A novel bridged nucleoside bearing a conformationally switchable sugar moiety in response to redox changes†

Takeshi Baba,‡ Tetsuya Kodama,‡ Kazuto Mori, Takeshi Imanishi and Satoshi Obika*

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A novel nucleoside analog with a disulfide bridge structure at the sugar moiety, which shows redox-responsive reversibility of the sugar conformation due to formation and scission of the disulfide bond, was designed and synthesized.

It is known that sugar moieties of nucleosides, nucleotides and nucleic acids exist in an equilibrium between S-type and N-type conformations.[1] These sugar conformations are recognized selectively by biomolecules, such as adenosine deaminase, DNA kinase, polymerase, RNase H and ATP receptors, to perform biological events based on the selected sugar conformation on the nucleoside, nucleotide or nucleic acid.[2–5] Restricting the nucleosugar conformations is thus an effective way to discover compounds having high affinity for biomolecules, and to inhibit enzymes[6,7] and/or enhance binding affinity for complementary strands.[8,9] Therefore, an intense effort has been made to develop a wide variety of conformationally restricted nucleosides, nucleotides and oligonucleotides.

Controlling the conformational change of small molecules by external stimuli is expected to be applied to nano-machines and nano-devices such as molecular switches.[10] In nucleic acid chemistry, photo-,[11] pH-,[12] and redox-responsive[13] nucleic acids have been reported; however, there are few examples of conformationally-switchable nucleosugar moieties to control the affinity for biomolecules and/or complementary strands.

Here, we designed a novel bridged nucleic acid (BNA) monomer bearing a disulfide bridged structure (disulfide-type BNA, Fig. 1). The disulfide bridge is expected to lock the sugar into the N-type conformation under oxidative conditions. On the other hand, cleavage of the bridge under reductive conditions should release the sugar conformational restrictions. In other words, the nucleosugar restriction of this disulfide-type BNA monomer could be controlled by changing the redox condition, making it applicable to a redox switch for various biomolecules.[14]

To synthesize the disulfide-type BNA monomer, we examined the effective introduction of sulfur functional groups into nucleosides (Scheme 1). At first, the known nucleoside derivative **1**[9] was treated with trifluoromethanesulfonyl chloride in the presence of triethylamine to afford 2,2′-anhydronucleoside **2**. Since it is well known that sulfur nucleophiles react with 2,2′-anhydronucleosides at the C2′-position,[15] 2,2′-anhydronucleoside **2** was subjected to potassium thioacetate at 120 °C to obtain bis(thioacetate) **4**. However, nucleoside **2** was converted to the 2′-thio-LNA derivative[16] **3** *via* monothioacetylated compound **2′**.

Therefore, another synthetic pathway (Scheme 2) was used to prepare the BNA monomer **8**. Hydroxyl groups of nucleoside **1** were triflated and the following alkali-mediated hydrolysis gave arabino-type nucleoside **5**. Subsequently, C2′-hydroxyl group was triflated to give ditriflate **6**.[17] As expected, the thioacetylation of **6** proceeded easily at room temperature and furnished desired **4** in 40% yield from nucleoside **1**. Subsequently, the acetyl groups of **4** were removed with aqueous ammonia solution under dilute conditions to afford disulfide-bridged nucleoside **7**. Finally, **7** was treated with boron trichloride to yield the desired BNA monomer **8**.

Next, structural changes of the BNA monomer under reductive and oxidative conditions were examined using ^{1}H-NMR spectroscopy (Fig. 2) and mass spectrometry (ESI†). Dithiothreitol (DTT) and hydrogen peroxide (H_2O_2) were used as redox reagents. In the absence of DTT, the 1′-H resonance of deuterated monomer **8-D** was observed at 6.58 ppm as a singlet (Fig. 2a). After adding DTT, the singlet resonance decreased significantly and the 1′-H resonance of the deuterated monomer **9-D** was observed at 5.95 ppm as a doublet (J = 10 Hz) (Fig. 2b). Furthermore, after adding H_2O_2, the doublet decreased and the singlet reappeared at 6.58 ppm (Fig. 2c). This observation suggested that the disulfide bridge of **8** cleaved and reformed reversibly in response to the reducing agent DTT and the oxidizing agent H_2O_2.[18] We also confirmed the change in the structure by electrospray ionization mass spectrometry (ESI†).[19] In the case of **8** in H_2O–MeOH solution, m/z 319 was observed as $[M + H]^+$. In the presence of excess DTT, m/z 321, 641 and 663 appeared, which were identical to $[M + H]^+$, $[2M + H]^+$ and $[2M + Na]^+$ for **9**, respectively. These results suggest that disulfide-type BNA **8** could function as a molecular switch by forming and cleaving the disulfide bridge reversibly in response to redox changes.

Next, redox reactions of BNA monomer **8** using DTT and H_2O_2 were repeated (Fig. 3), showing that the reaction

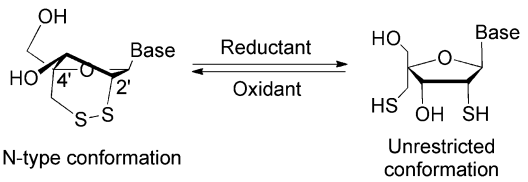
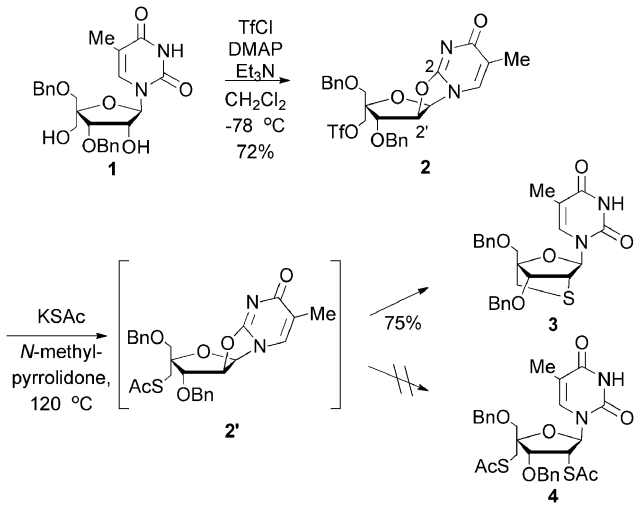


Fig. 1 Structure of disulfide-type BNA monomer and its change in structure by reductant and oxidant.

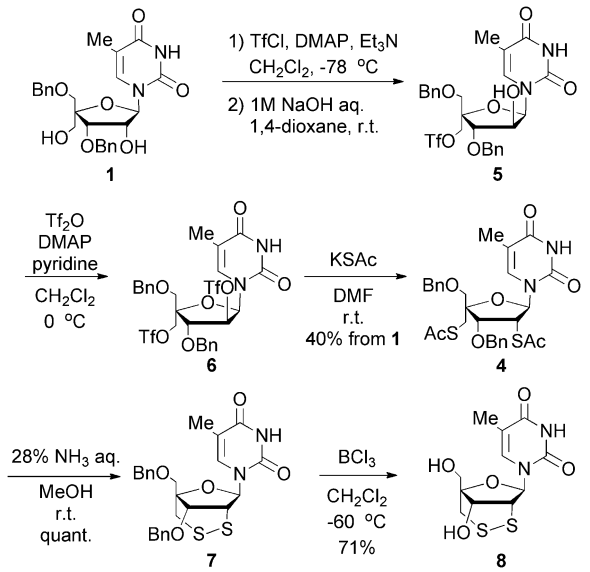
Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka, 565-0871, Japan. E-mail: obika@phs.osaka-u.ac.jp; Fax: +81 6 6879 8204; Tel: +81 6 6879 8200

† Electronic supplementary information (ESI) available: Full experimental procedures, characterization data of all new compounds, redox reactions using compound **8**. CCDC 768234. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0cc02484h

‡ Both authors contributed equally.



Scheme 1 Attempt to synthesize compound **4**.



Scheme 2 Synthesis of BNA monomer **8**.

successfully proceeded at least four times. No by-products such as sulfoxide or sulfone were observed during these repetitions.

The switching of the molecular structure was also performed with other redox agents: tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl), 2-mercaptoethanol, and sodium tetrahydroborate as reductants, and iodine and 2,2′-dithiodipyridine (2,2′-DTDP) as oxidants (ESI†). The conformational switching was also observed in the presence of these redox reagents. Under the presence of these redox reagents, sugar conformations of monomer **8** or **9** switched almost quantitatively between S-type and N-type, except for the case where 2-mercaptoethanol was used as a reductant.

The structure of monomer **8** was confirmed by X-ray crystallographic analysis (Fig. 4a) (see ESI†). Conformational parameters (pseudorotation phase angle and torsion angles) showed that the sugar conformation of **8** was fixed as the N-type.[20] Crystals of monomer **9** were not obtained because **9** was easily converted to **8** by air oxidation. Therefore, the optimized structure of **9** was calculated.[21] The structure

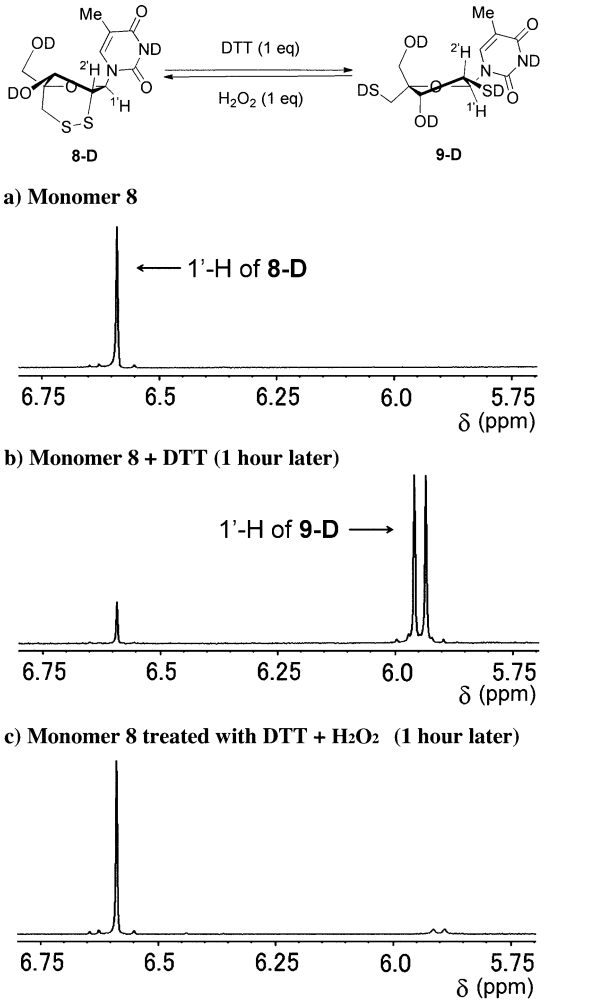


Fig. 2 Reversible redox reaction of BNA monomer **8** observed by ^{1}H NMR spectroscopy: (a) absence of DTT; (b) after reaction with DTT for 1 h; (c) after additional reaction with H_2O_2 for 1 h. Reaction was performed in the mixed solvent of deuterated methanol (CD_3OD) and deuterated PBS (D_2O was substituted for H_2O, pH 7.4).

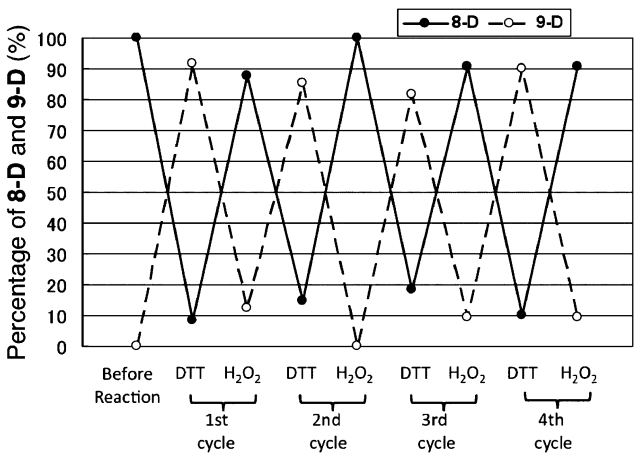


Fig. 3 Repetitive redox reaction of monomer **8**. Reaction conditions are shown in Fig. 2. The percentages of **8-D** (●) and **9-D** (○) were obtained by the integration values from the ^{1}H NMR spectra.

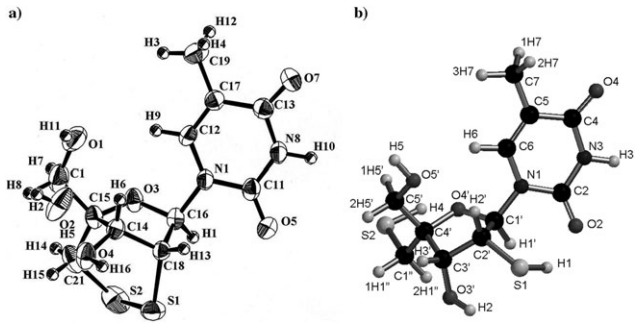


Fig. 4 (a) X-Ray crystal structure of BNA monomer **8** and (b) optimized structure of monomer **9**.[21]

showed that **9** formed the S-type sugar conformation (Fig. 4b). The dihedral angle (H1′–C1′–C2′–H2′ = 163.5°) obtained from the optimized structure was in good agreement with the coupling constant between 1′-H and 2′-H (J = 10 Hz) observed in Fig. 2b.[22] Furthermore, the dihedral angle (H2′–C2′–C3′–H3′ = −39.6°) was also in good agreement with the observed coupling constant (J = 5 Hz). These results clearly showed that the sugar moiety of **9** adopted the S-type sugar conformation.

In conclusion, we successfully synthesized a novel nucleoside with a disulfide bond forming a bridge between the 2′ and 4′ positions and showed that its sugar conformation was fixed to the N-type by X-ray crystallographic analysis. We also succeeded in switching the sugar conformation between the N-type and S-type reversibly by changing the redox conditions. An application study for antisense molecules, which change its own properties in response to redox conditions, is now ongoing, and will be reported elsewhere.

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19 Mass spectrum data of nucleosides **8**, **9** and deuterated nucleosides **8-D**, **9-D** are available in ESI†.
20 The selected conformational parameters obtained by X-ray crystallographic analysis of **8** are shown in Table S1 and compared with those of some 2′,4′-BNA analogs (Fig. S1) (see ESI†).
21 The calculation conditions for optimization and obtained parameters of the optimized conformer are presented in ESI†.
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