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Synthesis and conformation of a novel bridged nucleic acid having a *trans*-fused 3,5,8-trioxabicyclo[5.3.0]decane structure

Satoshi Obika, Tomohisa Osaki, Mitsuaki Sekiguchi, Roongjang Somjing, Yasuki Harada and Takeshi Imanishi*

Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan

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Abstract—A novel bridged nucleic acid analogue, 2′-deoxy-*trans*-3′,4′-BNA thymine monomer, was successfully synthesized. An ab initio calculation and X-ray structure analysis revealed that the *trans*-fused bicyclo[5.3.0]decane structure of the 2′-deoxy-*trans*-3′,4′-BNA effectively constrained the sugar puckering in $C_{2'}$-*endo* with appropriate γ, δ and χ angles.

The sugar moiety in nucleosides is well known to have relatively large conformational flexibility. We have synthesized the nucleic acid analogue, 2′,4′-BNA[1]/LNA,[2] of which sugar puckering is exactly restricted to N-type, and the 2′,4′-BNA oligonucleotides showed high binding affinity towards ssRNA[3] and dsDNA.[4] Thus, preorganization of a nucleoside sugar moiety in an appropriate conformation is one promising strategy to develop nucleic acid analogues with superior binding ability.

Oligonucleotides containing a nucleoside with *S*-type sugar conformation are expected to form a stable duplex of B-DNA and to be applicable to post-genome technologies, such as DNA microarray[5] and decoy nucleic acid.[6] Therefore, nucleoside analogues with a restricted *S*-type sugar conformation have been designed and synthesized to date (Fig. 1).[7–11] Some of them were introduced into oligonucleotides and the hybridizing properties were studied. However, these oligonucleotide analogues showed only moderate increase or considerable decrease in the duplex stability, probably due to insufficient and/or improper restriction of the sugar conformation. In the B-DNA, the furanose rings are puckered $C_{2'}$-*endo* or $C_{3'}$-*exo* (*S*-type), which is also exhibited by torsion angle δ ranged from 79° to 157°, and the γ angle is in the +sc range, 40°–73° (Fig. 2).[12] In addition, the χ angle around −100° is one important characteristic of the B-DNA.[12] Thus, besides the *S*-type sugar puckering, these torsion angles γ, δ and χ must be in the appropriate range to prepare an ideal nucleoside analogue for B-DNA modification.

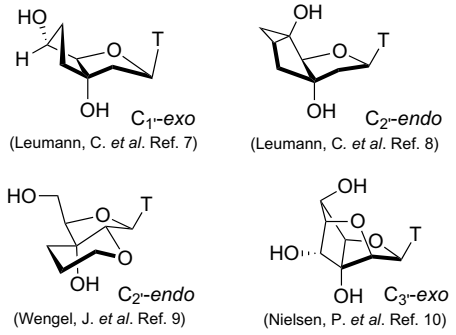

Figure 1. Selected bicyclic and tricyclic nucleoside analogues with *S*-type conformation.

Recently, we synthesized an *S*-type nucleoside analogue, *trans*-3′,4′-BNA **1**,[13] of which sugar puckering was restricted to $C_{3'}$-*exo* (Fig. 3). However, a *trans*-fused six-membered ring of **1** causes large δ angle (174.6°), and the steric hindrance of the 2′-substituent group results in low reactivity of 3′-OH (data not shown).[14] Here, we would like to describe the synthesis and structure of a

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* Corresponding author. Tel.: +81-6-6879-8200; fax: +81-6-6879-8204; e-mail: imanishi@phs.osaka-u.ac.jp

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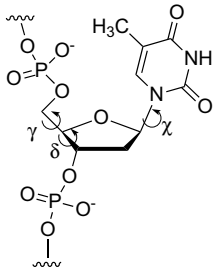


torsion angle	range
γ	40° ~ 73°
δ	79° ~ 157°
χ	-88° ~ -135°

Figure 2. The range of torsion angles γ, δ and χ for the B-DNA.[12]

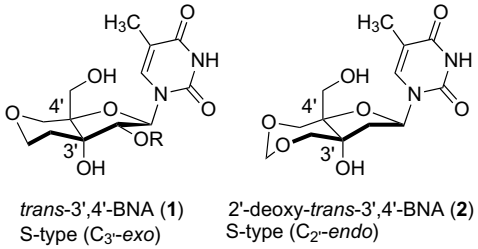
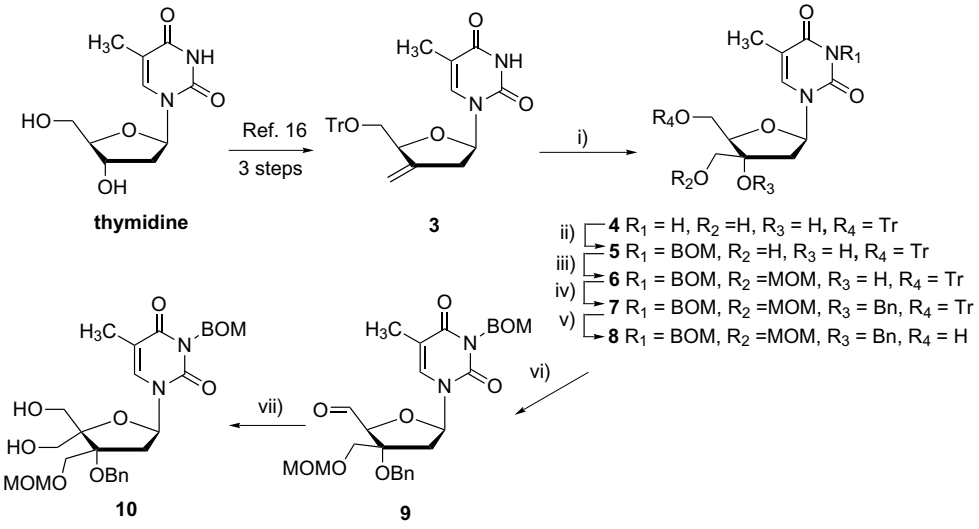


Figure 3. Structure of *trans*-3′,4′-BNA and 2′-deoxy-*trans*-3′,4′-BNA.

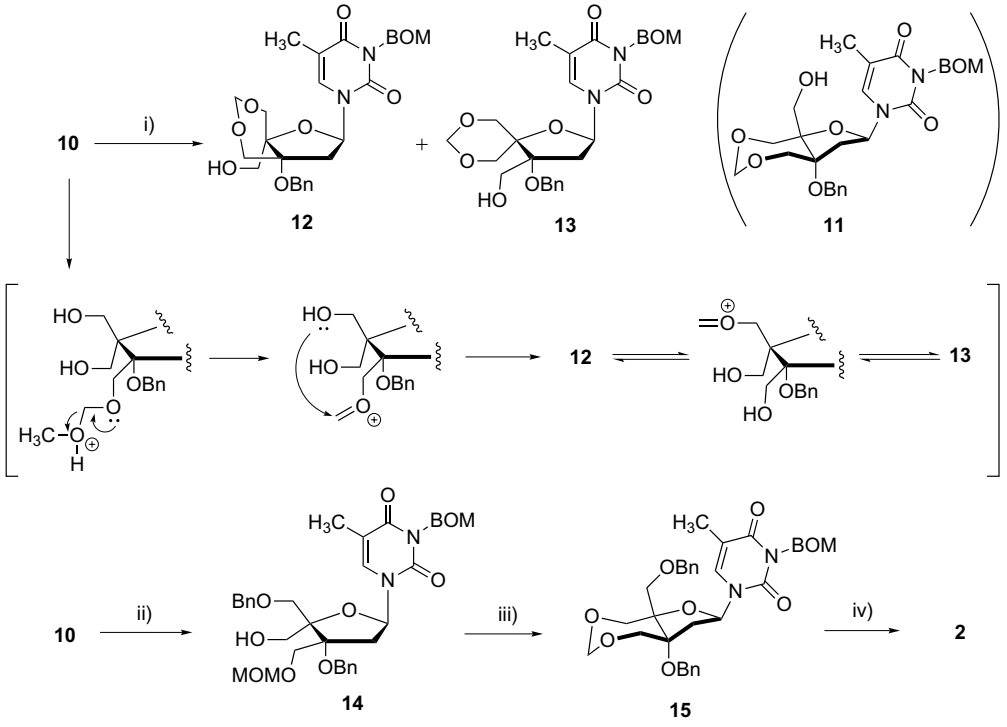
novel *S*-type nucleoside analogue, 2′-deoxy-*trans*-3′,4′-BNA **2**, which lacks 2′-substituents and has a *trans*-fused seven-membered ring containing a methylene acetal to adjust the δ angle.

At first, an ab initio calculation using 3-21G(*) basis set[15] for the 2′-deoxy-*trans*-3′,4′-BNA **2** was carried out to evaluate the conformation of the furanose ring and flexibility of the torsion angles γ and χ. The results show that the $C_{2'}$-*endo* sugar puckering is the most favorable with the δ angle of 159.4°. The torsion angles γ and χ in the optimized structure are 50.0° and −129.3°, respectively, and sufficient flexibility of γ and χ is also observed. Thus, it is shown that the 2′-deoxy-*trans*-3′,4′-BNA **2** fulfills the conformational criteria for an ideal *S*-type nucleoside analogue.

To successfully achieve the synthesis of 2′-deoxy-*trans*-3′,4′-BNA, we chose thymidine as starting material (Schemes 1 and 2). According to the literature,[16] thymidine was converted in a three-step sequence to the 3′-deoxy-3′-*C*-methylenethymidine derivative **3** (Scheme 1). Stereoselective catalytic osmium tetraoxide oxidation of **3** gave the diol **4** in 65% yield.[17] Protection of the thymine nucleobase in **4** using benzyl chloromethyl ether (BOMCl) afforded **5** in 89% yield, which was treated with chloromethyl methyl ether (MOMCl) to obtain **6** in 54% yield. The 3′-hydroxy group of **6** was protected with benzyl group to give **7** in 84% yield. Continuously, deprotection of the 5′-*O*-trytyl group gave the primary alcohol **8** in 90% yield. Dess–Martin oxidation of **8** affording the corresponding aldehyde **9** was followed by an aldol condensation using formaldehyde and reduction with sodium borohydride to give the diol **10** (63%, two steps). It was reported that the reaction of a MOM group with a neighboring hydroxyl group gave a cyclic methylene acetal under acidic conditions.[18] Therefore, we attempted to construct the *trans*-fused bicyclo[5.3.0]decane structure via a direct seven-membered ring formation of **11** under acidic conditions (Scheme 2). Although the formation of desired compound **11** was not observed, it was interesting that the spiro-type compound **13** was obtained in 53% yield along with the *cis*-fused compound **12** (28%) after sufficient reaction time at 80 °C.[19] This result implies that the *cis*-fused compound **12** migrated to the thermodynamically stable spiro-type compound **13** under the acidic reaction conditions.[20] To prevent the formation of *cis*- and spiro-type compounds, the 5′-hydroxy group in **10** was benzylated to give **14** in 49% yield. Then, the obtained **14**



Scheme 1. Reagents and conditions: (i) OsO_4, *N*-methylmorpholine-*N*-oxide, pyridine–H_2O–*tert*-BuOH, 75 °C (65%); (ii) BOMCl, DBU, DMF, 0 °C (89%); (iii) MOMCl, *i*-Pr_2NEt, pyridine–$ClCH_2CH_2Cl$, rt (54%); (iv) NaH, BnBr, *n*-Bu_4NI, DMF, rt (84%); (v) (+)-10-camphorsulfonic acid, CH_2Cl_2–MeOH, rt (90%); (vi) Dess–Martin periodinane, CH_2Cl_2, rt; (vii) 37% H_2CO aq, 1 M NaOH aq, THF, 15 °C, then $NaBH_4$, THF, 0 °C (63% from **8**).



Scheme 2. Reagents and conditions: (i) *p*-TsOH·H_2O, benzene, reflux (28% for **12**, 53% for **13**); (ii) NaH, BnBr, DMF, 0 °C (49%); (iii) *p*-TsOH·H_2O, $(CH_2O)_n$, $ClCH_2CH_2Cl$, reflux (47%); (iv) $HCOONH_4$, 20%$Pd(OH)_2$/C, EtOH, reflux (60%).

was allowed to react under acidic conditions; however, this reaction caused partial deprotection of the MOM group and the desired *trans*-fused compound **15** was not obtained. After some trials it was found that an addition of excess amount of paraformaldehyde under acidic conditions afforded the *trans*-fused compound **15** in 47% yield.[21] A Pd-mediated hydrogenolysis successfully gave the fully deprotected compound **2** in 60% yield.[22]

The *trans*-fused structure of **2** was confirmed by an X-ray crystallographic analysis (Fig. 4 and Table 1),[23] which also indicates that the furanose ring of **2** has a typical *S*-type conformation, $C_{2'}$-*endo* puckering (pseudorotation phase angle $P = 174.2°$). The maximum torsion angle

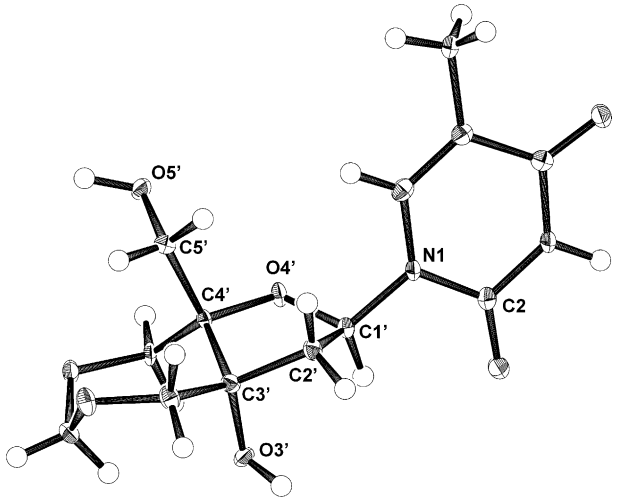


Figure 4. ORTEP drawing of 2′-deoxy-*trans*-3′,4′-BNA monomer **2**.

Table 1. Selected torsion angles, maximum torsion angle (ν_{max}) and pseudorotation phase angle (P) in the X-ray structure of **2**

ν_0 (C4′–O4′–C1′–C2′)	−19.0°
ν_1 (O4′–C1′–C2′–C3′)	40.1°
ν_2 (C1′–C2′–C3′–C4′)	−44.5°
ν_3 (C2′–C3′–C4′–O4′)	34.9°
ν_4 (C3′–C4′–O4′–C1′)	−10.2°
δ (O3′–C3′–C4′–C5′)	164.0°
γ (C3′–C4′–C5′–O5′)	−178.8°
χ (O4′–C1′–N1–C2)	−126.1°
ν_{max}	44.7°
P	174.2°

ν_{max} and δ angle of **2** are 44.7° and 164.0°, respectively. These angles are remarkably improved compared with those of the *trans*-3′,4′-BNA **1** (ν_{max}: 51.9° and δ: 174.6°)[13] and are in good agreement with those of natural B-DNA. In addition, the χ angle of −126.1° is also within the range of a typical B-type DNA.

In conclusion, we have successfully demonstrated the synthesis of a novel *S*-type nucleoside analogue, 2′-deoxy-*trans*-3′,4′-BNA **2**. By means of ab initio calculation and X-ray crystallography, **2** was found to fully satisfy the conformational requirements of B-DNA. Further studies on **2** and its oligonucleotide derivative are now in progress.

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14. The phosphitilation at the 3′-hydroxy group of **1** did not proceed efficiently, probably due to the steric hindrance of the 2′-substituent group (data not shown).
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19. Compound **12** and **13** were assigned by comparing with the analogues, which were synthesized through another route.
    Selected data for **12**: ^{1}H NMR ($CDCl_3$) δ 1.97 (3H, d, $J = 1$ Hz), 2.31 (1H, dd, $J = 6$, 8 Hz), 2.45 (1H, dd, $J = 7$, 14 Hz), 2.79 (1H, dd, $J = 6$, 14 Hz), 3.54 (1H, dd, $J = 8$, 11 Hz), 3.86 (1H, dd, $J = 6$, 11 Hz), 3.92 (2H, s), 4.11 (2H, s), 4.57 (2H, s), 4.66, 5.04 (2H, AB, $J = 6$ Hz), 4.70 (2H, s), 5.49 (2H, s), 6.28 (1H, t, $J = 6$ Hz), 7.27–7.41 (10H, m), 7.71 (1H, s). MS (EI): m/z 524 (M^+, 1.0), 91 (100). HRMS (EI): Calcd for $C_{28}H_{32}N_2O_8$ (M^+): 524.2153. Found: 524.2158.
    Selected data for **13**: ^{1}H NMR ($CDCl_3$) δ 1.94 (3H, d, $J = 1$ Hz), 1.96 (1H, dd, $J = 9$, 15 Hz), 2.76–2.79 (1H, br), 2.85 (1H, dd, $J = 5$, 14 Hz), 3.71–3.78 (3H, m), 4.03 (1H, dd, $J = 8$, 13 Hz), 4.17 (1H, dd, $J = 1$, 12 Hz), 4.49 (1H, br d, $J = 12$ Hz), 4.57, 4.72 (2H, AB, $J = 11$ Hz), 4.69 (2H, s), 4.81, 4.95 (2H, AB, $J = 6$ Hz), 5.47 (2H, s), 6.02 (1H, dd, $J = 5$, 9 Hz), 7.25–7.38 (11H, m). MS (EI): m/z 524 (M^+, 2.3), 418 (100). HRMS (FAB): Calcd for $C_{28}H_{33}N_2O_8$ (M^+H): 525.2237. Found: 525.2235.
20. Treatment of the spiro-type compound **13** under the acidic conditions afforded the *cis*-fused compound **12** (27%) along with the recoverd **13** (45%).
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22. Selected data for **2**: mp 244–247 °C (CH_3CN). ^{1}H 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, 4.05 (2H, AB, $J = 11$ Hz), 3.81, 3.91 (2H, AB, $J = 12$ Hz), 4.00, 4.15 (2H, AB, $J = 12$ 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). MS (FAB): m/z 315 (M^+H). HRMS (FAB): Calcd for $C_{13}H_{19}N_2O_7$ (M^+H): 315.1192. Found: 315.1216.
23. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC230411. 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 email: deposit@ccdc.cam.ac.uk].