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SYNTHESIS OF THE 5-METHYLURIDINE MONOMER OF 3′-*O*,4′-*C*-ETHYLENEOXY-BRIDGED NUCLEIC ACID

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Dedicated to Professor Dr. Masakatsu Shibasaki on the occasion of his 70th birthday

Abstract – A novel bridged nucleic acid, 3′-*O*,4′-*C*-ethyleneoxy-bridged nucleic acid (3′,4′-EoNA), forming 2′,5′-linkage with the flanking nucleotides in oligonucleotides was designed. The 3′,4′-EoNA is expected to improve the biophysical properties of the oligonucleotides (*e.g.*, binding affinity with complementary single-stranded oligonucleotides and resistance against nuclease digestion) because of the presence of the 6′-oxygen atom. In this study, the synthesis of the 5-methyluridine monomer of 3′,4′-EoNA was achieved via Lewis acid-mediated C4′-iodoethoxylation followed by intramolecular 1,4-dioxane ring formation. Here, we describe, in detail, the results of the study.

INTRODUCTION

In the past few decades, numerous structurally constrained nucleosides have been developed and used to improve the properties of oligonucleotides, such as the hybridization ability with complementary single-stranded oligonucleotides.[1] In particular, oligonucleotides modified by nucleosides with an additional bridge between the 2′- and 4′-positions, like 2′,4′-BNA[2]/LNA[3] (Figure 1A), can form a stable duplex with single-stranded RNA (ssRNA) because the sugar conformation is pre-organized to the N-type suitable for forming RNA duplex.

On the other hand, non-genetic 2′,5′-linked oligonucleotides (*iso*DNA and *iso*RNA, Figure 1B) have focused on structural features and unique biological activity. 2′,5′-Oligoadenylate 5′-triphosphate (2–5-A)

plays an important role in enhancing the ribonuclease L (RNase L)-mediated antiviral activity.[4] To develop an antiviral drug, various chemically modified 2–5-A derivatives have been synthesized.[5] Moreover, *iso*DNA and *iso*RNA have a potential for therapeutic applications (*e.g.*, antisense,[6] siRNA,[7] ribozyme,[8] and aptamer[9]) because of their excellent resistance against enzymatic digestion.[10]

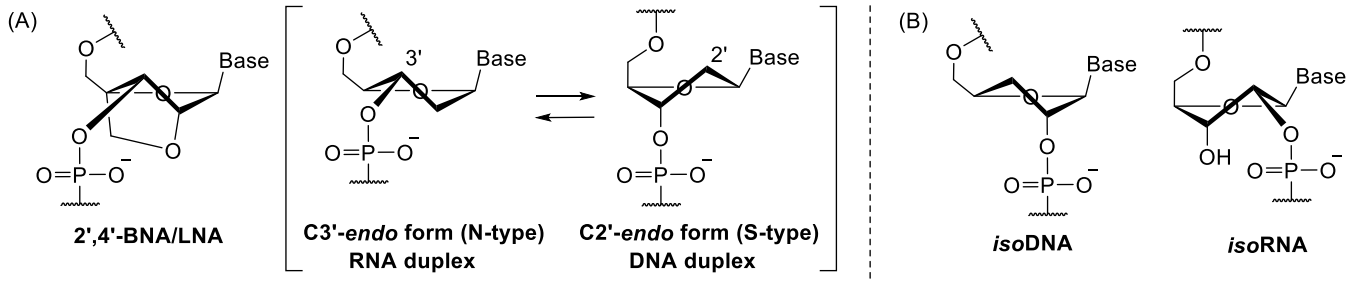


Figure 1. (A) Structure of 2',4'-BNA/LNA and equilibrium of sugar conformations of nucleotides and (B) Structures of *iso*DNA and *iso*RNA

*Iso*DNA and *iso*RNA also have a tendency to selectively and stably bind to ssRNA rather than ssDNA.[10a,11] As bridged analogues of *iso*DNA and *iso*RNA, several 3′-*O*,4′-*C*-bridged nucleic acids (3′,4′-BNAs) have also been developed (Figure 2A).[5f,12,13] However, the sugar conformation of all 3′,4′-BNAs is S-type which is the same as the sugar conformation in the DNA duplex; therefore, their modified oligonucleotides generally possess a lack of binding affinity to ssRNA. On the other hand, we recently developed EoNA[14] (Figure 2B) as 2′,4′-bridged nucleic acid with a 6′-oxygen atom and found that the presence of the 6′-oxygen atom significantly enhanced not only the binding affinity to ssRNA targets but also nuclease resistance, when compared with 2′,4′-BNA^{COC} [15] that has the same seven-membered bridge. Under such a background, we were interested in 3′,4′-BNA with a 6′-oxygen as a candidate for improving the properties of *iso*DNA- and *iso*RNA-type oligonucleotides. In this study, we designed and synthesized the 3′,4′-EoNA monomer **1** with a 1,4-dioxane bridge (Figure 2C).

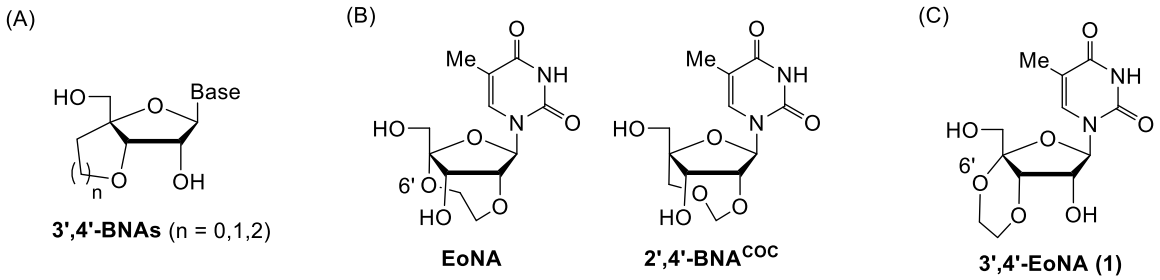
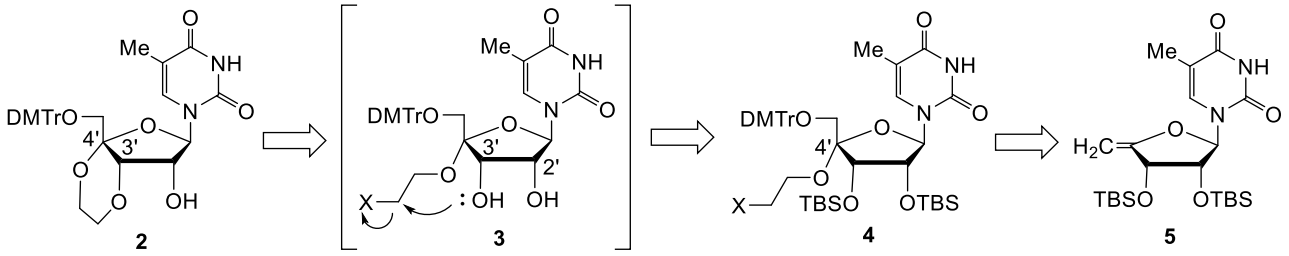


Figure 2. (A) Structures of 3'-*O*,4'-*C*-bridged nucleic acids (3',4'-BNAs) and (B) EoNA and 2',4'-BNA^{COC} and (C) 3',4'-EoNA

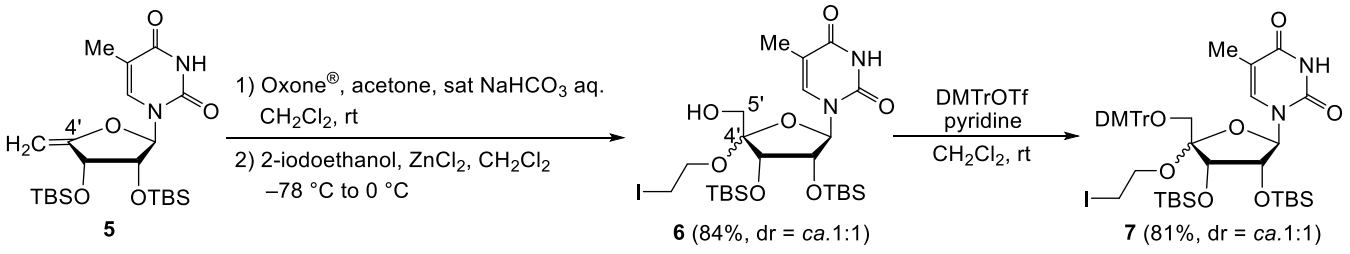
RESULTS AND DISCUSSION

The synthetic route for **2** possessing a 3′,4′-bridged structure was planned as shown in Scheme 1. This 1,4-dioxane ring formation was considered to be achieved by regioselective cyclization of diol **3**, which would be obtained by deprotection of **4**. Compound **4** would be prepared by epoxidation and a ring opening reaction from *exo*-olefin **5**[16] which can be synthesized from 5-methyluridine in 3 steps.



Scheme 1. Retrosynthetic strategy for 3',4'-EoNA derivative **2**

Initially, to introduce 2-iodoethoxy group at the C4′-position, *exo*-olefin **5** was treated with *m*CPBA in 2-iodoethanol; however, complex mixtures were obtained, and no desired product was detected. It was previously reported by Aso and Suemune et al. that synthesis of 4′-*C*-nitrobenzoyloxythymidine was achieved by $ZnCl_2$-mediated ring opening of epoxide.[17] Therefore, 4′-iodoethoxylation of **5** by epoxide ring-opening using $ZnCl_2$ was carried out. After epoxidation by *in situ* generated dimethyldioxirane (Scheme 2),[18] treatment with $ZnCl_2$ and 2-iodoethanol successfully yielded the desired **6** in 84% yield (*ca.* 1:1 inseparable diastereomixture). Then, considering the synthesis of EoNA-modified oligonucleotides, the primary alcohol in **6** was protected to give **7** in 81% yield by a 4,4′-dimethoxytrityl (DMTr) group commonly used as protecting group of 5′-hydroxyl group for automated DNA synthesis based on phosphoramidite chemistry.



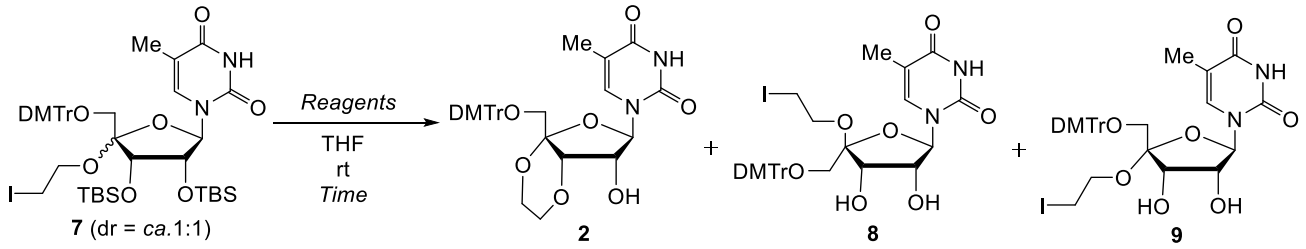
Scheme 2. Synthesis of C4'-(2-iodoethoxy)-5-methyluridine derivatives

By treatment with TBAF (2 eq.), compound **7** underwent not only deprotection of two TBS groups but also intramolecular cyclization to give desired **2** with 3′,4′-EoNA skeleton (entry 1 of Table 1). The cyclized product **2** and α-L-lyxofuranose derivative **8** were isolated in 55% and 42% yields, respectively.

Moreover, uncyclized product **9** and the EoNA derivative cyclized between the 2′- and 4′-positions were not observed at all. Reducing the amount of TBAF to 1 eq. yielded 3′,4′-EoNA derivative **2** (18%) and compound **8** (14%) together with a recovery of starting material **7** in 45% yield (entry 2). No monosilylated products were detected at all. In general, the acidity of the 2′-hydroxyl group of nucleosides is higher than that of the 3′-hydroxyl group. Thus, the results of entries 1 and 2 suggest that desilylation at the 2′-position causes migration of the remaining 3′-TBS group to 2′-hydroxyl group, cyclization between the resulting 3′-hydroxyl group and 4′-iodoethoxy group, and successive desilylation of the migrated TBS group to give **2**.

Acetic acid was added to suppress the basicity of TBAF (entries 3 and 4). In using a 1:1 mixture of TBAF and acetic acid, the effect of acetic acid was not observed. In contrast, the five-fold amount of acetic acid (10 eq.) yielded a ca. 1:3 inseparable diastereomixture of 2′,3′-diol **8** and uncyclized **9** in 28% yield, and no cyclized **2** was obtained, although the desilylation reaction was quite slow. This result indicated that the basicity of the reaction system played a key role to form the intramolecular 1,4-dioxane ring. Using an excess amount of 3HF-triethylamine and HF-pyridine instead of TBAF resulted in no reaction and recovered the starting material **7** (entries 5 and 6). These results demonstrated that the desired **2** was successfully obtained under the conditions shown in entry 1.

Table 1. Reactions of **7** with fluoride reagents



Entry	Reagents	Time	Isolated yields
1	TBAF (2 eq)	3 h	55% (**2**), 42% (**8**)
2	TBAF (1 eq)	3 h	18% (**2**), 14% (**8**), 45% (starting material **7**)
3	TBAF (2 eq), AcOH (2 eq)	3 h	50% (**2**), 21% (**8**)
4	TBAF (2 eq), AcOH (10 eq)	7 d	28% (**8**:**9** = *ca.*1:3), 47% (starting material **7**)
5	3HF-Et_3N (excess)	24 h	No reaction
6	HF-pyridine (excess)	24 h	No reaction

The structure of compound **2** was confirmed by NOESY and HMBC measurements (Figure 3). The stereochemistry of 4′-carbon was determined by NOESY, and the 1,4-dioxane ring was confirmed by

HMBC. On the other hand, the C4′-configuration of **8** was determined by NOESY correlations shown in Figure 4.

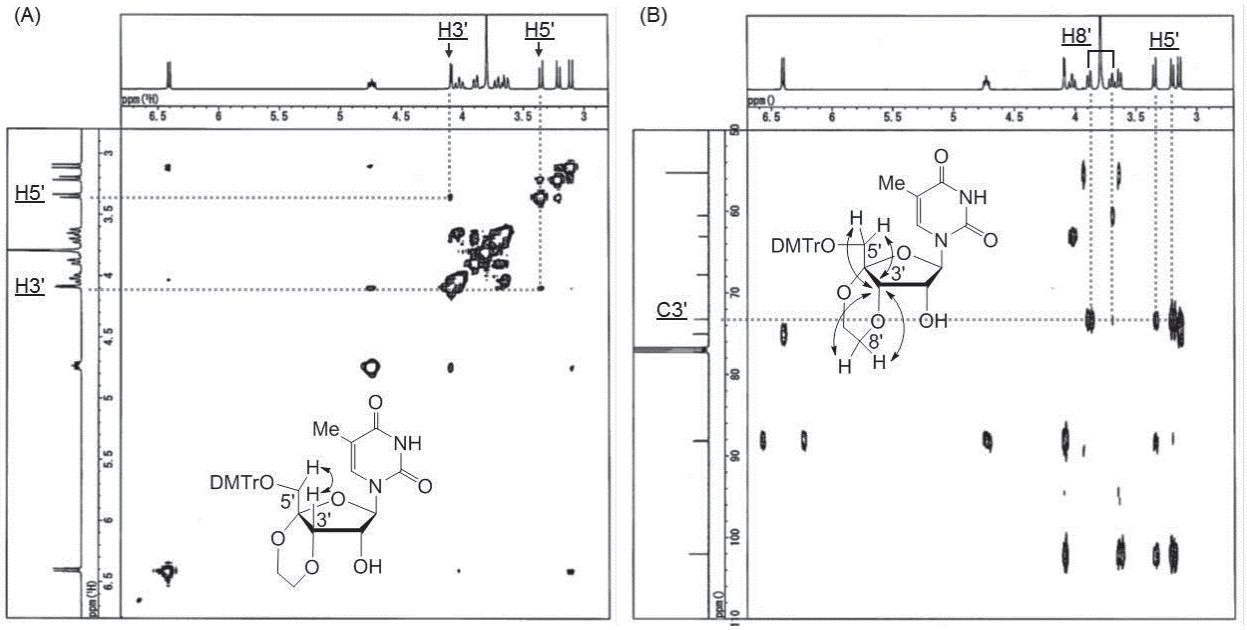


Figure 3. (A) NOESY and (B) HMBC spectra of compound **2**

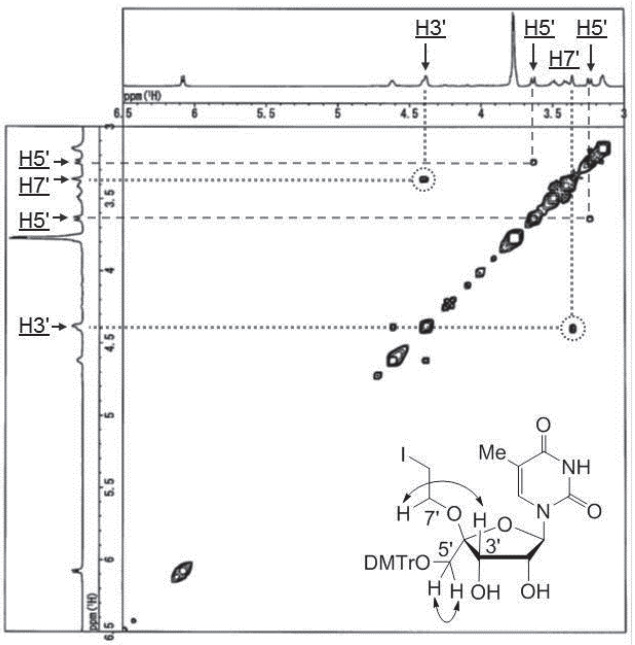
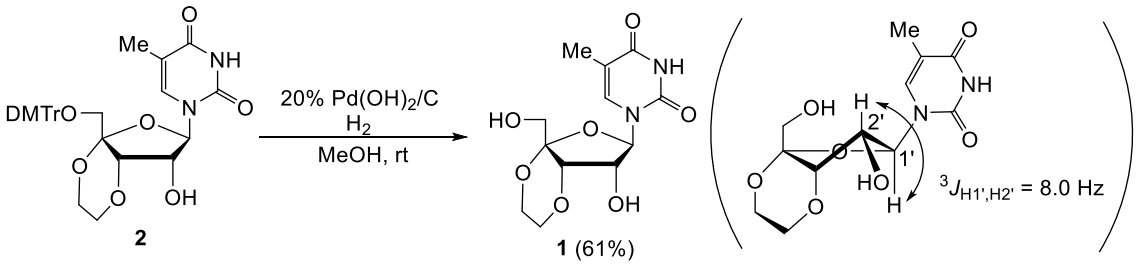


Figure 4. NOESY spectrum of compound **8**

Finally, the 5′-DMTr group was removed by hydrogenolysis to give the desired 3′,4′-EoNA monomer **1** in 61% yield (Scheme 3). Then, the conformational analysis of **1** was carried out by ^{1}H NMR. In general, the sugar conformation of nucleoside is easily determined from the coupling constant between ribose

protons in ^{1}H NMR spectrum.[19] As a result, $^3J_{H1'H2'}$ of 3′,4′-EoNA monomer **1** was 8.0 Hz in CD_3OD, which means that the sugar conformation of **1** was S-type in the same way as other 3′,4′-BNA analogues. Moreover, compound **1** having the acetal moiety (O4′-C4′-O6′) was confirmed to be stable under acidic conditions like 80% AcOH aqueous solution.



Scheme 3. Hydrogenolysis of compound **2**

In summary, we designed 3′,4′-EoNA with a 6′-oxygen atom, and the synthesis of the DMTr derivative of 3′,4′-EoNA was achieved in 7 steps from 5-methyluridine via Lewis acid-mediated C4′-alkoxylation and intramolecular 1,4-dioxane ring formation. To our knowledge, this 3′,4′-EoNA is the first 3′,4′-bridged nucleic acid possessing 6′-oxygen atom, although various 3′,4′-bridged nucleic acids have been developed to date. In the future, the biophysical properties of 3′,4′-EoNA-modified oligonucleotides, prepared by phosphitylation of compound **2** followed by oligonucleotide synthesis, will be explored in detail. We also plan to investigate applications of 3′,4′-EoNA to *iso*DNA- and *iso*RNA-based technologies including the novel antiviral 2′,5′-oligoadenylate.

EXPERIMENTAL

General Methods. All moisture-sensitive reactions were conducted in well-dried glassware under an Ar atmosphere. Anhydrous CH_2Cl_2 and pyridine were used as purchased. All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. ^{1}H NMR spectra were recorded at 500 MHz and ^{13}C NMR spectra were recorded at 125 MHz. Chemical shift values are expressed in δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard, and residual solvents for ^{1}H NMR, and $CDCl_3$ (δ = 77.0 ppm) and CD_3OD (δ = 49.0 ppm) for ^{13}C NMR. Fast atom bombardment mass spectra (FAB-MS) were recorded in positive-ion mode on a JEOL JMS-700 mass spectrometer. For column chromatography, silica gel PSQ 60B was used. The progress of the reaction was monitored by analytical thin-layer chromatography (TLC) on precoated glass plates.

1-[2-*O*,3-*O*-Di-*tert*-butyldimethylsilyl-4-*C*-(2-iodoethoxy)-β-D-ribofuranosyl]thymine and 1-[2-*O*,3-*O*-di-*tert*-butyldimethylsilyl-4-*C*-(2-iodoethoxy)-α-L-lyxofuranosyl]thymine (6)

Acetone (4.0 mL) and sat. $NaHCO_3$ aq. (20 mL) were added to a solution of compound **5**[16] (300 mg, 0.640 mmol) in CH_2Cl_2 (6.0 mL). Then, a solution of Oxone® (787 mg, 1.28 mmol) in H_2O (10 mL) was dropwise added to this solution at 0 °C. The reaction mixture was stirred at room temperature for 3 h. The resulting solution was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue (310 mg) was dissolved in anhydrous CH_2Cl_2 (10 mL) under Ar atmosphere, then 2-iodoethanol (0.50 mL, 6.40 mmol) and $ZnCl_2$ (1 M in THF, 0.64 mL, 0.64 mmol) was added to this solution at −78 °C. The reaction mixture was stirred at 0 °C for 1 h. After being quenched with sat. $NaHCO_3$ aq. at 0 °C, the mixture was filtered through a pad of Celite®. The filtrate was diluted with EtOAc. The solution was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue (488 mg) was purified by column chromatography (silica gel 10 g, *n*-hexane:EtOAc = 5:1 to 2:1) to give a diastereomixture of **6** as a white powder (353 mg, 84%, 2 steps from **5**).

IR (ATR): ν_{max} 3458, 2991, 1754, 1742, 1704, 1468, 1373, 1245 cm^{-1}.^{1}H NMR ($CDCl_3$): δ −0.06 (s, 1.5H), 0.05 (s, 1.5H), 0.08 (s, 1.5H), 0.10 (s, 1.5H), 0.10 (s, 1.5H), 0.12 (s, 1.5H), 0.16 (s, 1.5H), 0.16 (s, 1.5H), 0.87 (s, 4.5H), 0.90 (s, 4.5H), 0.95 (s, 9H), 1.92 (s, 1.5H), 1.94 (s, 1.5H), 2.42 (t, *J* = 5.5 Hz, 0.5H), 3.19–3.22 (m, 1H), 3.34–3.40 (m, 1.5H), 3.58 (dd, *J* = 8.5, 12.0 Hz, 0.5H), 3.72 (d, *J* = 6.5, 12.0 Hz, 0.5H), 3.80–3.90 (m, 1H), 3.96–4.05 (m, 2H), 4.16 (d, *J* = 4.0 Hz, 0.5H), 4.32 (d, *J* = 5.5 Hz, 0.5H), 4.49 (dd, *J* = 4.0, 5.5 Hz, 0.5H), 4.75 (t, *J* = 6.0 Hz, 0.5H), 5.48 (d, *J* = 6.0 Hz, 0.5H), 5.98 (d, *J* = 4.0 Hz, 1H), 7.12 (s, 0.5H), 7.46 (s, 0.5H), 9.27 (brs, 0.5H), 9.41 (brs, 0.5H). MS (FAB): *m/z* = 695 [MK^+]. HRMS (FAB): calcd for $C_{24}H_{45}IKN_2O_7Si_2$ [MK^+], 695.1447; found, 695.1441.

1-[2-*O*,3-*O*-Di-*tert*-butyldimethylsilyl-5-*O*-(4,4′-dimethoxytrityl)-4-*C*-(2-iodoethoxy)-β-D-ribofuranosyl]thymine and 1-[2-*O*,3-*O*-di-*tert*-butyldimethylsilyl-5-*O*-(4,4′-dimethoxytrityl)-4-*C*-(2-iodoethoxy)-α-L-lyxofuranosyl]thymine (7)

Under Ar atmosphere, AgOTf (637 mg, 2.48 mmol) was added to a solution of DMTrCl (840 mg, 2.48 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was stirred at room temperature for 0.5 h. Then, this reaction mixture was dropwised to a solution of compound **6** (1.48 g, 2.25 mmol) and pyridine (0.36 mL, 4.50 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h. After being quenched with MeOH and diluted with CH_2Cl_2, it was then washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue (2.12 g) was purified by column chromatography (silica gel 60 g, *n*-hexane:EtOAc = 5:1 to 3:1) to give a diastereomixture of **7** as a white powder (1.74 g, 81%).

IR (ATR): ν_{max} 2983, 1738, 1447, 1372, 1302, 1237 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ −0.16 (s, 1.5H), −0.07 (s, 1.5H), −0.06 (s, 1.5H), 0.01 (s, 1.5H), 0.00 (s, 3H), 0.02 (s, 1.5H), 0.02 (s, 1.5H), 0.69 (s, 4.5H), 0.79 (s,

4.5H), 0.87 (s, 4.5H), 0.89 (s, 4.5H), 1.56 (s, 1.5H), 2.05 (s, 1.5H), 2.97 (d, *J* = 11.5 Hz, 0.5H), 3.08–3.11 (m, 1H), 3.18–3.23 (m, 1H), 3.29–3.31 (m, 0.5H), 3.39 (d, *J* = 10.0 Hz, 0.5H), 3.76 (s, 3H), 3.78 (s, 3H), 3.80–3.89 (m, 2H), 4.04–4.14 (m, 1.5H), 4.42 (dd, *J* =7.5, 9.0 Hz, 0.5H), 4.49 (dd, *J* = 6.0, 9.5 Hz, 0.5H), 6.18 (d, *J* = 7.5 Hz, 0.5H), 6.23 (d, *J* = 6.0 Hz, 1H), 6.81–7.46 (m, 13H), 7.54, (s, 0.5H), 7.61 (s, 0.5H), 7.98 (brs, 0.5H), 8.03 (brs, 0.5H). MS (FAB): *m/z* = 997 [MK^+]. HRMS (FAB): calcd for $C_{45}H_{63}IKN_2O_9Si_2$ [MK^+], 997.2754; found, 997.2750.

1-[5-*O*-(4,4′-Dimethoxytrityl)-3-*O*,4-*C*-ethyleneoxy-β-D-ribofuranosyl]thymine (2), **1-[5-*O*-(4,4′-dimethoxytrityl)-4-*C*-(2-iodoethoxy)-α-L-lyxofuranosyl]thymine (8)**, and **1-[5-*O*-(4,4′-dimethoxytrityl)-4-*C*-(2-iodoethoxy)-β-D-ribofuranosyl]thymine (9)**

Entry 1 of Table 1: TBAF (1 M in THF, 0.42 mL, 0.42 mmol) was added to a solution of compound **7** (200 mg, 0.209 mmol) in THF (2.0 mL) at room temperature. After being stirred at room temperature for 3 h, the reaction mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue (180 mg) was purified by column chromatography (silica gel 15 g, $CHCl_3$:MeOH = 50:1 to 20:1) to give compound **2** (69.5 mg, 55%) as a white powder and **8** (64.0 mg, 42%) as a white powder.

Entry 2 of Table 1: TBAF (1 M in THF, 0.21 mL, 0.21 mmol) was added to a solution of compound **7** (200 mg, 0.209 mmol) in THF (2.0 mL) at room temperature. After being stirred at room temperature for 3 h, the reaction mixture was then diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue (190 mg) was purified by column chromatography (silica gel 15 g, $CHCl_3$:MeOH = 50:1 to 20:1) to give compound **2** (22.7 mg, 18%) as a white powder and **8** (21.3 mg, 14%) as a white powder, and to recover the starting material **7** (90.6 mg, 45%).

Entry 3 of Table 1: A mixture of TBAF (1 M in THF, 0.27 mL, 0.27 mmol) and AcOH (16 μL, 0.271 mmol) was added to a solution of compound **7** (130 mg, 0.136 mmol) in THF (2.0 mL) at room temperature. After being stirred at room temperature for 3 h, the reaction mixture was then diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue (140 mg) was purified by column chromatography (silica gel 15 g, $CHCl_3$:MeOH = 50:1 to 20:1) to give compound **2** (41.0 mg, 50%) as a white powder and **8** (21.2 mg, 21%) as a white powder.

Entry 4 of Table 1: A mixture of TBAF (1 M in THF, 0.39 mL, 0.39 mmol) and AcOH (0.11 mL, 1.93 mmol) was added to a solution of compound **7** (185 mg, 0.193 mmol) in THF (2.0 mL) at room temperature. After being stirred at room temperature for 7 d, the reaction mixture was then diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue (175 mg) was purified by column chromatography (silica gel 15 g, $CHCl_3$:MeOH = 50:1 to 20:1) to give a diastereomixture of **8** and **9** (**8**:**9** = *ca.*1:3, 38.9 mg, 28%) as a white powder and to recover the starting

material **7** (87.1 mg, 47%).

Compound **2**: Mp 114–116 °C. IR (ATR): ν_{max} 3481, 2984, 1737, 1449, 1373, 1298, 1234 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.35 (s, 3H), 3.08 (d, *J* = 11.0 Hz, 1H), 3.21 (d, *J* = 10.0 Hz, 1H), 3.35 (d, *J* =10.0 Hz, 1H), 3.62–3.74 (m, 2H), 3.80 (s, 6H), 3.89 (d, *J* = 12.0 Hz, 1H), 3.98–4.06 (m, 1H), 4.09 (d, *J* = 4.0 Hz, 1H), 4.74 (ddd, *J* = 4.0, 8.0, 10.0 Hz, 1H), 6.41 (d, *J* = 8.0 Hz, 1H), 6.39–7.37 (m, 13H), 7.62, (s, 1H), 8.04 (brs, 1H). ^{13}C NMR ($CDCl_3$): δ 11.3, 55.3, 60.6, 63.1, 67.9, 73.4, 75.1, 88.1, 88.2, 102.1, 111.7, 113.4, 127.4, 128.2, 130.19, 130.21, 134.4, 134.6, 135.6, 143.5, 151.2, 158.89, 158.91, 163.5. MS (FAB): *m/z* = 641 [MK^+]. HRMS (FAB): calcd for $C_{33}H_{34}KN_2O_9$ [MK^+], 641.1901; found, 641.1901.

Compound **8**: Mp 102–105 °C. IR (ATR): ν_{max} 3195, 2930, 1688, 1610, 1508, 1464, 1251 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.90 (s, 3H), 3.15 (m, 1H), 3.24 (d, *J* = 10.5 Hz, 1H), 3.36–3.49 (m, 3H), 3.64 (d, *J* = 10.5 Hz, 1H), 3.77 (s, 3H), 3.78 (s, 3H), 4.38 (m, 1H), 4.62 (m, 1H), 6.08 (d, *J* = 7.0 Hz, 1H), 6.84–7.49 (m, 14H), 9.67 (brs, 1H). ^{13}C NMR ($CDCl_3$): δ 2.9, 12.5, 55.2, 58.7, 62.5, 74.5, 74.6, 86.3, 89.4, 109.8, 111.7, 113.3, 127.0, 127.9, 129.1, 130.0, 135.0, 135.2, 135.4, 144.2, 151.6, 158.6, 163.7. MS (FAB): *m/z* = 769 [MK^+]. HRMS (FAB): calcd for $C_{33}H_{35}IKN_2O_9$ [MK^+], 769.1024; found, 769.1017.

Compound **9**: Mp 124–126 °C. IR (ATR): ν_{max} 3405, 3021, 1691, 1607, 1508, 1464, 1250, 1216 cm^{-1}. (major peak of the diastereomixture obtained in entry 4): ^1H NMR ($CDCl_3$): δ 1.27 (s, 3H), 3.20 (d, *J* = 10.0 Hz, 1H), 3.32–3.35 (m, 2H), 3.62–3.71 (m, 2H), 3.79 (s, 6H), 3.88 (d, *J* = 11.5 Hz, 1H), 4.02 (t, *J* = 11.0 Hz, 1H), 4.09 (d, *J* = 4.0 Hz, 1H), 4.73 (m, 1H), 6.42 (d, *J* = 8.0 Hz, 1H), 6.41–7.40 (m, 13H), 7.62 (s, 1H), 8.89 (brs, 1H).

1-(3-*O*,4-*C*-Ethyleneoxy-β-D-ribofuranosyl)thymine (1)

20% $Pd(OH)_2$ on carbon (48.9 mg) was added to a solution of compound **2** (84.0 mg, 0.139 mmol) in MeOH (5.0 mL) at room temperature. This suspension was stirred at room temperature for 12 h under H_2 atmosphere. After the reaction mixture was filtered through a pad of Celite®, the filtrate was concentrated *in vacuo*. The residue (92.1 mg) was purified by column chromatography (silica gel 5.0 g, $CHCl_3$:MeOH = 30:1 to 10:1) to give compound **1** as a white powder (25.5 mg, 61%).

Mp 92–94 °C. IR (ATR): ν_{max} 3315, 2944, 2832, 1683, 1449, 1411, 1219 cm^{-1}. ^1H NMR (CD_3OD): δ 1.89 (s, 3H), 3.51 (d, *J* = 10.0 Hz, 1H), 3.59–3.70 (m, 3H), 3.87 (d, *J* = 10.0 Hz, 1H), 4.00–4.02 (m, 2H), 4.68 (dd, *J* = 4.0, 8.0 Hz, 1H), 6.38 (d, *J* = 8.0 Hz, 1H), 7.98 (s, 1H). ^{13}C NMR (CD_3OD): δ 11.0, 60.3, 62.8, 65.6, 73.2, 73.8, 87.7, 102.4, 110.7, 136.9, 151.7, 164.8. MS (FAB): *m/z* = 339 [MK^+]. HRMS (FAB): calcd for $C_{12}H_{16}KN_2O_7$ [MK^+], 339.0595; found, 339.0600.

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