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Development of a novel nucleoside analogue with S-type sugar conformation: 2′-deoxy-*trans*-3′,4′-bridged nucleic acids

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Abstract—Two novel *trans*-3′,4′-bridged nucleic acid (*trans*-3′,4′-BNA) monomers, one with a 3,5,8-trioxabicyclo[5.3.0]decane structure and the other with a 4,7-dioxabicyclo[4.3.0]nonane structure, were successfully synthesized from thymidine. The locked *trans*-fused ring structures of the nucleoside analogues were confirmed by X-ray crystallography, which also indicated that their furanose rings had a typical S-type conformation involving $C_{2'}$-*endo* or $C_{3'}$-*exo* sugar puckering, respectively, and the same ring conformation as that observed in the B-type helical structure of the DNA duplex.

1. Introduction

The five-membered furanose ring in nucleosides or nucleotides is conformationally very flexible and exists in an equilibrium of two major conformers (N- and S-forms). Pre-organization of the nucleoside sugar conformation in an appropriate form is very effective in forming a stable duplex or triplex with single- or double-stranded nucleic acids. The recently developed 2′-*O*,4′-*C*-methylene bridged nucleic acid (2′,4′-BNA[1a–c]/LNA[2]) is an artificial nucleoside with an additional ring between the O2′ and C4′ atoms, so that the sugar moiety is tightly restricted in a typical N-type sugar conformation. Oligonucleotides containing 2′,4′-BNA monomers show significantly enhanced binding affinity not only toward single-stranded RNA complements but also towards double-stranded DNA complements. Various other N-type nucleoside analogues have also been synthesized.[1d–f]

Several nucleoside analogues with S-type sugar conformation also have been prepared, and the hybridizing ability of their oligonucleotide derivatives has been evaluated.[3a–i] However, a considerable decrease in duplex stability with complementary DNA strands was observed in many cases, probably due to inadequate restriction of the sugar moiety and/or steric repulsion between added structural components and neighboring nucleotide residues in the B-type duplex.[4] Ideal artificial nucleosides, which fulfill the structural requirements for stable B-type duplex formation have not yet been developed, and much work is still being carried out in this area.

Previously, we prepared *trans*-3′,4′-bridged nucleic acid (*trans*-3′,4′-BNA) monomers **1** and **2**, which have a 4,7-dioxabicyclo[4.3.0]nonane structure and a methoxy group at the C2′ position, as S-type nucleoside analogues (Fig. 1).[5a–c] The sugar moiety of these artificial nucleosides is restricted to the S-type conformation by the presence of an additional *trans*-fused ring between the C3′ and C4′ atoms of the nucleosides; the additional ring is expected to be positioned outside the B-type DNA duplex without any steric repulsion.

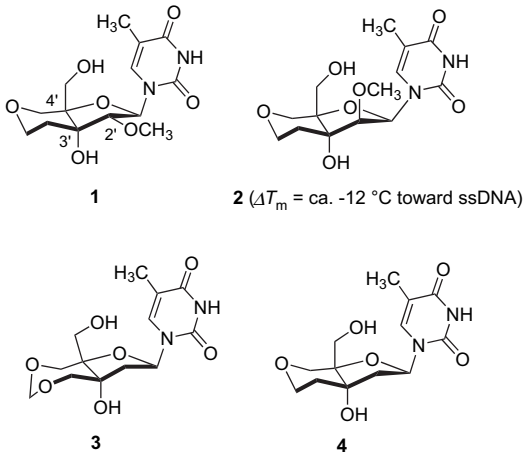

Figure 1. Structures of *trans*-3′,4′-bridged nucleic acids.

Keywords: BNA; DNA structure mimic; Conformation; X-ray crystallography.
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The phosphoramidite derivative of *arabino*-type monomer **2** was successfully synthesized and incorporated into oligonucleotides. The modified oligonucleotides containing monomer **2** were found to show low binding affinity towards complementary ssDNA and ssRNA in comparison with the corresponding natural oligonucleotides,[5c] suggesting that the methoxy group at the C2′ (β) position adversely contacts neighboring residues during formation of the duplex. In addition, previous research on artificial nucleosides restricted to an N-type sugar conformation has shown that the size of the additional ring and the position of oxygen atom on the bridged moiety affect duplex stability.[1b–f]

Based on these considerations, we designed de novo two 2′-deoxy type *trans*-3′,4′-BNA analogues **3**[6] and **4**, which we synthesized from thymidine. One had a 3,5,8-trioxabicyclo[5.3.0]decane structure and the other a 4,7-dioxabicyclo[4.3.0]nonane structure (Fig. 1). The syntheses of **3** and **4** are described below.

2. Results and discussion

2.1. Synthesis of the common intermediate A

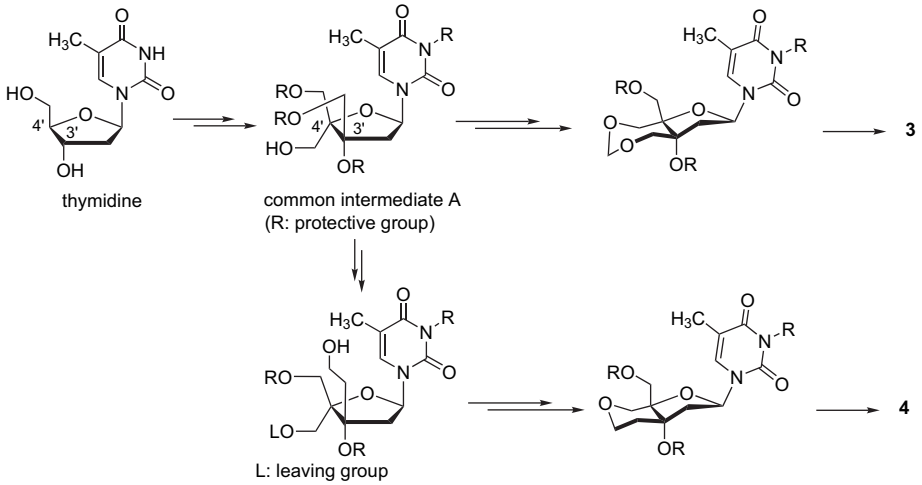
In the synthetic strategy for **3** and **4**, thymidine is used as a starting material, and hydroxymethyl groups are introduced at the C3′ and C4′ positions to give the common intermediate A, as outlined in Scheme 1. The *trans*-fused structure of **3** may be obtained by acetalization and that of **4** may be obtained via a nucleophilic reaction.

First, we prepared the precursor **12** of intermediate A as shown in Scheme 2. The 3′-deoxy-3′-*C*-methylenethymidine derivative **5**, prepared from thymidine according to known procedures,[7] was oxidized using a catalytic amount of osmium tetroxide to give the diol **6** with high stereoselectivity. After protection of the thymine nucleobase in **6** using benzyl chloromethyl ether (BOMCl), the obtained product **7** was treated with dibutyltindimethoxide and chloromethyl methyl ether (MOMCl) to give **8** in good yield.[8] Protection of the 3′-hydroxyl group in **8** with a benzyl group and subsequent removal of the trityl group at C5′ proceeded efficiently to give the primary alcohol **10** via **9**. Dess–Martin oxidation of **10** afforded the corresponding aldehyde **11**, followed by aldol condensation with formaldehyde and reduction with sodium borohydride to give the diol **12**, the precursor of intermediate A.

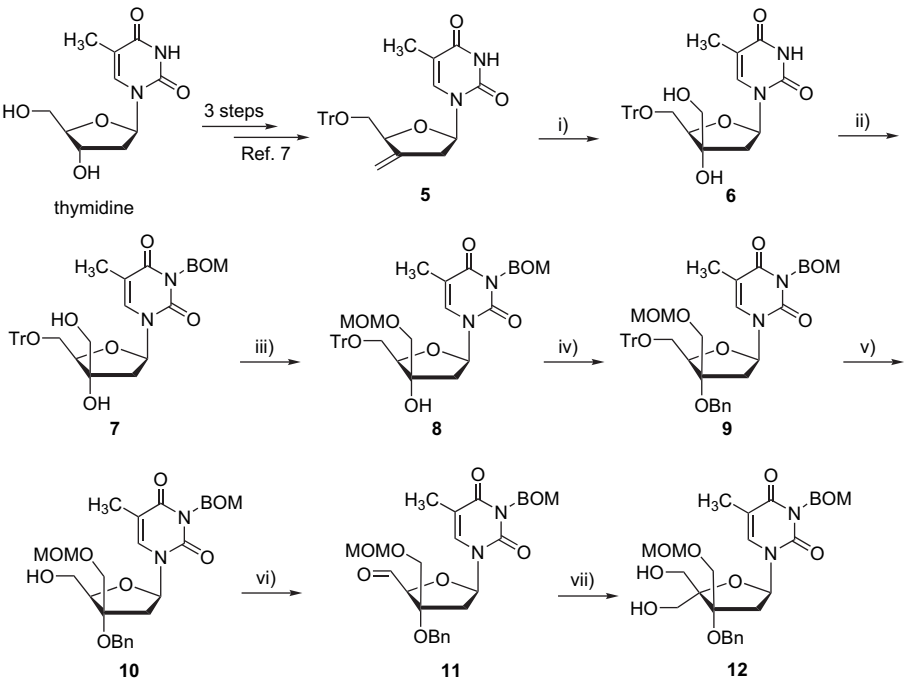
Three possible routes for efficient synthesis of the important intermediate A (**13**) were examined as shown in Scheme 3. In our previous communication, we reported that the 5′-hydroxyl group in **12** was protected with a benzyl group to give **13** (path A).[6] However, selective monobenzylation of the 5′-hydroxyl group did not proceed easily, and the desired product **13** was obtained in relatively low yield (49%) along with its isomer **14** (21%). To improve the yield and selectivity of this benzylation procedure, we attempted regioselective reduction of the corresponding benzylidene acetal **15** (path B), as described in our previous reports.[9] The reduction was carried out using several reagents (Table 1). Treatment of **15** with diisobutylaluminum hydride or sodium cyanoborohydride afforded complex mixtures (Runs 1 and 2); in contrast, the reaction of **15** with triethylsilane in the presence of boron trifluoride diethyl etherate or tin(IV) chloride gave the desired isomer **13**, but in low yield (Runs 3 and 4). Finally, it was found that path C was the most efficient for the preparation of **13**: treatment of **12** with 4,4′-dimethoxytrityl chloride resulted in exclusive tritylation of the hydroxyl group on the opposite side of the C5′ position to give **16**. After benzylation of the remaining 5′-hydroxyl group, the dimethoxytrityl group was efficiently deprotected under mildly acidic conditions to give the desired intermediate **13** in 83% yield over three steps. We inferred that formation of a hydrogen bond between the 5′-hydroxyl group and the oxygen atom at the C3′ substituent in **12** resulted in the opposite hydroxyl group becoming more reactive for tritylation with 4,4′-dimethoxytrityl chloride.

2.2. Synthesis of 3 from intermediate A (13)

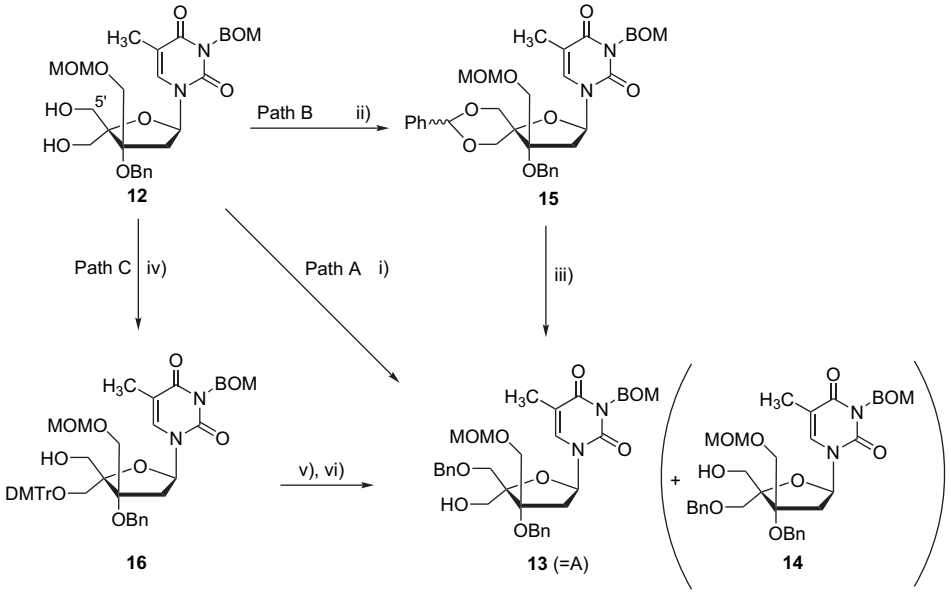
The target nucleoside **3** was synthesized from the intermediate **13** as shown in Scheme 4. We had already developed 2′,4′-BNA^{coc} by construction of a *cis*-methyleneoxymethylene (–C–O–C–) linkage between the O2′ and C4′ atoms.[10] The same conditions—treatment with paraformaldehyde and *p*-toluenesulfonic acid—were applied for direct construction of the *trans*-fused ring structure from **13**.



Scheme 1. Synthetic strategies for **3** and **4** from thymidine.



Scheme 2. Reagents and conditions: (i) OsO_4, *N*-methylmorpholine-*N*-oxide, pyridine/H_2O/*t*-BuOH, 75 °C (65%); (ii) BOMCl, DBU, DMF, 0 °C (89%); (iii) *n*-$Bu_2Sn(OMe)_2$, *i*-Pr_2NEt, MOMCl, 1,4-dioxane/CH_2Cl_2, reflux (93%); (iv) NaH, BnBr, *n*-Bu_4NI, DMF, room temperature (84%); (v) (+)-10-camphorsulphonic acid, CH_2Cl_2/MeOH, room temperature (90%); (vi) Dess–Martin periodinane, CH_2Cl_2, room temperature; (vii) 37% H_2CO aq, 1 M NaOH aq, THF, 15 °C, then $NaBH_4$, THF, 0 °C (63% from **10**).



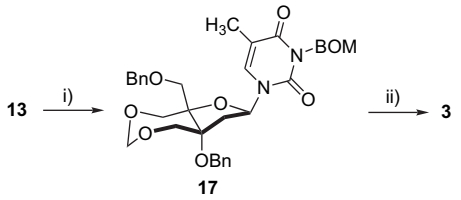
Scheme 3. Reagents and conditions: (i) NaH, BnBr, 0 °C (49%); (ii) benzaldehyde dimethylacetal, (+)-10-camphorsulphonic acid, CH_2Cl_2, room temperature quant; (iii) Et_3SiH, BF_3/Et_2O, CH_2Cl_2, 0 °C (30%); (iv) DMTrCl, Et_3N, CH_2Cl_2, room temperature; (v) NaH, BnBr, *n*-Bu_4NI, DMF, room temperature; (vi) (+)-10-camphorsulphonic acid, CH_2Cl_2/MeOH, room temperature (83% over three steps).

Table 1. Regioselective reduction of **15** for **13**

Run	Conditions	Yield (%)	
		13	**14**
1	DIBAL/H (5 equiv)	Complex mixtures	
2	$NaBH_3CN$ (5 equiv), $TiCl_4$ (5 equiv)	Complex mixtures	
3	Et_3SiH (10 equiv), BF_3/Et_2O (2 equiv)	30	—
4	Et_3SiH (10 equiv), $SnCl_4$ (2 equiv)	21	42

Fortunately, the expected nucleoside **17** was obtained successfully in 47% yield. We surmised that after initial removal of the MOM group in **13** under acidic conditions, a methylene bridged structure was immediately formed between two hydroxyl groups by excess paraformaldehyde to afford **17**.[11] Deprotection of the two benzyl groups and the BOM group in **17** using ammonium formate and 20% $Pd(OH)_2$–C gave the desired nucleoside analogue **3** in

moderate yield. All spectral data supported the structure assigned to **3**, and this structure was conclusively established by X-ray crystallographic analysis.[6]



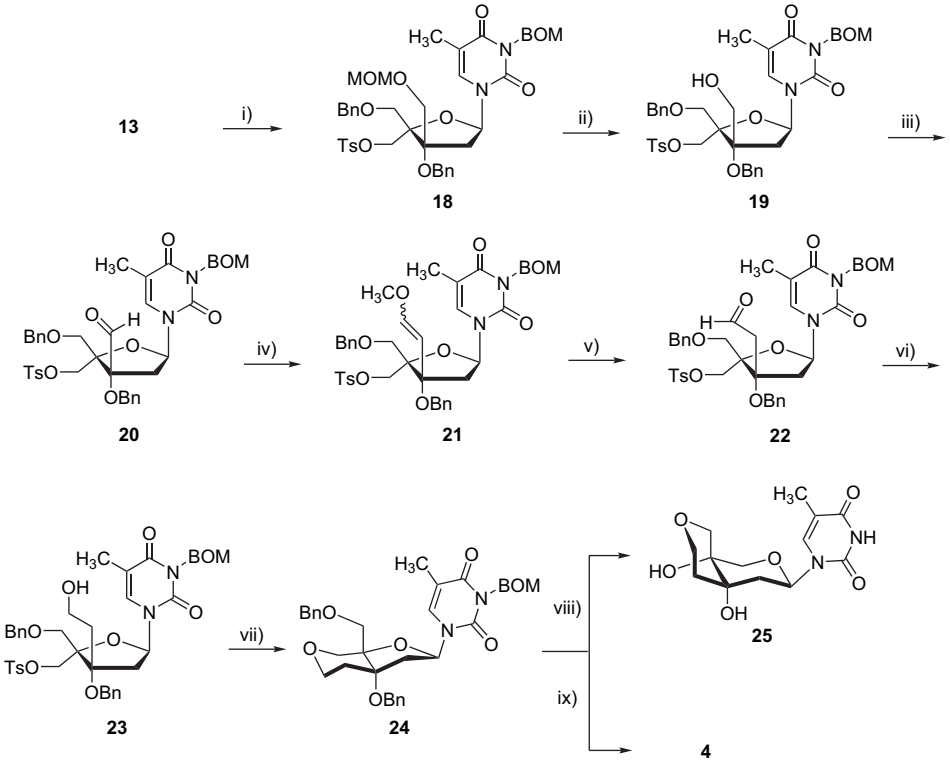
Scheme 4. Reagents and conditions: (i) *p*-TsOH·H_2O, $(CH_2O)_n$, $ClCH_2CH_2Cl$, reflux (47%); (ii) $HCOONH_4$, 20% $Pd(OH)_2$–C, EtOH, reflux (60%).

2.3. Synthesis of 4 from intermediate A (13)

Another type of *trans*-3′,4′-BNA **4**, whose structure contained a six-membered additional ring, was also synthesized from **13** (Scheme 5). After the introduction of a tosyl group as a leaving group in **13**, the MOM group was deprotected by TMSBr to give the alcohol **19** in good yield. Oxidation of **19** gave the corresponding aldehyde **20**, which was subjected to the Wittig reaction to give the enol ether **21**. Treatment of **21** with mercury(II) acetate[12] and subsequent reduction[13] of the resulting compound **22** gave the alcohol **23**, as expected, in fair yield. A base-mediated intramolecular S_N2 reaction of **23** proceeded smoothly on treatment with NaHMDS to give **24** in excellent yield. NMR analysis showed that the values of the coupling constants between the C1′ proton and C2′ protons of **24** were similar to those of **17** (C1′ H: J=6, 8 Hz for **24** and J=5, 9 Hz for **17**), which confirmed the successful formation of the *trans*-fused structure. Surprisingly, treatment of **24** with 20% $Pd(OH)_2$–C and cyclohexene in ethyl acetate gave the single compound **25**. The coupling constants between the C1′ proton and C2′ protons of **25** (C1′ H: J=2, 11 Hz) were remarkably different from those of **3** (C1′ H: J=6, 9 Hz). The *cis*-fused structure of the unexpected nucleoside **25** was confirmed by X-ray crystallographic analysis (Fig. 2, top).[14,15] A possible mechanism for the formation of **25** is shown in Scheme 6; this nucleoside is obtained via protonation at the O4′ position of **4** and furanose ring opening followed by formation of the more stable pyranose ring. Therefore, in order to avoid protonation of **4**, we examined the use of ammonium formate instead of cyclohexene as an H_2 source. Fortunately, synthesis of **4** was accomplished by catalytic hydrogenolysis over $Pd(OH)_2$–C with ammonium formate in 39% yield. The structure of **4** was confirmed by spectral data and X-ray crystallographic analysis (Fig. 2, bottom).[14] X-ray structure investigation showed that the furanose rings of **3**[6] and **4** were restricted to typical S-type sugar conformations, $C_{2'}$-*endo* puckering (P=174°) and $C_{3'}$-*exo* puckering (P=194°) respectively. It was also clear that both nucleobases were in an *anti* orientation (χ:−126° for **3** and χ:−129° for **4**), appropriate for the formation of the B-type duplex.

3. Conclusion

We have successfully synthesized two 2′-deoxy-type *trans*-3′,4′-bridged nucleic acid monomers, with a 3,5,8-



Scheme 5. Reagents and conditions: (i) *p*-TsCl, Et_3N, DMAP, CH_2Cl_2, room temperature quant.; (ii) TMSBr, CH_2Cl_2, −30 °C (97%); (iii) Dess–Martin periodinane, CH_2Cl_2, room temperature; (iv) Ph_3PCH_2OMe·Cl, LiHMDS, THF, −78 °C (66% over two steps); (v) $Hg(OAc)_2$, TBAI, THF/H_2O, room temperature; (vi) $NaBH_4$, THF/H_2O, 0 °C (63% over two steps); (vii) NaHMDS, THF, reflux (92%); (viii) cyclohexene, 20% $Pd(OH)_2$–C, AcOEt, reflux (82%) for **25**; (ix) $HCOONH_4$, 20% $Pd(OH)_2$–C, EtOH, reflux (39%) for **4**.

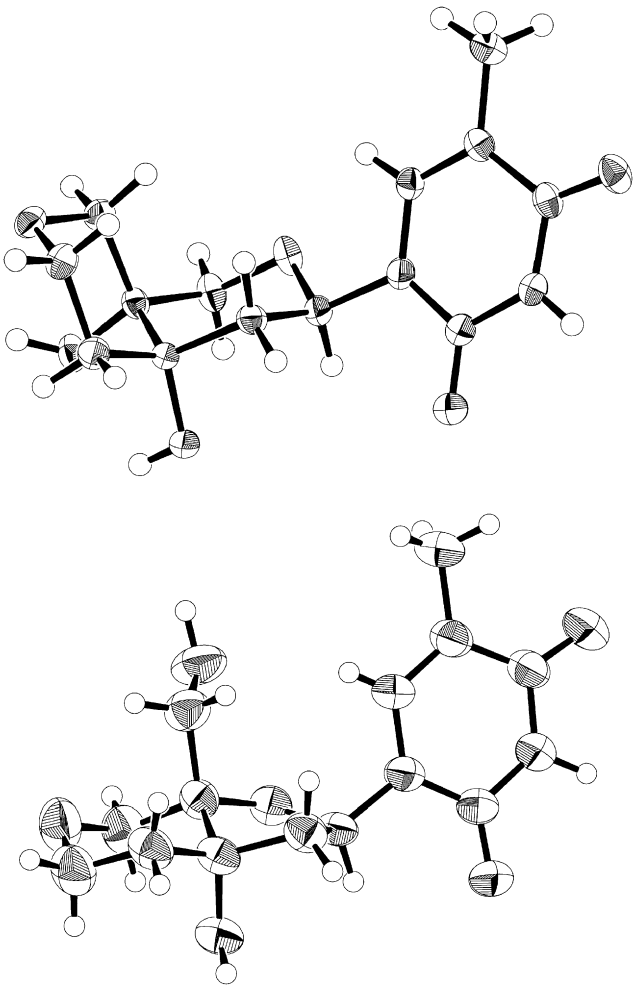
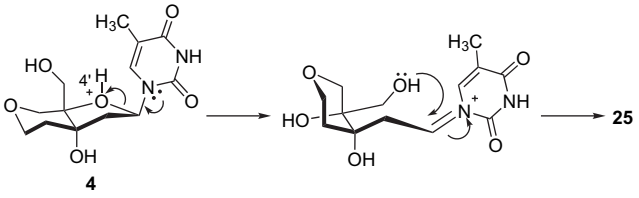


Figure 2. ORTEP drawings of **25** (top) and **4** (bottom).



Scheme 6. Proposed mechanism for the formation of **25**.

trioxabicyclo[5.3.0]decane structure and a 4,7-dioxabicyclo[4.3.0]nonane structure, respectively, using a common intermediate. It was shown by X-ray crystallography that these novel nucleosides had a typical S-type sugar conformation, satisfying the conformational requirements of the B-type DNA duplex. These nucleosides are strong candidates for ideal DNA structure mimics, which are required to investigate the properties of oligonucleotides.

4. Experimental

4.1. General

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

4.1.1. 3′-*C*-Hydroxymethyl-5′-*O*-tritylthymidine (6). To a stirred solution of **5** (5.7 g, 11.9 mmol) in *t*-BuOH were added *N*-methylmorpholine *N*-oxide (9.6 g, 82.1 mmol), pyridine (5.1 mL, 63.1 mmol), H_2O (6.7 mL), and OsO_4 (0.076 M solution in *t*-BuOH, 0.6 mL). The mixture was stirred under N_2 at 75 °C for 8 h. The reaction mixture was cooled to room temperature, and saturated $Na_2S_2O_3$ solution was added. The mixture was evaporated and extracted with AcOEt. The combined organic layers were washed with H_2O and saturated NaCl solution, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=20:1) to give **6** (4.0 g, 65%) as a colorless solid. Mp 112–114 °C. $[\alpha]_D^{26}$ −17.2 (*c* 1.0, $CHCl_3$). IR (KBr) ν_{max} 3423, 3052, 1686, 1474, 1443 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.36 (3H, d, *J*=1 Hz), 2.06 (1H, dd, *J*=9, 13 Hz), 2.36 (1H, dd, *J*=5, 13 Hz), 3.12 (1H, t, *J*=6 Hz), 3.24 (1H, dd, *J*=2, 11 Hz), 3.49–3.67 (3H, m), 4.04 (1H, s), 4.12 (1H, dd, *J*=2, 4 Hz), 6.44 (1H, dd, *J*=5, 9 Hz), 7.22–7.42 (15H, m), 7.64 (1H, d, *J*=1 Hz), 9.50 (1H, s). ^{13}C NMR ($CDCl_3$) δ 11.8, 41.6, 62.6, 65.0, 81.3, 84.1, 86.1, 88.1, 111.4, 127.5, 128.5, 135.6, 142.5, 150.5, 163.7. MS (FAB): *m*/*z* 515 (MH^+). Anal. Calcd for $C_{30}H_{30}N_2O_6$: C, 70.02; H, 5.88; N, 5.44. Found: C, 70.07; H, 6.01; N, 5.23.

4.1.2. 3-*N*-Benzyloxymethyl-3′-*C*-hydroxymethyl-5′-*O*-tritylthymidine (7). To a solution of **6** (302 mg, 0.59 mmol) in DMF (1 mL) was added DBU (0.11 mL, 0.76 mmol) under N_2 with ice-cooling. After stirring for 10 min, BOMCl (0.11 mL, 0.77 mmol) was added, and the mixture was stirred for 5 h at room temperature. The reaction mixture was partitioned with H_2O, and the mixture was extracted with AcOEt. The combined organic layers were washed with H_2O and saturated NaCl solution, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (AcOEt/*n*-hexane=1:1) to give **7** (332 mg, 89%) as a colorless solid. Mp 196–198 °C. $[\alpha]_D^{26}$ −8.4 (*c* 0.85, $CHCl_3$). IR (KBr) ν_{max} 3425, 3028, 2927, 1708, 1661, 1465 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.50 (3H, s), 1.95 (1H, dd, *J*=9, 13 Hz), 2.36 (1H, dd, *J*=5, 13 Hz), 2.54 (1H, t, *J*=7 Hz), 3.08 (1H, s), 3.29 (1H, dd, *J*=2, 11 Hz), 3.49 (1H, dd, *J*=7, 11 Hz), 3.54–3.61 (2H, m), 4.10 (1H, dd, *J*=2, 5 Hz), 4.69 (2H, s), 5.48 (2H, s), 6.43 (1H, dd, *J*=5, 9 Hz), 7.25–7.42 (20H, m), 7.51 (1H, s). ^{13}C NMR ($CDCl_3$) δ 12.5, 41.4, 62.6, 64.9, 70.6, 72.2, 81.0, 84.8, 86.0, 88.3, 110.6, 127.6, 127.7, 128.1, 128.3, 128.5, 134.2, 137.9, 142.6, 151.0, 163.4. MS (FAB): *m*/*z* 657 (M^+Na). Anal. Calcd for $C_{38}H_{38}N_2O_7 \cdot 1/5H_2O$: C, 72.45; H, 6.11; N, 4.29. Found: C, 72.40; H, 6.18; N, 4.29.

4.1.3. 3-*N*-Benzyloxymethyl-3′-*C*-methoxymethyloxymethyl-5′-*O*-tritylthymidine (8). To a stirred solution of **7**

(3.2 g, 5.04 mmol) in 1,4-dioxane (60 mL) was added *n*-$Bu_2Sn(OMe)_2$ (1.3 mL, 5.54 mmol) under N_2. The mixture was refluxed for 5 h. After evaporation of the solvent, the products were dissolved in CH_2Cl_2, and *i*-Pr_2NEt (1.3 mL, 7.56 mmol) and MOMCl (0.6 mL, 7.56 mmol) were added under ice-cooling. The whole was stirred for 2 h at the room temperature. After addition of saturated $NaHCO_3$ solution, the mixture was filtered with Celite, and the crude filtrate was extracted with AcOEt. The combined organic layers were washed with H_2O and saturated NaCl solution, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography ($CHCl_3$/AcOEt=4:1) to give **8** (3.17 g, 93%) as a colorless solid. Mp 59–61 °C. $[\alpha]_D^{26}$ −9.4 (*c* 0.75, $CHCl_3$). IR (KBr) ν_{max} 3464, 3015, 2928, 1712, 1665, 1457 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.23 (3H, s), 2.13 (1H, dd, *J*=10, 13 Hz), 2.38 (1H, dd, *J*=5, 13 Hz), 3.13 (1H, s), 3.23 (3H, s), 3.22–3.24 (1H, m), 3.42, 3.72 (2H, AB, *J*=10 Hz), 3.70–3.72 (1H, m), 4.12 (1H, s), 4.22, 4.38 (2H, AB, *J*=7 Hz), 4.72 (2H, s), 5.50 (2H, s), 6.58 (1H, dd, *J*=5, 10 Hz), 7.24–7.40 (20H, m), 7.82 (1H, s). ^{13}C NMR ($CDCl_3$) δ 12.0, 42.3, 55.5, 62.3, 70.6, 71.0, 72.1, 80.2, 84.7, 86.4, 87.9, 96.8, 110.6, 127.6, 127.6, 127.7, 128.0, 128.3, 128.9, 134.7, 138.0, 142.6, 151.1, 163.5. MS (FAB): *m*/*z* 701 (M^+Na). Anal. Calcd for $C_{40}H_{42}N_2O_8 \cdot 1/4H_2O$: C, 70.31; H, 6.27; N, 4.10. Found: C, 70.27; H, 6.26; N, 4.06.

4.1.4. 3′-*O*-Benzyl-3-*N*-benzyloxymethyl-3′-*C*-methoxymethyloxymethyl-5′-*O*-tritylthymidine (9). A solution of **8** (8.03 g, 11.8 mmol) in DMF (15 mL) was added dropwise to a stirred solution of 60% NaH (520 mg, 13.0 mmol) in DMF (10 mL) under N_2 and ice-cooling. After stirring for 15 min, BnBr (1.55 mL, 13.0 mmol) and *n*-Bu_4NI (436 mg, 1.18 mmol) were added to the mixture. After stirring for an additional 6 h at room temperature, the reaction mixture was partitioned with H_2O and extracted with AcOEt. Work-up as previously described and purification by silica gel column chromatography (AcOEt/*n*-hexane=1:3 to 1:2) afforded **9** (7.60 g, 84%) as a colorless solid. Mp 152–154 °C. $[\alpha]_D^{26}$ −25.3 (*c* 0.88, $CHCl_3$). IR (KBr) ν_{max} 3061, 3031, 2927, 2884, 1710, 1668, 1493, 1450 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.25 (3H, s), 2.08 (1H, dd, *J*=10, 13 Hz), 2.69 (1H, dd, *J*=5, 13 Hz), 3.20 (3H, s), 3.35 (1H, dd, *J*=3, 11 Hz), 3.53, 3.93 (2H, AB, *J*=11 Hz), 3.74 (1H, dd, *J*=4, 11 Hz), 4.00, 4.26 (2H, AB, *J*=7 Hz), 4.33 (1H, unresolved dd appears as a br t, *J*=3 Hz), 4.54, 4.59 (2H, AB, *J*=11 Hz), 4.71 (2H, s), 5.49 (2H, s), 6.51 (1H, dd, *J*=5, 9 Hz), 7.23–7.40 (25H, m), 7.82 (1H, d, *J*=1 Hz). ^{13}C NMR ($CDCl_3$) δ 12.0, 38.8, 55.6, 62.6, 65.3, 66.9, 70.6, 72.2, 84.6, 84.7, 85.3, 87.9, 96.4, 110.6, 127.3, 127.6, 127.6, 127.7, 128.0, 128.3, 128.4, 129.0, 134.7, 138.0, 138.0, 142.7, 151.1, 163.5. MS (FAB): *m*/*z* 791 (M^+Na). Anal. Calcd for $C_{47}H_{48}N_2O_8$: C, 73.42; H, 6.29; N, 3.64. Found: C, 73.17; H, 6.37; N, 3.59.

4.1.5. 3′-*O*-Benzyl-3-*N*-benzyloxymethyl-3′-*C*-(methoxymethyloxymethyl)thymidine (10). To a stirred solution of **9** (1.20 g, 1.56 mmol) in MeOH/CH_2Cl_2 (3:2, v/v, 5 mL) was added (+)-10-camphorsulfonic acid (72 mg, 0.31 mmol) at room temperature. After stirring for 9 h at the same temperature, the reaction mixture was partitioned with saturated $NaHCO_3$ and extracted with AcOEt. The usual work-up and purification by silica gel column chromatography (AcOEt/*n*-hexane=1:2) afforded **10** (739 mg, 90%) as a colorless solid. Mp 90–92 °C. $[\alpha]_D^{25}$ −5.4 (*c* 0.72, $CHCl_3$). IR (KBr) ν_{max} 3465, 3065, 3031, 2928, 1704, 1650, 1467 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.94 (3H, d, *J*=1 Hz), 2.24 (1H, dd, *J*=9, 13 Hz), 2.58 (1H, dd, *J*=5, 13 Hz), 2.98 (1H, t, *J*=6 Hz), 3.41 (3H, s), 3.84–4.01 (4H, m), 4.24 (1H, unresolved dd appears as br t, *J*=3 Hz), 4.60 (2H, s), 4.70 (4H, s), 5.49 (2H, s), 6.19 (1H, dd, *J*=5, 9 Hz), 7.25–7.39 (10H, m), 7.60 (1H, d, *J*=1 Hz). ^{13}C NMR ($CDCl_3$) δ 13.4, 38.0, 56.1, 62.1, 65.4, 66.8, 70.5, 72.2, 85.4, 85.6, 86.8, 97.0, 110.2, 127.2, 127.5, 127.6, 127.6, 128.2, 128.4, 135.6, 137.9, 151.0, 163.3. MS (FAB): *m*/*z* 527 (MH^+). Anal. Calcd for $C_{28}H_{34}N_2O_8$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.75; H, 6.49; N, 5.23.

4.1.6. 3′-*O*-Benzyl-3-*N*-benzyloxymethyl-4′-*C*-hydroxymethyl-3′-*C*-(methoxymethyloxymethyl)thymidine (12). A solution of **10** (3.4 g, 6.46 mmol) in CH_2Cl_2 (40 mL) was added to a suspension of Dess–Martin periodinane (3.6 g, 8.39 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred for 1 h at room temperature. The mixed solvent AcOEt/Et_2O (1:1, v/v, 10 mL) and saturated $Na_2S_2O_3$ solution were added to the reaction mixture, which was swirled until the suspension became clear. The mixture was extracted with the same mixed solvent (1:1 AcOEt/Et_2O, v/v). The combined organic layers were washed with H_2O and saturated NaCl solution, dried over Na_2SO_4, and concentrated under reduced pressure to give the crude **11** (3.6 g). To a stirred solution of **11** (3.6 g) in THF (60 mL) was added 37% aq formaldehyde (5.3 mL) and 1 M aq NaOH (3.3 mL) at 15 °C. The mixture was stirred for 2.5 h at the same temperature. $NaBH_4$ (978 mg, 26 mmol) was added to the reaction mixture with ice-cooling. After stirring for 2 h, the reaction mixture was neutralized with 0.5 M aq $KHSO_4$ and extracted with $CHCl_3$. The usual work-up and purification by silica gel column chromatography (AcOEt/*n*-hexane=2:1) afforded **12** (2.25 g, 63% from **10**) as a colorless oil. $[\alpha]_D^{23}$ +5.7 (*c* 1.32, $CHCl_3$). IR (KBr) ν_{max} 3436, 2929, 1706, 1661, 1464 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.92 (3H, s), 2.30 (1H, dd, *J*=9, 13 Hz), 2.50 (1H, dd, *J*=5, 14 Hz), 2.86 (1H, br s), 3.27 (1H, br s), 3.38 (3H, s), 3.62 (1H, t, *J*=8 Hz), 3.90–4.06 (5H, m), 4.55–4.70 (6H, m), 5.47 (2H, s), 6.08 (1H, dd, *J*=5, 9 Hz), 7.24–7.37 (10H, m), 7.62 (1H, s). ^{13}C NMR ($CDCl_3$) δ 13.2, 36.3, 56.2, 63.3, 64.5, 65.0, 65.3, 70.5, 72.2, 86.1, 86.8, 90.6, 96.9, 110.2, 127.1, 127.6, 127.7, 127.8, 128.3, 128.6, 135.8, 137.6, 137.9, 151.1, 163.4. MS (FAB): *m*/*z* 557 (MH^+). Anal. Calcd for $C_{29}H_{36}N_2O_9 \cdot 3/4H_2O$: C, 61.10; H, 6.63; N, 4.91. Found: C, 61.09; H, 6.35; N, 4.86.

4.1.7. 3′-*O*,5′-*O*-Dibenzyl-3-*N*-benzyloxymethyl-4′-*C*-hydroxymethyl-3′-*C*-(methoxymethyloxymethyl)thymidine (13) and 3′-*O*-benzyl-4′-*C*,3-*N*-dibenzyloxymethyl-3′-*C*-(methoxymethyloxymethyl)thymidine (14). A solution of **12** (509 mg, 0.91 mmol) in DMF (8 mL) was added dropwise to a stirred solution of 60% NaH (40 mg, 1.01 mmol) in DMF (5 mL) under ice-cooling. After stirring for 15 min, BnBr (0.12 mL, 1.01 mmol) was added, and the mixture was stirred for 2.5 h under ice-cooling. After addition of H_2O, the mixture was extracted with AcOEt. The usual work-up and purification by silica gel column chromatography (AcOEt/*n*-hexane=1:2) afforded **13** (291 mg,

49%) and **14** (122 mg, 21%) as colorless oils. Compound **13**: $[\alpha]_D^{21}$ +3.1 (*c* 0.98, $CHCl_3$). IR (KBr) ν_{max} 3479, 2951, 2887, 1707, 1664, 1460 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.64 (3H, d, *J*=1 Hz), 2.11 (1H, dd, *J*=10, 13 Hz), 2.67 (1H, dd, *J*=5, 13 Hz), 2.80 (1H, dd, *J*=3, 10 Hz), 3.34 (3H, s), 3.51 (1H, t, *J*=10 Hz), 3.82 (1H, d, *J*=11 Hz), 3.88 (1H, d, *J*=11 Hz), 3.99–4.07 (3H, m), 4.50–4.64 (6H, m), 4.67 (2H, s), 5,46 (2H, s), 6.38 (1H, dd, *J*=5, 10 Hz), 7.23–7.36 (15H, m), 7.80 (1H, d, *J*=1 Hz). ^{13}C NMR ($CDCl_3$) δ 13.0, 37.2, 56.3, 63.5, 64.3, 65.3, 70.4, 72.1, 73.1, 73.9, 84.1, 86.0, 90.2, 96.8, 110.0, 127.0,127.5, 127.6, 127.6, 127.8, 128.1, 128.2, 128.4, 128.6, 134.7, 136.6. MS (FAB): *m/z* 647 (MH^+). HRMS (FAB): calcd for $C_{36}H_{43}N_2O_9$ (MH^+): 647.2969; found: 647.2980. Compound **14**: $[\alpha]_D^{21}$ +23.0 (*c* 0.85, $CHCl_3$). IR (KBr) ν_{max} 3453, 2935, 1706, 1660, 1461, 1095 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.94 (3H, d, *J*=1 Hz), 2.17 (1H, dd, *J*=9, 14 Hz), 2.80 (1H, dd, *J*=5, 14 Hz), 3.20 (1H, dd, *J*=5, 7 Hz), 3.35 (3H, s), 3.81, 3.96 (2H, AB, *J*=11 Hz), 3.84, 3.98 (2H, AB, *J*=10 Hz), 3.87 (1H, dd, *J*=7, 12 Hz), 4.04 (1H, dd, *J*=5, 12 Hz), 4.48 (1H, d, *J*=12 Hz), 4.59–4.63 (5H, m), 4.69 (2H, s), 5.49 (2H, s), 6.21 (1H, dd, *J*=5, 9 Hz), 7.24–7.38 (15H, m), 7.82 (1H, d, *J*=1 Hz). ^{13}C NMR ($CDCl_3$) δ 13.4, 36.9, 56.0, 64.9, 65.0, 66.1, 70.4, 72.2, 72.5, 73.8, 86.1, 86.2, 89.9, 96.8, 109.9, 126.9, 127.4, 127.5, 127.5, 127.6, 128.2, 128.3, 128.3, 135.3, 137.7, 137.8, 137.9. MS (FAB): *m/z* 647 (MH^+). Anal. Calcd for $C_{36}H_{42}N_2O_9 \cdot 3/4H_2O$: C, 65.46; H, 6.37; N, 4.30. Found: C, 65.49; H, 6.64; N, 4.24.

4.1.8. 3′-*O*-Benzyl-3-*N*-benzyloxymethyl-4′-CH_2*O*,5′-*O*-benzylidene-3′-*C*-(methoxymethyloxymethyl)thymidine (15). To a solution of **12** (53 mg, 0.095 mmol) in CH_2Cl_2 (1 mL) were added benzaldehyde dimethyl acetal (68 μL, 0.48 mmol) and (+)-10-camphorsulfonic acid (4 mg, 0.019 mmol) at room temperature, and the mixture was stirred for 2 h at the same temperature. After addition of saturated $NaHCO_3$ solution, the mixture was extracted with AcOEt. The usual work-up and purification by silica gel column chromatography (AcOEt/*n*-hexane=2:3) afforded **15** (48 mg, 74% for *R*-isomer; 16 mg, 25% for *S*-isomer) as colorless solids. *R*-Isomer: Mp 54–56 °C. $[\alpha]_D^{21}$ +65.8 (*c* 1.13, $CHCl_3$). IR (KBr) ν_{max} 1044, 1078, 1271, 1458, 1660, 1706, 2933 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.88 (1H, dd, *J*=8, 15 Hz), 1.92 (3H, s), 3.17 (1H, dd, *J*=6, 15 Hz), 3.41 (3H, s), 3.49, 3.92 (2H, AB, *J*=12 Hz), 4.00 (1H, dd, *J*=3, 12 Hz), 4.26 (1H, d, *J*=12 Hz), 4.33 (1H, d, *J*=13 Hz), 4.50 (1H, dd, *J*=3, 13 Hz), 4.59 (2H, s), 4.63, 4.67 (2H, AB, *J*=7 Hz), 4.71 (2H, s), 5.49 (2H, s), 5.58 (1H, s), 6.05 (1H, br t, *J*=7 Hz), 7.21–7.40 (13H, m), 7.54 (2H, dd, *J*=1, 7 Hz), 7.83 (1H, s). ^{13}C NMR ($CDCl_3$) δ 13.5, 38.6, 56.2, 64.6, 65.4, 70.4, 70.9, 72.2, 84.1, 85.1, 86.7, 96.9, 101.2, 109.3, 125.9, 126.7, 127.0, 127.5, 127.6, 127.6, 128.2, 128.2, 128.4, 128.5, 129.0, 134.2, 137.5, 137.5, 137.9, 150.6, 163.4. MS (FAB): *m/z* 645 (MH^+). Anal. Calcd for $C_{36}H_{40}N_2O_9 \cdot 1/2H_2O$: C, 66.14; H, 6.25; N, 4.29. Found: C, 65.91; H, 6.20; N, 4.25. *S*-Isomer: Mp 43–45 °C. $[\alpha]_D^{21}$ +24.5 (*c* 0.76, $CHCl_3$). IR (KBr) ν_{max} 1038, 1098, 1459, 1664, 1706, 2881, 2948 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.97 (3H, s), 2.28 (1H, dd, *J*=10, 14 Hz), 3.04 (1H, dd, *J*=5, 14 Hz), 3.34 (3H, s), 3.72, 3.91 (2H, AB, *J*=12 Hz), 4.13, 4.15 (2H, AB, *J*=11 Hz), 4.38 (1H, dd, *J*=2, 12 Hz), 4.63 (2H, s), 4.70 (2H, s), 4.78, 4.87 (2H, AB, *J*=11 Hz), 4.83 (1H, dd, *J*=2, 11 Hz), 5.50 (2H, s), 5.55 (1H, s), 6.11 (1H, dd, *J*=5, 10 Hz), 7.13 (1H, s), 7.25–7.41 (13H, m), 7.46 (2H, m). ^{13}C NMR ($CDCl_3$) δ 13.6, 37.0, 55.8, 66.3, 68.7, 68.9, 70.5, 71.6, 72.2, 79.8, 85.3, 85.8, 96.0, 101.3, 110.3, 126.1, 127.0, 127.4, 127.5, 127.6, 128.2, 128.3, 128.3, 129.0, 133.6, 137.0, 138.3, 150.6, 163.1. MS (FAB): *m/z* 645 (MH^+). Anal. Calcd for $C_{36}H_{40}N_2O_9 \cdot 1/2H_2O$: C, 66.14; H, 6.25; N, 4.29. Found: C, 66.15; H, 6.26; N, 4.24.

4.1.9. 3′-*O*,5′-*O*-Dibenzyl-3-*N*-benzyloxymethyl-4′-*C*-hydroxymethyl-3′-*C*-(methoxymethyloxymethyl)thymidine (13). To a solution of **12** (580 mg, 1.04 mmol) in CH_2Cl_2 (18 mL) were added Et_3N (0.29 mL, 2.08 mmol) and 4,4′-dimethoxytrityl chloride (459 mg, 1.36 mmol) at room temperature, and the mixture was stirred for 10 min. After addition of saturated $NaHCO_3$ solution, the reaction mixture was extracted with AcOEt. The combined organic layers were washed with H_2O and saturated NaCl solution, dried over Na_2SO_4, and concentrated under reduced pressure. The yellow residue containing **16** was co-evaporated with toluene. To a suspension of 60% sodium hydride (83 mg, 2.08 mmol) in DMF (3 mL) was added the residue in DMF (7 mL) with ice-cooling. After stirring for 30 min, benzyl bromide (0.15 mL, 1.25 mmol) and *n*-Bu_4NI (192 mg, 0.52 mmol) were added and the mixture was stirred for 2 h at room temperature. After addition of H_2O, the mixture was extracted with Et_2O. The organic layer was washed with H_2O and saturated NaCl solution, dried over $MgSO_4$, and concentrated under reduced pressure. The yellow oily residue was dissolved without further treatment in CH_2Cl_2/MeOH (1:1, v/v, 14 mL), to which (+)-10-camphorsulfonic acid (73 mg, 0.31 mmol) was added, and the mixture was stirred for 10 min. After addition of saturated $NaHCO_3$ solution, the mixture was extracted with AcOEt. The usual work-up and purification by silica gel column chromatography (AcOEt/*n*-hexane=2:3) afforded **13** (559 mg, 83% over three steps).

4.1.10. 3′-*O*,5′-*O*-Dibenzyl-3-*N*-benzyloxymethyl-3′-*C*,4′-*C*-(2,4-dioxapentamethylene)thymidine (17). To a solution of **13** (229 mg, 0.35 mmol) in 1,2-dichloroethane (5 mL) were added paraformaldehyde (229 mg) and *p*-toluenesulfonic acid monohydrate (7 mg, 0.037 mmol), and the mixture was refluxed for 4.5 h. After addition of saturated $NaHCO_3$ solution, the mixture was extracted with $CHCl_3$. The usual work-up and purification by silica gel column chromatography (AcOEt/*n*-hexane=1:3 to 1:2) afforded **17** (103 mg, 47%) as a colorless oil. $[\alpha]_D^{26}$ +0.82 (*c* 0.50, $CHCl_3$). IR (KBr) ν_{max} 3064, 3031, 2954, 1957, 1704, 1660 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.71 (3H, d, *J*=1 Hz), 2.38 (1H, dd, *J*=9, 14 Hz), 2.87 (1H, dd, *J*=5, 13 Hz), 3.67 (1H, d, *J*=11 Hz), 3.74 (1H, d, *J*=13 Hz), 3.96 (1H, d, *J*=11 Hz), 4.26 (1H, d, *J*=11 Hz), 4.33 (1H, d, *J*=11 Hz), 4.37 (1H, d, *J*=13 Hz), 4.56–4.64 (4H, m), 4.67 (2H, s), 4.90, 5.33 (2H, AB, *J*=8 Hz), 5.45 (2H, s), 6.29 (1H, dd, *J*=5, 9 Hz), 7.23–7.40 (15H, m), 7.82 (1H, d, *J*=1 Hz). ^{13}C NMR ($CDCl_3$) δ 13.0, 37.2, 62.9, 63.7, 66.2, 70.4, 72.1, 73.3, 74.0, 77.2, 87.2, 88.0, 90.9, 93.1, 109.6, 127.4, 127.6, 127. 7, 127.7, 128.0, 128.1, 128.2, 128.6, 128.7, 134.8, 136.7, 137.0, 137.9, 150.9, 163.5. MS (FAB): *m/z* 615 (MH^+). Anal. Calcd for $C_{35}H_{38}N_2O_8 \cdot 3/4H_2O$: C, 66.92; H, 6.34; N, 4.46. Found: C, 66.99; H, 6.24; N, 4.41.

4.1.11. 3′-*C*,4′-*C*-(2,4-Dioxapentamethylene)thymidine (3). To a solution of **17** (26 mg, 0.053 mmol) in EtOH (1.5 mL) were added ammonium formate (33 mg, 0.53 mmol) and 20% $Pd(OH)_2$–C (26 mg), and the mixture was refluxed for 5.5 h. After filtration of the mixture, the filtrate was concentrated and purified by silica gel preparative TLC ($CHCl_3$/MeOH=10:1) to give **3** (10 mg, 60%) as a colorless solid. Mp 244–247 °C. $[\alpha]_D^{21}$ +75.8 (*c* 0.25, CH_3OH). IR (KBr) ν_{max} 3348, 3167, 3059, 2924, 2889, 2505, 2279, 1695, 1470 cm^{-1}. ^1H NMR (CD_3OD) δ 1.89 (3H, d, *J*=1 Hz), 2.36 (1H, dd, *J*=6, 13 Hz), 2.48 (1H, dd, *J*=9, 12 Hz), 3.73, 4.15 (2H, AB, *J*=11 Hz), 3.81 (1H, d, *J*=12 Hz), 3.91 (1H, d, *J*=13 Hz), 4.03 (2H, unresolved dd appears t, *J*=12 Hz), 4.85, 5.55 (2H, AB, *J*=8 Hz), 6.32 (1H, dd, *J*=6, 9 Hz), 8.15 (1H, d, *J*=1 Hz). ^{13}C NMR (CD_3OD) δ 12.6, 44.1, 63.7, 66.2, 70.9, 83.6, 88.5, 92.2, 94.1, 111.1, 138.7, 152.4, 166.6. MS (FAB): *m*/*z* 315 (MH^+). HRMS (FAB): calcd for $C_{13}H_{19}N_2O_7$ (MH^+): 315.1192; found: 315.1216. Anal. Calcd for $C_{13}H_{18}N_2O_7\cdot 3/2H_2O$: C, 45.74; H, 6.20; N, 8.20. Found: C, 46.08; H, 5.82; N, 8.28.

4.1.12. 3′-*O*,5′-*O*-Dibenzyl-3-*N*-benzyloxymethyl-3′-*C*-methoxymethyloxymethyl-4′-*C*-(*p*-toluenesulfonyloxymethyl)thymidine (18). To a solution of **13** (1.19 g, 1.84 mmol) in CH_2Cl_2 (18 mL) were added Et_3N (1.03 mL, 7.38 mmol), *p*-toluenesulfonyl chloride (527 mg, 2.77 mmol), and 4-dimethylaminopyridine (113 mg, 0.92 mmol) at room temperature, and the mixture was stirred for 13 h. After addition of saturated $NaHCO_3$ solution, the reaction mixture was extracted with AcOEt. The usual work-up and purification by silica gel column chromatography (AcOEt/*n*-hexane=1:2) afforded **18** as a colorless foam (1.49 g, quant). $[\alpha]_D^{23}$ −9.3 (*c* 1.00, $CHCl_3$). IR (KBr) ν_{max} 1098, 1177, 1268, 1362, 1460, 1658, 1710, 2890 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.62 (3H, d, *J*=2 Hz), 2.06 (1H, dd, *J*=10, 13 Hz), 2.40 (3H, s), 2.55 (1H, dd, *J*=5, 13 Hz), 3.31 (3H, s), 3.77, 3.95 (2H, AB, *J*=11 Hz), 3.84, 3.99 (2H, AB, *J*=11 Hz), 4.31, 4.43 (2H, AB, *J*=11 Hz), 4.45, 4.51 (2H, AB, *J*=6 Hz), 4.49 (2H, s), 4.54, 4.61 (2H, AB, *J*=11 Hz), 4.65 (2H, s), 5.44 (2H, s), 6.22 (1H, dd, *J*=5, 10 Hz), 7.19–7.38 (17H, m), 7.66 (1H, d, *J*=2 Hz), 7.71–7.74 (2H, m). ^{13}C NMR ($CDCl_3$) δ 13.0, 21.7, 36.9, 56.0, 64.4, 65.8, 70.4, 71.0, 71.8, 72.1, 73.9, 84.7, 86.7, 88.2, 96.8, 109.9, 126.9, 126.9, 127.5, 127.5, 127.6, 127.6, 127.7, 127.7, 128.0, 128.0, 128.1, 128.1, 128.2, 128.2, 128.6, 128.6, 129.6, 129.6, 132.1, 134.6, 136.5, 137.4, 137.4, 137.7, 144.7, 150.8, 163.2. MS (FAB): *m*/*z* 801 (MH^+). HRMS (FAB): calcd for $C_{43}H_{49}N_2O_{11}S$ (MH^+): 801.3057; found: 801.3093.

4.1.13. 3′-*O*,5′-*O*-Dibenzyl-3-*N*-benzyloxymethyl-3′-*C*-hydroxymethyl-4′-*C*-(*p*-toluenesulfonyloxymethyl)-thymidine (19). To a solution of **18** (1.26 g, 1.57 mmol) in CH_2Cl_2 (16 mL) was added TMSBr (2.49 mL, 18.9 mmol) at −30 °C, and the mixture was gradually warmed to 0 °C over 2 h. The mixture was then stirred for 22 h at 0 °C. After addition of saturated $NaHCO_3$ solution, the reaction mixture was extracted with AcOEt. The usual work-up and purification by silica gel column chromatography (AcOEt/*n*-hexane=2:3) afforded **19** as a colorless solid (1.15 g, 97%). Mp 60–63 °C. $[\alpha]_D^{23}$ +2.06 (*c* 0.96, $CHCl_3$). IR (KBr) ν_{max} 1096, 1176, 1266, 1362, 1455, 1658, 1706, 2929, 3428, 3745, 3857, 3921 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.67 (3H, d, *J*=1 Hz), 1.95 (1H, dd, *J*=10, 13 Hz), 2.20 (1H, dd, *J*=6, 7 Hz), 2.41 (3H, s), 2.55 (1H, dd, *J*=5, 13 Hz), 3.81, 3.88 (2H, AB, *J*=11 Hz), 3.80–3.88 (1H, m), 4.02 (1H, dd, *J*=6, 13 Hz), 4.31, 4.40 (2H, AB, *J*=11 Hz), 4.52 (2H, s), 4.55, 4.65 (2H, AB, *J*=11 Hz), 4.66 (2H, s), 5.44 (2H, s), 6.15 (1H, dd, *J*=5, 10 Hz), 7.20–7.38 (17H, m), 7.41 (1H, d, *J*=1 Hz), 7.71–7.74 (2H, m). ^{13}C NMR ($CDCl_3$) δ 13.0, 21.8, 36.3, 60.5, 64.5, 70.4, 70.7, 70.9, 72.2, 74.2, 84.7, 88.0, 88.1, 110.1, 127.0, 127.0, 127.6, 127.6, 127.6, 127.8, 127.9, 127.9, 128.2, 128.2, 128.3, 128.5, 128.5, 128.7, 128.7, 129.7, 129.7, 132.3, 134.1, 136.4, 137.2, 137.8, 144.8, 150.7, 163.2. MS (FAB): *m*/*z* 757 (MH^+). HRMS (FAB): calcd for $C_{41}H_{45}N_2O_{10}S$ (MH^+): 757.2795; found: 757.2741.

4.1.14. 3′-*O*,5′-*O*-Dibenzyl-3-*N*-benzyloxymethyl-3′-*C*-(2-methoxyethenyl)-4′-*C*-(*p*-toluenesulfonyloxymethyl)thymidine (21 *E*/*Z*). To a suspension of Dess–Martin periodinane (110 mg, 0.26 mmol) in CH_2Cl_2 (1.5 mL) was added a solution of compound **19** (151 mg, 0.20 mmol) in CH_2Cl_2 (1 mL), and the mixture was stirred for 1 h at room temperature. After addition of a mixture of saturated $Na_2S_2O_3$ and saturated $NaHCO_3$ solutions (1:2, v/v), the reaction mixture was stirred for a further 20 min and then extracted with Et_2O. The organic layer was washed with saturated $NaHCO_3$ solution, H_2O and saturated NaCl solution, dried over $MgSO_4$, and concentrated under reduced pressure to give crude compound **20**. To a suspension of (methoxymethyl)triphenyl phosphonium chloride (171 mg, 0.50 mmol) in THF (2.5 mL) was added LiHMDS (1.07 M in *n*-hexane, 0.411 mL, 0.44 mmol) with ice-cooling, and the mixture was stirred for 20 min at room temperature. To this mixture was added a solution of **20** in THF (1.5 mL) at −78 °C, and the mixture was gradually warmed to room temperature over 2 h. After addition of H_2O, the reaction mixture was extracted with AcOEt. The usual work-up and purification by silica gel column chromatography (AcOEt/*n*-hexane=2:5) afforded **21** as a colorless foam (103 mg, 66% in two steps, *E*:*Z*=7:4). IR (KBr) ν_{max} 1092, 1178, 1356, 1373, 1469, 1669, 1707, 2928, 3069 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.52 (12/11H, d, *J*=1 Hz), 1.64 (21/11H, d, *J*=1 Hz), 2.31 (7/11H, dd, *J*=10, 13 Hz), 2.37 (12/11H, s), 2.39 (21/11H, s), 2.54 (4/11H, dd, *J*=10, 14 Hz), 2.64 (7/11H, dd, *J*=5, 13 Hz), 2.89 (4/11H, dd, *J*=5, 14 Hz), 3.52 (21/11H, s), 3.61, 3.77 (14/11H, AB, *J*=10 Hz), 3.63 (12/11H, s), 3.69, 3.84 (8/11H, AB, *J*=11 Hz), 4.16 (4/11H, d, *J*=10 Hz), 4.18 (7/11H, d, *J*=10 Hz), 4.33–4.54 (59/11H, m), 4.65 (2H, s), 4.80 (7/11H, d, *J*=13 Hz), 5.44 (2H, s), 6.18 (4/11H, d, *J*=7 Hz), 6.25 (1H, dd, *J*=5, 10 Hz), 6.54 (7/11H, d, *J*=13 Hz), 7.18–7.37 (17H, m), 7.66–7.73 (3H, m). ^{13}C NMR ($CDCl_3$) δ 12.8, 13.0, 21.7, 21.7, 37.5, 39.9, 50.9, 56.4, 60.6, 64.3, 64.7, 69.4, 69.8, 70.4, 71.8, 72.1, 72.1, 73.8, 74.1, 84.8, 84.9, 85.5, 86.1, 88.2, 98.7, 100.1, 110.0, 116.0, 126.9, 127.0, 127.1, 127.3, 127.3, 127.5, 127.5, 127.6, 127.7, 127.9, 128.1, 128.1, 128.2, 128.3, 128.4, 128.6, 129.7, 129.7, 132.1, 134.6, 134.9, 136.8, 137.3, 137.8, 137.8, 138.0, 138.4, 144.8, 144.9, 150.7, 150.9, 150.9, 151.5, 163.3, 163.3. MS (FAB): *m*/*z* 783 (MH^+). HRMS (FAB): calcd for $C_{43}H_{47}N_2O_{10}S$ (MH^+): 783.2951; found: 783.2955.

4.1.15. 3′-*O*,5′-*O*-Dibenzyl-3-*N*-benzyloxymethyl-3′-*C*-(2-hydroxyethyl)-4′-*C*-(*p*-toluenesulfonyloxymethyl)thymidine (23). To a solution of **21** (528 mg, 0.67 mmol) in THF/H_2O (4:1, v/v, 11 mL) was added mercury(II) acetate

(860 mg, 2.70 mmol) at room temperature, and the mixture was stirred for 17 h. Next, *n*-Bu_4NI (2.50 g, 6.74 mmol) was added and the reaction mixture was stirred for a further 2 h. After addition of saturated NH_4Cl solution, the reaction mixture was extracted with AcOEt. The organic layer was washed with H_2O and saturated NaCl solution, dried over Na_2SO_4, and concentrated under reduced pressure to give **22**. To a solution of **22** in THF/H_2O (4:1, v/v, 11 mL) was added $NaBH_4$ (306 mg, 8.09 mmol) with ice-cooling, and the mixture was stirred for 15 min under the same conditions. After addition of saturated NH_4Cl solution, the reaction mixture was extracted with AcOEt. The usual work-up and purification by silica gel column chromatography (AcOEt/*n*-hexane=2:3) afforded **23** as a colorless foam (325 mg, 63% in two steps). $[\alpha]_D^{22}$ −9.92 (*c* 0.99, $CHCl_3$). IR (KBr) ν_{max} 1094, 1177, 1278, 1362, 1456, 1653, 1706, 2928, 3481, 3920 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.61 (3H, s), 2.10–2.33 (3H, m), 2.40 (3H, s), 2.75 (1H, dd, *J*=5, 14 Hz), 3.69–3.85 (2H, m), 3.83, 3.90 (2H, AB, *J*=11 Hz), 4.20, 4.40 (2H, AB, *J*=10 Hz), 4.40, 4.51 (2H, AB, *J*=11 Hz), 4.56, 4.61 (2H, AB, *J*=12 Hz), 4.65 (2H, s), 5.43 (2H, s), 6.13 (1H, dd, *J*=5, 10 Hz), 7.17–7.40 (17H, m), 7.60 (1H, s), 7.69–7.73 (2H, m). ^{13}C NMR ($CDCl_3$) δ 12.9, 21.7, 33.5, 40.2, 57.9, 64.0, 70.4, 70.5, 71.4, 72.1, 73.9, 83.9, 86.6, 88.5, 109.9, 126.8, 126.8, 127.5, 127.5, 127.5, 127.6, 127.6, 127.9, 127.9, 128.1, 128.1, 128.2, 128.4, 128.4, 128.6, 128.6, 129.7, 129.7, 132.0, 132.0, 134.5, 136.6, 137.0, 137.7, 144.9, 150.8, 163.2. MS (FAB): *m/z* 771 (MH^+). HRMS (FAB): calcd for $C_{42}H_{47}N_2O_{10}S$ (MH^+): 771.2951; found: 771.2944.

4.1.16. 3′-*O*,5′-*O*-Dibenzyl-3-*N*-benzyloxymethyl-3′-*C*,4′-*C*-ethoxymethylenethymidine (24). To a solution of **23** (33 mg, 0.043 mmol) in THF (0.8 mL) was added NaHMDS (1.1 M in THF, 0.086 mL, 0.094 mmol) at room temperature, and the mixture was refluxed for 3.5 h. After addition of saturated $NaHCO_3$ solution, the reaction mixture was extracted with AcOEt. The usual work-up and purification by silica gel column chromatography (AcOEt/*n*-hexane=1:2) afforded **24** as a colorless foam (24 mg, 92%). $[\alpha]_D^{21}$ −17.3 (*c* 0.89, $CHCl_3$). IR (KBr) ν_{max} 1025, 1095, 1165, 1216, 1277, 1367, 1457, 1657, 1704, 2877, 2955, 3031 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.76 (3H, d, *J*=1 Hz), 1.79–1.86 (1H, m), 2.12 (1H, dd, *J*=8, 13 Hz), 2.14–2.20 (1H, m), 3.18 (1H, dd, *J*=5, 14 Hz), 3.61, 3.78 (2H, AB, *J*=10 Hz), 3.78, 4.02 (2H, AB, *J*=10 Hz), 3.84, 4.29 (2H, AB, *J*=10 Hz), 4.43, 4.54 (2H, AB, *J*=11 Hz), 4.56 (2H, s), 4.69 (2H, s), 5.45, 5.49 (2H, AB, *J*=10 Hz), 6.03 (1H, dd, *J*=6, 8 Hz), 7.20–7.38 (15H, m), 7.88 (1H, d, *J*=1 Hz). ^{13}C NMR ($CDCl_3$) δ 13.2, 28.3, 38.1, 61.5, 63.3, 64.9, 70.3, 72.1, 72.1, 73.6, 81.0, 85.2, 87.9, 108.6, 127.1, 127.1, 127.2, 127.2, 127.5, 127.5, 127.5, 127.6, 127.8, 128.1, 128.1, 128.4, 128.4, 128.4, 128.4, 134.9, 137.1, 137.4, 137.9, 150.7, 163.4. MS (FAB): *m/z* 599 (MH^+). HRMS (FAB): calcd for $C_{35}H_{39}N_2O_7$ (MH^+): 599.2757; found: 599.2782.

4.1.17. 1-(2′-Deoxy-3′-*C*,4′-*C*-ethoxymethylene-β-D-*ribo*-pyranosyl)thymine (25). To a solution of **24** (120 mg, 0.20 mmol) in AcOEt (2.0 mL) were added 20% $Pd(OH)_2$–C (120 mg) and cyclohexene (1.5 mL, 15.0 mmol) at room temperature. The mixture was refluxed for 14 h. After filtration of the mixture, the filtrate was concentrated and purified by silica gel column chromatography ($CHCl_3$/MeOH=20:1) to give **25** as a colorless solid (49 mg, 82%). Mp 76–78 °C. $[\alpha]_D^{22}$ +73.3 (*c* 0.70, MeOH). IR (KBr) ν_{max} 1046, 1116, 1264, 1319, 1382, 1474, 1690, 3328 cm^{-1}. ^1H NMR (CD_3OD) δ 1.44 (1H, m), 1.71 (1H, dd, *J*=2, 14 Hz), 1.95 (3H, d, *J*=1 Hz), 2.23 (1H, dt, *J*=6, 13 Hz), 2.61 (1H, dd, *J*=11, 14 Hz), 3.46, 3.94 (2H, AB, *J*=12 Hz), 3.66, 4.10 (2H, AB, *J*=13 Hz), 3.75 (1H, ddd, *J*=6, 12, 13 Hz), 3.91 (1H, m), 6.11 (1H, dd, *J*=2, 11 Hz), 7.66 (1H, d, *J*=1 Hz). ^{13}C NMR (CD_3OD) δ 12.6, 37.2, 38.1, 66.3, 69.3, 70.3, 70.7, 72.5, 80.9, 112.0, 138.2, 152.2, 166.4. MS (FAB): *m/z* 299 (MH^+). HRMS (FAB): calcd for $C_{13}H_{19}N_2O_6$ (MH^+): 299.1243; found: 299.1244.

4.1.18. 3′-*C*,4′-*C*-Ethoxymethylenethymidine (4). To a solution of **24** (49 mg, 0.082 mmol) in EtOH (2.7 mL) were added 20% $Pd(OH)_2$–C (100 mg) and ammonium formate (1.03 g, 16.4 mmol) at room temperature. The mixture was refluxed for 6 h. After filtration of the mixture, the filtrate was concentrated and purified by silica gel column chromatography ($CHCl_3$/MeOH=15:1) to give **4** as a colorless solid (10 mg, 39%). Mp 215–217 °C. $[\alpha]_D^{25}$ +26.6 (*c* 0.22, MeOH). IR (KBr) ν_{max} 1037, 1102, 1267, 1479, 1686, 2540, 2961, 3076, 3327 cm^{-1}. ^1H NMR (CD_3OD) δ 1.75 (1H, dd, *J*=3, 13 Hz), 1.86–1.97 (1H, m), 1.89 (3H, d, *J*=1 Hz), 2.26 (1H, dd, *J*=8, 13 Hz), 2.56 (1H, dd, *J*=6, 13 Hz), 3.46 (1H, dd, *J*=1, 11 Hz), 3.75 (1H, dd, *J*=5, 11 Hz), 3.82 (1H, d, *J*=10 Hz), 3.92–4.01 (1H, m), 4.00 (1H, dd, *J*=1, 10 Hz), 4.17 (1H, d, *J*=11 Hz), 6.18 (1H, dd, *J*=6, 8 Hz), 8.17 (1H, d, *J*=1 Hz). ^{13}C NMR (CD_3OD) δ 12.6, 34.0, 44.3, 62.9, 63.2, 65.2, 76.6, 86.2, 88.8, 110.4, 138.8, 152.4, 166.8. MS (FAB): *m/z* 299 (MH^+). HRMS (FAB): calcd for $C_{13}H_{19}N_2O_6$ (MH^+): 299.1243; Found: 299.1234.

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References and notes

1. (a) Obika, S.; Nanbu, D.; Hari, Y.; Morio, K.; In, Y.; Ishida, T.; Imanishi, T. *Tetrahedron Lett.* **1997**, *38*, 8735–8738; (b) Imanishi, T.; Obika, S. *J. Synth. Org. Chem., Jpn.* **1999**, *57*, 969–980; (c) Imanishi, T.; Obika, S. *Chem. Commun.* **2002**, 1653–1659; (d) Freier, S. M.; Altmann, K.-H. *Nucleic Acids Res.* **1997**, *25*, 4429–4443; (e) Wang, G.; Gunic, E.; Girardet, J.-L.; Stoisavljevic, V. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1147–1150; (f) Morita, K.; Hasegawa, C.; Kaneko, M.; Tsutsumi, S.; Sone, J.; Ishikawa, T.; Imanishi, T.; Koizumi, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 73–76.
2. The 2′,4′-BNA is also called 'LNA' by Wengel, J. et al. See: (a) Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455–456; (b) Vester, B.; Wengel, J. *Biochemistry* **2004**, *43*, 13233–13241.
3. (a) Tarköy, M.; Bolli, M.; Schweizer, B.; Leumann, C. *Helv. Chim. Acta* **1993**, *76*, 481–510; (b) Tarköy, M.; Bolli, M.; Leumann, C. *Helv. Chim. Acta* **1994**, *77*, 716–744; (c) Bolli, M.; Trafelet, H. U.; Leumann, C. *Nucleic Acids Res.* **1996**, *24*, 4660–4667; (d) Steffens, R.; Leumann, C. *J. Am. Chem. Soc.* **1997**, *119*, 11548–11549; (e) Steffens, R.; Leumann, C.

Helv. Chim. Acta **1997**, *80*, 2426–2439; (f) Steffens, R.; Leumann, C. *J. Am. Chem. Soc.* **1999**, *121*, 3249–3255; (g) Nielsen, P.; Pfundheller, H. M.; Olsen, C. E.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3423–3433; (h) Ravn, J.; Thorup, N.; Nielsen, P. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1855–1861; (i) Vijgen, S.; Nauwelaerts, K.; Wang, J.; Aerschot, A. V.; Lagoja, I.; Herdewijn, P. *J. Org. Chem.* **2005**, *70*, 4591–4597.

4. Leumann's group has synthesized the novel nucleosides, namely bicyclo-DNA and tricyclo-DNA. Oligonucleotides containing the nucleosides showed slightly enhanced duplex stability in limited sequences. See Ref. 3c,d.
5. (a) Obika, S.; Sekiguchi, M.; Osaki, T.; Shibata, N.; Masaki, M.; Hari, Y.; Imanishi, T. *Tetrahedron Lett.* **2002**, *43*, 4365–4368. P. Nielsen's group also reported the synthesis of **1** independently, and reference to *Chem. Commun.* **2002**, 1888–1889; (b) Sekiguchi, M.; Osaki, T.; Harada, Y.; Obika, S.; Imanishi, T. *Nucleic Acids Res. Suppl.* **2003**, *3*, 111–112; (c) Sekiguchi, M.; Obika, S.; Harada, Y.; Osaki, T.; Somjing, R.; Mitsuoka, Y.; Shibata, Y.; Masaki, M.; Imanishi, T. *J. Org. Chem.* **2006**, *71*, 1306–1316.
6. Preliminary results for the synthesis of **3** have been reported: Obika, S.; Osaki, T.; Sekiguchi, M.; Somjing, R.; Harada, Y.; Imanishi, T. *Tetrahedron Lett.* **2004**, *45*, 4801–4804.
7. (a) Froehlich, M. L.; Swartling, D. J.; Lind, R. E.; Mott, A. W.; Bergstrom, D. E. *Nucleosides Nucleotides* **1989**, *8*, 1529–1535; (b) Sharma, M.; Bobek, M. *Tetrahedron Lett.* **1990**, *31*, 5839–5842.
8. Martinelli, M. J.; Vaidyanathan, R.; Khau, V. V. *Tetrahedron Lett.* **2000**, *41*, 3773–3776.
9. (a) See Ref. 1a; (b) Hari, Y.; Obika, S.; Sakaki, M.; Morio, K.; Yamagata, Y.; Imanishi, T. *Tetrahedron* **2002**, *58*, 3051–3063.
10. (a) Hari, Y.; Osaki, T.; Eguchi, K.; Obika, S.; Imanishi, T. *Nucleic Acids Res. Suppl.* **2002**, *2*, 147–148; (b) Hari, Y.; Obika, S.; Ohnishi, R.; Eguchi, K.; Osaki, T.; Ohishi, H.; Imanishi, T. *Bioorg. Med. Chem.* **2006**, *14*, 1029–1038.
11. Compound **17** was not obtained on treatment of **13** with *p*-toluenesulfonic acid in the absence of paraformaldehyde.[6] See also: Nouguier, R.; Gras, J.-L.; Mchich, M. *Tetrahedron* **1988**, *44*, 2943–2950.
12. Sawada, D.; Kanai, M.; Shibasaki, M. *J. Am. Chem. Soc.* **2000**, *122*, 10521–10532.
13. Jennings, B. M.; Liu, M. T. H. *J. Am. Chem. Soc.* **1976**, *98*, 6417–6418.
14. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC637463 and CCDC637464 for **4** and **25**, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].
15. It has been reported that a furanosyl to pyranosyl ring-isomerization was observed under basic conditions. See: Ueda, T.; Shuto, S. *Nucleosides Nucleotides* **1984**, *3*, 295–302.