar conformation.

Experimental Section

3-*O*-Benzyl-1,2-*O*-isopropylidene-3-*C*-vinyl-α-D-allofuranose (4). A solution of compound **3**[26] (990 mg, 2.63 mmol) in 80% aqueous acetic acid (15 mL) was stirred at 40 °C for 3 h. After neutralization with 10 M aqueous sodium hydroxide, the reaction mixture was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 3:2) to give compound **4** as a colorless oil (793 mg, 90%). $[\alpha]^{27}_D$: +68.6 (*c* 0.76, $CHCl_3$). IR ν_{max} (KBr): 3432, 2934, 1638, 1455, 1380 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.39 (3H, s), 1.63 (3H, s), 2.17 (1H, t, J = 6 Hz), 2.67 (1H, t, J = 2 Hz), 3.63−3.82 (3H, m), 4.15 (1H, d, J = 8 Hz), 4.61, 4.70 (2H, ABq, J = 11 Hz), 4.66 (1H, d, J = 4 Hz), 5.38 (1H, d, J = 18 Hz), 5.55 (1H, d, J = 11 Hz), 5.82 (1H, d, J = 4 Hz), 5.94 (1H, dd, J = 11, 18 Hz), 7.26−7.38 (5H, m). ^{13}C NMR ($CDCl_3$): δ 26.7, 26.9, 64.2, 67.9, 70.2, 79.6, 80.9, 104.5, 118.9, 127.5, 127.7, 128.3, 134.2, 137.5. Mass (FAB): *m*/*z* 337 $(M + H)^+$. Anal. Calcd for $C_{18}H_{24}O_6 \cdot {}^1/_4H_2O$: C, 63.42; H, 7.24. Found: C, 63.35; H, 7.10.

3-*O*-Benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-3-*C*-vinyl-α-D-ribofuranose (5). To a stirred solution of compound **4** (682 mg, 2.03 mmol) in THF−H_2O (1:8, 9.0 mL) was added $NaIO_4$ (564 mg, 2.64 mmol) at 0 °C. After the mixture was stirred for 45 min, ethylene glycol (0.2 mL) was added and stirred for more 15 min. The reaction mixture was filtered and extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue (640 mg) was dissolved in THF−H_2O (1:1, 12 mL), and aqueous 37% formaldehyde (0.8 mL, 10.5 mmol) and 1 M

(35) Gotfredsen, C. H.; Jacobsen, J. P.; Wengel, J. *Tetrahedron Lett.* **1994**, *35*, 6941−6944.

(36) Dickerson, R. E.; Drew, H. R. *J. Mol. Biol.* **1981**, *149*, 761−786. Drew, H. R.; Wing, R. M.; Takano, T.; Broka, C.; Tanaka, S.; Itakura, K.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2179−2183.

(37) Drew, H. R.; Dickerson, R. E. *J. Mol. Biol.* **1981**, *151*, 535−556.

aqueous sodium hydroxide (4.2 mL, 4.2 mmol) were added. The whole was stirred at room temperature for 18 h. After neutralization with diluted hydrochloric solution, the reaction mixture was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:1) to give compound **5** as a colorless oil (562 mg, 82% over two steps). $[\alpha]^{27}_D$: +10.9 (*c* 0.92, $CHCl_3$). IR ν_{max} (KBr): 3420, 2985, 1637, 1457, 1381 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.37 (3H, s), 1.67 (3H, s), 2.48 (1H, brs), 2.59 (1H, brs), 3.67, 3.75 (2H, ABq, J = 12 Hz), 4.07, 4.33 (2H, ABq, J = 12 Hz), 4.57, 4.71 (2H, ABq, J = 11 Hz), 4.73 (1H, d, J = 4 Hz), 5.32 (1H, d, J = 18 Hz), 5.50 (1H, d, J = 11 Hz), 5.88 (1H, d, J = 4 Hz), 5.93 (1H, dd, J = 11, 18 Hz), 7.25−7.36 (5H, m). ^{13}C NMR ($CDCl_3$): δ 25.9, 26.4, 63.8, 65.5, 67.7, 82.22, 87.0, 87.6, 104.6, 113.1, 117.5, 127.3, 127.7, 128.4, 135.5, 137.8. Mass (FAB): *m*/*z* 337 $(M + H)^+$. Anal. Calcd for $C_{18}H_{24}O_6 \cdot {}^1/_5H_2O$: C, 63.59; H, 7.23. Found: C, 63.59; H, 7.10.

3,5-Di-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-3-*C*-vinyl-α-D-ribofuranose (6). To a suspension of 60% sodium hydride (75 mg, 1.89 mmol) in anhydrous DMF (3.0 mL) was added compound **5** (455 mg, 1.35 mmol) in anhydrous DMF (7.0 mL) at 0 °C and the mixture stirred for 30 min. Benzyl bromide (0.17 mL, 1.42 mmol) was added and the mixture stirred at room temperature for 1.5 h. Methanol was added to the mixture and evaporated to dryness under reduced pressure. The residue was dissolved in ether, washed with H_2O and saturated aqueous NaCl, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:3) to give compound **6** as a colorless oil (314 mg, 54%). $[\alpha]^{26}_D$: +26.4 (*c* 1.12, $CHCl_3$). IR ν_{max} (KBr): 3560, 2938, 1605, 1455, 1379 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.36 (3H, s), 1.67 (3H, s), 3.54 (2H, s), 4.14, 4.23 (2H, ABq, J = 12 Hz), 4.48, 4.57 (2H, ABq, J = 12 Hz), 4.56, 4.71 (2H, ABq, J = 12 Hz), 4.70 (1H, d, J = 4 Hz), 5.26 (1H, d, J = 18 Hz), 5.40 (1H, d, J = 11 Hz), 5.84 (1H, dd, J = 11, 18 Hz), 5.89 (1H, d, J = 4 Hz), 7.22−7.36 (10H, m). ^{13}C NMR ($CDCl_3$): δ 26.1, 26.5, 62.1, 67.4, 71.2, 73.5, 82.5, 87.1, 88.5, 104.7, 113.1, 117.3, 127.0, 127.3, 127.3, 127.4, 128.1, 128.2, 135.8, 138.2, 138.3. Mass (FAB): *m*/*z* 427 $(M + H)^+$. Anal. Calcd for $C_{25}H_{30}O_6 \cdot {}^1/_5H_2O$: C, 69.81; H, 7.12. Found: C, 69.80; H, 7.10.

3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-4-*C*-(*p*-toluenesulfonyloxymethyl)-3-*C*-vinyl-α-D-ribofuranose (7). To a solution of compound **6** (510 mg, 1.2 mmol) in anhydrous CH_2Cl_2 (10 mL) were added Et_3N (5.0 mL, 36 mmol), *p*-TsCl (343 mg, 1.8 mmol), and DMAP (73 mg, 0.60 mmol) at room temperature, and the mixture was stirred for 12 h. Saturated aqueous $NaHCO_3$ was added, and the mixture was stirred for a further 30 min and then extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:3) to give compound **7** as a light yellow solid (639 mg, 92%). Mp: 82−84 °C. $[\alpha]^{28}_D$: +8.0 (*c* 1.06, $CHCl_3$). IR ν_{max} (KBr): 2933, 1361, 1178 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.32 (3H, s), 1.46 (3H, s), 2.31 (3H, s), 3.32, 3.57 (2H, ABq, J = 9 Hz), 4.32 (2H, s), 4.69 (1H, d, J = 4 Hz), 4.60, 4.76 (2H, ABq, J = 11 Hz), 4.52, 4.64 (2H, ABq, J = 10 Hz), 5.25 (1H, d, J = 18 Hz), 5.39 (1H, d, J = 11 Hz), 5.81 (1H, d, J = 4 Hz), 5.89 (1H, dd, J = 11, 18 Hz), 7.14−7.30 (12H, m), 7.75 (2H, d, J = 9 Hz). ^{13}C NMR ($CDCl_3$): δ 21.6, 26.1, 26.1, 67.0, 68.9, 69.1, 73.1, 82.3, 86.8, 86.9, 104.6, 113, 118.3, 125.6, 127.0, 127.0, 127.2, 128.0, 128.0, 129.4, 133.1, 135.7, 138.0, 138.5, 144.1. Mass (FAB): *m*/*z* 603 $(M + Na)^+$. Anal. Calcd for $C_{30}H_{36}O_8S$: C, 66.19; H, 6,25; S, 5.52. Found: C, 66.10; H, 6.29; S, 5.29.

3,5-Di-*O*-benzyl-3-*C*-hydroxyethyl-1,2-*O*-isopropylidene-4-*C*-(*p*-toluenesulfonyloxymethyl)-α-D-ribofuranose (8). Method A (via Hydroboration/Oxidation Approach). To a stirred solution of compound **7** (590 mg, 1.02 mmol) and chlorotris(triphenylphosphine)rhodium (I) (18 mg, 0.02 mmol) in anhydrous THF (15 mL) was added dropwise thexylborane (0.42 M in THF, 3.6 mL, 1.52 mmol) at 0 °C, and the whole was stirred at room temperature for 2 h. A mixture of 1 M aqueous sodium hydroxide (1.6 mL) and 30% hydrogen peroxide (0.6 mL) was added, and the whole was heated under reflux for 1.5 h. After filtration through Celite, the mixture was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:1) to give compound **8** as a colorless oil (340 mg, 56%).

Method B (via Oxidative Cleavage of Allyl Group). To a stirred solution of compound **13** (1.2 g, 2.0 mmol) in THF−H_2O (1:1, 12 mL) were added $NaIO_4$ (1.3 g, 6.0 mmol) and OsO_4 (0.07 M in *tert*-butyl alcohol, 0.28 mL, 0.02 mmol), and the mixture was stirred at room temperature for 2 h. After filtration, the filtrate was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in THF−H_2O (1:1, 12 mL) and sodium tetrahydroborate (75 mg, 2.0 mmol) was added at 0 °C. After the mixture was stirred for 1 h at the same temperature, acetone was added, and the mixture was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:1) to give compound **8** as a colorless oil (754 mg, 63%). $[\alpha]^{21}_D$: +15.4 (*c* 0.83, $CHCl_3$). IR ν_{max} (KBr): 3490, 2967, 1599, 1354 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.28 (3H, s), 1.41 (3H, s), 1.91 (1H, dt, J = 14, 7 Hz), 2.22 (1H, dt, J = 15, 6 Hz), 2.31 (3H, s), 3.38, 3.66 (2H, ABq, J = 10 Hz), 3.75 (1H, dd, J = 7, 11 Hz), 3.79 (1H, dd, J = 6, 11 Hz), 4.35, 4.79 (2H, ABq, J = 10 Hz), 4.39, 4.42 (2H, ABq, J = 11 Hz), 4.58, 4.69 (2H, ABq, J = 12 Hz), 4.66 (1H, d, J = 4 Hz), 5.71 (1H, d, J = 4 Hz), 7.14−7.35 (12H, m), 7.69 (2H, d, J = 8 Hz). ^{13}C NMR ($CDCl_3$): δ 21.6, 25.9, 25.9, 33.8, 57.8, 66.4, 67.3, 69.1, 73.6, 83.8, 85.9, 86.7, 103.3, 113.2, 126.5, 127.1, 127.5, 127.6, 127.9, 128.1, 128.2, 129.5, 132.7, 137.3, 138.1, 144.3. Mass (FAB): *m*/*z* 621 $(M + Na)^+$. Anal. Calcd for $C_{32}H_{38}O_9S \cdot {}^1/_4H_2O$: C, 63.72; H, 6.43; S, 5.36. Found: C, 63.79; H, 6.43; S, 5.13.

3-*C*-Allyl-3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose (11). A solution of compound **10**[29] (4.9 g, 13 mmol) in 80% aqueous acetic acid (85 mL) was stirred at 40 °C for 3 h. After neutralization with 10 M aqueous sodium hydroxide, the reaction mixture was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue (4.5 g) was dissolved in THF−H_2O (1:1, 60 mL), and sodium periodate (3.6 g, 17 mmol) was added at 0 °C. The mixture was stirred at the same temperature for 45 min. Ethylene glycol (1.0 mL) was added and stirred for further 15 min. After being filtered, the solution was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in THF−H_2O (1:1, 60 mL), and aqueous 37% formaldehyde (4.7 mL, 63 mmol) and 1 M aqueous sodium hydroxide (25 mL, 25 mmol) were added. The whole was stirred at room temperature for 18 h. After neutralization with diluted hydrochloric solution, the reaction mixture was extracted with EtOAc. The organic layer was washed with H_2O and saturated NaCl solution, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:1) to give compound **11** as a colorless oil (2.9 g, 65% over three steps). $[\alpha]^{26}_D$: −6.4 (*c* 0.82, $CHCl_3$). IR ν_{max} (KBr): 3415, 2942, 1637, 1381, 1211, 1019 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.33 (3H, s), 1.65 (3H, s), 2.45 (1H, d, J = 7 Hz), 2.50 (1H, d, J = 7 Hz), 2.65 (1H, ddt, J = 6, 15, 2 Hz), 2.76 (1H, dd, J = 8, 15 Hz), 3.86, 3.90 (2H, ABX, J = 12, 6 Hz), 4.12, 4.17 (2H, ABX, J = 12, 7 Hz), 4.54 (1H, d, J = 4 Hz), 4.65, 4.72 (2H, ABq, J = 10 Hz), 5.20−5.30 (2H, m), 5.72 (1H, d, J = 4 Hz), 5.88−6.04 (1H, m), 7.28−7.39

(5H, m). ^{13}C NMR ($CDCl_3$): δ 25.8, 26.3, 37.0, 63.6, 64.0, 67.3, 83.1, 86.0, 87.4, 104.1, 112.6, 119.6, 127.4, 127.7, 128.3, 131.8, 137.9. Mass (FAB): *m*/*z* 373 $(M + Na)^+$. HRMS (FAB): calcd for $C_{19}H_{26}O_6$ $(M + H)^+$ 373.1627, found 373.1613. Anal. Calcd for $C_{18}H_{24}O_6 \cdot {}^1/_{10}H_2O$: C, 64.79; H, 7.50. Found: C, 64.52; H, 7.48.

3-*C*-Allyl-3,5-di-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose (12). To a suspension of 60% sodium hydride (2.83 g, 70.8 mmol) in anhydrous DMF (250 mL) was added dropwise compound **11** (17.8 g, 50.5 mmol) in anhydrous DMF (250 mL) at 0 °C. After the solution was stirred for 2 h, benzyl bromide (6.24 mL, 53.1 mmol) was added, and the mixture was stirred at room temperature for 1.5 h. MeOH was added, evaporated to dryness under reduced pressure, and the residue was extracted with ether and H_2O. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over $MgSO_4$, and concentrated under reduced pressure. The resultant residue was purified by flash silica gel column chromatography (EtOAc/$CHCl_3$ = 1:30) to give compound **12** as a colorless oil (14.5 g, 65%). $[\alpha]^{25}_D$: +14.2 (*c* 0.82, $CHCl_3$). IR ν_{max} (KBr): 3564, 2936, 1637, 1380, 1106 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.31 (3H, s), 1.63 (3H, s), 2.24 (1H, dd, *J* = 5, 9 Hz), 2.49 (1H, dd, *J* = 6, 15 Hz), 2.79 (1H, dd, *J* = 7, 15 Hz), 3.57, 3.71 (2H, ABq, *J* = 10 Hz), 3.91 (1H, dd, *J* = 9, 12 Hz), 4.29 (1H, dd, *J* = 5, 12 Hz), 4.53 (1H, d, *J* = 4 Hz), 4.54, 4.60 (2H, ABq, *J* = 12 Hz), 4.66, 4.71 (2H, ABq, *J* = 11 Hz), 5.14−5.19 (2H, m), 5.71 (1H, d, *J* = 4 Hz), 5.84−5.99 (1H, m), 7.28−7.33 (10H, m). ^{13}C NMR ($CDCl_3$): δ 25.9, 26.4, 36.4, 61.6, 67.8, 68.8, 73.7, 83.4, 85.5, 88.4, 103.7, 112.7, 119.2, 127.0, 127.2, 127.5, 127.6, 128.2, 132.2, 137.8, 138.3. Mass (FAB): *m*/*z* 447 $(M+Li)^+$. HRMS (FAB): calcd for $C_{26}H_{32}O_6Na$ $(M + Na)^+$ 463.2097, found 463.2093.

3-*C*-Allyl-3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-4-*C*-(*p*-toluenesulfonyloxymethyl)-α-D-ribofuranose (13). To a solution of compound **12** (14.5 g, 30.0 mmol) in anhydrous CH_2Cl_2 (280 mL) were added Et_3N (18.4 mL, 132 mmol), *p*-TsCl (9.42 g, 49.4 mmol), and DMAP (2.02 g, 16.5 mmol) at room temperature, and the mixture was stirred for 19 h. Saturated aqueous $NaHCO_3$ was added, and the reaction mixture was stirred for a further 30 min and extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:4) to give compound **13** as a white solid (18.3 g, 93%). Mp: 115−116 °C. $[\alpha]^{25}_D$: +17.2 (*c* 0.89, $CHCl_3$). IR ν_{max} (KBr): 2936, 1361, 1177 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.27 (3H, s), 1.40 (3H, s), 2.31 (3H, s), 2.44 (1H, dd, *J* = 7, 15 Hz), 2.76 (1H, dd, *J* = 7, 15 Hz), 3.42, 3.65 (2H, ABq, *J* = 10 Hz), 4.37, 4.76 (2H, ABq, *J* = 10 Hz), 4.38, 4.41 (2H, ABq, *J* = 12 Hz), 4.50 (1H, d, *J* = 4 Hz), 4.59, 4.70 (2H, ABq, *J* = 12 Hz), 5.10−5.16 (2H, m), 5.65 (1H, d, *J* = 4 Hz), 5.76−5.92 (1H, m), 7.12−7.31 (12H, m), 7.69 (2H, d, *J* = 8 Hz). ^{13}C NMR ($CDCl_3$): δ 21.6, 25.9, 26.0, 35.8, 66.3, 67.2, 69.2, 73.4, 83.5, 85.5, 86.7, 103.5, 113.0, 119.4, 126.6, 127.0, 127.4, 127.5, 128.0, 128.1, 128.2, 129.5, 131.7, 132.9, 137.5, 138.3, 144.2. Mass (FAB): *m*/*z* 617 $(M + Na)^+$. Anal. Calcd for $C_{33}H_{38}O_8S$: C, 66.65; H, 6,44; S, 5.39. Found: C, 66.49; H, 6.43; S, 5.31.

(1*R*,6*S*,7*R*,8*R*)-6-Benzyloxy-1-benzyloxymethyl-7,8-*O*-isopropyridene-3,9-dioxabicyclo[4.3.0]nonane (14). To a solution of compound **8** (182 mg, 0.30 mmol) in anhydrous THF (8.0 mL) was added NaHMDS (1.0 M in THF, 1.2 mL, 1.2 mmol), and the mixture was heated under reflux for 12 h. Saturated aqueous $NaHCO_3$ was added, and the solution was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure to give compound **14** as a white solid (110 mg, 86%). Mp: 105−106 °C. $[\alpha]^{22}_D$: +4.9 (*c* 0.70, $CHCl_3$). IR ν_{max} (KBr): 2943, 1456, 1377, 1210 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.32 (3H, s), 1.35 (3H, s), 1.83−1.97 (1H, m), 2.36−2.41 (1H, m), 3.53, 4.53 (2H, ABq, *J* = 10 Hz), 3.56, (1H, dt, *J* = 12, 3 Hz), 3.70, 4.03 (2H, ABq, *J* = 11 Hz), 3.66−3.74 (1H, m), 4.51, 4.57 (2H, ABq, *J* = 12 Hz), 4.63, 5.07 (2H, ABq, *J* = 12 Hz), 4.99 (1H, d, *J* = 5 Hz), 5.99 (1H, d, *J* = 5 Hz), 7.19−7.41 (10H, m). ^{13}C NMR ($CDCl_3$): δ 26.3, 27.2, 28.4, 62.4, 65.3, 66.4, 73.8, 74.7, 77.9, 85.7, 88.0, 105.2, 115.6, 126.3, 126.8, 127.5, 127.8, 128.1, 128.4, 137.6, 139.0. Mass (EI): *m*/*z* 91 (Bn^+, 100), 426 (M^+, 0.2). Anal. Calcd for $C_{25}H_{30}O_6 \cdot {}^1/_3H_2O$: C, 69.43; H, 7.15. Found: C, 69.38; H, 7.02.

3-*C*-Acetoxyethyl-1,2-di-*O*-acetyl-3,5-di-*O*-benzyl-4-*C*-(*p*-toluenesulfonyloxymethyl)-D-ribofuranose (16). To a stirred solution of compound **8** (140 mg, 0.23 mmol) in acetic acid (1.0 mL) were added acetic anhydrate (0.14 mL, 1.5 mmol) and concd H_2SO_4 (1 drop) at room temperature. After being stirred for 6 h, the solution was neutralized with $NaHCO_3$, and the mixture was extracted with EtOAc and H_2O. The organic layer was washed with saturated aqueous $NaHCO_3$, H_2O, and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:2) to give anomeric mixture **16** (α:β = ca. 1:3) as a colorless oil (154 mg, 98%). ^{1}H NMR ($CDCl_3$): δ 1.91 (3/4H, s), 1.93 (9/4H, s), 1.99 (3/4H, s), 2.01 (9/4H, s), 2.04 (3/4H, s) 2.05 (9/4H, s), 2.22−2.28 (1H, m), 2.39 (3H, s), 2.42−2.47 (1H, m), 3.44 (3/4H, d, *J* = 10 Hz), 3.49 (1/4H, d, *J* = 11 Hz), 3.73 (1H, m), 4.18−4.28 (4H, m), 4.44−4.65 (15/4H, m), 4.83 (1/4H, d, *J* = 11 Hz), 5.49 (1/4H, d, *J* = 5 Hz), 5.53 (3/4H, d, *J* = 1 Hz), 5.89 (3/4H, d, *J* = 1 Hz), 6.32 (1/4H, d, *J* = 5 Hz), 7.07 (6/4H, d, *J* = 8 Hz), 7.19−7.37 (42/4H, m), 7.69 (2H, d, *J* = 8 Hz). Mass (FAB): *m*/*z* 707 $(M + Na)^+$. HRMS (FAB): calcd for $C_{35}H_{40}O_{12}SNa$ $(M + Na)^+$ 707.2138, found 707.2140. Anal. Calcd for $C_{35}H_{40}O_{12}S \cdot {}^1/_5H_2O$: C, 61.07; H, 5.92; S, 4.68. Found: C, 61.07; H, 5.94; S, 4.56.

3′-*C*-Acetoxyethyl-2′-*O*-acetyl-3′,5′-di-*O*-benzyl-5-methyl-4′-*C*-(*p*-toluenesulfonyloxymethyl)uridine (17). To a solution of compound **16** (5.05 g, 7.38 mmol) in anhydrous 1,2-dichloroethane (80 mL) were added thymine (1.4 g, 11.1 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (7.18 mL, 29.5 mmol), and the mixture was heated under reflux for 2 h. The solution was cooled to 0 °C, and TMSOTf (2.27 mL, 12.5 mmol) was added dropwise. The mixture was heated under reflux for further 7 h. Saturated aqueous $NaHCO_3$ was added, and the solution was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:1) to give compound **17** as a white foam (5.24 g, 95%). Mp: 68−72 °C. $[\alpha]^{21}_D$: −41.7 (*c* 0.79, $CHCl_3$). IR ν_{max} (KBr): 3033, 1746, 1693, 1367, 1230 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.42 (3H, d, *J* = 1 Hz), 2.03 (3H, s), 2.09 (3H, s) 2.42 (3H, s), 2.34−2.42 (2H, m), 3.85, 3.92 (2H, ABq, *J* = 11 Hz), 4.09, 4.23 (2H, ABq, *J* = 11 Hz), 4.11−4.22 (2H, m), 4.63, 4.73 (2H, ABq, *J* = 12 Hz), 4.64, 5.00 (2H, ABq, *J* = 12 Hz), 5.79 (1H, d, *J* = 9 Hz), 6.21 (1H, d, *J* = 9 Hz), 7.20−7.56 (12H, m), 7.67 (1H, s), 7.68 (2H, d, *J* = 6 Hz), 7.95 (1H, brs). ^{13}C NMR ($CDCl_3$): δ 11.9, 20.8, 21.0, 21.8, 28.1, 59.4, 67.0, 70.0, 71.2, 73.9, 77.6, 83.1, 83.1, 87.3, 111.4, 126.8, 127.3, 127.6, 127.9, 128.2, 128.4, 128.7, 129.8, 131.6, 135.5, 136.2, 137.4, 145.1, 150.6, 163.1, 169.5, 170.6. Mass (FAB): *m*/*z* 751 $(M + H)^+$. Anal. Calcd for $C_{38}H_{42}N_2O_{12}S$: C, 60.43; H, 5.67; N, 3.71; S, 4.27. Found: C, 60.43; H, 5.67; N, 3.77; S, 4.03.

3′-*C*-Acetoxyethyl-3′,5′-di-*O*-benzyl-5-methyl-4′-*C*-(*p*-toluenesulfonyloxymethyl)uridine (18). To a stirred solution of compound **17** (1.32 g, 1.76 mmol) in THF (25 mL) was added 40% aqueous $MeNH_2$ (1.5 mL, 18 mmol) at 0 °C and was stirred for 1 h. Further 40% aqueous $MeNH_2$ (1.5 mL) was added and stirred for 1 h. The solvent was evaporated under reduced pressure, and the residue was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by flash silica gel column chromatography ($CHCl_3$/MeOH = 40:1) to give compound **18** as a white foam (1.08 g, 87%). Mp: 69−71 °C. $[\alpha]^{21}_D$: −10.6 (*c* 0.73, $CHCl_3$). IR ν_{max} (KBr): 3381, 3064, 1741, 1704, 1366, 1234 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.49 (3H, s), 2.08 (3H, s), 2.40 (3H, s), 2.40−2.47 (2H, m), 3.72, 3.81 (2H, ABq, *J*

= 11 Hz), 4.06, 4.20 (2H, ABq, J = 10 Hz), 4.38 (1H, d, J = 8 Hz), 4.29−4.40 (2H, m), 4.54 (1H, d, J = 11 Hz), 4.61−4.65 (2H, m), 4.88 (1H, d, J = 11 Hz), 5.89 (1H, d, J = 8 Hz), 7.16−7.36 (12H, m), 7.49 (1H, s), 7.69 (2H, d, J = 8 Hz), 8.20 (1H, brs). ^{13}C NMR ($CDCl_3$): δ 12.0, 21.1, 21.8, 28.9, 60.0, 67.1, 69.5, 71.7, 74.0, 79.1, 83.6, 86.7, 87.6, 111.2, 127.0, 127.6, 127.7, 127.9, 128.3, 128.4, 128.7, 129.9, 131.5, 135.6, 136.3, 137.1, 145.3, 155.0, 163.2, 170.7. Mass (FAB): m/z 709 $(M + H)^+$. HRMS (FAB) calcd for $C_{36}H_{41}N_2O_{11}S$ $(M + H)^+$ 709.2432, found 709.2424.

3′-*C*-Acetoxyethyl-3′,5′-di-*O*-benzyl-2′-*O*-(2-methoxypropyl)-5-methyl-4′-*C*-(*p*-toluenesulfonyloxymethyl)uridine (19). To a solution of compound **18** (4.77 g, 6.74 mmol) in anhydrous CH_2Cl_2 (67 mL) were added 2-methoxypropene (9.69 mL, 0.10 mol) and *p*-TsOH·H_2O (25.6 mg, 0.14 mmol) at 0 °C. After the mixture was stirred for 3 h, saturated aqueous $NaHCO_3$ was added, and the mixture was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:1) to give compound **19** as a white foam (4.72 g, 90%). Mp: 72−74 °C. $[\alpha]^{25}_D$: −37.6 (c 0.71, $CHCl_3$). IR ν_{max} (KBr): 2989, 1692, 1367, 1235, 1178 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.24 (6H, s), 1.49 (3H, s), 2.07 (3H, s) 2.20−2.28 (1H, m), 2.42 (3H, s), 2.53−2.61 (1H,m), 3.00 (3H, s), 3.88, 3.94 (2H, ABq, J = 11 Hz), 4.10 (1H, d, J = 11 Hz), 4.19−4.26 (3H, m), 4.55−4.62 (3H, m), 4.68 (1H, d, J = 11 Hz), 5.18 (1H, d, J = 11 Hz), 6.14 (1H, d, J = 8 Hz), 7.21−7.36 (12H, m), 7.49 (1H, s), 7.69 (2H, d, J = 8 Hz), 8.06 (1H, s). ^{13}C NMR ($CDCl_3$): δ 11.9, 21.1, 21.8, 24.7, 25.3, 27.9, 50.1, 59.8, 67.0, 70.7, 71.9, 74.0, 78.5, 83.4, 84.0, 86.0, 101.9, 110.8, 126.8, 127.3, 127.7, 127.9, 128.3, 128.3, 128.7, 129.7, 131.9, 136.5, 136.6, 138.3, 145.0, 150.7, 163.2, 170.7. Mass (FAB): m/z 781 $(M + H)^+$. Anal. Calcd for $C_{40}H_{48}O_{12}N_2S\cdot{}^1/_5H_2O$: C, 61.24; H, 6.22; N, 3.57; S, 3.93. Found: C, 61.20; H, 6.15; N, 3.49; S, 4.11.

3′,5′-Di-*O*-benzyl-3′-*C*-hydroxyethyl-2′-*O*-(2-methoxypropyl)-5-methyl-4′-*C*-(*p*-toluenesulfonyloxymethyl)uridine (20). To a solution of compound **19** (974 mg, 1.25 mmol) in THF−MeOH (3:1, 18 mL) was added 2 M aqueous NaOH (1.3 mL, 2.6 mmol) at 0 °C. After being stirred for 1.5 h, H_2O was added, and the solution was extracted with CH_2Cl_2. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:1) to give compound **20** as a white foam (806 mg, 87%). Mp: 90−92 °C. $[\alpha]^{25}_D$ −52.3 (c 0.90, $CHCl_3$). IR ν_{max} (KBr): 3417, 3190, 2990, 1692, 1364, 1176 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.24 (3H, s), 1.27 (3H, s), 1.48 (3H, d, J = 1 Hz), 1.68 (1H, brs), 2.04−2.18 (1H, m), 2.43 (3H, s), 2.51−2.57 (1H, m), 3.00 (3H, s), 3.77−3.81 (2H, m), 3.84, 4.01 (2H, ABq, J = 11 Hz), 4.14, 4.37 (2H, ABq, J = 11 Hz), 4.54, 5.15 (2H, ABq, J = 12 Hz), 4.58, 4.65 (2H, ABq, J = 11 Hz), 4.64 (1H, d, J = 8 Hz), 6.12 (1H, d, J = 8 Hz), 7.17−7.39 (12H, m), 7.50 (1H, d, J = 1 Hz), 7.69 (2H, d, J = 8 Hz), 8.06 (1H, brs). ^{13}C NMR ($CDCl_3$): δ 11.9, 21.8, 24.7, 25.3, 31.2, 50.0, 57.7, 66.8, 71.2, 71.8, 74.0, 78.4, 84.0, 84.2, 86.1, 101.8, 110.8, 126.7, 127.2, 127.6, 127.9, 128.2, 128.6, 129.7, 132.0, 136.7, 136.7, 138.6, 144.9, 150.7, 163.3. Mass (FAB): m/z 739 $(M + H)^+$. Anal. Calcd for $C_{38}H_{46}N_2O_{11}S\cdot{}^1/_2H_2O$: C, 61.03; H, 6.33; N, 3.75; S, 4.34. Found: C, 61.06; H, 6.25; N, 3.62; S, 4.24.

3′,5′-Di-*O*-benzyl-3′-*C*,4′-*C*-ethoxymethylene-2′-*O*-(2-methoxypropyl)-5-methyluridine (21). To a stirred solution of compound **20** (728 mg, 0.99 mmol) in anhydrous THF (16 mL) was added NaHMDS (1.0 M in THF, 3.0 mL, 3.0 mmol), and the mixture was heated under reflux for 6 h. After the solution was cooled to 0 °C, saturated aqueous $NaHCO_3$ was added, and the mixture was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was recrystallized from EtOAc−*n*-hexane to give compound **21** as a white solid (400 mg, 73%). Mp: 172−175 °C. $[\alpha]^{26}_D$ −129.8 (c 0.81, $CHCl_3$). IR ν_{max} (KBr): 3178, 2955, 1695, 1460, 1088 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.29 (3H, s), 1.38 (3H, s), 1.56 (3H, s), 1.93−2.05 (1H, m), 2.30−2.35 (1H, m), 3.01 (3H, s), 3.25 (1H, d, J = 10 Hz), 3.77−3.85 (3H, m), 4.29−4.36 (2H, m), 4.58, 5.33 (2H, ABq, J = 11 Hz), 4.67 (2H, s), 5.42 (1H, d, J = 8 Hz), 6.56 (1H, d, 8 Hz), 7.26−7.47 (10H, m), 7.96 (1H, s). ^{13}C NMR ($CDCl_3$): δ 12.1, 24.4, 26.4, 27.8, 49.0, 62.1, 66.1, 66.4, 73.9, 75.5, 76.5, 79.1, 83.6, 86.6, 101.0, 110.6, 127.3, 127.5, 127.7, 128.1, 128.3, 128.6, 136.6, 137.5, 138.4, 151.1, 163.4. Mass (FAB) m/z 567 $(M + H)^+$. Anal. Calcd for $C_{31}H_{38}N_2O_8\cdot{}^1/_3H_2O$: C, 65.02; H, 6.81; N, 4.89. Found: C, 64.98; H, 6.81; N, 4.83.

3′,5′-Di-*O*-benzyl-3′-*C*,4′-*C*-ethoxymethylene-5-methyluridine (22). To a solution of compound **21** (466 mg, 0.84 mmol) in THF−MeOH (9.0 mL, 2:1) was added *p*-TsOH·H_2O (64 mg, 0.34 mmol) at 0 °C, and the whole was stirred for 1 h. Saturated aqueous $NaHCO_3$ was added, and the solution was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:1) to give compound **22** as a white foam (355 mg, 85%). Mp: 87−89 °C. $[\alpha]^{27}_D$: −45.5 (c 0.66, $CHCl_3$). IR ν_{max} (KBr): 3429, 1687, 1489, 1271, 1071 cm^{-1}. ^{1}H NMR (acetone-d_6): δ 1.62 (3H, s), 1.93−2.01 (1H, m), 2.48−2.54 (1H, m), 3.61, 4.21 (2H, ABq, J = 9 Hz), 3.69−3.86 (2H, m), 3.95, 4.02 (2H, ABq, J = 10 Hz), 4.60, 4.66 (2H, ABq, J = 12 Hz), 4.72, 5.43 (2H, ABq, J = 11 Hz), 5.03 (1H, dd, J = 3, 7 Hz), 5.20 (1H, d, J = 3 Hz), 6.10 (1H, d, J = 7 Hz), 7.26−7.51 (10H, m), 7.91 (1H, s), 10.1 (1H, brs). ^{13}C NMR ($CDCl_3$): δ 12.5, 27.7, 62.4, 65.1, 66.7, 71.0, 73.7, 80.0, 83.0, 86.0, 94.5, 109.9, 127.1, 127.2, 127.9, 128.2, 128.4, 136.4, 137.0, 138.9, 152.1, 163.7. Mass (FAB): m/z 495 $(M + H)^+$. HRMS (FAB): calcd for $C_{27}H_{30}O_7N_2$ $(M + H)^+$ 495.2132, found 495.2137.

3′,5′-Di-*O*-benzyl-3-*N*-benzyloxymethyl-3′-*C*,4′-*C*-ethoxymethylene-5-methyluridine (23). To a solution of compound **22** (106 mg, 0.21 mmol) in anhydrous DMF (2 mL) were added DBU (0.063 mL, 0.42 mmol) and benzyl chloromethyl ether (0.044 mL, 0.32 mmol) at 0 °C. After the mixture was stirred for 45 min, MeOH was added, and the solution was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:2) to give compound **23** as a white foam (116 mg, 90%). Mp: 48−49 °C. $[\alpha]^{26}_D$: −32.9 (c 0.77, $CHCl_3$). IR ν_{max} (KBr): 3430, 2954, 2874, 1698, 1657, 1458, 1071 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.77 (3H, s), 1.77−1.85 (1H, m), 2.47 (1H, d, J = 14 Hz), 3.41, 3.97 (2H, ABq, J = 10 Hz), 3.65−3.73 (1H, m), 3.84−3.88 (1H, m), 3.92, 4.31 (2H, ABq, J = 10 Hz), 4.47 (2H, s), 4.54 (1H, d, J = 5 Hz), 4.62, 5.39 (2H, ABq, J = 11 Hz), 4.70 (2H, s), 5.11 (1H, s), 5.45, 5.49 (2H, ABq, J = 9 Hz), 5.69 (1H, d, J = 5 Hz), 7.25−7.44 (16H, m), 7.83 (1H, s). ^{13}C NMR ($CDCl_3$): δ 13.3, 27.8, 62.4, 64.9, 66.8, 70.6, 70.7, 72.4, 73.6, 80.0, 83.2, 86.0, 94.8, 109.4, 127.1, 127.2, 127.3, 127.5, 127.6, 127.8, 128.2, 128.2, 128.4, 134.8, 137.1, 137.9, 138.9, 152.8, 163.0. Mass (FAB): m/z 615 $(M + H)^+$. Anal. Calcd for $C_{35}H_{38}N_2O_8\cdot{}^1/_{10}H_2O$: C, 68.19; H, 6.23; N, 4.54. Found: C, 68.01; H, 6.25; N, 4.49.

3′,5′-Di-*O*-benzyl-3-*N*-benzyloxymethyl-3′-*C*,4′-*C*-ethoxymethylene-2′-*O*-methyl-5-methyluridine (24). To a stirred suspension of 60% sodium hydride (9.0 mg, 0.23 mmol) in anhydrous DMF (0.6 mL) was added dropwise a solution of compound **23** (109 mg, 0.18 mmol) in anhydrous DMF (1.4 mL) at 0 °C. After the mixture was stirred for 30 min, MeI (0.06 mL, 0.9 mmol) was added and stirred for 1 h at room temperature. MeOH was added and evaporated to dryness under reduced pressure, and the residue was extracted with EtOAc and H_2O. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 1:2) to give compound **24** as a white foam (97 mg, 86%). Mp: 44−45

°C. $[\alpha]^{24}_D$: −89.1 (*c* 0.80, $CHCl_3$). IR ν_{max} (KBr): 2953, 2885, 1712, 1669, 1456, 1090 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.65 (3H, s), 2.02−2.14 (1H, m), 2.45 (1H, brd, J = 14 Hz), 3.31, 4.33 (2H, ABq, J = 10 Hz), 3.44 (3H, s), 3.78 (2H, d, J = 8 Hz), 3.86, 4.28 (2H, ABq, J = 11 Hz), 4.65−4.67 (5H, m), 4.87 (1H, d, J = 8 Hz), 5.29 (1H, d, J = 11 Hz), 5.47, 5.50 (2H, ABq, J = 10 Hz), 6.55 (1H, d, J = 8 Hz), 7.23−7.45 (15H, m), 7.80 (1H, s). ^{13}C NMR ($CDCl_3$): δ 13.1, 27.6, 59.4, 61.8, 66.1, 66.2, 70.7, 72.1, 73.9, 74.9, 79.1, 83.5, 86.6, 87.7, 110.3, 127.3, 127.3, 127.4, 127.4, 127.6, 128.1, 128.2, 128.3, 128.6, 135.5, 136.8, 137.9, 138.4, 151.8, 163.3. Mass (FAB): *m*/*z* 629 $(M + H)^+$. Anal. Calcd for $C_{36}H_{40}N_2O_8 \cdot {}^1/_5H_2O$: C, 68.38; H, 6.44; N, 4.43. Found: C, 68.31; H, 6.43; N, 4.45.

3′-*C*,4′-*C*-Ethoxymethylene-2′-*O*-methyl-5-methyluridine (1). To a suspension of 20% palladium hydroxide over carbon (150 mg) in EtOH (1.0 mL) was added a solution of compound **24** (278 mg, 0.44 mmol) in EtOH (3.0 mL) and cyclohexene (2.2 mL, 22 mmol). The mixture was heated under reflux for 7 h and filtered. The solution was evaporated under reduced pressure, and the residue was recrystallized from EtOH to give compound **1** (57 mg, 39%) as a white solid. Mp: 256−259 °C. $[\alpha]^{27}_D$: −49.7 (*c* 0.53, CH_3OH). IR ν_{max} (KBr): 3238, 2966, 1705, 1469 cm^{-1}. ^1H NMR (CD_3OD): δ 1.84 (1H, dd, J = 3, 13 Hz), 1.90 (3H, d, J = 1 Hz), 2.07 (1H, dt, J = 13, 6 Hz), 3.42 (3H, s), 3.47 (1H, d, J = 9 Hz), 3.75 (1H, dd, J = 5, 11 Hz), 3.95−4.08 (4H, m), 4.63 (1H, d, J = 7 Hz), 6.17 (1H, d, J = 7 Hz), 8.10 (1H, d, J = 1 Hz). ^{13}C NMR ($CDCl_3$): δ 12.6, 33.5, 59.2, 63.0, 64.7, 65.9, 84.7, 85.7, 89.7, 111.8, 139.5, 153.0, 166.1. Mass (FAB): *m*/*z* 329 $(M + H)^+$. Anal. Calcd for $C_{14}H_{20}N_2O_7$: C, 51.22; H, 6.14; N, 8.53. Found: C, 51.25; H, 6.12; N, 8.31.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*C*,4′-*C*-ethoxymethylene-2′-*O*-methyl-5-methyluridine (29). To a solution of compound **1** (58 mg, 0.18 mmol) in anhydrous pyridine (0.8 mL) were added dimethoxytrityl chloride (88 mg, 0.26 mmol) and DMAP (4 mg, 0.04 mmol) at 50 °C, and the mixture was stirred for 2.5 h. Saturated aqueous $NaHCO_3$ was added, and the solution was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by flash silica gel column chromatography (EtOAc/*n*-hexane = 3:1) to give compound **29** as a white foam (87 mg, 77%). Mp: 127−129 °C. $[\alpha]^{24}_D$: −49.4 (*c* 0.91, $CHCl_3$). IR ν_{max} (KBr): 3458, 3195, 3007, 2953, 2837, 1691, 1508, 1464, 1254 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.28 (3H, s), 1.45−1.53 (2H, m), 2.94 (1H, s), 3.40−3.48 (2H, m), 3.51 (2H, s), 3.80 (6H, s), 3.91 (1H, dt, J = 10, 4 Hz), 4.09 (1H, d, J = 9 Hz), 4.74 (1H, d, J = 6 Hz), 6.10 (1H, d, J = 6 Hz), 6.84 (4H, dd, J = 2, 8 Hz), 7.24−7.39 (9H, m), 7.98 (1H, d, J = 1 Hz), 8.18 (1H, brs). ^{13}C NMR ($CDCl_3$): δ 11.4, 32.4, 55.3, 59.2, 61.8, 65.8, 65.9, 75.8, 83.8, 84.3, 88.3, 88.5, 111.3, 113.1, 113.1, 127.5, 127.9, 128.8, 130.7, 130.7, 134.7, 137.3, 142.2, 158.9. Mass (FAB): *m*/*z* 631 $(M + H)^+$. Anal. Calcd for $C_{35}H_{38}N_2O_9 \cdot {}^1/_2H_2O$: C, 65.72; H, 6.14; N, 4.38. Found: C, 65.72; H, 6.15; N, 4.31.

3′,5′-Di-*O*-benzyl-2′-deoxy-3′-*C*,4′-*C*-ethoxymethylene-5-methyl-2′-oxouridine (25). To a suspension of Dess−Martin periodinane (1.26 g, 2.97 mmol) in CH_2Cl_2 (10 mL) was added a solution of compound **22** (979 mg, 1.98 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred at room temperature for 30 min. The mixture of saturated aqueous $Na_2S_2O_3$ and $NaHCO_3$ (2:1) was added, and the solution was extracted with CH_2Cl_2. The combined organic layer was washed with saturated aqueous $Na_2S_2O_3$, H_2O, and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/*n*-hexane = 1:1) to give compound **25** (945 mg, 97%) as a white solid. Mp: 86−88 °C. $[\alpha]^{22}_D$: −121.7 (*c* 1.00, $CHCl_3$). IR ν_{max} (KBr): 3032, 2879, 1785, 1691, 1458, 1389, 1281, 1211, 1090, 1014 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.49 (3H, s), 1.93−2.05 (1H, m), 2.27−2.32 (1H, m), 3.50, 4.23 (2H, ABq, J = 11 Hz), 3.75−3.82 (1H, m), 3.82−3.91 (1H m), 3.83, 4.52 (2H, ABq, J = 11 Hz), 4.34 (2H, t, J = 10 Hz), 4.52 (2H, s), 6.51 (1H, s), 7.21−7.40 (10H, m), 7.45 (1H, s), 8.52 (1H, brs). ^{13}C NMR ($CDCl_3$): δ 12.0, 24.0, 61.4, 65,1, 65.4, 74.1, 74.1, 78.6, 82.0, 82.0, 127.2, 109.8, 127.8, 128.3, 128.3, 128.7, 128.7, 135.7, 135.8, 137.2, 151.2, 163.0, 196.5. Mass (FAB): *m*/*z* 493 $(M + H)^+$. Anal. Calcd for $C_{27}H_{28}N_2O_7 \cdot {}^3/_4H_2O$: C, 64.09; H, 5.88; N, 5.54. Found: C, 64.11; H, 6.12; N, 5.27.

1-(3,5-Di-*O*-benzyl-3-*C*,4-*C*-ethoxymethylene-β-D-*arabino*-pentofuranosyl)thymine (26). To a solution of compound **25** (65 mg, 0.13 mmol) in anhydrous THF (1.5 mL) was added DIBALH (0.53 mL, 0.53 mmol) at −78 °C, and the whole was stirred for 5 h at the same temperature. The reaction mixture was partitioned with saturated aqueous NH_4Cl, filtered through Celite, and extracted with EtOAc. The combined organic layer was washed with H_2O and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 40:1) to give compound **26** (44 mg, 67%) as a white solid. Mp: 133−135 °C. $[\alpha]^{24}_D$: −34.7 (*c* 0.87, $CHCl_3$). IR ν_{max} (KBr): 3342, 2956, 1695, 1478, 1392, 1279, 1173, 1065 cm^{-1}. ^1H NMR (acetone-d_6): δ 1.70 (3H, d, J = 1 Hz), 1.96−2.06 (1H, m), 2.23−2.29 (1H, m), 3.68−3.77 (1H, dt, J = 11, 3 Hz), 3.82−3.87 (1H, dd, J = 4, 11 Hz), 4.05, 4.09 (2H, ABq, J = 9 Hz), 4.14, 4.25 (2H, ABq, J = 10 Hz), 4.56, 4.70 (2H, ABq, J = 11 Hz), 4.58−4.72 (2H, m), 4.88 (1H, brt, J = 5 Hz), 5.25 (1H, d, J = 6 Hz), 6.10 (1H, d, J = 4 Hz), 7.26−7.45 (10H, m), 7.90 (1H, d, J = 1 Hz) 10.05 (1H, brs). ^{13}C NMR (acetone-d_6): δ 165.3, 151.7, 140.0, 139.7, 138.4, 129.8, 129.7, 129.0, 128.9, 128.8, 109.2, 92.2, 87.1, 84.3, 74.3, 73.1, 70.3, 65.8, 65.5, 62.6, 26.7, 13.4. Mass (EI): *m*/*z* 494 (M^+). Anal. Calcd for $C_{27}H_{30}N_2O_7 \cdot {}^1/_2H_2O$: C, 64.40; H, 6.21; N, 5.56. Found: C, 64.16; H, 6.17; N, 5.41.

1-(3,5-Di-*O*-benzyl-3-*C*,4-*C*-ethoxymethylene-β-D-*arabino*-pentofuranosyl)-3-*N*-benzyloxymethylthymine (27). To a solution of compound **26** (600 mg, 1.2 mmol) in anhydrous DMF (12 mL) were added DBU (0.2 mL, 1.33 mmol) and benzyl chloromethyl ether (0.25 mL, 1.82 mmol) at 0 °C, and the mixture was stirred for 3 h. The reaction mixture was partitioned with MeOH, evaporated under reduced pressure, and extracted with CH_2Cl_2. The organic layer was washed with H_2O and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/*n*-hexane = 2:3) to give compound **27** (89 mg, 68%) as a white solid. Mp: 72−74 °C. $[\alpha]^{24}_D$: −1.88 (*c* 0.89, $CHCl_3$). IR ν_{max} (KBr): 3407, 3072, 3030, 2956, 1700, 1657, 1460, 1369, 1278, 1166, 1065 cm^{-1}. ^1H NMR (acetone-d_6): δ 1.74 (3H, d, J = 2 Hz), 2.00−2.07 (1H, m), 2.24−2.28 (1H, m), 3.74 (1H, dt, J = 11, 4 Hz), 3.85 (1H, dd, J = 5, 11 Hz), 4.07, 4.10 (2H, ABq, J = 10 Hz), 4.12, 4.21 (2H, ABq, J = 10 Hz), 4.60, 4.69 (2H, ABq, J = 12 Hz), 4.61, 4.67 (2H, ABq, J = 12 Hz), 4.65 (2H, s), 4.94 (1H, dd, J = 5, 6 Hz), 5.19 (1H, d, J = 6 Hz), 5.40, 5.46 (2H, ABq, J = 9 Hz), 6.11 (1H, d, J = 5 Hz), 7.24−7.45 (15H, m), 7.95 (1H, d, J = 2 Hz). ^{13}C NMR ($CDCl_3$): δ 13.3, 25.6, 61.4, 64.2, 65.2, 69.7, 70.3, 70.3, 72.3, 72.9, 73.4, 82.6, 86.2, 91.9, 108.6,-127.1, 127.1, 127.7, 127.7, 127.8, 127.8, 128.2, 128.4, 128.5, 135.7, 137.2, 137.2, 137.7, 151.0, 163.5. Mass (EI): *m*/*z* 614 (M^+). Anal. Calcd for $C_{35}H_{38}N_2O_8 \cdot {}^3/_4H_2O$: C, 66.92; H, 6.33; N, 4.46. Found: C, 66.90; H, 6.18; N, 4.38.

1-(3,5-Di-*O*-benzyl-3-*C*,4-*C*-ethoxymethylene-2-*O*-methyl-β-D-*arabino*-pentofuranosyl)-3-*N*-benzyloxymethylthymine (28). To a suspension of 60% NaH (85 mg, 2.2 mmol) in anhydrous DMF (5.0 mL) was added dropwise a solution of compound **27** (675 mg, 1.1 mmol) in anhydrous DMF (5.0 mL) at 0 °C. After being stirred for 30 min, MeI (0.34 mL, 5.5 mmol) was added and the mixture stirred for 30 min. The reaction mixture was partitioned with MeOH, evaporated under reduced pressure, and extracted with EtOAc. The combined organic layer was washed with H_2O and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/*n*-hexane = 2:3) to give compound **28** (645 mg, 93%) as a white solid. Mp: 86−88 °C. $[\alpha]^{22}_D$: +12.2 (*c* 0.79,

$CHCl_3$). IR ν_{max} (KBr): 3073, 2951, 1703, 1658, 1457, 1370, 1280, 1241, 1204, 1164, 1093 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.80 (3H, d, J = 1 Hz), 1.90−2.02 (1H, m), 2.13−2.19 (1H, m), 3.35 (3H, s), 3.71 (1H, m), 3.87 (1H, m), 3.82, 4.01 (2H, AB, J = 10 Hz), 4.18 (2H, AB, J = 9 Hz), 4.37 (1H, d, J = 5 Hz), 4.53 (2H, s), 4.54, 4.65 (2H, AB, J = 12 Hz), 4.74 (2H, s), 5.51 (2H, s), 6.06 (1H, d, J = 5 Hz), 7.24−7.39 (15H, m), 7.91 (1H, d, J = 1 Hz). ^{13}C NMR ($CDCl_3$): δ 13.3, 25.7, 60.5, 61.2, 64.3, 64.6, 68.2, 70.2, 72.0, 73.1, 81.5, 82.1, 86.0, 90.3, 108.3, 126.8, 127.1, 127.5, 127.8, 128.1, 128.3, 128.5, 135.5, 136.9, 137.9, 150.7, 163.7. Mass (EI): m/z 628 (M^+). Anal. Calcd for $C_{36}H_{40}N_2O_8 \cdot {}^1/_3H_2O$: C, 68.12; H, 6.46; N, 4.13. Found: C, 67.91; H, 6.35; N, 4.34.

1-(3-*C*,4-*C*-Ethoxymethylene-2-*O*-methyl-β-D-*arabino*-pentofuranosyl)thymine (2). To a suspension of 20% palladium hydroxide over carbon (48 mg) in MeOH (1.0 mL) was added compound **28** (96 mg, 0.15 mmol) in MeOH (2.0 mL) and ammonium formate (4.8 g, 7.6 mmol). The mixture was heated under reflux for 7.5 h and filtered. The solution was evaporated under reduced pressure and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 20:1) to give compound **2** (32 mg, 63%) as a white solid. Mp: 241−243 °C. $[\alpha]^{22}_D$: +19.9 (*c* 1.00, MeOH). IR ν_{max} (KBr): 3441, 1651, 1479, 1285, 1034 cm^{-1}.^1H NMR (CD_3OD): δ 1.74−1.88 (1H, m), 1.88 (3H, d, J = 1 Hz), 1.97−2.09 (1H, m), 3.32 (3H, s), 3.77−3.83 (1H, m), 3.86−3.90 (1H, m), 3.93, 4.07 (2H, AB, J = 11 Hz), 3.95 (1H, d, J = 5 Hz), 3.98 (2H, s), 6.13 (1H, d, J = 5 Hz), 8.07 (1H, d, J = 1 Hz). ^{13}C NMR ($CDCl_3$): δ 12.6, 16.6, 31.7, 60.2, 60.8, 62.4, 65.2, 77.7, 86.8, 87.6, 91.0, 109.5, 139.5, 152.2, 166.8. Mass (EI): m/z 328 (M^+). Anal. Calcd for $C_{14}H_{20}N_2O_7 \cdot {}^1/_2H_2O$: C, 49.80; H, 6.27; N, 8.30. Found: C, 49.62; H, 6.11; N, 8.20.

1-[5-*O*-(4,4′-Dimethoxytrityl)-3-*C*,4-*C*-ethoxymethylene-2-*O*-methyl-β-D-*arabino*-pentofuranosyl]thymine (30). To a suspension of silver trifluoromethanesulfonate (1.55 g, 6.0 mmol) in anhydrous CH_2Cl_2 (1.0 mL) was added dropwise 4,4′-dimethoxytrityl chloride (2.1 g, 6.0 mmol) in anhydrous CH_2Cl_2 (5.0 mL), and then the mixture was stirred for 1 h at room temperature. The supernatant fluid (2.0 mL, 3.4 equiv) was added to a solution of compound **2** (198 mg, 0.60 mmol) in anhydrous CH_2Cl_2−pyridine (1:1, 2.5 mL), and the mixture was stirred for 5 h at room temperature. The reaction mixture was partitioned with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 30:1) to give compound **30** (382 mg, 100%) as a white solid. Mp: 270−272 °C. $[\alpha]^{25}_D$: −31.3 (*c* 0.60, $CHCl_3$). IR ν_{max} (KBr): 3397, 2957, 1689, 1608, 1509, 1465, 1279, 1250, 1176, 1106,1038 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.44 (3H, d, J = 1 Hz), 1.64−1.69 (1H, m), 1.70 (3H, s), 1.76−1.88 (1H, m), 2.81 (1H, brs), 3.14 (3H, s), 3.64 (1H, m), 3.70 (1H, m), 3.76, 4.09 (2H, ABq, J = 14 Hz), 3.77 (6H, s), 3.84−3.94 (2H, m), 6.05 (1H, d, J = 4 Hz), 6.77−6.82 (4H, m), 7.21−7.42 (9H, m), 7.94 (1H, d, J = 1 Hz), 9.25 (1H, brs). ^{13}C NMR ($CDCl_3$): δ 12.2, 31.0, 55.3, 55.3, 60.0, 60.8, 61.3, 64.8, 77.5, 85.0, 85.3, 86.7, 90.0, 109.2, 112.8, 112.9, 126.9, 127.6, 128.4, 130.1, 130.2, 136.1, 136.1, 137.5, 144.8, 150.3, 158.4, 158.4, 164.4. Mass (FAB): m/z 631 (M + H)$^+$. HRMS (FAB): calcd for $C_{35}H_{39}N_2O_9$ (M + H)$^+$ 631.2656, found 631.2640.

1-[3-*O*-(*N*,*N*′-Diisopropylamino-β-cyanoethoxyphosphino)-5-*O*-(4,4′-dimethoxytrityl)-3-*C*,4-*C*-ethoxymethylene-2-*O*-methyl-β-D-*arabino*-pentofuranosyl]thymine (31). To a solution of compound **30** (121 mg, 0.19 mmol) in anhydrous MeCN−THF (1:1, 2.0 mL) were added 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (0.15 mL, 0.46 mmol) and 4,5-dicyanoimidazole (46 mg, 0.39 mmol), and the whole was stirred for 15 h at 50 °C. The reaction mixture was partitioned with saturated $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/*n*-hexane = 2:3) to give compound **31** (88 mg, 55%) as a white solid. Mp: 75−77 °C. ^{31}P NMR ($CDCl_3$): δ 141.4, 142.7. Mass (FAB): m/z 831 (M + H)$^+$. HRMS (FAB): calcd for $C_{44}H_{56}N_4O_{10}P$ (M + H)$^+$ 831.3735, found 831.3715.

Oligonucleotide Synthesis. Synthesis of ODNs containing *trans*-3′,4′-BNA monomer **2** (**ODN2**−**ODN4**) was performed in 0.2 μmol scale on an automated DNA synthesizer using the phosphoramidite approach. As an activator, 5-ethylthio-1*H*-tetrazole (ETT) was used for every coupling step. The *trans*-3′,4′-BNA amidite **30** was manually coupled as follows. Just before the coupling step for **30**, the column reactor was detached from the DNA synthesizer. The column was filled with 0.1 M solution of **30** in MeCN (0.05 mL) and 0.25 M solution of ETT in MeCN (0.05 mL) and vigorously shaken for 24 h at 50 °C. The column was then attached to the DNA synthesizer, and the reaction cycle was continued after the coupling step of **30**. Every ODN synthesis was performed on DMTr-ON mode. Cleavage from the CPG support and removal of protecting groups were accomplished by using 28% aqueous ammonia (55 °C for 12 h). The crude ODNs bearing a DMTr group were detritylated and purified with a C_{18} solid-phase extraction cartridge [washed with 10% MeCN aqueous solution, detritylated with 2% TFA aqueous solution, and eluted with 35% MeOH aqueous solution]. The obtained ODNs were again purified by reversed-phase HPLC [buffer A, 0.1 M TEAA; buffer B, 0.1 M TEAA/MeCN = 1:1, B 12% to 22.6%/20 min, linear gradient; flow rate, 3.0 mL/min]. The composition of the **ODN2**−**ODN4** was confirmed by MALDI-TOF-MS analysis: m/z **ODN2** [M − H]$^-$ calcd 3718.51, found 3719.35; **ODN3** [M − H]$^-$ calcd 3718.51, found 3718.14; **ODN4** [M − H]$^-$ calcd 3718.51, found 3718.92.

UV Melting Experiments. The UV melting profiles were recorded in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl at a scan rate of 0.5 °C/min with detection at 260 nm. The final concentration of each ODN was 4.0 μM. The melting temperatures were obtained as the maxima of the first derivative of the melting curves.

Circular Dichroism (CD) Spectroscopy. CD spectra were recorded at 10 °C in the quartz cuvette of 1 cm optical path length. The samples were prepared in the same manner as described in the UV melting experiments. The molar ellipticity was calculated from the equation $[\theta] = \theta/cl$, where θ is the relative intensity, c is the sample concentration, and l is the cell path length in centimeters.

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Supporting Information Available: General experimental procedures, copies of ^1H and ^{13}C NMR of compounds **1**, **2**, **4**−**8**, **11**−**14**, **17**−**30**, HPLC chromatograms, and MALDI-TOF-MS spectra for **ODN2**−**ODN4**. UV melting profiles for the duplexes between **ODN1**−**ODN4** and DNA or RNA complement and the duplexes between **ODN2** and DNA complement with or without one base mismatch. This material is available free of charge via the Internet at http://pubs.acs.org.

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