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Synthesis of a novel bridged nucleoside bearing a fused-azetidine ring, 3′-amino-3′,4′-BNA monomer

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Abstract—A novel bridged nucleic acid monomer, 3′-amino-3′-deoxy-5-methyl-3′-*N*,4′-*C*-methyleneuridine, was successfully synthesized via a useful and convenient azetidine ring formation under Staudinger's conditions. A ^{1}H NMR experiment and a PM3 calculation revealed that the sugar moiety of the novel bridged nucleic acid monomer, 3′-amino-3′,4′-BNA, was restricted to S-type conformation.

The synthesis of novel nucleoside analogues is of great interest because of their application as a key intermediate for an antisense and/or an antigene molecule to regulate target gene expression,[1] as well as their direct use for an anti-tumor or an antiviral compound. In recent studies for developing an ideal and a practical antisense molecule, the oligonucleotide analogues having 'non-genetic' 2′,5′-phosphodiester linkages were found to be a good candidate as an antisense molecule due to their RNA selective hybridization property and enzymatic stability.[2] Furthermore, one of the 2′,5′-linked oligonucleotides, 2-5A (2′,5′-oligoadenylate 5′-triphosphate), is well known to activate a RNase L to prevent viral infection;[3] therefore, intensive efforts towards the development of various 2-5A analogues have been demonstrated to date.[4]

3′-Amino-3′-deoxynucleoside is an essential component of oligonucleotides N3′→P5′ phosphoramidate that are well known to have high binding affinity with ssRNA, ssDNA and dsDNA.[5] The sugar moiety of 3′-amino-3′-deoxynucleoside prefers N-type puckering due to weak *gauche* effect between the 3′-nitrogen and the 4′-oxygen. This preference in the sugar puckering mode is thought to be one major reason for the superior binding ability of oligonucleotides N3′→P5′ phosphoramidate.[6]

Recently, we achieved the synthesis and development of a novel class of nucleic acid analogues, bridged nucleic acid (BNA),[7] such as 2′-*O*,4′-*C*-methylene BNA (2′,4′-BNA)[8–12] and 3′-*O*,4′-*C*-methylene BNA (3′,4′-BNA),[13,14] as shown in Figure 1. The sugar conformation of 3′,4′-BNA was found to be restricted in S-type puckering mode, while that of 2′,4′-BNA is locked in N-type. In addition, the 3′,4′-BNA oligonucleotides, including a 2′,5′-phosphodiester linkage and an oxetane-fused ribofuranose ring, showed favorable features as an antisense molecule.[15]

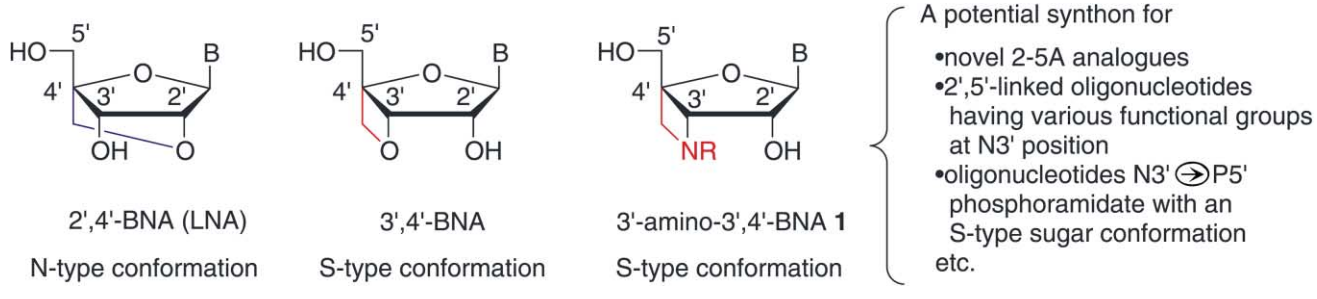

Figure 1. Structures of bridged nucleic acids (BNAs).

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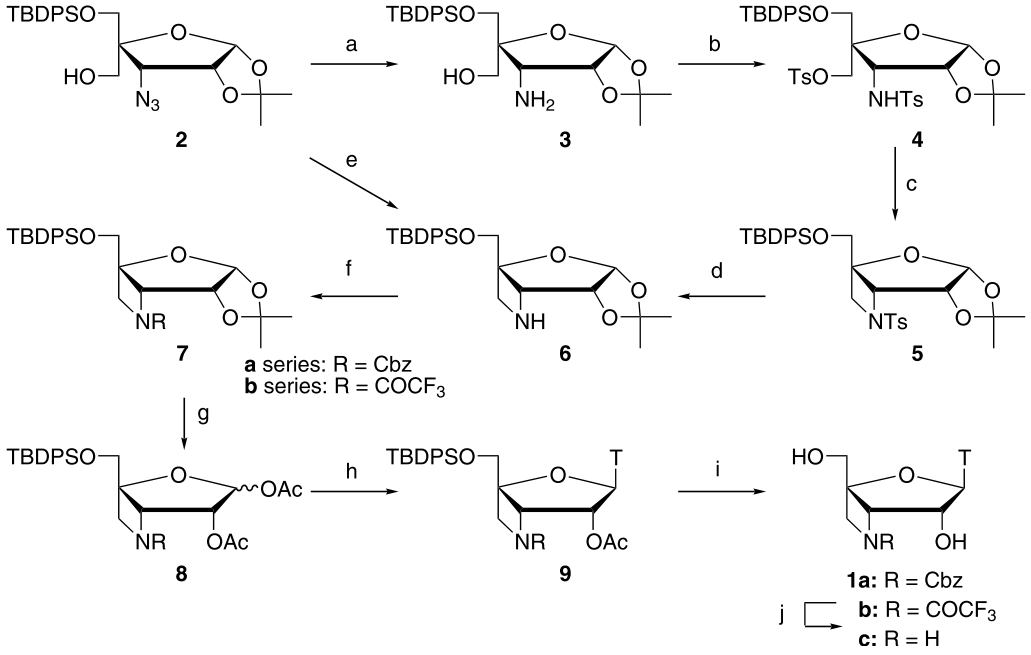
On the other hand, 3′-amino-3′,4′-BNA monomer **1**, which has an azetidine-fused furanose ring, is one of the isosteres of the 3′,4′-BNA monomer, and is potentially capable of introducing some functional groups at the 3′-nitrogen atom, such as a fluorescent or a chemiluminescent probe, an intercalator and DNA scissors.[16] Furthermore, the 3′-amino-3′,4′-BNA monomer **1** is applicable to the oligonucleotides N3′→P5′ phosphoramidate with S-type sugar conformation, when **1** is introduced into oligonucleotides via the 3′-amino and 5′-hydroxy groups. The effect of S-type sugar conformation on hybridizing ability of the oligonucleotides N3′→P5′ phosphoramidate is of great interest. Thus, the 3′-amino-3′,4′-BNA monomer **1** would be an atractive and potential synthon for a novel class of antisense and antigene molecules.[17] Here, we would like to describe the synthesis and conformational analysis of a novel nucleoside analogue **1**.

The synthesis of **1** was successfully achieved by using 3-azido derivative **2**[10] as the starting material (Scheme 1). An azido group in **2** was reduced by the usual hydrogenolysis, and the resulting amino alcohol **3** was treated with *p*-toluenesulfonyl chloride to give **4** in 92% yield (two steps). Reaction of **4** with sodium hydride effectively caused an azetidine ring formation to afford **5** (90%), and reductive detosylation of **5** with sodium in amyl alcohol gave **6** in 66% yield. On the other hand, the azetidine derivative **6** was also obtained in 99% yield directly from azido alcohol **2** via the aza-ylide intermediate under Staudinger's conditions (triphenylphosphine, *o*-xylene, Δ).[18] To our best knowledge, this is the first example clearly exhibiting that a Staudinger reaction of 1,3-azido alcohol is a very useful entry for azetidines.[19] After benzyloxycarbonylation or trifluoroacetylation of the azetidine **6** (93–96%, **6**→**7**), acetolysis was performed to give the diacetate **8** in 82–83% yields. Coupling reactions of **8** with silylated thymine effectively proceeded to give the desired β-anomer of **9** (76–85%), exclusively. Deprotection of the 2′- and 5′-hydroxy groups in **9a** gave the nucleoside analogues **1a** in 86% yields (two steps). A similar reaction of **9b** proceeded only in 28% yield (two steps), probably due to instability of the trifluoroacetyl group in **9b** under the alkaline conditions. The treatment of **1b** with conc. ammonia gave the unsubstituted azetidine derivative **1c** in 87% yield. Thus, we achieved the synthesis of the novel azetidine-fused nucleoside analogue **1**.

Next, conformational analysis of 3′-amino-3′,4′-BNA monomer **1** was performed. On ^{1}H NMR measurements of **1a** and **1c**, relatively large $J_{1'2'}$ values (7.6 Hz for **1a** and 6.0 Hz for **1c**) were observed,[20] which mean that the azetidine derivative **1** was conformationally restricted in S-form (S%=95% for **1a** and 72% for **1c**),[21] in analogy with the parent oxetane derivative (S%=91–96% for 3′,4′-BNA monomers).[13,14] In addition, the conformational energy of **1c** was analyzed by means of the PM3 semi-empirical calculation.[22] The result demonstrated in Figure 2 clearly shows that the $C_{1'}$-*exo* puckering mode (conformation **A**, pseudorotational phase angle P=ca. 130°) is the most favorable form. The heat of formation of conformer **A** is ca. 4.6 kcal/mol lower than that of another conformer **B** ($C_{1'}$-*endo* form, P=ca. 300°), and relatively large energy



Scheme 1. *Reagents and conditions*: (a) H_2 (1 atm), 10% Pd–C, AcOEt, rt. (b) TsCl, DMAP, Et_3N, CH_2Cl_2, rt, (92%, two steps). (c) NaH, THF, rt, 90%. (d) Na, *n*-AmOH, 120–130°C, 66%. (e) Ph_3P, *o*-xylene, reflux, 99%. (f) CbzCl or $(CF_3CO)_2O$, DMAP, CH_2Cl_2, 0°C, 96% for **7a**, 93% for **7b**. (g) Ac_2O, AcOH, H_2SO_4, rt, 83% for **8a**, 82% for **8b**. (h) 2TMS·T, TMSOTf, $ClCH_2CH_2Cl$, rt, 76% for **9a**, 85% for **9b**. (i) K_2CO_3, MeOH, 0°C, then *n*-Bu_4NF, THF, rt, 86% for **1a**, 28% for **1b**. (j) NH_3 aq., *p*-dioxane, rt, 87%.

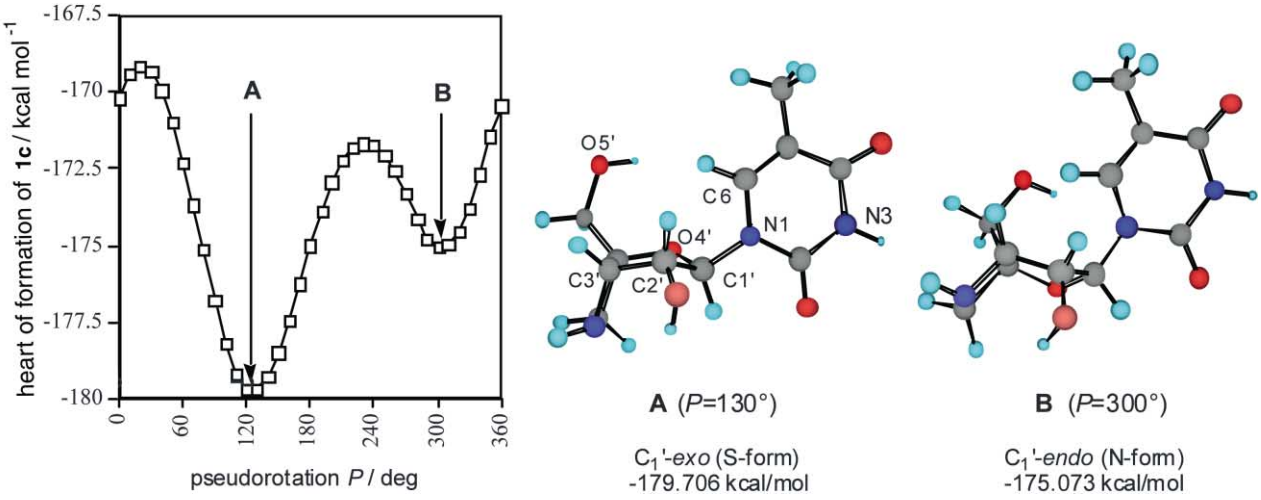


Figure 2. PM3 calculations of 3′-amino-3′,4′-BNA monomer **1c**.

barriers (P = ca. 20 and 230°) exist between the conformer **A** and **B**, which come from the restrained structure of the azetidine-fused furanose ring.

Thus, we successfully synthesized a novel azetidine-fused nucleoside analogue, 3′-amino-3′,4′-BNA monomer **1**, and also demonstrated that this compound had predominantly S-form sugar puckering. We believe that the nucleoside analogue **1** would be a potential synthon for a novel antiviral agent, a highly functional 2′,5′-linked oligonucleotide, and an oligonucleotide N3′→P5′ phosphoramidate with a restricted S-type sugar conformation and so on. Further studies on **1** are now in progress.

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20. The ^{1}H NMR spectra of **1a** and **1c** were measured in CD_3OD and D_2O (+5% DCl), respectively.
21. The percentage of S-conformation (S%) was calculated from the equation: $S\% = 100\times(J_{1'2'}-1)/6.9$. See: (a) Ref. 13; (b) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333–2344; (c) Altona, C. *Recl. Trav. Chim. Pays-Bas* **1982**, *101*, 413–433; (d) De Leeuw, F. A. A. M.; Altona, C. *J. Chem. Soc., Perkin Trans. 2* **1982**, 375–384.
22. The Spartan™ version 5.1 molecular orbital package (Wavefunction Inc.) utilizing the PM3 Hamiltonian was used for the semi-empirical MO calculations. All initial structures used for the MO calculation were generated by changing the dihedral angles ν_0–ν_4 with the constant ν_{max} value. Numerical calculations were performed on an Octane™ (SGI) workstation.