

Synthesis and hybridization property of a boat-shaped pyranosyl nucleic acid containing an exocyclic methylene group in the sugar moiety



Kazuto Mori^a, Tetsuya Kodama^{b,*}, Satoshi Obika^{a,*}

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

^b Graduate School of Pharmaceutical Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8601, Japan

ARTICLE INFO

Article history:

Received 6 November 2014

Revised 19 November 2014

Accepted 20 November 2014

Available online 28 November 2014

Keywords:

Nucleic acids

Carbohydrates

Oligonucleotides

ABSTRACT

A boat-shaped pyranosyl nucleic acid (BsNA) having an exocyclic methylene group in the sugar moiety was synthesized to investigate the possibility that the axial H3' of original BsNA is the cause of its duplex destabilization. The synthesized BsNA analog was chemically stable against various nucleophiles. From the thermal stability of duplex oligonucleotides including the BsNA analog, it was found that the duplex-forming ability can be sensitive to the size of functional groups at the 3'-position.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Many conformationally restricted nucleotides have been developed to date.¹ Especially, 2',4'-bridged nucleic acid (2',4'-BNA)²/locked nucleic acid (LNA)³ developed independently by our group and Wengel's group is used in therapeutic application⁴ and nanotechnology⁵ because of its high affinity with complementary single stranded DNA and RNA (ssDNA, ssRNA). Its outstanding duplex-forming ability is derived from the preorganized sugar conformation that mimics a nucleotide in an A-type RNA duplex. Although various 2',4'-BNA/LNA analogs have been developed in the past,¹ few analogs⁶ have higher binding affinities for complementary strands than that of original 2',4'-BNA/LNA. In addition, 2',4'-BNA^{COC}, whose sugar conformation is closest to a typical A-type RNA duplex in the 2',4'-BNA/LNA analogs, does not have the highest duplex-forming ability.⁷ Hence, it is necessary to develop a novel type of artificial nucleic acid on the basis of new strategy.

Recently, we designed and synthesized a boat-shaped glucopyranosyl nucleic acid (BsNA) **1**,⁸ which had a constrained pyranose as the basic skeleton (Fig. 1). Regrettably, the incorporation of BsNA **1-T** into oligonucleotides decreased the duplex-forming ability with a complementary ssDNA and ssRNA. Some factors can be attributed to this destabilization. One is the axial H3' which can

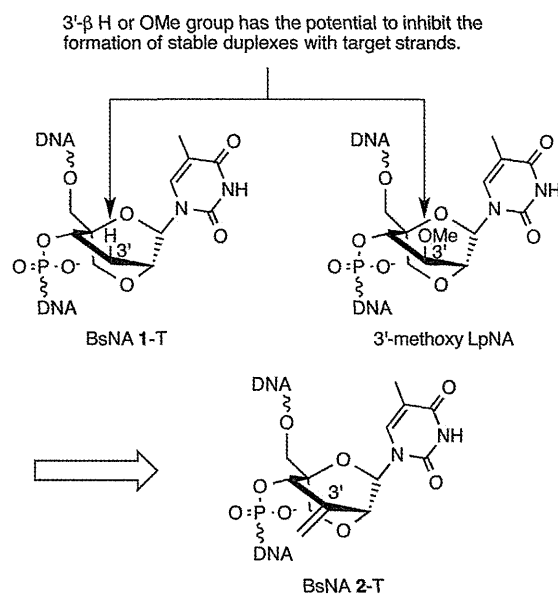


Figure 1. The structure of BsNA 1-T and 3'-methoxy LpNA and design of BsNA 2-T.

invade between neighboring nucleobases and inhibit π - π stacking interaction (Fig. 2a). Pedersen's group reported 3'-methoxy locked pyranosyl nucleic acid (LpNA)⁹ (Fig. 1), which had a low binding

* Corresponding authors. Tel./fax: +81 5 2789 2971 (T.K.); tel.: +81 6 6879 8200; fax: +81 6 6879 8204 (S.O.).

E-mail addresses: kodama@ps.nagoya-u.ac.jp (T. Kodama), obika@phs.osaka-u.ac.jp (S. Obika).

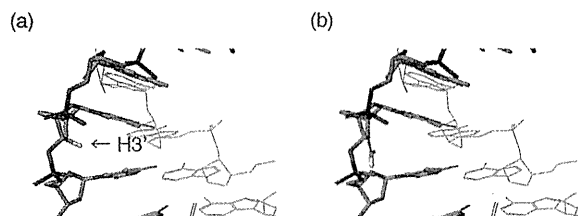


Figure 2. Representative low energy structure of an A-type DNA duplex comprising BsNA 1 (a) and the structure with an exocyclic methylene group (BsNA 2) modeled into the sugar moiety (b).

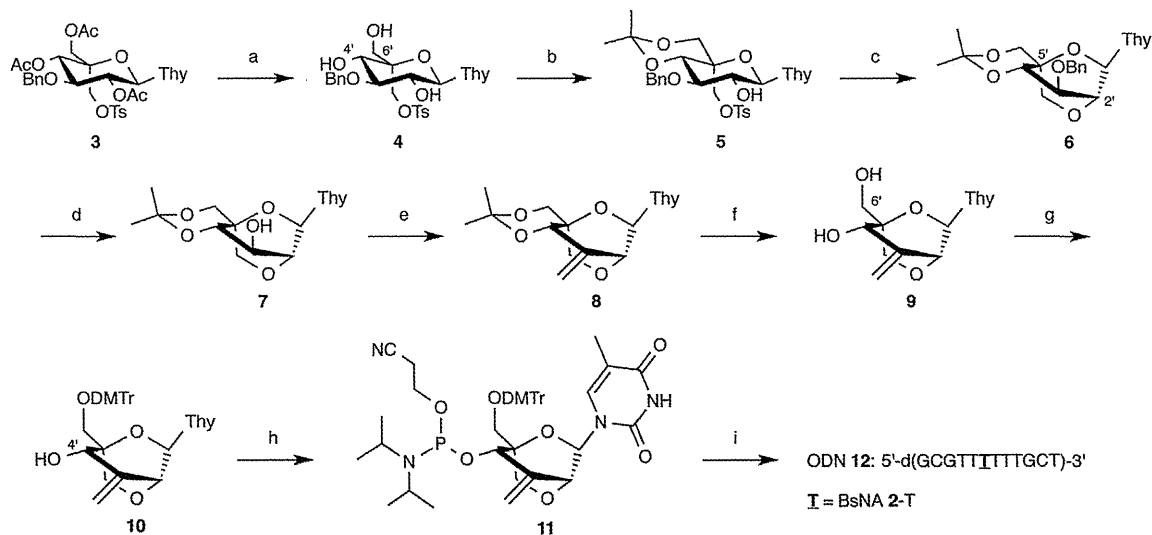
affinity with complementary strands as with the case of BsNA 1. They also suggested that the axial position of the C3'-OMe group can cause a destabilization of a duplex.

In this study, we newly designed and synthesized a BsNA analog that does not have the axial substituent at 3'-position. At first, replacing the C3' with oxygen atom was occurred to us, but it takes a great deal of exertion to synthesize that analog owing to its own acetal structure.¹⁰ Therefore, we decided to introduce an exocyclic methylene group to the C3' position to eliminate the axial H3' (Fig. 1). Recently, Seth's group also reported that an exocyclic methylene group could act as a bioisostere of the 2'-oxygen atom in 2',4'-BNA/LNA.^{6a} Since BsNA 2-T has no functional groups at the axial C3' position (Fig. 2b), the duplex forming ability will be improved if the axial H3' is the cause of the duplex destabilization.

2. Results and discussion

2.1. Synthesis of BsNA 2

BsNA 2-T was synthesized from known glucopyranoside **3**^{8a} as shown in Scheme 1. First, glucopyranoside **3** was deacetylated to give triol **4**. When glucopyranoside **3** was reacted with K₂CO₃ and MeOH, the reaction yield was very low because of the elimination of the tosylate group. This problem was negligible when **3** was treated with methylamine at 0 °C, and desired triol **4** was obtained at high yield. Next, the 4'- and 6'-hydroxy groups of triol **4** were protected as an isopropylidene acetal, and the resulting compound **5** was subjected to sodium hydride under moderate heating conditions to form the bridge between the C2'- and C5'-positions.



Scheme 1. Reagents and conditions: (a) 40% aq CH₃NH₂, THF, 0 °C, 95%; (b) 2,2-dimethoxypropane, CSA, DMF, rt, 88%; (c) NaH, DMF, 60 °C, 97%; (d) H₂, Pd(OH)₂/C, AcOEt, rt, 94%; (e) PDC, MS4A, CH₂Cl₂, rt, then Ph₃PCH₃Br, *n*-BuLi, THF, rt, 61%; (f) 60% aq AcOH, rt, 93%; (g) DMTrCl, pyridine, rt, 96%; (h) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, *N,N*-diisopropylethylamine, CH₃CN, 0 °C, 79%; (i) DNA synthesis. Thy = thymine-1-yl.

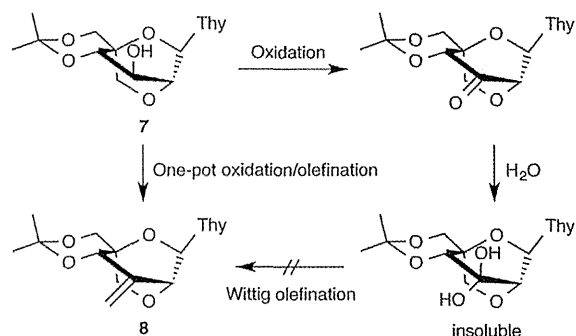


Figure 3. The reason for adopting one-pot oxidation/olefination procedure to obtain alkene 8.

Subsequently, the resultant compound **6** was exposed to hydrolysis conditions using palladium hydroxide to remove benzyl group. When alcohol **7** was oxidized, the resultant ketone was easily hydrated under an air atmosphere. The desired alkene **8** was not yielded from the hydrated compound using Wittig reagent (Fig. 3). Once the hydrate generated, it exhibited an insoluble property and the removal of water from the hydrate was difficult by typical dehydration procedures. Therefore, one-pot oxidation/olefination procedure was adopted. Alcohol **7** was firstly oxidized using PDC, and then methyl triphenyl phosphonium ylide was added to the reaction mixture to afford alkene **8**. Next, removal of the isopropylidene group with aqueous acetic acid furnished nucleoside **9**. Finally, tritylation of **9** at the 6'-hydroxy group with 4,4'-dimethoxytrityl chloride (DMTrCl) and phosphitylation at the 4'-hydroxy group of **10** with 2-cyanoethyl *N,N*-diisopropylaminochlorophosphoramidite afforded the desired phosphoramidite building block **11**.

Phosphoramidite **11** was introduced into oligodeoxynucleotide (ODN) using an automated DNA synthesizer. The sequence was the same as that of our previous work.^{8a} The concentration of phosphoramidite **11** was 0.1 M and the coupling time was prolonged to 8 min. 5-[3,5-Bis(trifluoromethyl)phenyl]-1*H*-tetrazole was used as an activator. Coupling yields were checked by trityl monitoring and were estimated to be over 95%. Synthesized ODN was cleaved from the solid support and deprotected by treatment with ammonium hydroxide solution. The obtained ODN **12** was purified by

Table 1
Evaluation of thermal denaturation temperatures (T_m values) of duplexes^a

| Oligodeoxynucleotides ^b | DNA complement | | RNA complement | |
|--------------------------------------|----------------|----------------|----------------|----------------|
| | T_m (°C) | ΔT_m^c | T_m (°C) | ΔT_m^c |
| Natural | 51 | – | 47 | – |
| 5'-d(GCGTTTITTTGCT)-3' (12) | 33 | –18 | 31 | –16 |
| 5'-d(GCGTTTITTTGCT)-3' (13) | 41 | –10 | 39 | –8 |

^a Target strand sequence: 5'-AGCAAAAACGC-3'. Thermal denaturation studies' conditions: 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl; each strand concentration = 4 μ M; scan rate of 0.5 °C min⁻¹ at 260 nm. T_m was determined by taking the first derivative of the melting curve. The number is the average of three independent measurements.

^b I = BsNA 2-T, T = BsNA 1-T.

^c ΔT_m s are calculated relative to T_m values of unmodified DNA–DNA and DNA–RNA duplexes.

reverse-phase HPLC and characterized by MALDI-TOF mass spectrometry.

2.2. Chemical stability of BsNA 2

BsNA **2** has a reactive allylphosphate ester which could be cleaved by nucleophiles.¹¹ This structure is described as an important feature in the biological activity of some nucleoside antibiotics¹² or antineoplastic agent¹³ possibly due to enhanced chemical reactivity. Therefore, there was concern that the 3' oxygen atom was eliminated during the deprotection process of ODN synthesis using ammonium hydroxide. However, such a cleaved ODN fragment was not observed and BsNA **2** proved to be stable against amines. We evaluated its chemical stability in some detail. Figure S1 shows the reverse-phase HPLC profile of the mixture after treatment of ODN **12** with 1 mM glutathione and 10 mM MgCl₂ for 12 h. The peak corresponding to ODN **12** did not disappear, and the notable new peak derived from the ODN was not observed. As a result, BsNA **2** was stable on the conditions that it was heated or treated with thiol and divalent metal ion, and the worrying strand-breakage was not occurred.

2.3. Evaluation of duplex-forming ability of BsNA 2

We evaluated the affinity of the synthesized ODN with complementary ssDNA and ssRNA through UV melting experiments. The UV melting profiles are shown in Figures S2 and S3, and the thermal denaturation temperatures (T_m values) are summarized in Table 1. ODN **12** did not improve the thermal stabilities of the duplexes with ssDNA and ssRNA ($\Delta T_m = -18, -16$); indeed, the duplex forming ability of ODN **12** was still lower than that of ODN **13**. This additional destabilization can be explained by a steric clash between the introduced exocyclic methylene group and the neighboring nucleotide which reduced π - π stacking in a different way from that of BsNA **1**. It was reported that an exocyclic methylene group can be sterically hindered in the hybridization when it is located in the major groove of the duplex.¹⁴ The C3'-exocyclic methylene group of BsNA **2** might act in that manner.

Given the fact that the introduction of the exocyclic methylene group to the C3' position lead to the further decrease of the binding affinity of BsNA **1**, one could anticipate that the binding affinity is sensitive to the size of functional groups at the 3'-position and will be improved when replacing the C3' with smaller groups. However, it should be noted that the duplex-forming ability of BsNAs is too lower than is assumed based on the past report¹⁴ and other factors, for example, the nucleobase lean and the nucleobase orientation, may be attributed to the destabilization of the duplexes. It is difficult to make conclusions about the factor affecting the binding affinity of BsNAs and the investigation of the above hypotheses is still in progress.

3. Conclusion

We successfully synthesized a BsNA analog bearing an exocyclic methylene group in the sugar moiety. The synthesized BsNA analog was chemically stable. The binding affinity of the analog with ssDNA and ssRNA was still lower than that of original BsNA possibly due to a steric clash between the introduced exocyclic methylene group and the neighboring nucleotide. Therefore, it is expected that the duplex-forming ability is sensitive to the size of functional groups at 3'-position and replacing the C3' with smaller groups will lead to an improvement of it. However, other structural properties cannot be ruled out as the cause of the duplex destabilization and the investigation of these hypotheses is currently underway in our laboratory.

4. Experimental

4.1. General

Dichloromethane, DMF and pyridine were distilled from CaH₂ and the other reagents used as received from commercial suppliers. ¹H NMR (400 and 300 MHz), ¹³C NMR (100.5 and 75.5 MHz) and ³¹P NMR (161.8 MHz) were recorded on JEOL JNM-ECS-400 or JNM-ECS-300 spectrometers. Chemical shifts are reported in parts per million referenced to internal tetramethylsilane (0.00 ppm), residual CHCl₃ (7.26 ppm), CH₃CN (1.94 ppm), or DMSO (2.50 ppm) for ¹H NMR, and chloroform-*d*₁ (77.16 ppm) or acetonitrile-*d*₃ (118.26 ppm) for ¹³C NMR. Relative to 85% H₃PO₄ as external standard for ³¹P NMR. IR spectra were recorded on a JASCO FT/IR-4200 spectrometers. Optical rotations were recorded on a JASCO DIP-370 instrument. Mass spectra were measured on JEOL JMS-600 or JMS-700 mass spectrometers. MALDI-TOF mass spectra were recorded on a Bruker Daltonics Autoflex II TOF/TOF mass spectrometer. For column chromatography, Fuji Silysia PSQ-100B or FL-100D silica gel was used. For high performance liquid chromatography (HPLC), SHIMADZU LC-6AD, SPD-10AV_{VP} and CTO-10AV_{VP} were used. Thermal denaturation experiments were carried out on SHIMADZU UV-1650 and UV-1800 spectrometers equipped with a T_m analysis accessory.

4.2. 1-(3-O-Benzyl-5-C-(tosyloxymethyl)- β -D-glucopyranosyl)thymine 4

To a solution of compound **3** (689 mg, 1.0 mmol) in THF (10 mL) was added aqueous 40% methylamine (4.2 mL, 50 mmol) at 0 °C and the resultant mixture was stirred at 0 °C for 7 h. Further aqueous 40% methylamine (1.7 mL, 20 mmol) was added to the mixture and the mixture was stirred at 0 °C for 4 h. Again, aqueous 40% methylamine (0.8 mL, 10 mmol) was added to the mixture and the mixture was stirred at 0 °C for 2 h. After removal of THF under reduced pressure, the mixture was separated with H₂O and AcOEt and the aqueous layer was extracted with AcOEt. The combined organic layer was dried over Na₂SO₄, and concentrated. The crude product was purified by column chromatography (SiO₂, *n*-hexane/AcOEt = 1:5) to give compound **4** (535 mg, 95%) as a white foam. [α]_D²² –13.4 (c 1.00, CH₃OH); IR ν_{max} (KBr): 1691, 3332 cm⁻¹; ¹H NMR (400 MHz, CD₃CN) δ 1.84 (3H, d, *J* = 1 Hz), 2.44 (3H, s), 3.07 (1H, br s), 3.42 (1H, d, *J* = 12 Hz), 3.52 (1H, d, *J* = 12 Hz), 3.60–3.87 (5H, m), 4.06 (1H, d, *J* = 11 Hz), 4.45 (1H, d, *J* = 11 Hz), 4.72 (1H, d, *J* = 11 Hz), 4.84 (1H, d, *J* = 11 Hz), 5.86 (1H, d, *J* = 9 Hz), 7.27–7.45 (8H, m), 7.83 (2H, d, *J* = 9 Hz), 9.24 (1H, br s); ¹³C NMR (100.5 MHz, CD₃CN) δ 2.4, 21.6, 64.1, 68.6, 71.2, 72.8, 75.5, 79.8, 80.2, 82.4, 111.6, 128.3, 128.7, 128.9, 129.1, 131.0, 132.9, 137.0, 140.0, 146.6, 151.7, 164.4; MS (EI) *m/z* (*I*_{rel},%) 562 (0.1), 480 (1.0), 390 (5.2), 264 (4.4), 172 (6.0), 91 (100), 65

(10.8); HRMS (EI): Calcd for $C_{26}H_{30}N_2O_{10}S$ $[M]^+$: 562.1621. Found: 562.1643.

4.3. 1-(3-O-Benzyl-4,6-O-isopropylidene-5-C-(tosyloxymethyl)- β -D-glucopyranosyl)thymine 5

To a solution of compound 4 (514 mg, 0.91 mmol) in DMF (9.1 mL) were added 2,2-dimethoxypropane (1.1 mL, 9.1 mmol) and (+)-10-camphorsulfonic acid (21 mg, 0.09 mmol) and the resultant mixture was stirred at room temperature for 13 h under a N_2 atmosphere. Further 2,2-dimethoxypropane (0.2 mL, 1.8 mmol) was added to the mixture and the mixture was stirred at room temperature for 2 h. After addition of saturated aq $NaHCO_3$, the reaction mixture was extracted with AcOEt, the organic layer was dried over Na_2SO_4 , and concentrated. The crude product was purified by column chromatography (SiO_2 , n -hexane/AcOEt = 2:5) to give compound 5 (481 mg, 88%) as a white foam. $[\alpha]_D^{22}$ -2.6 (c 1.00, $CHCl_3$); IR ν_{max} (KBr): 1598, 1704, 2992, 3221 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ 1.31 (3H, s), 1.46 (3H, s), 1.86 (3H, s), 2.41 (3H, s), 3.60 (1H, d, $J = 11$ Hz), 3.78–3.91 (4H, m), 4.03 (1H, br s), 4.55 (1H, d, $J = 11$ Hz), 4.69 (1H, d, $J = 11$ Hz), 4.75 (1H, d, $J = 11$ Hz), 4.81 (1H, d, $J = 11$ Hz), 5.97 (1H, d, $J = 9$ Hz), 7.08 (1H, s), 7.24–7.33 (7H, m), 7.83 (2H, d, $J = 8$ Hz), 9.35 (1H, br s); ^{13}C NMR (100.5 MHz, CD_3CN) δ 12.4, 19.1, 21.6, 29.2, 64.7, 65.1, 72.4, 73.7, 74.8, 75.6, 78.1, 80.2, 101.5, 112.1, 128.3, 128.6, 128.9, 129.0, 131.0, 132.8, 136.7, 139.8, 146.6, 151.7, 164.5; MS (EI) m/z ($I_{rel},\%$) 602 (0.6), 430 (1.4), 353 (3.1), 172 (3.7), 155 (8.6), 127 (8.4), 91 (100), 65 (9.1); HRMS (EI): Calcd for $C_{29}H_{34}N_2O_{10}S$ $[M]^+$: 602.1934. Found: 602.1935.

4.4. 1-(3-O-Benzyl-4,6-O-isopropylidene-2-O,5-C-methano- β -D-glucopyranosyl)thymine 6

To a solution of compound 5 (478 mg, 0.79 mmol) in DMF (8 mL) was added sodium hydride (95 mg, 60% in oil, 2.4 mmol) and the resultant mixture was stirred at 60 °C for 10 min under a N_2 atmosphere. After addition of saturated aq NH_4Cl , the reaction mixture was extracted with AcOEt. The organic layer was washed with H_2O , and brine, dried over Na_2SO_4 , and concentrated. The crude product was purified by column chromatography (SiO_2 , n -hexane/AcOEt = 1:2) to give compound 6 (329 mg, 97%) as a white foam. $[\alpha]_D^{23}$ $+14.0$ (c 1.00, $CHCl_3$); IR ν_{max} (KBr): 1682, 2993 cm^{-1} ; 1H NMR (300 MHz, $CDCl_3$) δ 1.44 (3H, s), 1.55 (3H, s), 1.79 (3H, d, $J = 1$ Hz), 3.65 (1H, d, $J = 15$ Hz), 3.81 (1H, ddd, $J = 2, 5, 5$ Hz), 3.86 (1H, dd, $J = 2, 13$ Hz), 3.95 (1H, d, $J = 15$ Hz), 4.06 (1H, dd, $J = 2, 5$ Hz), 4.39–4.50 (4H, m), 6.14 (1H, t, $J = 2$ Hz), 7.10–7.13 (2H, m), 7.23–7.32 (4H, m), 9.14 (1H, br s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.7, 18.8, 28.9, 62.9, 65.8, 65.9, 68.6, 72.2, 75.1, 78.6, 83.4, 100.1, 109.1, 127.6, 128.2, 128.6, 135.5, 136.9, 150.2, 164.0; MS (EI) m/z ($I_{rel},\%$) 430 (16.8), 415 (4.4), 167 (15.2), 133 (6.2), 127 (10.0), 111 (8.5), 97 (11.4), 91 (100), 69 (7.1); HRMS (EI): Calcd for $C_{22}H_{26}N_2O_7$ $[M]^+$: 430.1740. Found: 430.1770.

4.5. 1-(4,6-O-Isopropylidene-2-O,5-C-methano- β -D-glucopyranosyl)thymine 7

To a solution of compound 6 (134 mg, 0.31 mmol) in AcOEt (6.2 mL) was added 20% $Pd(OH)_2/C$ (67 mg). The reaction mixture was stirred under H_2 atmosphere at room temperature for 20 h, filtered and concentrated. The crude product was purified by column chromatography (SiO_2 , n -hexane/AcOEt = 1:5) to give compound 7 (100 mg, 94%) as a white foam. $[\alpha]_D^{24}$ -60.1 (c 1.00, $CHCl_3$); IR ν_{max} (KBr): 1700, 2993, 3186 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ 1.43 (3H, s), 1.66 (3H, s), 1.83 (3H, s), 3.66 (1H, d, $J = 11$ Hz), 3.83 (1H, d, $J = 10$ Hz), 4.04 (1H, d, $J = 11$ Hz), 4.24 (2H, s), 4.43 (1H, d, $J = 10$ Hz), 4.50 (1H, m), 4.99 (1H, s), 5.97 (1H, d, $J = 1$ Hz), 7.51

(1H, s), 10.33 (1H, s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.8, 19.1, 29.0, 63.2, 65.8, 66.9, 68.7, 71.7, 74.6, 84.2, 100.2, 109.8, 135.1, 150.7, 164.6; MS (EI) m/z ($I_{rel},\%$) 340 (19.3), 325 (13.4), 214 (100), 185 (13.7), 167 (35.9), 157 (19.9), 139 (13.2), 127 (46.2), 111 (22.5), 97 (24.0), 83 (26.8), 69 (37.6), 59 (50.6); HRMS (EI): Calcd for $C_{15}H_{20}N_2O_7$ $[M]^+$: 340.1271. Found: 340.1273.

4.6. 1-(3-Deoxy-3-exomethylene-4,6-O-isopropylidene-2-O,5-C-methano- β -D-glucopyranosyl)thymine 8

To a solution of compound 7 (89 mg, 0.26 mmol) in CH_2Cl_2 (2.6 mL) were added MS4A (180 mg) and pyridinium dichromate (120 mg, 0.32 mmol) and the resultant mixture was stirred at room temperature for 2 h under a N_2 atmosphere. In a separate flask, triphenylphosphonium bromide (476 mg, 1.33 mmol) was stirred at -78 °C under a N_2 atmosphere in dry THF (3.1 mL), and n -butyllithium (1.65 M in n -hexane, 0.76 mL, 1.25 mmol) was added dropwise to give a yellow slurry. After stirring the phosphonium ylide for 1 h at 0 °C, it was cooled to -78 °C and was added to the PDC reaction mixture at -78 °C. The reaction mixture was warmed slowly to rt and stirred for 3 h. After filtration of the mixture through a Celite pad, the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2 , n -hexane/AcOEt = 1:1) to give compound 8 (53 mg, 61%) as a white foam. $[\alpha]_D^{18}$ $+20.8$ (c 1.00, $CHCl_3$); IR ν_{max} (KBr): 1681, 1696, 2880, 2995, 3190 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ 1.48 (3H, s), 1.62 (3H, s), 1.95 (3H, d, $J = 1$ Hz), 3.66 (1H, d, $J = 11$ Hz), 3.95–3.98 (2H, m), 4.45 (1H, dd, $J = 2, 10$ Hz), 4.56 (1H, d, $J = 10$ Hz), 5.44 (1H, d, $J = 3$ Hz), 5.51 (1H, d, $J = 3$ Hz), 6.21 (1H, d, $J = 2$ Hz), 7.23 (1H, d, $J = 1$ Hz), 8.89 (1H, br s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.0, 19.0, 28.9, 63.2, 66.3, 69.2, 69.6, 70.9, 84.2, 100.5, 110.4, 119.7, 133.9, 138.1, 150.1, 163.9; MS (EI) m/z ($I_{rel},\%$) 336 (3.1), 321 (5.1), 279 (2.8), 182 (75.2), 167 (69.2), 152 (9.6), 124 (97.8), 95 (100), 67 (68.5); HRMS (EI): Calcd for $C_{16}H_{20}N_2O_6$ $[M]^+$: 336.1321. Found: 336.1320.

4.7. 1-(3-Deoxy-3-exomethylene-2-O,5-C-methano- β -D-glucopyranosyl)thymine 9

Compound 8 (55 mg, 0.16 mmol) was dissolved in AcOH/ H_2O (3:2, 2.4 mL) and stirred at room temperature for 10 h. The solvent was removed under reduced pressure and the residue co-evaporated with toluene. The crude product was purified by column chromatography (SiO_2 , $CHCl_3/MeOH = 15:1$) to give compound 9 (45 mg, 93%) as a white foam. $[\alpha]_D^{23}$ $+67.9$ (c 1.00, CH_3OH); IR ν_{max} (KBr): 1695, 3391 cm^{-1} ; 1H NMR (300 MHz, DMSO- d_6) δ 1.75 (3H, d, $J = 1$ Hz), 3.55 (1H, d, $J = 16$ Hz), 3.55 (1H, d, $J = 16$ Hz), 3.61 (1H, d, $J = 16$ Hz), 3.90–3.93 (1H, m), 3.99 (1H, d, $J = 13$ Hz), 4.20 (1H, d, $J = 3$ Hz), 4.33 (1H, m), 4.88 (1H, br s), 5.29 (1H, s), 5.36 (1H, s), 5.75 (1H, br s), 5.98 (1H, d, $J = 3$ Hz), 7.32 (1H, d, $J = 1$ Hz); ^{13}C NMR (75.5 MHz, CD_3CN) δ 12.5, 62.2, 64.6, 67.2, 72.3, 79.4, 83.8, 109.7, 119.7, 135.9, 143.9, 151.0, 164.7; MS (FAB) m/z 297 $[M+H]^+$; HRMS (FAB): Calcd for $C_{13}H_{17}N_2O_6$ $[M+H]^+$: 297.1081. Found: 297.1109.

4.8. 1-(3-Deoxy-6-O-(4,4'-dimethoxytrityl)-3-exomethylene-2-O,5-C-methano- β -D-glucopyranosyl)thymine 10

To a solution of compound 9 (45 mg, 0.15 mmol) in pyridine (1.5 mL) was added 4,4'-dimethoxytrityl chloride (78 mg, 0.23 mmol) and the resultant mixture was stirred at room temperature for 4 h under a N_2 atmosphere. After addition of H_2O , the reaction mixture was extracted with AcOEt, the organic layer was dried over Na_2SO_4 , and concentrated. The crude product was purified by column chromatography (SiO_2 , 0.5% triethylamine in n -hexane/AcOEt = 1:1) to give compound 10 (86 mg, 96%) as a white foam. $[\alpha]_D^{24}$ $+39.6$ (c 1.00, $CHCl_3$); IR ν_{max} (KBr): 1509, 1607,

1693, 2836, 2934, 3003, 3185, 3402 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 1.93 (3H, s), 2.38 (1H, d, *J* = 6 Hz), 3.23 (1H, d, *J* = 14 Hz), 3.40 (1H, d, *J* = 14 Hz), 3.80 (6H, s), 3.89 (1H, d, *J* = 13 Hz), 4.09 (1H, d, *J* = 13 Hz), 4.46 (1H, d, *J* = 3 Hz), 4.62 (1H, m), 5.37 (1H, d, *J* = 2 Hz), 5.53 (1H, m), 6.16 (1H, d, *J* = 3 Hz), 6.84–6.87 (4H, m), 7.22–7.46 (10H, m), 9.07 (1H, br s); ¹³C NMR (75.5 MHz, CDCl₃) δ 12.9, 30.1, 55.3, 62.2, 64.4, 67.2, 71.1, 78.0, 83.4, 86.6, 109.8, 113.4, 119.9, 127.2, 128.0, 128.1, 130.0, 130.1, 134.7, 135.3, 135.4, 141.8, 144.5, 150.2, 158.7 (1), 158.7 (4), 164.2; MS (FAB) *m/z* 599 [M+Na]⁺; HRMS (FAB): Calcd for C₃₄H₃₅N₂O₈ [M+H]⁺: 599.2388. Found: 599.2427.

4.9. 1-[4-O-(2-Cyanoethoxy(diisopropylamino)phosphino)-3-deoxy-6-O-(4,4'-dimethoxytrityl)-3-exomethylene-2-O,5-C-methano-β-D-glucopyranosyl]thymine **11**

To a solution of compound **10** (74 mg, 0.12 mmol) in dry CH₃CN (1.2 mL) were added *N,N*-diisopropylethylamine (63 μL, 0.36 mmol) and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (40 μL, 0.18 mmol) and the resultant mixture was stirred at 0 °C for 2.5 h under a N₂ atmosphere. Further *N,N*-diisopropylethylamine (21 μL, 0.12 mmol) and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (13 μL, 0.06 mmol) were added to the mixture and the mixture was stirred at 0 °C for 1.5 h. The reaction mixture was concentrated and the obtained crude product was purified by column chromatography (SiO₂, 0.5% triethylamine in *n*-hexane/AcOEt = 1:1) to give compound **11** (76 mg, 79%) as a white foam. ³¹P NMR (161.8 MHz, CDCl₃) δ 149.3, 149.8; MS (FAB) *m/z* 799 [M+H]⁺; HRMS (FAB): Calcd for C₄₃H₅₂N₄O₉P [M+H]⁺: 799.3466. Found: 799.3491.

4.10. Oligodeoxynucleotide **12**

Synthesis of ODN **12** modified with BsNA **2** was performed on an automated DNA synthesizer (Gene Design nS-8) on a 0.2 μmol scale using a phosphoramidite coupling protocol and 5-[3,5-bis(trifluoromethyl)phenyl]-1*H*-tetrazole as the activator. The concentration of each phosphoramidite was 0.1 M and the coupling times were 8 min. Coupling yields were checked by trityl monitoring and were estimated to be over 95%. The CPG solid supported ODN (DMTr-ON) was treated with concentrated ammonium hydroxide solution at 55 °C for 12 h, and then concentrated. The crude ODN was roughly purified and detritylated with a Sep-Pak Plus C₁₈ Environmental Cartridge, and then carefully by RP-HPLC using Waters XBridge™ OST C18 2.5 μm (10 × 50 mm) with a linear gradient of CH₃CN (6–12% over 30 min) in 0.1 M triethylammonium acetate buffer (pH = 7.0). The purity of the ODN was analyzed by RP-HPLC on a Waters XBridge™ Shield RP 18 2.5 μm

(4.6 × 50 mm) and characterized by MALDI-TOF mass spectrometry. The isolation yield calculated from the UV absorbance at 260 nm was 27%. MALDI-TOF-MS data ([M-H]⁻): found 3688.5 (calcd 3687.4).

Acknowledgments

This study was supported by the Japan Society for the Promotion of Science (JSPS), the Ministry of Education, Culture, Sports, Science, and Technology in Japan (MEXT), and the Advanced Research for Medical Products Mining Programme of the National Institute of Biomedical Innovation (NIBIO). K.M. is grateful for a Research Fellowship for Young Scientist from JSPS.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmc.2014.11.030>.

References and notes

- (a) Rahman, S. M. A.; Imanishi, T.; Obika, S. *Chem. Lett.* **2009**, 38, 512; (b) Obika, S.; Rahman, S. M. A.; Fujisaka, A.; Kawada, Y.; Baba, T.; Imanishi, T. *Heterocycles* **2010**, 81, 1347; (c) Prakash, T. P. *Chem. Biodivers.* **2011**, 8, 1616.
- Obika, S.; Nanbu, D.; Hari, Y.; Morio, K.; In, Y.; Ishida, T.; Imanishi, T. *Tetrahedron Lett.* **1997**, 38, 8735.
- Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455.
- (a) Veedu, R. N.; Wengel, J. *Chem. Biodivers.* **2010**, 7, 536; (b) Yamamoto, T.; Nakatani, M.; Narukawa, K.; Obika, S. *Future Med. Chem.* **2011**, 3, 339.
- Østergaard, M. E.; Hrdlicka, P. J. *Chem. Soc. Rev.* **2011**, 40, 5771.
- (a) Seth, P. P.; Allerson, C. R.; Berdeja, A.; Siwkowski, A.; Pallan, P. S.; Gaus, H.; Prakash, T. P.; Watt, A. T.; Egli, M.; Swayze, E. E. *J. Am. Chem. Soc.* **2010**, 132, 14942; (b) Hanessian, S.; Schroeder, B. R.; Giacometti, R. D.; Merner, B. L.; Østergaard, M. E.; Swayze, E. E.; Seth, P. P. *Angew. Chem., Int. Ed.* **2012**, 51, 11242; (c) Morihiro, K.; Kodama, T.; Kentefu; Moai, Y.; Veedu, R. N.; Obika, S. *Angew. Chem., Int. Ed.* **2013**, 52, 5074.
- (a) Hari, Y.; Obika, S.; Ohnishi, R.; Eguchi, K.; Osaki, T.; Ohishi, H.; Imanishi, T. *Bioorg. Med. Chem.* **2006**, 14, 1029; (b) Mitsuoka, Y.; Kodama, T.; Ohnishi, R.; Hari, Y.; Imanishi, T.; Obika, S. *Nucleic Acids Res.* **2009**, 37, 1225.
- (a) Mori, K.; Kodama, T.; Obika, S. *Org. Lett.* **2011**, 13, 6050; (b) Migawa, M. T.; Seth, P. P.; Swayze, E. E.; Ross, B. S.; Song, Q.; Han, M. PCT Int. Appl. WO 2011/017521 A2, 2011.
- Bomholt, N.; Jørgensen, P. T.; Pedersen, E. B. *Bioorg. Med. Chem. Lett.* **2011**, 21, 7376.
- Madsen, A. S.; Wengel, J. *J. Org. Chem.* **2012**, 77, 3878.
- Matsuda, A.; Sasaki, T. *Cancer Sci.* **2004**, 95, 105.
- (a) Buchanan, J. G.; Wightman, R. H. In *Topics in Antibiotic Chemistry*; Sammes, P. G., Ed.; Ellis Horwood: West Sussex, 1982; Vol. 6, p 229; (b) Nakagawa, F.; Okazaki, T.; Naito, A.; Iijima, Y.; Yamazaki, M. *J. Antibiot.* **1985**, 38, 823; (c) Takahashi, S.; Nakagawa, F.; Kawazoe, K.; Furukawa, Y.; Sato, S.; Tamura, C.; Naito, T. *J. Antibiot.* **1985**, 38, 830.
- Takenuki, K.; Matsuda, A.; Ueda, T.; Sasaki, T.; Fujii, A.; Yamaguchi, K. *J. Med. Chem.* **1988**, 31, 1063.
- Seth, P. P.; Allerson, C. R.; Berdeja, A.; Swayze, E. E. *Bioorg. Med. Chem. Lett.* **2011**, 21, 588.

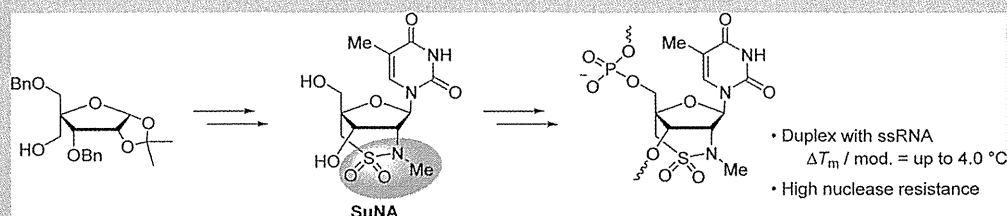
Sulfonamide-Bridged Nucleic Acid: Synthesis, High RNA Selective Hybridization, and High Nuclease Resistance

Yasunori Mitsuoka,^{†,‡} Yuko Fujimura,[‡] Reiko Waki,[†] Akira Kugimiya,[‡] Tsuyoshi Yamamoto,[†] Yoshiyuki Hari,[†] and Satoshi Obika^{*,†}

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

[‡]Discovery Research Laboratory for Innovative Frontier Medicines, Shionogi & Co., Ltd., 3-1-1 Futaba-cho, Toyonaka, Osaka 561-0825, Japan

S Supporting Information



- Duplex with ssRNA
 $\Delta T_m / \text{mod.} = \text{up to } 4.0 \text{ } ^\circ\text{C}$
- High nuclease resistance

ABSTRACT: 2'-N,4'-C-(N-Methylamino)sulfonylmethylene-bridged thymidine (SuNA), which has a six-membered linkage including a sulfonamide moiety, was synthesized and introduced into oligonucleotides. The oligonucleotides containing SuNA exhibited excellent nuclease resistance, a high affinity toward single-stranded RNA, and a low affinity toward single-stranded DNA compared to the natural oligonucleotide.

The first systemic antisense drug, Kynamro, was approved by the FDA in 2013,¹ and many other antisense oligonucleotides are in clinical trials. For the practical application of antisense methodology, chemical modification is essential to achieve a strong interaction with single-stranded RNA (ssRNA) in a sequence-specific manner. In addition, high resistance against enzymatic degradation is also required for in vivo applications. Among numerous chemical modifications, the introduction of a bridged structure between the 2'- and 4'-positions generally increases affinity toward ssRNA and improves resistance to nuclease degradation.² Since the discovery of the 2'-O,4'-C-methylene-bridged nucleic acid (2',4'-BNA³/LNA⁴), which is a typical example of these bridged compounds, many bridged nucleic acids have been developed.^{5–11} Previous studies have revealed that nuclease resistance can be enhanced by increasing the ring size of the bridge moieties because of increasing steric hindrance (i.e., 2',4'-BNA/LNA < ENA,⁵ 2',4'-BNA^{NC6} < 2',4'-BNA^{COC7}) (Figure 1). However, this decreases binding affinity because of insufficient restriction of the sugar conformation (i.e., 2',4'-BNA/LNA > ENA, 2',4'-BNA^{NC} > 2',4'-BNA^{COC}). Thus, a balance between these two properties would be very important for the development of practical antisense oligonucleotides.

Recently, we synthesized several 2',4'-BNAs possessing amide or urea moieties in their bridged structure (Figure 1). They possessed increased nuclease resistance and/or RNA selectivity compared to analogues with the same-membered bridged structure, maintaining high affinity toward ssRNA (i.e., 2',4'-BNA/LNA vs AmNA,⁸ ENA vs six-membered AmNA,⁹ 2',4'-BNA^{COC} vs urea-BNA¹⁰). These properties suggest that

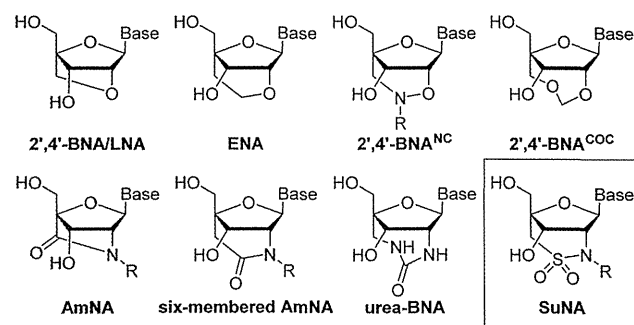


Figure 1. Structures of 2',4'-BNA/LNA, ENA, 2',4'-BNA^{NC}, 2',4'-BNA^{COC}, AmNA, six-membered AmNA, urea-BNA, and SuNA designed in the present study.

the exocyclic carbonyl groups inhibit the interaction between the oligonucleotides and nuclease and destabilize the duplex formed with single-stranded DNA (ssDNA). These are expected to derive from steric and electronic properties of the exocyclic carbonyl groups. However, how the bridged moiety itself affects the hybridization properties and the nuclease resistance of the oligonucleotides remains unknown.

This study used a sulfonamide structure, which is often seen in bioactive compounds or drugs,¹² to evaluate the relationship between ring size and hybridization properties with ssRNA and between bulkiness of the bridge structure and nuclease resistance. Ring size of the bridge structure containing a

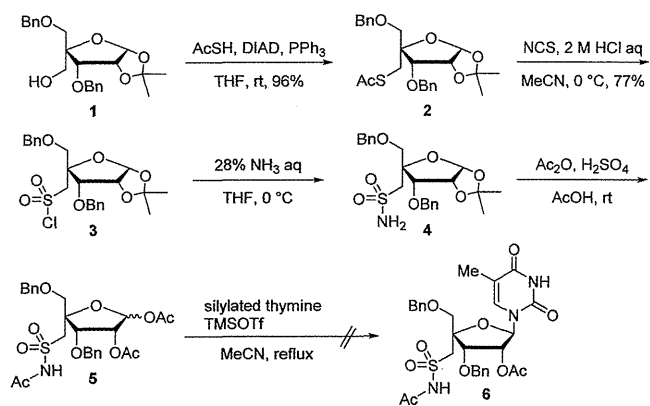
Received: September 13, 2014

Published: October 24, 2014

sulfonamide moiety should be larger than that of a same-membered bridged structure because sulfur is larger than oxygen, carbon, or nitrogen. Moreover, a sulfonamide moiety is more bulky than an amide or urea structure. Thus, 2',4'-*N*,4'-*C*-(*N*-methylamino)sulfonylmethylene-bridged thymidine (SuNA-T) (Figure 1) was synthesized, and the properties of the SuNA-T-modified oligonucleotides were examined.

Initially, the construction of a sulfonamide-bridged structure from **1**,¹³ a common precursor for the synthesis of 2',4'-BNA/LNA, was attempted without any *N*-substituents (Scheme 1). A

Scheme 1. Synthesis of Intermediates and Introduction of Base



thioacetyl group was introduced into **1** through a Mitsunobu reaction to afford **2**, which was then converted to the sulfonyl chloride derivative **3**.¹⁴ Treatment of **3** with ammonia gave the sulfonamide derivative **4**. In the acetylation of **4**, the sulfonamide and two hydroxy groups were acetylated to afford triacetate **5**. Although the coupling reaction of **5** with silylated thymine, prepared in situ from thymine and *N,O*-bis(trimethylsilyl)-acetamide, was attempted, a complex mixture resulted instead of the desired product **6**. Reactivity of the acylsulfonamide group of **5** may cause many side reactions. This result implied that the construction of a sulfonamide-bridged structure without any *N*-substituents was difficult via this synthetic route.

To avoid the side reactions promoted by the acylsulfonamide group of **5**, a methyl group was introduced into the nitrogen atom of the acylsulfonamide group (Scheme 2). Treatment of **3** with methylamine and subsequent acetylation afforded triacetate **7**. As expected, the coupling reaction of **7** with silylated thymine was successful and provided the desired product **8** in good yield (74% in three steps), indicating **7** is a good precursor for coupling reactions with silylated nucleobases. After removal of the acetyl groups of **8**, the 2'-hydroxyl group was inverted by mesylation, followed by treatment with NaOH to afford compound **9**. Triflation of **9** and subsequent treatment with K₂CO₃ resulted in intramolecular cyclization to give the desired product **10**. Benzyl groups were removed by hydrogenolysis to afford SuNA-T monomer **11**. Finally, dimethoxytritylation of **11** with 4,4'-dimethoxytrityl chloride followed by phosphitylation gave the phosphoramidite **13**.

The structure of SuNA-T monomer **11**¹⁵ was confirmed by X-ray crystallography (Figure 2a, Table 1). The crystal structure of **11** revealed that the pseudorotation phase angle *P* was 16°, which supports its *N*-type sugar pucker. Moreover, the ν_{\max} and δ values of **11** were 44° and 80°, respectively. The ν_{\max} values, which represent the maximum degree of the sugar puckering mode (*N/S*-type), indicated that the sugar conformation of

Scheme 2. Synthesis of Phosphoramidite 13

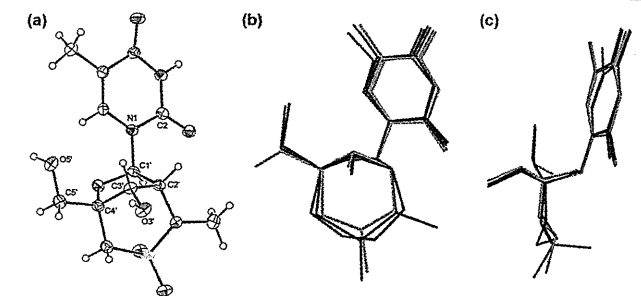
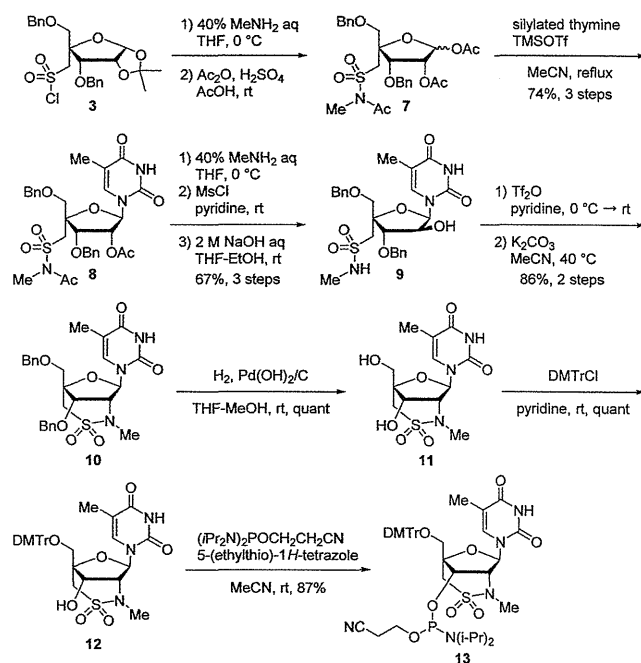


Figure 2. (a) X-ray structure of SuNA-T (**11**). (b, c) Superpositions of X-ray structure of SuNA-T (**11**) (red), 2',4'-BNA^{NC}[NMe] (green), and 2',4'-BNA^{COC} (blue).

Table 1. Selected Parameters from X-ray Analysis

| | δ (deg) | ν_{\max} (deg) | <i>P</i> (deg) |
|-------------------------------|----------------|--------------------|----------------|
| 2',4'-BNA | 66 | 57 | 17 |
| ENA | 76 | 48 | 15 |
| 2',4'-BNA ^{NC} [NMe] | 75 | 49 | 23 |
| SuNA-T (11) | this work | 80 | 44 |
| 2',4'-BNA ^{COC} | 78 | 38 | 17 |

SuNA (ν_{\max} 44°) was between the six-membered bridge (ENA and 2',4'-BNA^{NC}) and the seven-membered bridge (2',4'-BNA^{COC}) (Figure 2b,c). This may be due to the large sulfur atom. The bridge structure of SuNA is more bulky than that of 2',4'-BNA^{NC}[NMe], which has the same six-membered bridged structure, because of the two oxygen atoms and methyl group of the sulfonamide moiety (Figure 2b,c).

Phosphoramidite **13** was incorporated into oligonucleotides using an automated DNA synthesizer with standard phosphoramidite chemistry, except for a prolonged coupling time of 16 min with 5-(ethylthio)-1*H*-tetrazole as an activator, conditions similar to those for 2',4'-BNA^{COC} (see the Supporting Information). The sulfonamide bridge was stable under conventional conditions, that is, aqueous ammonia and

methylamine at room temperature, for cleavage from the resin and removal of protecting groups.

The duplex-forming abilities of the modified oligonucleotides 15–19 with ssDNA and ssRNA were evaluated by UV melting experiments and compared with those of the corresponding natural DNA 14 (Table 2). The T_m values for duplexes formed

Table 2. T_m Values ($^{\circ}\text{C}$) of Oligonucleotides with Complementary DNA and RNA^a

| oligonucleotides | T_m ($\Delta T_m/\text{mod.}$) | | $T_m(\text{RNA}) - T_m(\text{DNA})$ |
|----------------------------|------------------------------------|-----------|-------------------------------------|
| | ssDNA | ssRNA | |
| 5'-GCGTTTTTTGCT-3' (14) | 53 | 49 | -4 |
| 5'-GCGTTTTTTGCT-3' (15) | 49 (-4.0) | 51 (+2.0) | +2 |
| 5'-GCGTTTTTTGCT-3' (16) | 47 (-3.0) | 54 (+2.5) | +7 |
| 5'-GCGTTTTTTGCT-3' (17) | 46 (-2.3) | 60 (+3.7) | +14 |
| 5'-GCGTTTTTTGCT-3' (18) | 48 (-1.7) | 57 (+2.7) | +9 |
| 5'-GCGTTTTTTGCT-3' (19) | 53 (0.0) | 73 (+4.0) | +20 |

^aUV melting profiles were measured in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl at a scan rate of 0.5 $^{\circ}\text{C}/\text{min}$ at 260 nm. The concentration of the oligonucleotide was 4 μM for each strand. $\underline{\text{T}}$ = SuNA-T. The sequences of target DNA and RNA complements were 5'-d(AGCAAAAACGC)-3' and 5'-r(AGCAAAAACGC)-3'.

by 15–19 with ssRNA were higher than that of the duplex formed by the natural DNA 14 and ssRNA. Changes in ΔT_m /modification values ranged from +2.0 $^{\circ}\text{C}$ to +4.0 $^{\circ}\text{C}$. This stabilization is between the six-membered bridge (ENA; +3.5 $^{\circ}\text{C}$ to +5.2 $^{\circ}\text{C}$, 2',4'-BNA^{NC}; +4.7 $^{\circ}\text{C}$ to +5.8 $^{\circ}\text{C}$, the six-membered AmNA; +1.0 $^{\circ}\text{C}$ to +4.7 $^{\circ}\text{C}$) and the seven-membered bridge (2',4'-BNA^{COC}; +1.0 $^{\circ}\text{C}$ to +2.0 $^{\circ}\text{C}$ and urea-BNA; +1.0 $^{\circ}\text{C}$ to +2.3 $^{\circ}\text{C}$). This tendency seems to correlate the ν_{max} values of the sugar conformations. In contrast, the oligonucleotides 15–19 destabilized the duplex with ssDNA. In the case of 17 and 19, the differences in T_m values with ssRNA and with ssDNA were 14 and 20 $^{\circ}\text{C}$, respectively. The oligonucleotides modified by SuNA-T monomer 11 exhibited greater RNA selective hybridization ability than the six-membered AmNA and urea-BNA. This result indicates that a bulky bridge structure destabilized the duplex with ssDNA more efficiently than a small bridge structure, because the bulky bridge would make a steric clash with the C5' atom of the 3'-neighboring residue when it is located in the narrow minor groove of the B-form DNA duplex.

The enzymatic stability of the modified oligonucleotides was evaluated using a 3'-exonuclease. A comparison of oligonucleotides 20–24 is shown in Figure 3. Under the conditions used in this experiment, natural oligonucleotide 20 and the 2',4'-BNA(LNA)-modified oligonucleotide 21 were completely degraded within 2 and 10 min, respectively. In contrast, the SuNA-modified oligonucleotide 24 significantly enhanced stability against the 3'-exonuclease. This ability was comparable to that of the 2',4'-BNA^{COC}-modified oligonucleotide 23, which had a seven-membered bridge structure and was better than that of the 2',4'-BNA^{NC}[NMe]-modified oligonucleotide 22, which had a six-membered bridge structure. These results revealed that the six-membered bridged structure possessing a

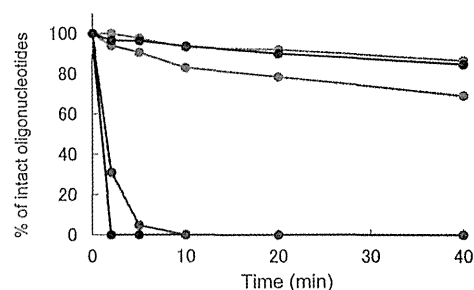


Figure 3. Hydrolysis of oligonucleotides (750 pmol) conducted at 37 $^{\circ}\text{C}$ in buffer (100 μL) containing 50 mM Tris-HCl (pH 8.0), 10 mM MgCl_2 , and phosphodiesterase I (4.0 $\mu\text{g}/\text{mL}$). Sequences: 5'-d(TTTTTTTTTT)-3', $\underline{\text{T}}$ = natural (black, 20), 2',4'-BNA/LNA (pink, 21), 2',4'-BNA^{NC}[NMe] (green, 22), 2',4'-BNA^{COC} (blue, 23), SuNA (red, 24).

sulfonamide moiety inhibited degradation by a 3'-exonuclease as well as the seven-membered bridge structure did. Previous modeling studies suggested that the appropriate bridged structure between the 2'- and 4'-positions causes a steric challenge to nuclease binding, and a steric clash with the metal ion in the active site of the nuclease and consequently this provides high nuclease resistance.¹⁶ We suppose that the sulfonamide bridge can emphasize the steric clash with the nuclease surface and the metal ion and lead to high enzymatic stability.

For the practical application of antisense methodology, the degradation of the complementary RNA through the RNase H mechanism is very important.^{17,18} Hence, the SuNA-modified gapter 25, which is 16-mer length having 7-mer central DNA gap and fully modified with the phosphorothioate linkages, was synthesized (Table S1, Supporting Information), and the degradation of complementary RNA in the 25/RNA heteroduplex was examined in the presence of RNase H (Figure 4).^{11g} Under the conditions used in this experiment,

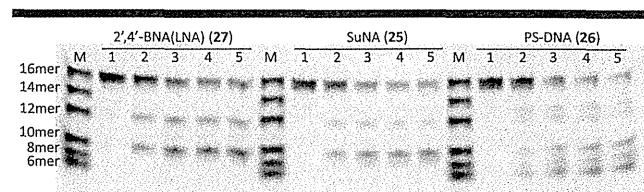


Figure 4. *E. coli* RNase H activity analysis of 5'-Cy3-labeled RNA forming duplexes with phosphorothioated DNA (PS-DNA) 26, the 2',4'-BNA(LNA)-modified gapter 27, and the SuNA-modified gapter 25 using 25% denaturing PAGE containing 7 M urea. Lanes 1–5 represent digestion time at 0, 5, 15, 30, and 60 min, respectively. Conditions of cleavage reaction: 5'-Cy3-labeled RNA (0.5 μM) and 25-27 (10 μM) in reaction buffer containing 40 mM Tris-HCl (pH 7.2), 150 mM NaCl, 4 mM MgCl_2 , and 1 mM DTT at 37 $^{\circ}\text{C}$; 0.01 U/ μL of RNase H. M: Marker.

degradation of RNA in the 25/RNA heteroduplex was observed. A similar degradation was shown in the phosphorothioated DNA (PS-DNA) 26/RNA and the 2',4'-BNA(LNA)-modified gapter 27/RNA heteroduplex.

In conclusion, a novel bridged nucleic acid monomer 11, 2'-N,4'-C-(N-methylamino)sulfonylmethylene-bridged thymidine (SuNA-T), has been designed and successfully synthesized. This is the first example of a nucleic acid analogue with a sulfonamide-type bridged structure between the 2'- and 4'-positions. The SuNA-modified oligonucleotides produced

stable duplexes with ssRNA and unstable duplexes with ssDNA. In addition, oligonucleotides containing this analogue have increased stability against nuclease degradation, similar to a seven-membered bridge structure, maintaining high affinity with ssRNA. These investigations reveal that decreasing the ring size, which means increasing the ν_{\max} value, increases binding affinity to ssRNA, and that a bulkier bridge structure produces greater nuclease resistance. These results suggest that the SuNA modification provides valuable information that can be applied to antisense technology. In addition, the SuNA-modified gapmer exhibited the degradation of complementary RNA through the RNase H mechanism, and further biological studies are in progress.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details, representative UV melting data, ^1H , ^{13}C , and ^{31}P spectra of all new compounds, and HPLC charts and MALDI-TOF-MS spectra of new oligonucleotides. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: obika@phs.osaka-u.ac.jp.

Notes

The authors declare no competing financial interest.

■ REFERENCES

- Lee, R. G.; Crosby, J.; Baker, B. F.; Graham, M. J.; Crooke, R. M. *J. Cardiovasc. Transl. Res.* **2013**, *6*, 969.
- Reviews: (a) Kaur, H.; Babu, B. R.; Maiti, S. *Chem. Rev.* **2007**, *107*, 4672. (b) Obika, S.; Rahman, S. M. A.; Fujisaka, A.; Kawada, Y.; Baba, T.; Imanishi, T. *Heterocycles* **2010**, *81*, 1347.
- (a) Obika, S.; Nanbu, D.; Hari, Y.; Morio, K.; In, Y.; Ishida, T.; Imanishi, T. *Tetrahedron Lett.* **1997**, *38*, 8735. (b) Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.; Morio, K.; Doi, T.; Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401.
- (a) Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455. (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.
- (a) Morita, K.; Hasegawa, C.; Kaneko, M.; Tsutsumi, S.; Sone, J.; Ishikawa, T.; Imanishi, T.; Koizumi, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 73. (b) Morita, K.; Takagi, M.; Hasegawa, C.; Kaneko, M.; Tsutsumi, S.; Sone, J.; Ishikawa, T.; Imanishi, T.; Koizumi, M. *Bioorg. Med. Chem.* **2003**, *11*, 2211.
- (a) Miyashita, K.; Rahman, S. M. A.; Seki, S.; Obika, S.; Imanishi, T. *Chem. Commun.* **2007**, 3765. (b) Rahman, S. M. A.; Seki, S.; Obika, S.; Yoshikawa, H.; Miyashita, K.; Imanishi, T. *J. Am. Chem. Soc.* **2008**, *130*, 4886.
- (a) Hari, Y.; Obika, S.; Ohnishi, R.; Eguchi, K.; Osaki, T.; Ohishi, H.; Imanishi, T. *Bioorg. Med. Chem.* **2006**, *14*, 1029. (b) Mitsuoka, Y.; Kodama, T.; Ohnishi, R.; Hari, Y.; Imanishi, T.; Obika, S. *Nucleic Acids Res.* **2009**, *37*, 1225.
- Yahara, A.; Shrestha, A. R.; Yamamoto, T.; Hari, Y.; Osawa, T.; Yamaguchi, M.; Nishida, M.; Kodama, T.; Obika, S. *ChemBioChem* **2012**, *13*, 2513.
- Hari, Y.; Osawa, T.; Kotobuki, Y.; Shrestha, A. R.; Yahara, A.; Obika, S. *Bioorg. Med. Chem.* **2013**, *21*, 4405.
- Nishida, M.; Baba, T.; Kodama, T.; Yahara, A.; Imanishi, T.; Obika, S. *Chem. Commun.* **2010**, 46, 5283.
- For other nucleic acids bridged between the 2'- and 4'-positions, see: (a) Kumar, R.; Singh, S. K.; Koshkin, A. A.; Rajwanshi, V. K.; Meldgaard, M.; Wengel, J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2219.
- (b) Singh, S. K.; Kumar, R.; Wengel, J. *J. Org. Chem.* **1998**, *63*, 10035.
- (c) Varghese, O. P.; Barman, J.; Pathmasiri, W.; Plashkevych, O.; Honcharenko, D.; Chattopadhyaya, J. *J. Am. Chem. Soc.* **2006**, *128*, 15173. (d) Albæk, N.; Petersen, M.; Nielsen, P. *J. Org. Chem.* **2006**, *71*, 7731. (e) Srivastava, P.; Barman, J.; Pathmasiri, W.; Plashkevych, O.; Wenska, M.; Chattopadhyaya, J. *J. Am. Chem. Soc.* **2007**, *129*, 8362. (f) Seth, P. P.; Siwkowski, A.; Allerson, C. R.; Vasquez, G.; Lee, S.; Prakash, T. P.; Wancewicz, E. V.; Wittchell, D.; Swayze, E. E. *J. Med. Chem.* **2009**, *52*, 10. (g) Zhou, C.; Liu, Y.; Andaloussi, M.; Badgujar, N.; Plashkevych, O.; Chattopadhyaya, J. *J. Org. Chem.* **2009**, *74*, 118. (h) Seth, P. P.; Vasquez, G.; Allerson, C. A.; Berdeja, A.; Gaus, H.; Kinberger, G. A.; Prakash, T. P.; Migawa, M. T.; Bhat, B.; Swayze, E. E. *J. Org. Chem.* **2010**, *75*, 1569. (i) Seth, P. P.; Allerson, C. R.; Berdeja, A.; Siwkowski, A.; Pallan, P. S.; Gaus, H.; Prakash, T. P.; Watt, A. T.; Egli, M.; Swayze, E. E. *J. Am. Chem. Soc.* **2010**, *132*, 14942. (j) Shrestha, A. R.; Hari, Y.; Yahara, A.; Osawa, T.; Obika, S. *J. Org. Chem.* **2011**, *76*, 9891. (k) Hari, Y.; Morikawa, T.; Osawa, T.; Obika, S. *Org. Lett.* **2013**, *15*, 3702. (l) Morihira, K.; Kodama, T.; Kentefu; Moai, Y.; Veedu, R. N.; Obika, S. *Angew. Chem., Int. Ed.* **2013**, *52*, 5074.
- Reviews: (a) Supuran, C. T.; Casini, A.; Scozzafava, A. *Med. Res. Rev.* **2003**, *23*, 535. (b) Kamal, A.; Faazil, S.; Malik, M. S. *Expert Opin. Ther. Patents* **2014**, *24*, 339. (c) Carta, F.; Supuran, C. T.; Scozzafava, A. *Future Med. Chem.* **2014**, *6*, 1149.
- Waga, T.; Nishizaki, T.; Miyakawa, I.; Ohru, H.; Meguro, H. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 1433.
- Nishiguchi, A.; Maeda, K.; Miki, S. *Synthesis* **2006**, 4131.
- CCDC 1018300 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.
- Pallan, P. S.; Allerson, C. R.; Berdeja, A.; Seth, P. P.; Swayze, E. E.; Prakash, T. P.; Egli, M. *Chem. Commun.* **2012**, 48, 8195.
- Lima, W.; Wu, H.; Crooke, S. T. In *Antisense Drug Technology: Principles, Strategies and Applications*, 2nd ed.; Crooke, S. T., Ed.; CRC Press: Boca Raton, 2007; pp 47–74.
- Bennett, C. F. In *Antisense Drug Technology: Principles, Strategies and Applications*, 2nd ed.; Crooke, S. T., Ed.; CRC Press: Boca Raton, 2007; pp 273–304.

Communication

C5-Azobenzene-substituted 2'-Deoxyuridine-containing Oligodeoxynucleotides for Photo-Switching Hybridization

Shohei Mori ¹, Kunihiko Morihira ^{1,2,*} and Satoshi Obika ^{1,2,*}

¹ Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan; E-Mail: mori-s@phs.osaka-u.ac.jp

² National Institute of Biomedical Innovation (NIBIO), 7-6-8 Saito-Asagi, Osaka 567-0085, Japan

* Authors to whom correspondence should be addressed; E-Mails: morihira@phs.osaka-u.ac.jp (K.M.); obika@phs.osaka-u.ac.jp (S.O.); Tel.: +81-6-6879-8202 (K.M.); +81-6-6879-8200 (S.O.); Fax: +81-6-6879-8204 (K.M & S.O.).

Received: 3 March 2014; in revised form: 15 April 2014 / Accepted: 17 April 2014 /

Published: 22 April 2014

Abstract: A new photoisomeric nucleoside dU^{Az} bearing an azobenzene group at the C5-position of 2'-deoxyuridine was designed and synthesized. Photoisomerization of dU^{Az} in oligodeoxynucleotides can be achieved rapidly and selectively with 365 nm (forward) and 450 nm (backward) irradiation. Thermal denaturation experiments revealed that dU^{Az} stabilized the duplex in the *cis*-form and destabilized it in the *trans*-form with mismatch discrimination ability comparable to thymidine. These results indicate that dU^{Az} could be a powerful material for reversibly manipulating nucleic acid hybridization with spatiotemporal control.

Keywords: azobenzene; molecular switch; nucleoside; oligonucleotide; photochromism

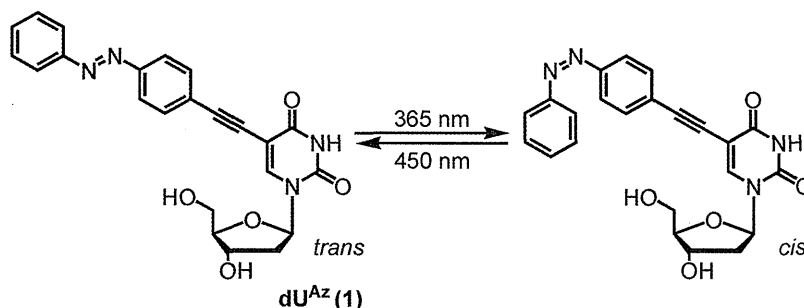
1. Introduction

Regulation of nucleic acid hybridization by some external stimuli is a rewarding challenge due to its potential to control gene expression flow from DNA to protein at a predetermined place and time. This technique could allow for spatiotemporal controllable pharmacotherapy based on nucleic acid agents. The regulation of nucleic acid hybridization is also important in the field of nanotechnology, such as in the construction of DNA-origami [1–3]. Modified oligonucleotides (ONs) that can reversibly alter the hybridization ability by noninvasive external stimuli are therefore necessary. The most promising

external stimulus is light, due to the possibility of accurately controlling the location, dosage and time of the irradiation. For example, Asanuma *et al.* have reported reversible photoregulation of DNA duplex formation via installation of azobenzene moieties on ONs [4,5]. Azobenzene and its derivatives are commonly adopted due to their rapid photoisomerization and drastic changes in geometry and dipole moment [6,7].

In this study, we describe a new type of azobenzene-modified nucleoside that reversibly changes its properties upon photoisomerization by ultraviolet (365 nm) or visible light (450 nm). There are several positions to attach a photochromic moiety to a nucleoside, and we have selected the C5 position of 2'-deoxyuridine (dU^{Az} , Figure 1) [8]. It is predicted that the azobenzene moiety of dU^{Az} is projected into the major groove of the double helix via a rigid ethynyl linker. We assumed that the duplexes containing *trans*- dU^{Az} would be destabilized because the hydrophobic azobenzene moiety extends to the outside of the groove [9] which surrounded by a highly polar aqueous phase, and interferes with hydration and the formation of interstrand cation bridges to stabilize the duplexes [10,11]. Meanwhile, *cis*- dU^{Az} -modification would not affect the duplex stability due to compact conformation of the azobenzene moiety. In other words, the affinity of ONs containing dU^{Az} for complementary single-stranded DNA or RNA may be reversibly changed, triggered by light.

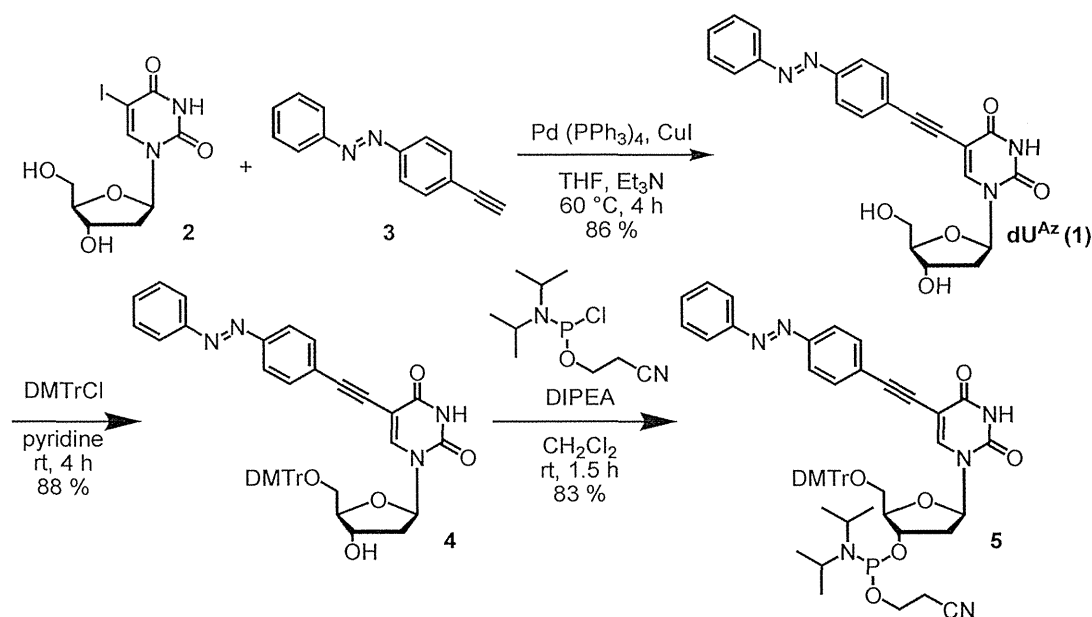
Figure 1. Photoisomeric nucleoside used in this study.



2. Results and Discussion

2.1. Synthesis of dU^{Az} Phosphoramidite and dU^{Az} -Modified Oligodeoxynucleotides

The synthetic route of dU^{Az} phosphoramidite is outlined in Scheme 1. dU^{Az} nucleoside **1** was synthesized from the corresponding 2'-deoxy-5-iodouridine (**2**) through a palladium-catalyzed cross-coupling reaction [12] with 4-ethynylazobenzene **3** [13]. Tritylation at the primary hydroxyl group of **1** with DMTrCl and phosphitylation at the secondary hydroxyl group yielded phosphoramidite **5**. The amidite **5** was incorporated into the oligodeoxynucleotide using conventional solid-phase phosphoramidite synthesis and purified by reverse-phase HPLC (29% yield). The ON sequences used in this study are shown in Table 1.

Scheme 1. Route for the synthesis of **dU^{Az}** phosphoramidite.**Table 1.** The oligonucleotides used in this study.

| ON | Sequence | |
|----|--------------------------------------|--------------------------------------|
| 6 | 5'-d(GCGTTTTTTGCT)-3' | control DNA |
| 7 | 5'-d(GCGTTU ^{Az} TTTGCT)-3' | dU^{Az} -modified DNA |
| 8 | 5'-d(AGCAAAAACGC)-3' | full match DNA |
| 9 | 5'-d(AGCAA <u>A</u> AACGC)-3' | mismatch DNA (T) |
| 10 | 5'-d(AGCAA <u>A</u> CAACGC)-3' | mismatch DNA (C) |
| 11 | 5'-d(AGCAA <u>A</u> GAACGC)-3' | mismatch DNA (G) |
| 12 | 5'-r(AGCAAAAACGC)-3' | full match RNA |
| 13 | 5'-r(AGCAA <u>A</u> AACGC)-3' | mismatch RNA (U) |
| 14 | 5'-r(AGCAA <u>A</u> CAACGC)-3' | mismatch RNA (C) |
| 15 | 5'-r(AGCAA <u>A</u> GAACGC)-3' | mismatch RNA (G) |

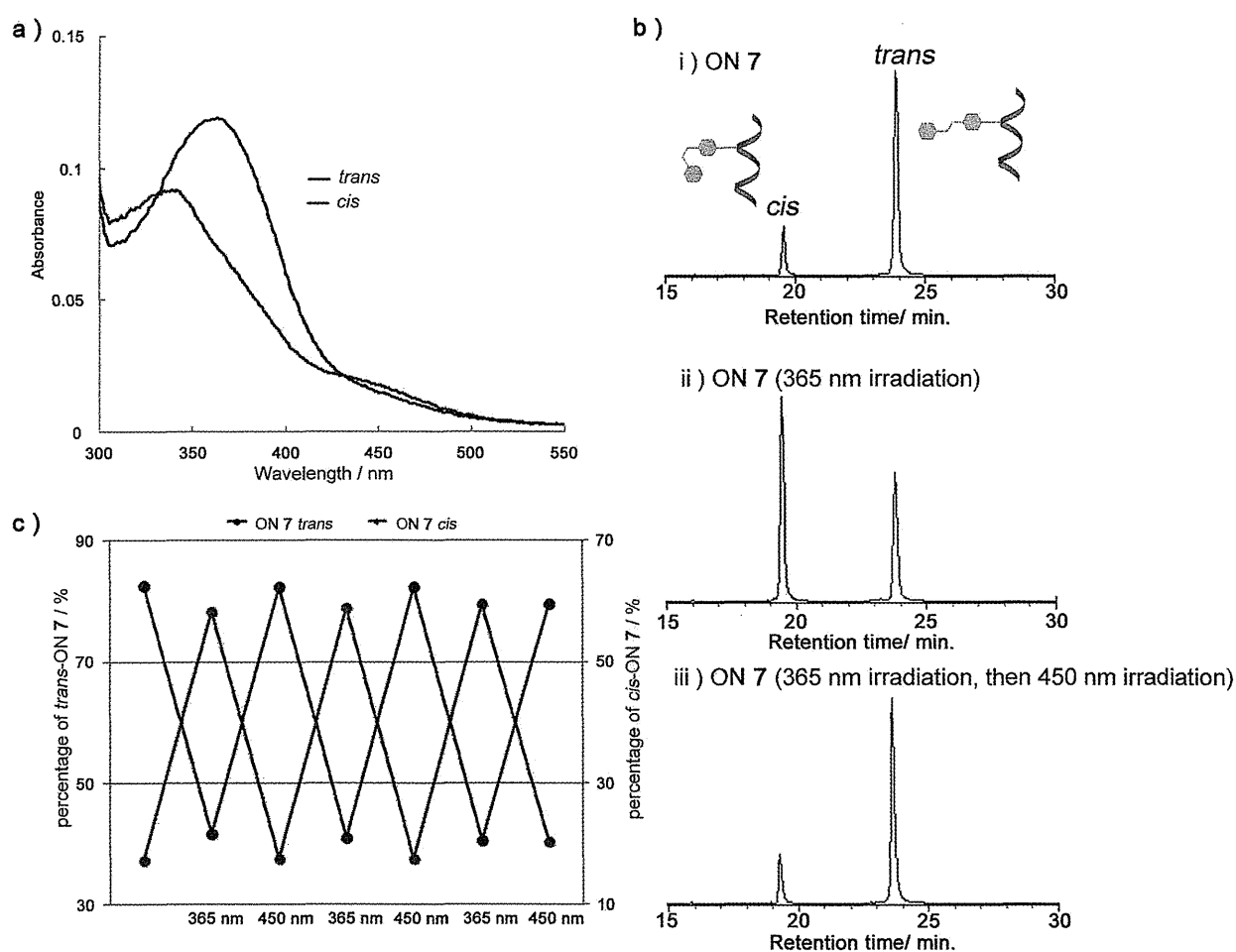
2.2. Photoisomerization Property of **dU^{Az}**

We initially investigated the efficiency of the **dU^{Az}** *cis-trans* photoisomerization property in ON by UV spectra and HPLC analysis. UV spectra of *trans/cis* ON **7**, showed that photoisomerization of *trans-dU^{Az}* to *cis-dU^{Az}* decreased absorbance at 365 nm and increased absorbance at 310 nm and 450 nm (Figure 2a). The λ_{max} of *cis*-form (340 nm) was blue-shifted compared to that of the *trans*-form (365 nm), as was the case with previous reports [6,7,14]. The *trans*-form **dU^{Az}** was photoisomerized to the *cis*-form by a 10-second irradiation of 365 nm monochromic light with 60% conversion, as determined by the HPLC peak areas (Figure 2b). In addition, subsequent 10-second irradiation of 450 nm yielded the *trans* form isomer with 80%. The HPLC analysis showed no side products from the reactions.

Even when the photoirradiation was repeated three times, the efficiency of the **dU^{Az}** *cis-trans* photoisomerization was not attenuated (Figure 2c). It can therefore be concluded that **dU^{Az}** has a rapid

and highly efficient *cis-trans* photoisomerization property and the potential to work as a photo-switch for various biomolecules.

Figure 2. Photoisomerization properties of dU^{Az} in oligodeoxynucleotide. (a) Absorbance spectra of *trans*- (black line) and *cis*- (red line) ON 7. (b) HPLC analysis of the photoisomerization of ON 7; (i) Before irradiation; (ii) after 365 nm irradiation for 10 s; (iii) subsequent irradiation at 450 nm, 10 s. (c) Repetitive photoisomerization of ON 7 induced by alternative light irradiation at 365 nm and 450 nm. The percentages of *trans*- (black line) and *cis*- (red line) ON 7 obtained from the HPLC peak areas are shown. Conditions: ON 7 (4.0 μM), NaCl (100mM) in sodium phosphate buffer (10 mM, pH 7.0) was irradiated at room temperature.



We investigated the differences in the thermal stability of 12-bp duplexes containing dU^{Az} in the *trans*- and *cis*-forms by monitoring the melting temperature (T_m) following the way of azobenzene-modified nucleoside containing ONs (Table 2) [15,16]. DNA duplex 7/8 showed a modest T_m difference (ΔT_m) between the *trans*- and *cis*-forms, namely, the T_m value of the *cis*-form was 2 $^{\circ}\text{C}$ higher than that of the *trans*-form. On the other hand, the ON 7/RNA 12 duplex showed a larger T_m difference. The T_m value of the *cis*-form was 5 $^{\circ}\text{C}$ higher than that of the *trans*-form. It is noteworthy that the *cis*-ON 7/RNA 12 duplex showed a T_m value comparable to that of natural DNA 6/RNA 12

duplex. According to past studies, the *cis*-form photochromic moieties generically destabilize the duplex because of its interference with the vicinity bases stacking interaction [4,5,17–19]. In this study, ON containing **dU^{Az}** showed a higher hybridization ability when **dU^{Az}** is *cis*-form rather than *trans*-form, unlike ONs containing the exiting photochromic nucleoside. Brown *et al.* have reported that hydrophobic buta-1,3-diynyl anthracene in ON leads to significant destabilization of the duplex, probably because the aromatic moiety is exposed to the aqueous environment [9]. The azobenzene moiety of *trans*-**dU^{Az}** also would extend to the outside of the major groove, a highly polar aqueous phase. This may have an impact on the groove hydration and the formation of interstrand cation bridges, and lead to destabilization of the duplex containing *trans*-**dU^{Az}**.

Table 2. UV-melting points of 12-bp duplexes. ^a

| Duplex | T_m [°C] | | ΔT_m [°C] ^b ($T_{m\ cis} - T_{m\ trans}$) |
|--------|---------------------------|-------------------------|---|
| | <i>trans</i> ^c | <i>cis</i> ^d | |
| 6/8 | | 52 | - |
| 7/8 | 47 | 49 | 2 |
| 6/12 | | 47 | |
| 7/12 | 42 | 47 | 5 |

^a All T_m values for the duplexes (4.0 μ M) were determined in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl. The T_m values given are the average of at least three data points; ^b The change in the T_m value induced by the *cis-trans* photoisomerization; ^c The percentage of *trans* isomer was *ca.* 80%; ^d The percentage of *cis* isomer was *ca.* 60%.

Finally, we investigated the mismatch discrimination ability of ON containing **dU^{Az}**. The T_m values of mismatched DNA duplexes containing **dU^{Az}** were found to be 14 or 15 °C lower than that of ON7/DNA8 in both *trans*- and *cis*-form (Table 3). Toward complementary ssRNA, ON containing **dU^{Az}** could also discriminate mismatched bases comparable to ON7 (Table S1 in Supplementary Material). These results indicate that the mismatch discrimination ability of ON containing *trans/cis*-**dU^{Az}** is not spoiled by the C5-substituted-azobenzene moiety of **dU^{Az}**.

Table 3. UV-melting points of DNA duplexes with a mismatched base pair. ^a

| Duplex | Base pair | T_m [°C] | | ΔT_m [°C] ^b | |
|--------|--------------------------|---------------------------|-------------------------|--------------------------------|-------------------------|
| | | <i>trans</i> ^c | <i>cis</i> ^d | <i>trans</i> ^c | <i>cis</i> ^d |
| 6/9 | T:T | | 40 | | -12 |
| 6/10 | T:C | | 37 | | -15 |
| 6/11 | T:G | | 41 | | -11 |
| 7/9 | U^{Az} :T | 33 | 35 | -14 | -14 |
| 7/10 | U^{Az} :C | 33 | 34 | -14 | -15 |
| 7/11 | U^{Az} :G | 33 | 35 | -14 | -14 |

^a All T_m values for the duplexes (4.0 μ M) were determined in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl. The T_m values given are the average of at least three data points; ^b ΔT_m values are calculated relative to the T_m values of matched DNA 6/DNA 8 (52 °C) or ON 7/DNA 8 (47 °C for *trans* and 49 °C for *cis*) duplexes.; ^c The percentage of *trans* isomer was *ca.* 80%; ^d The percentage of *cis* isomer was *ca.* 60%.

We achieved synthesis of the photoisomeric nucleoside, dU^{Az} , for which the hybridization can be controlled by using different wavelengths of light. The ΔT_m value between the *trans*- and *cis*-form is more remarkable in the DNA/RNA duplex than the DNA duplex. Although dU^{Az} photoisomerization induced modest T_m differences, the modification of ONs with multiple dU^{Az} units or the introduction of substituents to the azobenzene moiety [20] could enhance the ΔT_m value between the *trans*- and *cis*-forms. Our strategy indicated the possibility of photo-switches based on dU^{Az} -modified ONs for the development of unique molecular machines and the control of various biological phenomena.

3. Experimental

3.1. General

Reagents and solvents were purchased from commercial suppliers and were used without purification unless otherwise specified. All experiments involving air and/or moisture-sensitive compounds were carried out under N_2 or Ar atmosphere. All reactions were monitored with analytical TLC (Merck Kieselgel 60 F254). Column chromatography was carried out with a Fuji Silysia FL-100D. Physical data were measured as follows: NMR spectra were recorded on a JEOL JNM-ECS-500 spectrometer in CDCl_3 or $\text{DMSO}-d_6$ as the solvent with tetramethylsilane as an internal standard. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. Optical rotations were recorded on a JASCO P-2200 instrument. FAB mass spectra were measured on a JEOL JMS-700 mass spectrometer.

3.2. Preparation of 5-(4-Phenyldiazenylphenyl)ethynyl-2'-deoxyuridine (1)

Under an argon atmosphere, 4-ethynylazobenzene (**3** [13], 1.06 g, 5.12 mmol), $\text{Pd}(\text{PPh}_3)_4$ (592 mg, 0.512 mmol), and CuI (113 mg, 0.512 mmol) was dissolved in dry DMF (50 mL). Then, Et_3N (3.6 mL) and 2'-deoxy-5-iodouridine (**2**, 1.81 g, 5.12 mmol) were added. The reaction mixture was stirred at 60 °C for 4 h. The resultant mixture was filtered over Celite. The filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography and eluted with $\text{CHCl}_3/\text{MeOH}$ (20:1), to give compound **1** (1.80 g, 81%) as a light-orange powder: M.p. 208–210 °C; IR (KBr): ν 3439 (NH, OH), 1617 (C=O), 1289 (N=N) cm^{-1} ; $[\alpha]_D^{24}$ –3.7 (c 1.00, DMSO); $^1\text{H-NMR}$ (500 MHz, $\text{DMSO}-d_6$): δ 11.7 (1H, brs, NH), 8.47 (1H, s, H-6), 7.94–7.90 (4H, m), 7.69–7.57 (5H, m), 6.14 (1H, t, $J = 6.5$ Hz, H-1'), 5.27 (1H, d, $J = 4.0$ Hz, H-3'), 5.20 (1H, t, $J = 5.0$ Hz, C-H4'), 4.30–4.26 (1H, m, OH), 3.82 (1H, m, OH), 3.71–3.58 (2H, m, H-5'), 2.21–2.17 (2H, m, H-2'); $^{13}\text{C-NMR}$ (125 MHz, $\text{DMSO}-d_6$): δ 161.3, 151.9, 151.0, 149.4, 132.2, 131.8, 129.5, 125.4, 122.9, 122.6, 97.8, 91.5, 87.6, 85.6, 84.9, 69.8, 60.8, 40.2; FAB-LRMS $m/z = 433$ (MH^+); FAB-HRMS calcd for $\text{C}_{23}\text{H}_{21}\text{N}_4\text{O}_5$ 433.1506, found 433.1524.

3.3. Preparation of 5'-O-(4,4'-Dimethoxytrityl)-5-(4-phenyldiazenylphenyl)ethynyl-2'-deoxyuridine (4)

To a solution of compound **1** (141 mg, 0.324 mmol) in dry pyridine (3 mL) was added DMTrCl (131 mg, 0.389 mmol) at room temperature, and the reaction mixture was stirred for 4 h. The reaction was quenched by the addition of MeOH with 10 min stirring. The solvent was removed *in vacuo*, and the residue was partitioned between CHCl_3 and H_2O . The separated organic layer was washed with H_2O , followed by brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography and eluted with $\text{CHCl}_3/\text{MeOH}$ (20:1 with 0.5%

Et₃N) to give Compound **4** (239 mg, 88%) as an orange foam: IR (KBr): ν 3437, 3410(NH, OH), 1701 (C=O), 1272 (N=N) cm⁻¹; [α]_D²⁴ 36.2 (c 1.00, CHCl₃); ¹H-NMR (500 MHz, CDCl₃): δ 8.51 (1H, brs, NH), 8.29 (1H, s, H-6), 7.90 (2H, d, J = 7.5 Hz), 7.70 (2H, d, J = 8.5 Hz), 7.52–7.45 (5H, m), 7.37–7.28 (6H, m), 7.16 (1H, dd, J = 6.5 and 1.0 Hz), 7.10 (2H, d, J = 8.0 Hz), 6.82–6.79 (4H, m), 6.38 (1H, dd, J = 7.5, 6.5 Hz, H-1'), 4.60–4.59 (1H, m, H-3'), 4.14–4.13 (1H, m, H-4'), 3.70 (3H, s, OMe), 3.69 (3H, s, OMe), 3.50 (1H, dd, J = 8.0 and 3.0 Hz, H-5'), 3.34 (1H, dd, J = 8.0 and 3.0 Hz, H-5'), 2.57–2.53 (1H, m, H-2'), 2.40–2.34 (1H, m, H-2'), 2.09 (1H, brs, OH); ¹³C-NMR (125 MHz, CDCl₃): δ 158.6, 152.6, 151.7, 148.8, 144.3, 135.4, 132.4, 131.3, 129.9, 129.1, 128.1, 127.9, 127.1, 125.1, 122.9, 122.5, 113.4, 100.4, 93.6, 87.2, 86.7, 85.9, 82.2, 72.4, 63.3, 55.2, 41.7; FAB-LRMS m/z = 757 (MNa⁺); FAB-HRMS calcd for C₄₄H₃₈N₄O₇Na 757.2633, found 757.2633.

3.4. Preparation of 3-O-{2-Cyanoethyl(diisopropylamino)phosphino}-5'-O-(4,4'-Dimethoxytrityl)-5-(4-phenyldiazenylphenyl)ethynyl-2'-deoxyuridine (**5**)

To a solution of compound **4** (188 mg, 0.26 mmol) in dry MeCN (5 mL) was added *N,N*-diisopropylamine (0.13 mL, 0.76 mmol) and 2-cyanoethyl-*N,N'*-diisopropylchlorophosphoramidite (0.09 mL, 0.40 mmol) at room temperature, and the reaction mixture was stirred for 1.5 h. The resultant mixture was partitioned between AcOEt and H₂O. The separated organic layer was washed with saturated aqueous NaHCO₃, followed by brine. The organic layer was dried (Na₂SO₄) and concentrated in vacuo. The residue was purified by silica gel column chromatography and eluted with CHCl₃/MeOH (20:1 with 0.5% Et₃N), to give a 17:3 diastereomeric mixture of **5** (324 mg, 82%) as an orange foam: IR (KBr): ν 3610 (NH), 1699 (C=O), 1272 (N=N) cm⁻¹; [α]_D²⁴ 32.5 (c 1.00, CHCl₃); ¹H-NMR (500 MHz, CDCl₃): δ 9.08 (1H, brs, NH), 8.35 (0.85H, s, H-6), 8.30 (0.15H, s, H-6), 7.89 (2H, d, J = 7.5 Hz), 7.67 (2H, d, J = 8.5 Hz), 7.55–7.04 (14H, m), , 6.67–6.75 (4H, m), , 6.35 (1H, dd, J = 7.5, 6.0 Hz, H-1'), 4.68–4.61 (1H, m, H-3'), 4.26 (1H, m, H-4'), 3.70 (3H, s, OMe), 3.69 (3H, s, OMe), 3.67–3.53 (5H, m, CH₂CH₂CN, H-5'), 3.31 (1H, dd, J = 8.5, 2.5 Hz, H-5'), 2.65–2.56 (1H, m, H-2'), 2.47–2.36 (3H, m, H-2', ((CH₃)₂CH)₂N), 1.18 (12H, d, J = 6.5 Hz, ((CH₃)₂CH)₂N); ¹³C-NMR (125 MHz, CDCl₃): δ 161.2, 158.5(9), 158.5(6), 152.6, 151.5, 149.1, 144.35, 142.5, 135.4, 132.3, 132.0, 131.1, 130.0 (d, J (C, P) = 6.0 Hz), 129.1, 128.7, 128.0, 127.9, 127.0, 125.1, 122.8, 122.4, 120.5, 117.3, 113.3, 100.3, 93.4, 86.3 (d, J (C, P) = 3.5 Hz), 85.9, 82.4, 77.3, 77.0, 76.8, 73.4, 73.2, 63.0, 58.2, 58.1, 55.1, 43.2 (d, J (C, P) = 13.0 Hz), 40.8 (d, J (C, P) = 5.0 Hz), 25.6, 24.5(9), 24.5(3), 24.4(8), 20.2 (d, J (C, P) = 7.0 Hz); ³¹P-NMR (200 MHz, CDCl₃): δ 149.09, 148.66; FAB-LRMS m/z = 957 (MNa⁺); FAB-HRMS calcd for C₅₃H₅₅N₆O₈PNa 957.3711, found 957.3711.

3.5. Synthesis of dU^{Az}-Modified Oligodeoxynucleotides

Solid-phase oligonucleotide synthesis was performed on an nS-8 Oligonucleotides Synthesizer (GeneDesign, Inc., Osaka, Japan) using commercially available reagents and phosphoramidites with 5-(bis-3, 5-trifluoromethylphenyl)-1*H*-tetrazole (0.25 M concentration in acetonitrile) as the activator. dU^{Az} phosphoramidite was chemically synthesized as described above. All of the reagents were assembled, and the oligonucleotides were synthesized according to the standard synthesis cycle (trityl on mode). Cleavage from the solid support and deprotection were accomplished with concentrated ammonium hydroxide solution at 55 °C for 12 h. The crude oligonucleotides were purified with

Sep-Pak Plus C18 cartridges (Waters) followed by RP-HPLC on a XBridge™ OST C18 Column, 2.5 μm , 10 \times 50 mm (Waters) using MeCN in 0.1 M triethylammonium acetate buffer (pH 7.0). The purified oligonucleotides were quantified by UV absorbance at 260 nm and confirmed by MALDI-TOF mass spectrometry (Table 4).

Table 4. Yields and MALDI-TOF MS data of dU^{Az} -modified oligonucleotide.

| Oligodeoxynucleotide | Yield | MALDI-TOF MS | |
|--------------------------------------|-------|-------------------------|------------------------|
| | | Calcd. $[\text{M-H}]^-$ | found $[\text{M-H}]^-$ |
| 5'-d(GCGTTU ^{Az} TTTGCT)-3' | 7 | 29% | |
| | | 3822.6 | 3822.4 |

3.6. UV Melting Experiments

Melting temperatures (T_m) were determined by measuring the change in absorbance at 260 nm as a function of temperature using a Shimadzu UV-Vis Spectrophotometer UV-1650PC equipped with a T_m analysis accessory TMSPC-8. Equimolecular amounts of the target DNA/RNA and oligonucleotides were dissolved in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl to give a final strand concentration of 4.0 μM . The melting samples were denatured at 100 °C and annealed slowly to room temperature. Absorbance was recorded in the forward and reverse directions at temperatures of 5 to 90 °C at a rate of 0.5 °C/min.

3.7. Photoisomerization of dU^{Az}

The *trans*-to-*cis* isomerization was performed with a UV-LED lamp (ZUV-C30H; OMRON) and a ZUV-L10H lens unit (760 mW/cm²). The *cis*-to-*trans* isomerization was performed with a Xenon lamp (MAX-303; Asahi Spectra Co., Ltd., Tokyo, Japan) and XHQA420 optical filter. Absorbance spectra of *trans*-*cis* ON 7 were measured by a Shimadzu UV-Vis Spectrophotometer UV-1650PC. Conditions: ON 7 (4.0 μM), NaCl (100mM) in sodium phosphate buffer (10 mM, pH 7.0).

4. Conclusions

We have synthesized a new photoisomeric nucleoside, C5-azobenzene-modified 2'-deoxyuridine dU^{Az} using Sonogashira-type cross-coupling as a key step. dU^{Az} showed very rapid reversible *cis*-*trans* photoisomerization with monochromic light at the appropriate wavelength in oligodeoxynucleotide. dU^{Az} -modified oligodeoxynucleotide showed an interesting duplex-forming property, namely, the T_m values of both the dU^{Az} -modified ON/DNA and dU^{Az} -modified ON/RNA were higher for the *cis*-form than for the *trans*-form, unlike conventional azobenzene-modified ONs. Additionally, it was revealed that installation of dU^{Az} into oligodeoxynucleotide had little influence on the mismatch recognition ability.

Supplementary Materials

Supplementary materials can be accessed at: <http://www.mdpi.com/1420-3049/19/4/5109/s1>.

Acknowledgments

This work was supported by the Japan Society for the Promotion of Science (JSPS), the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and the Advanced Research for Medical Products Mining Programme of the National Institute of Biomedical Innovation (NIBIO).

Author Contributions

K.M. and S.O. designed the research. S.M. and K.M. performed the experiments and analyzed the data. S.M. was mainly responsible for writing the manuscript, with contributions from K.M. and S.O.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Kuzuya, A.; Komiyama, M. DNA origami: Fold, stick, and beyond. *Nanoscale* **2010**, *2*, 309–321.
2. Topping, T.; Voigt, N.V.; Nangreave, J.; Yan, H.; Gothelf, K.V. DNA origami: A quantum leap for self-assembly of complex structures. *Chem. Soc. Rev.* **2011**, *40*, 5636–5646.
3. Pinheiro, A.V.; Han, D.; Shin, W.M.; Yan, H. Challenges and opportunities for structural DNA nanotechnology. *Nat. Nanotechnol.* **2011**, *6*, 763–772.
4. Asanuma, H.; Ito, T.; Yoshida, T.; Liang, X.; Komiyama, M. Photoregulation of the formation and dissociation of a DNA duplex by using the *cis-trans* isomerization of azobenzene. *Angew. Chem. Int. Ed.* **1999**, *38*, 2393–2395.
5. Asanuma, H.; Liang, X.; Yoshida, T.; Komiyama, M. Photocontrol of DNA duplex formation by using azobenzene-bearing oligonucleotides. *ChemBioChem* **2001**, *2*, 39–44.
6. Beharry, A.A.; Woolley, G.A. Azobenzene photoswitches for biomolecules. *Chem. Soc. Rev.* **2011**, *40*, 4422–4437.
7. Dhammika, H.M.; Burdette, S.C. Photoisomerization in different classes of azobenzene. *Chem. Soc. Rev.* **2012**, *41*, 1809–1825.
8. Barrois, S.; Wagenknecht, H.A. Diarylethene-modified nucleotides for switching optical properties in DNA. *Beilstein. J. Org. Chem.* **2012**, *8*, 905–914.
9. Xiao, Q.; Ranasinghe, R.T.; Tang, A.M.P.; Brown, T. Naphthalenyl- and anthracenyl-ethynyl dT analogues as base discriminating fluorescent nucleosides and intramolecular energy transfer donors in oligonucleotide probes. *Tetrahedron* **2007**, *63*, 3483–3490.
10. Franklin, R.E.; Gosling, R.G. Molecular configuration in sodium thymonucleate. *Nature* **1953**, *171*, 740–741.
11. Anderson, C.F.; Record, M.T., Jr. Salt-nucleic acid interactions. *Annu. Rev. Phys. Chem.* **1995**, *46*, 657–700.
12. Sonogashira, K.; Tohda, Y.; Hagihara, N. A convenient synthesis of acetylenes: Catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes and bromopyridines. *Tetrahedron Lett.* **1975**, *16*, 4467–4470.

13. Shirai, Y.; Sasaki, T.; Guerrero, J.M.; Yu, B.; Hodge, P.; Tour, J.M. Synthesis and photoisomerization of Fullerene- and oligo(phenylene ethynylene)-azobenzene derivatives. *ACS Nano* **2008**, *2*, 97–106.
14. Matharu, A.S.; Jeeva, S.; Ramanujam, P.S. Liquid crystals for holographic optical data storage. *Chem. Soc. Rev.* **2007**, *36*, 1868–1880.
15. Liang, X.; Asanuma, H.; Komiyama, M. Photoregulation of DNA triplex formation by azobenzene. *J. Am. Chem. Soc.* **2002**, *124*, 1877–1883.
16. Nishioka, H.; Liang, X.; Asanuma, H. Effect of the *ortho* modification of azobenzene on the photoregulatory efficiency of DNA hybridization and the thermal stability of its *cis* form. *Chem. Eur. J.* **2010**, *16*, 2054–2062.
17. Asanuma, H.; Yoshida, T.; Ito, T.; Komiyama, M. Photo-responsive oligonucleotides carrying azobenzene at the 2'-position of uridine. *Tetrahedron Lett.* **1999**, *40*, 7995–7998.
18. Patnaik, S.; Kumar, P.; Garg, B.S.; Gandni, R.P.; Gupta, K.C. Photomodulation of PS-modified oligonucleotides containing azobenzene substituent at pre-selected positions in phosphate backbone. *Bioorg. Med. Chem.* **2007**, *15*, 7840–7849.
19. Ogasawara, S.; Maeda, M. Straightforward and reversible photoregulation of hybridization by using a photochromic nucleoside. *Angew. Chem. Int. Ed.* **2008**, *47*, 8839–8842.
20. Nishioka, H.; Liang, X.; Kashida, H.; Asanuma, H. 2',6'-Dimethylazobenzene as an efficient and thermo-stable photoregulator for the photoregulation of DNA hybridization. *Chem. Commun.* **2007**, 4354–4356.

Sample Availability: Samples of the compounds are not available from the authors.

© 2014 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).

Photoinduced changes in hydrogen bonding patterns of 8-thiopurine nucleobase analogues in a DNA strand†

Cite this: *Org. Biomol. Chem.*, 2014, **12**, 2468

Kunihiko Morihiko,^{a,b} Tetsuya Kodama,^c Shohei Mori^a and Satoshi Obika^{*a,b}

Hydrogen bonds (H-bonds) formed between nucleobases play an important role in the construction of various nucleic acid structures. The H-donor and H-acceptor pattern of a nucleobase is responsible for selective and correct base pair formation. Herein, we describe an 8-thioadenine nucleobase analogue and an 8-thiohypoxanthine nucleobase analogue with a photolabile 6-nitroveratryl (NV) group on the sulfur atom (**SA^{NV}** and **SH^{NV}**, respectively). Light-triggered removal of the NV group causes tautomerization and a change in the H-bonding pattern of **SA^{NV}** and **SH^{NV}**. This change in the H-bonding pattern has a strong effect on base recognition by 8-thiopurine nucleobase analogues. In particular, base recognition by **SH^{NV}** is clearly shifted from guanine to adenine upon photoirradiation. These results show that a photoinduced change in the H-bonding pattern is a unique strategy for manipulating nucleic acid assembly with spatiotemporal control.

Received 5th December 2013,
Accepted 13th February 2014

DOI: 10.1039/c3ob42427h

www.rsc.org/obc

Introduction

The complementarity of natural A–T and G–C base pairs in DNA is the principal mechanism for the preservation and flow of genetic information. The hydrogen-bonding (H-bonding) patterns of the four natural nucleobases play an important role in the selective and correct formation of base pairs. These H-bonding interactions can result in the formation of higher order complexes of nucleic acids, depending on the sequence. Therefore, the control of H-bonding interactions using external stimuli is important for regulating biological processes and for the possibility of developing unique DNA-based molecular machines. Various external stimuli have been used to this end; light is an ideal trigger because the timing, location, and intensity of the irradiation can be easily controlled. Among such strategies, nucleobase caging strategies involving the installation of a photolabile group are very important. Photolabile caging groups perturb the H-bonding capabilities of the

nucleobases. Photoirradiation reinstates the H-bonding capabilities and allows nucleobase interaction in the “OFF to ON” direction. Nucleobase-caged nucleosides can be widely used for the photoregulation of antisense oligodeoxynucleotides (ODNs),^{1,2} siRNAs,^{3,4} aptamers,⁵ ribozymes^{6,7} and deoxyribozymes,^{8,9} diagnostic ODNs,¹⁰ DNA architectures,¹¹ and DNA logic gates.^{12,13}

Recently, we reported the synthesis and properties of a unique light-responsive nucleobase analogue derived from 2-mercaptobenzimidazole (**SB^{NV}**) (Fig. 1a).¹⁴ **SB^{NV}** is modified with a photolabile 6-nitroveratryl (NV) group,¹⁵ and the nitrogen at the 3-position serves as an H-acceptor (A). **SB^{NV}** can selectively form a base pair with guanine even before photoirradiation, unlike conventional caged nucleobases. Light-triggered removal of the NV group causes tautomerization of the nucleobase, and changes the role of the 3-nitrogen atom from H-A to H-donor (D). Following this change in the H-bonding pattern, base recognition by **SB^{NV}** can be shifted from guanine to adenine. We also demonstrated that a light-triggered strand exchange reaction targeting different mRNA fragment sequences could be achieved using ODNs containing **SB^{NV}**. These results indicate that a photoinduced change in the H-bonding pattern of a nucleobase is a good strategy for manipulating nucleic acid assemblies in a spatially and temporally controlled manner. In this paper, to further investigate the effect of this change in H-bonding pattern of nucleobases on the base recognition ability, we designed new light-responsive nucleoside analogues bearing the NV group: 8-thioadenine and 8-thiohypoxanthine (**SA^{NV}** and **SH^{NV}**, respectively;

^aGraduate 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

^bNational Institute of Biomedical Innovation (NIBIO), 7-6-8 Saito-Asagi, Ibaraki, Osaka 567-0085, Japan

^cGraduate School of Pharmaceutical Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8601, Japan

† Electronic supplementary information (ESI) available: NMR spectra of new compounds, HPLC and MALDI-TOF MS analysis of modified ODNs, photoreaction of modified ODNs and UV melting curves for modified duplexes. See DOI: 10.1039/c3ob42427h