Pergamon

Tetrahedron Letters 43 (2002) 4365–4368

TETRAHEDRON LETTERS

Synthesis and conformation of a novel bridged nucleoside with S-type sugar puckering, *trans*-3′,4′-BNA monomer

Satoshi Obika, Mitsuaki Sekiguchi, Tomohisa Osaki, Nao Shibata, Miyuki Masaki, Yoshiyuki Hari and Takeshi Imanishi*

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

Received 29 March 2002; revised 17 April 2002; accepted 19 April 2002

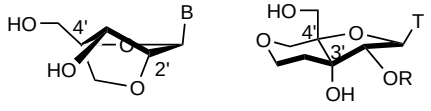
Abstract—A novel bridged nucleoside bearing a 4,7-dioxabicyclo[4.3.0]nonane skeleton, *trans*-3′,4′-BNA monomer, was successfully synthesized. A ^{1}H NMR experiment and an X-ray crystallographic analysis revealed that the sugar puckering of the 3′,4′-BNA monomer was restricted to an S-type ($C_{3'}$-*exo*) conformation.

The importance of the synthesis of novel nucleoside analogues is increasing more and more in the post-genome-sequencing era. The nucleic acid analogues which strongly bind with their RNA complements are very useful as antisense molecules to regulate the targeted gene expression.[1] On the other hand, the analogues acquiring both strong and sequence selective binding ability towards the DNA complements are expected to be applicable to some other beneficial technologies such as DNA microarray[2] and a decoy nucleic acid.[3]

Double-stranded RNA and DNA are well-known to prefer forming A-type and B-type helical structures, respectively. In each structure, ribonucleosides predominantly exist in N-type sugar conformation, while deoxyribonucleosides have S-type puckering.[4] Preorganization of nucleic acids into the proper shape would be a most promising strategy to prepare novel nucleic acid analogues having strong binding affinity towards their DNA and/or RNA complements.[5] In fact, we have achieved the synthesis of a novel class of nucleic acid analogue, 2′-*O*,4′-*C*-methylene bridged nucleic acid (2′,4′-BNA[6]/LNA[7]), in which the sugar moiety was strictly locked in N-type ($C_{3'}$-*endo*) conformation, and we successfully demonstrated that the introduction of 2′,4′-BNA monomers into oligonucleotides significantly enhanced their binding affinity towards RNA complements.[8,9]

Nowadays, some nucleoside analogues with a bicyclic or tricyclic sugar moiety, of which the conformation was restricted in S-type (DNA-type), were synthesized (Fig. 2), and the hybridization property of the oligonucleotides containing these conformationally restrained nucleoside analogues was evaluated.[10–13] However, only moderate enhancement of the duplex stability by these nucleoside modifications was observed, probably due to insufficient and/or improper restriction of the sugar conformation, or steric repulsion between the additional ring structure and neighboring nucleotide residues.

To solve these problems, we designed *trans*-3′,4′-BNA monomer **1**, a novel nucleoside analogue having a 4,7-dioxabicyclo[4.3.0]nonane skeleton as a sugar moiety (Fig. 1). From preliminary molecular modeling experiments, it was presumed that the ribofuranose ring of **1** was strictly restrained in S-type ($C_{3'}$-*exo*) conformation and that the *trans*-fused six-membered ring of **1** was located outside of the helix structure when it was



2′,4′-BNA (LNA)
N-type conformation
($C_{3'}$-*endo*)

trans-3′,4′-BNA
1a: R = H
1b: R = Me
S-type conformation
($C_{3'}$-*exo*)

Figure 1. Structures of 2′,4′-BNA and *trans*-3′,4′-BNA.

Keywords: nucleic acid analogues; nucleosides; conformation; X-ray crystal structures.

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

0040-4039/02/$ - see front matter
PII: S0040-4039(02)00784-0

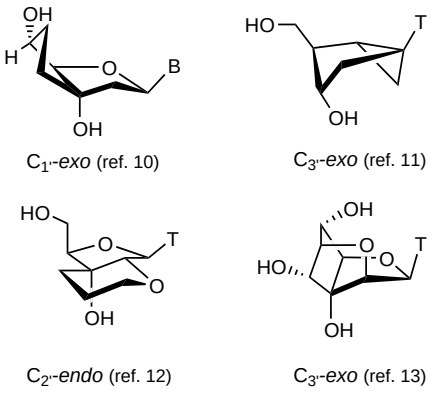


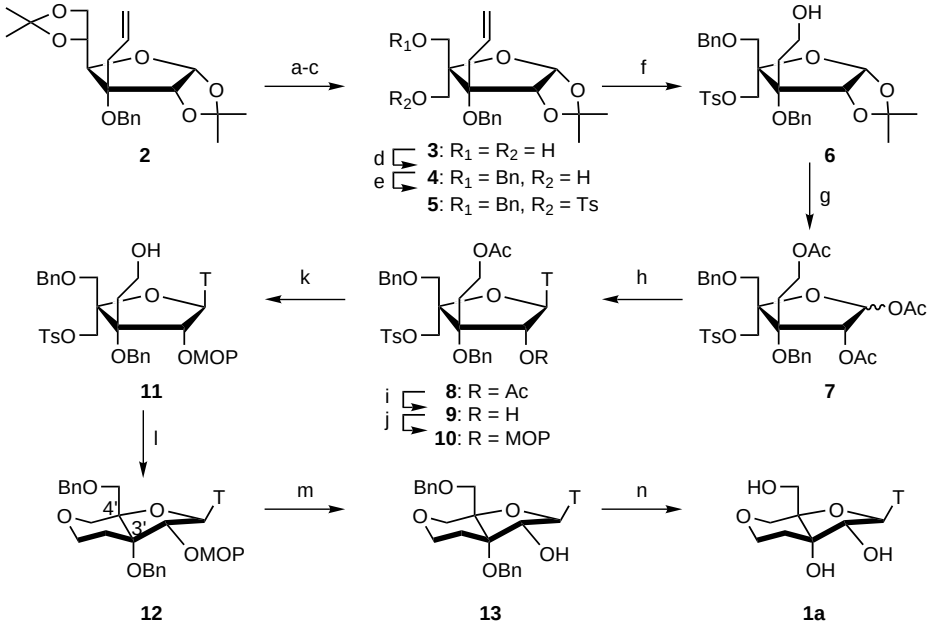
Figure 2. Selected bicyclic and tricyclic nucleoside analogues with S-type conformation.

introduced into a B-type DNA duplex. Here, we describe the synthesis and conformation of the *trans*-3′,4′-BNA **1**.

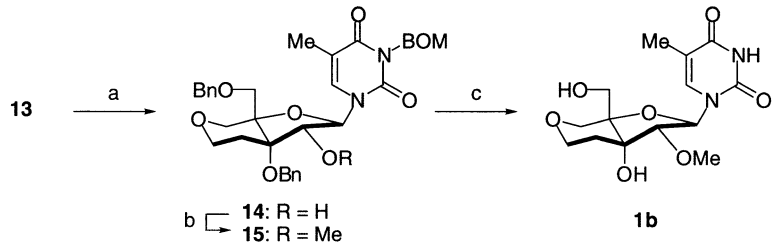
The synthesis of **1a** was successfully achieved by using the known D-allofuranose derivative **2**[14] as the starting material (Scheme 1). Introduction of a hydroxymethyl group at the C4 position of **2** proceeded by the conventional aldol-Canizzaro reaction to give **3** in 65% yield (three steps). The diol **3** was selectively benzylated at the 5-hydroxy group to afford **4** in 56% yield, which was treated with *p*-toluenesulfonyl chloride to give **5** in 80% yield. Reaction of **5** with OsO_4 and $NaIO_4$ in THF/H_2O followed by reduction with $NaBH_4$ gave primary alcohol **6** in 63% overall yield. Treatment of **6** with Ac_2O, AcOH and a catalytic amount of conc. H_2SO_4 afforded the triacetate **7** in 98% yield as an anomeric mixture. Coupling reaction of **7** with silylated thymine effectively proceeded to give the desired β-anomer of **8** in 87% yield. After several attempts to distinguish the two acetoxy groups in **8**, it was found that the 2′-*O*-acetyl group in **8** was selectively removed by treatment with aqueous $MeNH_2$. The 2′-hydroxy group of the resulting **9** was protected with 2-methoxy-2-propyl (MOP) group to give **10** in 74% overall yield. After deacetylation of **10**, ring-closure reaction successfully proceeded on treatment with NaHMDS to give **12** in 64% overall yield. Deprotection of the 2′-hydroxy group in **12** with *p*-toluensulfonic acid followed by Pd-mediated hydrogenolysis afforded the desired ribonucleoside analogue **1a**, which has a *trans*-fused 4,7-dioxabicyclo[4.3.0]nonane structure.[15]

Next, the 2′-*O*-methyl congener **1b** was also synthesized as shown in Scheme 2. The thymine nucleobase of **13** was protected with a benzyloxymethyl (BOM) group, and the resulting **14** was treated with NaH and MeI to give the 2′-*O*-methyl derivative **15** in 77% overall yield. Deprotection of both 3′-*O*- and 5′-*O*-benzyl groups and the BOM group at the nucleobase afforded **1b** in 39% yield.[16]

On ^1H NMR measurements of **1a** and **1b**, relatively large $J_{1'2'}$ values (6.7 Hz for **1a** and 7.1 Hz for **1b**) were observed, which means that the *trans*-3′,4′-BNA monomers **1** were conformationally restricted in S-type (S%=83% for **1a** and 89% for **1b**).[17] In addition, an X-ray structure investigation (Fig. 3 and Table 1) clearly shows that **1b** existed in a typical S-type confor-



Scheme 1. *Reagents and conditions*: (a) 80% AcOH aq., 60°C. (b) $NaIO_4$, THF/H_2O, 0°C. (c) 37% HCHO aq., 1N NaOH aq., THF/H_2O, rt, 65% (three steps). (d) NaH, BnBr, DMF, 0°C, 56%. (e) TsCl, Et_3N, DMAP, CH_2Cl_2, rt, 80%. (f) (1) OsO_4, $NaIO_4$, THF/H_2O, rt, (2) $NaBH_4$, THF/H_2O, 0°C, 63% (two steps). (g) Ac_2O, AcOH, H_2SO_4, rt, 98%. (h) thymine, BSA, TMSOTf, $ClCH_2CH_2Cl$, reflux, 87%. (i) 40% $MeNH_2$ aq., THF, 0°C, 87%. (j) 2-Methoxypropene, $TsOH/H_2O$, CH_2Cl_2, 0°C, 85%. (k) 2N NaOH aq. MeOH/THF, rt, 87%. (l) 1 M NaHMDS, THF, reflux, 73%. (m) $TsOH/H_2O$, THF/MeOH, 0°C, 85%. (n) 20% $Pd(OH)_2/C$, cyclohexene, EtOH, reflux, 69%.



Scheme 2. *Reagents and conditions*: (a) BOMCl, DBU, DMF, 0°C, 90%. (b) MeI, NaH, DMF, rt, 86%. (c) 20% $Pd(OH)_2/C$, cyclohexene, EtOH, reflux, 39%.

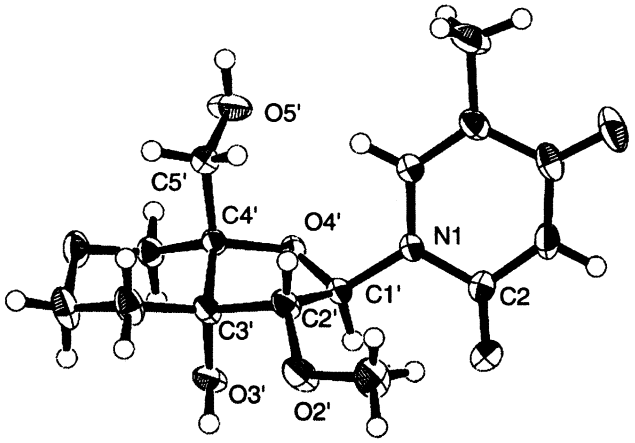


Figure 3. ORTEP drawing of **1b**.

Table 1. Selected torsion angles, pseudorotation phase angle (P) and maximum torsion angle (v_{max}) of **1b** determined from X-ray structure

v_0 (C4′–O4′–C1′–C2′)	−3.8°
v_1 (O4′–C1′–C2′–C3′)	30.6°
v_2 (C1′–C2′–C3′–C4′)	−44.3°
v_3 (C2′–C3′–C4′–O4′)	44.3°
v_4 (C3′–C4′–O4′–C1′)	−25.0°
δ (O3′–C3′–C4′–C5′)	174.6°
γ (C3′–C4′–C5′–O5′)	167.9°
χ (O4′–C1′–N1–C2)	−125.8°
v_{max}	51.9°
P	211.4°

Crystal data for **1b**: $C_{14}H_{20}N_2O_7$, $M=328.32$, colorless prism, 0.35×0.35×0.20 mm, monoclinic, $P2_1(\#4)$, $a=6.034(3)$, $b=10.040(5)$, $c=12.562(6)$ Å, $\beta=103.905(6)°$, $V=738.7(6)$ Å^3, $T=293$ K, $Z=2$, μ(Mo Kα)$=1.19$ cm^{-1}, 5023 reflections measured, 2204 reflections observed, $R=0.041$, $R_w=0.127$, CCDC 182207.

mation ($C_{3'}$-*exo* puckering mode, sugar pseudorotation phase angle[4] $P=211.4°$).[18,19] The maximum torsion angle,[4] v_{max}, of **1b** was also calculated to be 51.9° from the observed endocyclic sugar torsion angles. These features are certainly attributable to the constraints of the *trans*-fused six-membered ring.

Thus, we have successfully demonstrated the synthesis of a novel bridged nucleoside analogue, *trans*-3′,4′-BNA monomer **1**, of which the sugar conformation was effectively restricted in $C_{3'}$-*exo* puckering mode by a *trans*-fused six-membered ring. Further studies on **1** and their oligonucleotide derivatives are now in progress.

Acknowledgements

We thank A. Yamano (Rigaku Corp.) for his help with X-ray crystallographic analysis. Part of this work was supported by a Grant for Research on Health Sciences focusing on Drug Innovation from The Japan Health Sciences Foundation, Industrial Technology Research Grant Program in '00 from New Energy and Industrial Technology Development Organization (NEDO) of Japan, a Grant-in-Aid from Japan Society for the Promotion of Science, and a Grant-in-Aid from the Ministry of Education, Science, and Culture, Japan.

References

1. (a) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543–584; (b) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123–6194; (c) Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993**, *36*, 1923–1937; (d) *Clinical Trials of Genetic Therapy with Antisense DNA and DNA Vectors*; Wickstrom, E., Ed.; Marcel Dekker: New York, 1998; (e) *Applied Antisense Oligonucleotide Technology*; Stein, C. A.; Krieg, A. M., Eds.; Wiley-Liss: New York, 1998.
2. For reviews: (a) Niemeyer, C. M.; Blohm, D. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 2865–2869; (b) Cheung, V. G.; Morley, M.; Aguilar, F.; Massimi, A.; Kucherlapati, R.; Childs, G. *Nature Genet.* **1999**, *21*, 15–19; (c) Lockhart, D. J.; Winzeler, E. A. *Nature* **2000**, *405*, 827–836.
3. For reviews: (a) Cho-Chung, Y. S.; Park, Y. G.; Lee, Y. N. *Curr. Opin. Mol. Ther.* **1999**, *1*, 386–392; (b) Morishita, R.; Aoki, M.; Kaneda, Y. *Curr. Drug. Targets* **2000**, *1*, 15–23.
4. Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984 and references cited therein.
5. (a) Herdewijn, P. *Liebigs Ann.* **1996**, 1337–1348; (b) Kool, E. T. *Chem. Rev.* **1997**, *97*, 1473–1487; (c) Herdewijn, P. *Biochim. Biophys. Acta* **1999**, *1489*, 167–179; (d) Leumann, C. J. *Bioorg. Med. Chem.* **2002**, *10*, 841–854.
6. (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.

7. The 2′,4′-BNA was also called 'LNA' by Wengel et al. See: (a) Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455–456; (b) 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; (c) Koshkin, A. A.; Nielsen, P.; Meldgaard, M.; Rajwanshi, V. K.; Singh, S. K.; Wengel, J. *J. Am. Chem. Soc.* **1998**, *120*, 13252–13253; (d) Wengel, J. *Acc. Chem. Res.* **1999**, *32*, 301–310; (e) Meldgaard, M.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3539–3554.
8. Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.; Morio, K.; Doi, T.; Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401–5404.
9. In addition to the unprecedented duplex-forming ability of the BNA modified oligonucleotides, we also reported their strong and sequence-selective triplex-forming ability. See: (a) Obika, S.; Hari, Y.; Morio, K.; Imanishi, T. *Tetrahedron Lett.* **2000**, *41*, 221–224; (b) Obika, S.; Hari, Y.; Sugimoto, T.; Sekiguchi, M.; Imanishi, T. *Tetrahedron Lett.* **2000**, *41*, 8923–8927; (c) Torigoe, H.; Hari, Y.; Sekiguchi, M.; Obika, S.; Imanishi, T. *J. Biol. Chem.* **2001**, *276*, 2354–2360; (d) Obika, S.; Uneda, T.; Sugimoto, T.; Nanbu, D.; Minami, T.; Doi, T.; Imanishi, T. *Bioorg. Med. Chem.* **2001**, *9*, 1001–1011; (e) Obika, S.; Hari, Y.; Sekiguchi, M.; Imanishi, T. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 2079–2081; *Angew. Chem.* **2001**, *113*, 2149–2151; (f) Obika, S.; Onoda, M.; Morita, K.; Andoh, J.; Koizumi, M.; Imanishi, T. *Chem. Commun.* **2001**, 1992–1993; (g) Torigoe, H.; Obika, S.; Imanishi, T. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1235–1238.
10. (a) Tarkoy, M.; Bolli, M.; Schweizer, B.; Leumann, C. *Helv. Chim. Acta* **1993**, *76*, 481–510; (b) Tarkoy, M.; Bolli, M.; Leumann, C. *Helv. Chim. Acta* **1994**, *77*, 716–744.
11. Altmann, K.-H.; Imwinkelried, R.; Kesselring, R.; Rihs, G. *Tetrahedron Lett.* **1994**, *35*, 7625–7628.
12. Nielsen, P.; Pfundheller, H. M.; Olsen, C. E.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3423–3433.
13. Ravn, J.; Thorup, N.; Nielsen, P. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1855–1861.
14. Bar, N. C.; Roy, A.; Achari, B.; Mandal, S. B. *J. Org. Chem.* **1997**, *62*, 8948–8951.
15. Selected data for **1a**: mp 244–246°C (*i*Pr–OH), ^{1}H NMR (CD_3OD): δ 1.76 (1H, dd, $J=4$, 13 Hz), 1.88 (3H, d, $J=1$ Hz), 1.91 (1H, ddd, $J=6$, 13, 14 Hz), 3.58, 4.06 (2H, ABq, $J=10$ Hz), 3.77 (1H, dd, $J=5$, 11 Hz), 3.86, 3.98 (2H, ABq, $J=12$ Hz), 4.00 (1H, dt, $J=11$, 4 Hz), 4.73 (1H, d, $J=7$ Hz), 5.98 (1H, d, $J=7$ Hz), 8.06 (1H, d, $J=1$ Hz). HRMS (FAB): calcd for $C_{13}H_{18}N_2NaO_7$ $(M+Na)^+$: 337.1012. Found: 337.1011.
16. Selected data for **1b**: mp 256–259°C (MeOH), ^{1}H 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). 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%.
17. The precise ^{1}H NMR measurements were carried out in CD_3OD, and the percentage of S-type conformation (S%) was calculated from the equation: $S\% = 100\times(J_{1'2'}-1)/6.9$. See: (a) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333–2344; (b) Altona, C. *Recl. Trav. Chim. Pays-Bas* **1982**, *101*, 413–433; (c) De Leeuw, F. A. A. M.; Altona, C. *J. Chem. Soc., Perkin Trans. 2* **1982**, 375–384.
18. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC182207. 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].
19. In the X-ray structure of **1b**, the torsion angle γ (C3′–C4′–C5′–O5′) was 169.7°, which differs from the γ angle in a canonical B-type DNA (γ=ca. 60°). However, we found that the latter conformer (γ=ca. 60°) of **1b** is more stable than the former conformer (γ=ca. 180°) (ΔE=ca. 3 kcal/mol) from molecular orbital calculations (HF/6-31G*//HF/3-21G(*)).