Chemosensors **2015**, *3*, 36-54; doi:10.3390/chemosensors3020036

OPEN ACCESS

chemosensors

ISSN 2227-9040

www.mdpi.com/journal/chemosensors

Article

Synthesis and Properties of 2'-Deoxyuridine Analogues Bearing Various Azobenzene Derivatives at the C5 Position

Shohei Mori [1], Kunihiko Morihiro [1,2,*], Yuuya Kasahara [1,2], Shin-ichi Tsunoda [2] , and Satoshi Obika [1,2,*]

[1] Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan; E-Mails: mori-s@phs.osaka-u.ac.jp (S.M.); y-kasahara@nibio.go.jp (Y.K.)

[2] National Institute of Biomedical Innovation (NIBIO), 7-6-8 Saito-Asagi, Osaka 567-0085, Japan; E-Mail: tsunoda@nibio.go.jp

***** Authors to whom correspondence should be addressed; E-Mails: k-morihiro@nibio.go.jp (K.M.); obika@phs.osaka-u.ac.jp (S.O.); Tel.: +81-72-641-9882 (K.M.); +81-6-6879-8200 (S.O.); Fax: +81-72-641-9884 (K.M.); +81-6-6879-8204 (S.O.).

Academic Editor: Patrick Hrdlicka

Received: 4 September 2014 / Accepted: 9 March 2015 / Published: 27 March 2015

Abstract: Nucleic acids that change their properties upon photo-irradiation could be powerful materials for molecular sensing with high spatiotemporal resolution. Recently, we reported a photo-isomeric nucleoside bearing azobenzene at the C5 position of 2'-deoxyuridine (**dU^{Az}**), whose hybridization ability could be reversibly controlled by the appropriate wavelength of light. In this paper, we synthesized and evaluated **dU^{Az}** analogues that have various *para*-substitutions on the azobenzene moiety. Spectroscopic measurements and HPLC analyses revealed that the *para*-substitutions of the azobenzene moiety strongly affect the photo-isomerization ability and thermal stability of the *cis*-form. The results suggest that proper substitution of the azobenzene moiety can improve the properties of **dU^{Az}** as a light-responsive nucleic acid probe.

Keywords: azobenzene; nucleoside; nucleic acid probe; oligonucleotide; photochromism

1. Introduction

Much attention has been focused on chemically modified nucleic acids that can alter their properties by some external stimuli [1–3]. Photo-responsive nucleic acids have been realized by regulation of hydrogen bonding [4] and stacking effects [5] between nucleobases. Light is considered to be a promising external stimulus due to the possibility of accurately controlling the location, dosage, and time of the irradiation. Various photochromic derivatives have been attached to nucleic acids to photo-control their properties [6]. For example, diarylethene-modified oligonucleotides (ONs) showed changes in their optical properties [7,8] and hybridization abilities [8] by photo-isomerization. However, the changes in hybridization abilities were moderate and the diarylethene modification itself destabilized the DNA duplex in both forms. To obtain a significant melting temperature difference (ΔT_m) upon photo-isomerization, ONs containing *cis-trans* type photochromic derivatives have been reported. Owing to their dynamic structural changes upon photo-irradiation, *cis-trans* type photochromic derivatives could greatly influenced the stability of nucleic acid duplexes. Although stilbene-type derivative-modified ONs have been utilized for photo-control of duplex formation, G-quadruplex formation, and gene expression, they require short wavelength photo-irradiation for *cis* to *trans* isomerization [9–11]. This would be disadvantageous for biomolecules due to photo-damaging reactions including the formation of pyrimidine dimers [12]. Azobenzene derivative-modified ONs are quite promising photochromic ONs because they can rapidly photo-isomerize by longer wavelengths of light [13,14]. Asanuma *et al.* have demonstrated that ONs modified with azobenzene moieties could be utilized as photo-responsive tweezers, RNA scission, and engines [15]. Recently, we reported that the ON containing C5-azobenzene-substituted 2'-deoxyuridine (**dU^{Az}**) could be photo-isomerized from *trans* to *cis* with an efficiency of 60% by UV light (365 nm) and from *cis* to *trans* with an efficiency of 80% by visible light (450 nm) [16]. The ON containing **dU^{Az}** showed interesting hybridization properties, namely, the T_m values of the duplexes formed between **dU^{Az}**-modified ON and complementary DNA or RNA were higher after UV irradiation than before irradiation. This may be because the hydrophobic azobenzene in the *trans* form extends to the outside of the groove and interferes with hydration and the formation of interstrand cation bridges to stabilize the duplexes. Meanwhile, *cis*-**dU^{Az}** did not affect the duplex stability due to the compact conformation of the azobenzene moiety. These results indicated that **dU^{Az}** could be a potential building block to control nucleic acid hybridization with high spatiotemporal resolution.

In this study, we describe the synthesis and properties of **dU^{Az}** analogues bearing various *para*-substituted azobenzene derivatives (Figure 1). The isomerization properties of photochromic compounds have been proposed to be strongly influenced by substituents [17]. Thus, the introduction of electron-donating or -withdrawing substituents into the azobenzene moiety could improve the properties of **dU^{Az}** and create unique photo-sensors for various biomolecules.

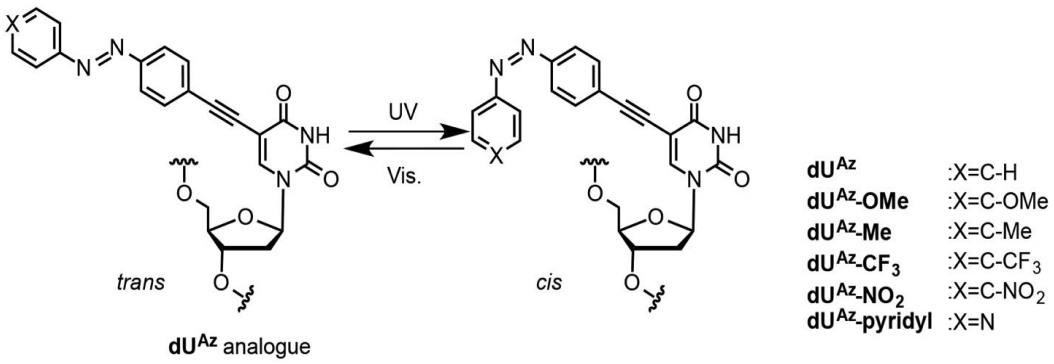


Figure 1. *Para*-substituted **dU^{Az}** analogues used in this study.

2. Experimental Section

2.1. 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; Merck, Darmstadt, Germany). Column chromatography was carried out using FL-100D silica gel (Fuji Silysia, Aichi, Japan). Physical data were measured as follows. NMR spectra were recorded on JNM-ECS-300, JNM-ECS-400, and JNM-ECS-500 spectrometers (JEOL, Tokyo, Japan) using $CDCl_3$ or DMSO-d_6 as solvents with tetramethylsilane as an internal standard. IR spectra were recorded on a FT/IR-4200 spectrophotometer (JASCO, Tokyo, Japan). Optical rotations were recorded on a JASCO P-2200 instrument. FAB mass spectra were measured on a JEOL JMS-700 mass spectrometer. Solid-phase ON synthesis was performed on an nS-8 Oligonucleotide Synthesizer (GeneDesign, Osaka, Japan). MALDI-TOF mass spectra were recorded on an ultrafleXtreme mass spectrometer (Bruker Daltonics, Billerica, MA, USA) for oligonucleotides and on a JMS-S3000 (JEOL, Tokyo, Japan) for small molecules. Photo-irradiation experiments were conducted with a Xenon lamp (MAX-303; Asahi Spectra, Tokyo, Japan) using HQBP 450-VIS ø 25 and 365-VIS ø 25 as the optical filters. UV/Vis absorption measurements and UV melting experiments were performed using a UV-1650PC UV-Vis spectrophotometer equipped with a TMSPC-8 T_m analysis accessory (Shimadzu, Kyoto, Japan). ITC experiments were performed using a Microcal iTC$_{200}$ (Malvern Instruments, Worcestershire, UK).

*2.2. Preparation of 4-[4-(trimethylsilyl)ethynylphenylazo]pyridine (**2**)*

Under an argon atmosphere, 4-(4-iodophenylazo)pyridine **1** [18] (950 mg, 3.07 mmol) was dissolved in dry THF (30 mL). Pd(PPh$_3$)$_4$ (358 mg, 0.310 mmol), CuI (59.0 mg,0.310 mmol), Et_3N (2.1 mL, 15.4 mmol), and trimethylsilylacetylene (1.1 mL, 7.68 mmol) were then added. The reaction mixture was stirred at 60 ℃ for 6 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 hexane/AcOEt (8:1) to give compound **2** (485 mg, 57%) as a red oil. IR (KBr): ν 2156 (C≡C), 1250 (N=N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.81 (2H, dd, J = 2.0, 4.5 Hz), 7.91 (2H, dd, J = 1.5, 6.5 Hz), 7.70 (2H, dd, J = 1.5, 4.5 Hz), 7.63 (2H, dd, J = 2.0, 6.5 Hz), 0.28 (9H, s); ^{13}C-NMR

(100 MHz, $CDCl_3$): δ 151.5, 151.4, 133.0, 132.5, 130.4, 129.3, 123.5, 123.4, 116.33, 116.31; FAB-HRMS *m/z* (MH^+) calcd for $C_{16}H_{18}N_3Si$: 280.1265; found 280.1272.

*2.3. Preparation of 4-(4-ethynylphenylazo)pyridine (**5**)*

Under an argon atmosphere, to a solution of compound **2** [19] (319 mg, 1.14 mmol) in dry THF (5 mL) were added K_2CO_3 (79 mg, 0.57 mmol) and MeOH (5 mL) and the reaction mixture was stirred for 2 h at room temperature. The resultant mixture was diluted with AcOEt (30 mL) and 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 hexane/AcOEt (3:1) to give compound **5** (205 mg, 86%) as a red powder. M.p. 160 ℃ (decomposed); IR (KBr): *v* 2092 (C≡C), 1263 (N=N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.82 (2H, dd, *J* = 1.5, 4.5 Hz), 7.92 (2H, d, *J* = 8.0 Hz), 7.71 (2H, dd, *J* = 1.5, 4.5 Hz), 7.66 (2H, d, *J* = 9.0 Hz), 3.28 (1H, s); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 157.0, 151.8, 151.4, 133.1, 126.2, 123.3, 116.2, 83.0, 80.3; FAB-HRMS *m/z* (MH^+) calcd for $C_{13}H_{10}N_3$: 208.0869; found 208.0855.

*2.4. Preparation of 4-ethynyl-4'-methoxyazobenzene (**6**)*

Under an argon atmosphere, to a solution of compound **3** [19] (750 mg, 2.44 mmol) in dry THF (10 mL) were added K_2CO_3 (169 mg, 1.22 mmol) and MeOH (10 mL) and the reaction mixture was stirred for 2 h at room temperature. The resultant mixture was diluted with AcOEt (40 mL) and 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 hexane/CH_2Cl_2 (4:1) to give compound **6** (576 mg, 99%) as an orange powder. M.p. 76–78 ℃; IR (KBr): *v* 2102 (C≡C), 1252 (N=N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.81 (2H, d, *J* = 8.5 Hz), 7.73 (2H, d, *J* = 8.0 Hz), 7.51 (2H, d, *J* = 8.0 Hz), 6.89 (2H, d, *J* = 9.0 Hz), 3.76 (3H, s), 3.11 (1H, s); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 162.3, 152.3, 146.9, 132.9, 124.9, 123.9, 122.4, 114.2, 83.4, 79.1, 55.5; FAB-HRMS *m/z* (MH^+) calcd for $C_{15}H_{13}N_2O$: 237.1022; found 237.1021.

*2.5. Preparation of 4-ethynyl-4'-methylazobenzene (**7**)*

Under an argon atmosphere, to a solution of compound **4** [19] (403 mg, 1.38 mmol) in dry THF (6 mL) were added K_2CO_3 (95 mg, 0.69 mmol) and MeOH (6 mL) and the reaction mixture was stirred for 2 h at room temperature. The resultant mixture was diluted with AcOEt (20 mL) and 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 hexane to give compound **7** (203 mg, 92%) as an orange powder. M.p. 138–140 ℃; IR (KBr): *v* 2156 (C≡C), 1250 (N=N) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.87–781 (4H, m), 7.61 (2H, d, *J* = 8.5 Hz), 7.30 (2H, d, *J* = 8.5 Hz), 3.21 (1H, s), 2.42 (3H, s); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 152.2, 150.7, 142.0, 132.9, 129.8, 124.3, 123.0, 122.7, 83.3, 79.3, 21.5; MALDI-TOF-HRMS *m/z* (MH^+) calcd for $C_{15}H_{13}N_2$: 221.1073; found 221.1075.

*2.6. Preparation of 5-[4-(4-pyridyl)diazenylphenyl]ethynyl-2'-deoxyuridine (**11**)*

Under an argon atmosphere, compound **5** (281 mg, 1.35 mmol) was dissolved in dry DMF (15 mL). $Pd(PPh_3)_4$ (156 mg, 0.135 mmol), CuI (26 mg, 0.135 mmol), Et_3N (940 μL), and 2'-deoxy-5-iodouridine **10** (466 mg, 1.35 mmol) were then added. The reaction mixture was stirred at 60 ℃ for 12 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 DMF to give compound **11** (295 mg, 50%) as a red powder. M.p. 235 ℃ (decomposed); IR (KBr): *v* 3250 (NH, OH), 1636 (C=O); $[\alpha]_D^{24}$ −19.6 (c 1.00, DMSO); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.7 (1H, br s, NH), 8.82 (2H, d, J = 4.5 Hz), 8.48 (1H, s, H-6), 7.96 (2H, d, J = 8.0 Hz), 7.75–7.68 (4H, m), 6.13 (1H, t, J = 6.0 Hz, H-1'), 5.27 (1H, d, J = 4.0 Hz, OH), 5.21 (1H, t, J = 4.5 Hz, OH), 4.29–4.25 (1H, m, H-3'), 3.84–3.82 (1H, m. H-4'), 3.67–3.58 (2H, m, H-5'), 2.21–2.17 (2H, m, H-2'); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 161.3, 156.5, 151.5, 150.8, 149.4, 144.68, 132.3, 126.8, 123.5, 115.9, 97.7, 91.4, 87.6, 85.4, 85.0, 69.8, 60.8, 40.3; MALDI-TOF-HRMS *m/z* (MH^+) calcd for $C_{22}H_{20}N_5O_5$: 434.1459; found 434.1451.

*2.7. Preparation of 5-[4-(4-methoxyphenyl)diazenylphenyl]ethynyl-2'-deoxyuridine (**12**)*

Under an argon atmosphere, compound **6** (303 mg, 1.28 mmol) was dissolved in dry DMF (15 mL). $Pd(PPh_3)_4$ (148 mg, 0.128 mmol), CuI (24 mg, 0.128 mmol), Et_3N (1 mL), and 2'-deoxy-5-iodouridine **10** (441 mg, 1.28 mmol) were then added. The reaction mixture was stirred at 60 ℃ for 12 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 $CHCl_3$/MeOH (10:1) to give compound **12** (360 mg, 61%) as a light-orange powder. M.p. 253 ℃ (decomposed); IR (KBr): *v* 3345 (NH, OH), 2102 (C≡C), 1638 (C=O) cm^{-1}; $[\alpha]_D^{24}$ −21.6 (c 1.00, DMSO); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.7 (1H, br s, NH), 8.47–8.44 (1H, m, H-6), 7.90–7.85 (4H, m), 7.64 (2H, d, J = 6.0 Hz), 7.13 (2H, d, J = 5.0 Hz), 6.18–6.11 (1H, m, H-1'), 5.31–5.25 (1H, m,OH), 5.24–5.18 (1H, m, OH), 4.32–4.24 (1H, m, H-3'), 3.89–3.81 (4H, m, H-4', Ph-OMe), 3.70–3.59 (2H, m, H-5'), 2.23–2.14 (2H, m, H-2'); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 162.3, 161.3, 151.2, 149.4, 146.2, 144.3, 132.2, 124.8, 122.6, 114.7, 97.9, 91.6, 87.6, 85.1, 84.9, 69.9, 60.8, 55.6, 40.3; FAB-HRMS *m/z* (MH^+) calcd for $C_{24}H_{23}N_4O_6$: 463.1612; found 463.1613.

*2.8. Preparation of 5-[4-(4-methylphenyl)diazenylphenyl]ethynyl-2'-deoxyuridine (**13**)*

Under an argon atmosphere, compound **7** (280 mg, 1.27 mmol) was dissolved in dry DMF (13 mL). $Pd(PPh_3)_4$ (173 mg, 0.127 mmol), CuI (29 mg, 0.127 mmol), Et_3N (1.33 mL), and 2'-deoxy-5-iodouridine **10** (292 mg, 0.846 mmol) were then added. The reaction mixture was stirred at 60 ℃ for 12 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 $CHCl_3$/MeOH (10:1) to give compound **13** (284 mg, 75%) as a light-orange powder. M.p. 255 ℃ (decomposed); IR (KBr): *v* 3345 (NH, OH), 2091 (C≡C), 1641 (C=O) cm^{-1}; $[\alpha]_D^{24}$ −20.6 (c 1.00, DMSO); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.7 (1H, br s, NH), 8.47–8.44 (1H, m, H-6), 7.92–7.79 (4H, m), 7.69–7.63 (2H, m), 7.43–7.38 (2H, m), 6.17–6.11 (1H, m, H-1'), 5.30–5.24 (1H, m, OH), 5.22–5.16 (1H, m, OH), 4.30–4.23 (1H, m, H-3'), 3.85–3.79 (1H, m, H-4'), 3.71–3.57 (2H, m, H-5'), 2.40 (3H, s), 2.22-2.13 (2H, m, H-2'); ^{13}C-NMR

(125 MHz, DMSO-d_6): δ 161.3, 151.1, 150.1, 149.4, 144.4, 142.2, 132.2, 130.0, 125.1, 122.8, 97.7, 91.4, 87.6, 85.4, 85.0, 69.8, 60.8, 40.3, 21.1; MALDI-TOF-HRMS *m/z* (MH^+) calcd for $C_{24}H_{23}N_4O_5$: 447.1663; found 447.1663.

*2.9. Preparation of 5-[4-(4-trifluoromethylphenyl)diazenylphenyl]ethynyl-2'-deoxyuridine (**14**)*

Under an argon atmosphere, compound **8** [20] (369 mg, 1.35 mmol) was dissolved in dry DMF (15 mL). $Pd(PPh_3)_4$ (156 mg, 0.135 mmol), CuI (26 mg, 0.135 mmol), Et_3N (1 mL), and 2'-deoxy-5-iodouridine **10** (465 mg, 1.35 mmol) were then added. The reaction mixture was stirred at 60 ℃ for 12 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 $CHCl_3$/MeOH (10:1) to give compound **14** (415 mg, 62%) as a light-orange powder. M.p. 255 ℃ (decomposed); IR (KBr): *v* 3365 (NH, OH), 1659 (C=O) cm^{-1}; $[\alpha]_D^{24}$ −16.7 (c 1.00, DMSO); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.8 (1H, br s, NH), 8.50 (1H, s, H-6), 8.08 (2H, d, *J* = 8.0 Hz), 7.99 (4H, d, *J* = 8.5 Hz), 7.71 (2H, d, *J* = 8.0 Hz), 6.15 (1H, t, *J* = 6.5 Hz, H-1'), 5.29 (1H, d, *J* = 3.5 Hz, OH), 5.24–5.21 (1H, m, OH), 4.29 (1H, s, H-3'), 3.84 (1H, d, *J* = 3.5 Hz, H-4'), 3.71–3.61 (2H, m, H-5'), 2.22–2.18 (2H, m, H-2'); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 161.3, 154.0, 150.8, 149.4, 144.6, 132.3, 130.9, 126.8, 126.3, 123.4, 123.2, 97.7, 91.4, 87.6, 86.2, 85.0, 79.2, 69.8, 60.8, 40.3; MALDI-TOF-HRMS *m/z* (MNa^+) calcd for $C_{24}H_{19}N_4O_5F_3Na$: 523.1200; found 523.1200.

*2.10. Preparation of 5-[4-(4-nitrophenyl)diazenylphenyl]ethynyl-2'-deoxyuridine (**15**)*

Under an argon atmosphere, compound **9** [21] (302 mg, 1.20 mmol) was dissolved in dry DMF (15 mL). $Pd(PPh_3)_4$ (139 mg, 0.120 mmol), CuI (23 mg, 0.120 mmol), Et_3N (1 mL), and 2'-deoxy-5-iodouridine **10** (414 mg, 1.20 mmol) were then added. The reaction mixture was stirred at 60 ℃ for 12 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 $CHCl_3$/MeOH (5:1) to give compound **15** (545 mg, 95%) as a red powder. M.p. 270 ℃ (decomposed); IR (KBr): *v* 3403 (NH, OH), 1631 (C=O) cm^{-1}; $[\alpha]_D^{24}$ −22.5 (c 1.00, DMSO); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.8 (1H, br s, NH), 8.50–8.44 (3H, m), 8.10–8.08 (2H, m), 8.02–7.97 (2H, m), 7.74–7.68 (2H, m), 6.17–6.11 (1H, m, H-1'), 5.30–5.28 (1H, m, OH), 5.22 (1H, d, *J* = 4.5 Hz, OH), 4.28 (1H, m, H-3'), 3.84 (1H, m, H-4'), 3.68–3.62 (2H, m, H-5'), 2.22–2.18 (2H, m, H-2'); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 161.3, 155.0, 150.9, 149.4, 148.6, 144.7, 132.3, 126.7, 125.1, 123.6, 123.5, 97.7, 91.5, 87.6, 86.5, 85.0, 69.8, 60.8, 40.3; MALDI-TOF-HRMS *m/z* (MNa^+) calcd for $C_{23}H_{19}N_5O_7Na$: 500.1177; found 500.1169.

*2.11. Preparation of 5'-O-(4,4'-dimethoxytrityl)-5-[4-(4-pyridyl)diazenylphenyl]ethynyl-2'-deoxyuridine (**16**)*

To a solution of compound **11** (147 mg, 0.339 mmol) in dry pyridine (4 mL) was added DMTrCl (138 mg, 0.406 mmol) at room temperature, and the reaction mixture was stirred for 16 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 $CHCl_3$/MeOH (20:1

with 0.5% Et_3N) to give compound **16** (223 mg, 89%) as a red foam. IR (KBr): *v* 3323 (NH, OH), 1705 (C=O), 1252 (N=N) cm^{-1}; $[\alpha]_D^{24}$ −4.3 (c 1.00, DMSO); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.7 (1H, br s, NH), 8.67 (2H, d, *J* = 4.5 Hz), 8.05 (1H, s, H-6), 7.68 (2H, d, *J* = 8.5 Hz), 7.59 (2H, d, *J* = 6.0 Hz), 7.28 (2H, d, *J* = 7.0 Hz), 7.17–7.09 (8H, m), 7.02–7.00 (1H, m), 6.70–6.68 (4H, m), 6.01 (1H, t, *J* = 6.5 Hz, H-1'), 5.23–5.21 (1H, m,OH), 4.19–4.17 (1H, m, H-3'), 3.82 (1H, s, H-4'), 3.49 (6H, s, OMe), 3.06–3.04 (2H, m, H-5'), 2.16–2.13 (2H, m, H-2'); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 161.3, 158.1, 156.5, 151.6, 150.7, 149.3, 144.7, 143.7, 135.5, 135.4, 132.2, 129.6, 127.9, 127.6, 126.7, 126.6, 123.1, 115.9, 113.2, 98.1, 86.1, 86.0, 85.8, 85.3, 70.35, 63.5, 54.96, 40.4; MALDI-TOF-HRMS *m/z* (MH^+) calcd for $C_{43}H_{38}N_5O_7$: 736.2766; found 736.2759.

*2.12. Preparation of 5'-O-(4,4'-dimethoxytrityl)-5-[4-(4-methoxyphenyl)diazenylphenyl] ethynyl-2'-deoxyuridine (**17**)*

To a solution of compound **12** (101 mg, 0.218 mmol) in dry pyridine (3 mL) was added DMTrCl (89 mg, 0.262 mmol) at room temperature, and the reaction mixture was stirred for 16 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 $CHCl_3$/MeOH (20:1 with 0.5% Et_3N) to give compound **17** (152 mg, 90%) as an orange foam. IR (KBr): *v* 3437, 3410 (NH, OH), 1701 (C=O), 1272 (N=N) cm^{-1}; $[\alpha]_D^{24}$ 28.4 (c 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 10.2 (1H, br s, NH), 8.28 (1H, s, H-6), 7.88 (2H, d, *J* = 7.0 Hz), 7.64 (2H, d, *J* = 7.0 Hz), 7.46 (2H, d, *J* = 7.0 Hz), 7.36 (4H, d, *J* = 7.5 Hz), 7.26–7.24 (3H, m), 7.11 (1H, d, *J* = 8.0 Hz), 6.81–6.74 (4H, m), 6.45–6.37 (1H, m, H-1'), 4.60–4.58 (1H, m, H-3'), 4.22–4.19 (1H, m, H-4'), 3.84 (3H, s), 3.49 (6H, s, OMe), 3.45 (1H, d, *J* = 10.0 Hz, H-5'), 3.31 (1H, d, *J* = 9.0 Hz, H-5'), 2.68–2.57 (1H, m, H-2'), 2.40–2.28 (1H, m, H-2'), 1.29–1.19 (1H, m, OH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 162.1, 161.8, 158.4, 151.6, 149.5, 146.9, 144.4, 135.5, 135.4, 132.3, 129.9, 129.8, 128.0, 127.8, 124.3, 122.1, 114.1, 113.2, 100.3, 93.5, 86.9, 86.0, 86.0, 82.2, 72.2, 60.3, 55.4, 55.0, 41.6; MALDI-TOF-HRMS *m/z* (MH^+) calcd for $C_{45}H_{41}N_4O_8$: 765.2919; found 765.2912.

*2.13. Preparation of 5'-O-(4,4'-dimethoxytrityl)-5-[4-(4-methylphenyl)diazenylphenyl] ethynyl-2'-deoxyuridine (**18**)*

To a solution of compound **13** (200 mg, 0.446 mmol) in dry pyridine (5 mL) was added DMTrCl (182 mg, 0.536 mmol) at room temperature, and the reaction mixture was stirred for 16 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 hexane/AcOEt (1:2) to give compound **18** (208 mg, 62%) as an orange foam. IR (KBr): *v* 3425 (NH, OH), 1707 (C=O), 1253 (N=N) cm^{-1}; $[\alpha]_D^{24}$ −27.9 (c 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.09 (1H, br s, NH), 8.27 (1H, s, H-6), 7.80 (2H, d, *J* = 8.0 Hz), 7.67 (2H, d, *J* = 7.5 Hz), 7.45 (2H, d, *J* = 8.0 Hz), 7.35 (4H, d, *J* = 8.5 Hz), 7.31–7.24 (4H, m), 7.15 (1H, t, *J* = 7.0 Hz), 7.11 (2H, d, *J* = 8.5 Hz), 6.81–6.78

(4H, m), 6.38 (1H, t, *J* = 4.0 Hz, H-1'), 4.60–4.57 (1H, m, H-3'), 4.17–4.09 (1H, m, H-4'), 3.77–3.65 (6H, m, OMe), 3.50–3.46 (1H, m, H-5'), 3.40–3.28 (1H, m, H-5'), 2.67–2.52 (2H, m, H-2'), 1.86–1.82 (1H, m, OH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 161.2, 158.6, 151.7, 150.7, 149.2, 144.4, 141.9, 135.4, 132.4, 130.0, 129.9, 129.8, 128.1, 127.9, 127.1, 124.7, 122.9, 122.3, 113.4, 100.4, 93.6, 87.1, 86.8, 85.9, 72.4, 63.5, 55.2, 41.2, 21.5; MALDI-TOF-HRMS *m/z* (MNa^+) calcd for $C_{45}H_{40}N_4O_7Na$: 771.2789; found 771.2780.

*2.14. Preparation of 5'-O-(4,4'-dimethoxytrityl)-5-[4-(4-trifluoromethylphenyl)diazenylphenyl] ethyn-yl-2'-deoxyuridine (**19**)*

To a solution of compound **14** (114 mg, 0.228 mmol) in dry pyridine (3 mL) was added DMTrCl (93 mg, 0.273 mmol) at room temperature, and the reaction mixture was stirred for 16 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 $CHCl_3$ with 0.5% Et_3N) to give compound **19** (118 mg, 65%) as an orange foam. IR (KBr): *v* 3372 (NH, OH), 1669 (C=O), 1253 (N=N) cm^{-1}; $[\alpha]_D^{24}$ 26.9 (c 1.00, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 10.04 (1H, br s, NH), 8.25 (1H, s, H-6), 7.85 (2H, d, *J* = 8.5 Hz), 7.69–7.55 (4H, m), 7.41–7.12 (8H, m), 7.07–6.95 (3H, m), 6.71–6.69 (4H, m), 6.36–6.29 (1H, m, H-1'), 4.55–4.47 (1H, m, H-3'), 4.15–4.07 (1H, m, H-4'), 3.57 (6H, s, OMe), 3.42–3.34 (1H, m, H-5'), 3.25–3.18 (1H, m, H-5'), 2.60–2.42 (2H, m, H-2’), 2.32–2.21 (1H, m), 0.99–0.90 (1H, m, OH); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.7, 158.5, 154.2, 151.2, 149.5, 135.5, 132.4, 129.88, 129.86, 128.0, 127.9, 127.0, 126.22, 126.18, 126.0, 125.9, 123.0, 122.7, 113.3, 100.2, 93.3, 87.0, 86.9, 86.1, 83.7, 83.1, 75.2, 72.3, 55.1, 24.7; MALDI-TOF-HRMS *m/z* (MNa^+) calcd for $C_{45}H_{37}N_4O_7F_3Na$: 825.2507; found 825.2497.

*2.15. Preparation of 5'-O-(4,4'-dimethoxytrityl)-5-[4-(4-nitrophenyl)diazenylphenyl] ethynyl-2'-deoxyuridine (**20**)*

To a solution of compound **15** (150 mg, 0.314 mmol) in dry pyridine (3 mL) was added DMTrCl (128 mg, 0.377 mmol) at room temperature, and the reaction mixture was stirred for 16 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 $CHCl_3$/MeOH (20:1 with 0.5% Et_3N) to give compound **20** (115 mg, 47%) as a red foam. IR (KBr): *v* 3452 (NH, OH), 1703 (C=O), 1251 (N=N) cm^{-1}; $[\alpha]_D^{24}$ −16.9 (c 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.98 (1H, br s, NH), 8.38-8.22 (3H, m), 7.95 (2H, d, *J* = 7.0 Hz), 7.69 (2H, d, *J* = 7.0 Hz), 7.49-7.14 (8H, m), 7.06 (2H, d, *J* = 7.0 Hz), 6.83-6.72 (5H, m), 6.43-6.38 (1H, m, H-1'), 4.61-4.57 (1H, m, H-3'), 4.21-4.16 (1H, m, H-4'), 3.65 (6H, s, OMe), 3.50-3.45 (1H, m, H-5'), 3.33-3.26 (1H, m, H-5'), 2.66-2.59 (1H, m, H-2'), 2.34-2.27 (1H, m, H-2'), 1.28-1.17 (1H, m, OH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 161.6, 158.5, 155.5, 151.2, 148.6, 144.4, 135.5, 135.4, 132.4, 132.3, 129.9, 128.0, 127.9,

127.8, 127.0, 124.6, 124.5, 123.4, 123.3, 123.0, 122.9, 113.3, 113.2, 100.1, 87.0, 86.9, 72.2, 55.1; MALDI-TOF-HRMS *m/z* (MNa^+) calcd for $C_{44}H_{37}N_5O_9Na$: 802.2483; found 802.2501.

*2.16. Preparation of 3'-O-[2-cyanoethyl(diisopropylamino)phosphino]-5'-O-(4,4'-dimethoxytrityl)-5-[4-(4-pyridyl)diazenylphenyl]ethynyl-2'-deoxyuridine (**21**)*

Under an argon atmosphere, to a solution of compound **16** (223 mg, 0.312 mmol) in dry CH_2Cl_2 (3 mL) was added *N*,*N*-diisopropylamine (160 μL, 0.935 mmol) and 2-cyanoethyl-*N*,*N*'-diisopropylchlorophosphoramidite (105 μL, 0.468 mmol) at room temperature, and the reaction mixture was stirred for 3 h. The resultant mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$, 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 $CHCl_3$/MeOH (20:1 with 0.5% Et_3N) to give a 1:1 diastereomeric mixture of **21** (219 mg, 75%) as a red foam. IR (KBr): *ν* 3603 (NH), 1712 (C=O), 1251 (N=N) cm^{-1}; $[\alpha]_D^{24}$ 32.3 (c 1.00, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): *δ* 8.84 (2H, m), 8.42 (0.3H, s, H-6), 8.37 (0.7H, s, H-6), 7.74–7.70 (4H, m), 7.51–7.50 (2H, m), 7.41–7.35 (4H, m), 7.31–7.16 (3H, m), 7.04 (2H, dd, *J* = 9.0, 8.5 Hz), 6.83–6.77 (4H, m), 6.42–6.36 (1H, m, H-1'), 4.72–4.61 (1H, m, H-3'), 4.27–4.19 (1H, m, H-5'), 3.93–3.74 (1H, m, H-5'), 3.71 (6H, s, OMe), 3.67–3.49 (4H, m, CH_2CH_2CN), 3.36–3.24 (1H, m, H-4'), 2.72–2.52 (2H, m, H-2'), 2.49–2.35 (2H, m), 1.28 (8.4 H, t, *J* = 7.0 Hz, $((CH_3)_2CH)_2N$), 1.17 (3.6H, t, *J* = 7.0 Hz, $((CH_3)_2CH)_2N$); ^{13}C-NMR (125 MHz, $CDCl_3$): *δ* 158.5, 157.1, 151.1, 144.33, 144.29, 132.8, 135.5, 135.4, 135.3, 132.4, 132.3, 129.9, 128.0, 127.9, 127.8, 127.0, 126.8, 122.91, 122.89, 117.5, 116.2, 113.2, 100.1, 93.0, 87.0, 85.8, 63.1, 58.4, 58.2, 58.1, 57.9, 55.1, 43.23, 43.17, 43.07, 43.01, 40.9, 24.6, 24.54, 24.48, 24.44, 24.39, 21.5, 20.4, 20.3, 20.2; ^{31}P-NMR (120 MHz, $CDCl_3$): *δ* 148.8, 148.4; MALDI-TOF-HRMS *m/z* (MNa^+) calcd for $C_{52}H_{54}N_7O_8NaP$: 958.3664; found 958.3647.

*2.17. Preparation of 3'-O-[2-cyanoethyl(diisopropylamino)phosphino]-5'-O-(4,4'-dimethoxytrityl)-5-[4-(4-methoxyphenyl)diazenylphenyl]ethynyl-2'-deoxyuridine (**22**)*

Under an argon atmosphere, to a solution of compound **17** (152 mg, 0.199 mmol) in dry CH_2Cl_2 (2 mL) was added *N*,*N*-diisopropylamine (103 μL, 0.600 mmol) and 2-cyanoethyl-*N*,*N*'-diisopropylchlorophosphoramidite (67 μL, 0.299 mmol) at room temperature, and the reaction mixture was stirred for 3 h. The resultant mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$, 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 $CHCl_3$/MeOH (20:1 with 0.5% Et_3N) to give a 3:1 diastereomeric mixture of **22** (72 mg, 38%) as an orange foam. IR (KBr): *ν* 3624 (NH), 1700 (C=O), 1253 (N=N) cm^{-1}; $[\alpha]_D^{24}$ 19.4 (c 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): *δ* 9.76 (1H, br s, NH), 8.37–8.28 (1H, m, H-6), 7.89 (2H, d, *J* = 7.5 Hz), 7.66 (2H, t, *J* = 8.0 Hz), 7.51–7.47 (2H, m), 7.41–7.31 (4H, m), 7.31–7.25 (2H, m), 7.19–7.12 (1H, m), 7.08 (2H, dd, *J* = 6.0, 10.0 Hz), 7.01–6.96 (2H, m), 6.84–6.78 (4H, m), 6.41–6.31 (1H, m, H-1'), 4.70–4.62 (1H, m, H-3'), 4.31–4.29 (0.25H, m, H-4'), 4-18-416 (0.75H, m, H-4'), 3.87–3.82 (4H, m, CH_2CH_2CN), 3.82 (3H, s), 3.68 (6H, s, OMe), 3.64–3.48 (2H, m, H-5'), 2.76–2.58 (2H, m, H-2'), 2.47–2.35 (2H, m, $((CH_3)_2CH)_2N$), 1.28 (9H, t, *J* = 7.0 Hz, $((CH_3)_2CH)_2N$), 1.17 (3H, t, *J* = 7.0 Hz, $((CH_3)_2CH)_2N$); ^{13}C-NMR (125 MHz, $CDCl_3$): *δ* 162.0, 158.4,

151.6, 149.2, 146.8, 144.2, 144.2, 135.28, 135.25, 132.18, 132.17, 129.8, 129.78, 129.75, 127.9, 127.85, 127.82, 127.7, 126.9, 124.6, 124.3, 121.97, 121.95, 117.3, 114.1, 113.2, 100.2, 100.1, 93.3, 86.8 (d, *J* (C, P) = 3.5 Hz), 77.2, 77.0, 76.7, 63.0, 62.9, 58.2, 58.1, 55.4, 54.9, 44.9 (d, *J* (C, P) = 5.0 Hz), 24.4, 24.3, 22.8, 22.7, 20.2 (d, *J* (C, P) = 7.0 Hz); ^{31}P-NMR (200 MHz, $CDCl_3$): δ 149.5, 149.1; MALDI-TOF-HRMS *m/z* (MNa^+) calcd for $C_{54}H_{57}N_6O_9NaP$: 987.3817; found 987.3826.

*2.18. Preparation of 3'-O-[2-cyanoethyl(diisopropylamino)phosphino]-5'-O-(4,4'-dimethoxytrityl)-5-[4-(4-methylphenyl)diazenylphenyl]ethynyl-2'-deoxyuridine (**23**)*

Under an argon atmosphere, to a solution of compound **18** (170 mg, 0.227 mmol) in dry CH_2Cl_2 (3 mL) was added *N*,*N*-diisopropylamine (119 µL, 0.681 mmol) and 2-cyanoethyl-*N*,*N*'-diisopropylchlorophosphoramidite (78 µL, 0.341 mmol) at room temperature, and the reaction mixture was stirred for 3 h. The resultant mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$, 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 hexane/AcOEt (1:1) to give a 2:1 diastereomeric mixture of **23** (120 mg, 55%) as an orange foam. IR (KBr): *v* 3610 (NH), 1699 (C=O), 1272 (N=N) cm^{-1}; $[\alpha]_D^{24}$ 29.6 (c 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.74 (1H, br s, NH), 8.35–8.26 (1H, m, H-6), 7.84–7.77 (2H, m), 7.71–7.63 (2H, m), 7.51–7.44 (2H, m), 7.41–7.33 (4H, m), 7.32–7.25 (5H, m), 7.12–7.03 (2H, m), 6.84–6.75 (4H, m), 6.41–6.31 (1H, m, H-1'), 4.72–4.60 (1H, m, H-3'), 4.29–4.19 (1H, m, H-4'), 3.93–3.74 (4H, m, CH_2CH_2CN), 3.70 (4H, s, OMe), 3.63 (2H, s, OMe), 3.37–3.12 (2H, m, H-5'), 2.72–2.50 (2H, m, H-2'), 2.50 (3H, s), 2.41–2.26 (2H, m, $((CH_3)_2CH)_2N$), 1.22–1.14 (8H, m, $((CH_3)_2CH)_2N$), 1.12–1.06 (4H, m, $((CH_3)_2CH)_2N$); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 153.6, 146.7, 145.7, 139.3, 137.4, 136.8, 130.4, 127.4, 124.93, 124.91, 124.74, 124.72, 123.0, 122.9, 122.0, 121.9, 119.8, 117.9, 119.3, 112.5, 108.3, 88.5, 82.1, 80.9 (d, *J* (C, P) = 3.5 Hz), 68.8, 68.7, 58.2, 53.4, 53.3, 50.2, 48.4, 38.2, 35.9, 35.8, 19.5 (d, *J* (C, P) = 7.0 Hz), 16.5, 15.4, 15.3; ^{31}P-NMR (200 MHz, $CDCl_3$): δ 149.6, 149.2; MALDI-TOF-HRMS *m/z* (MNa^+) calcd for $C_{54}H_{57}N_6O_8NaP$: 971.3868; found 971.3875.

*2.19. Preparation of 3'-O-[2-cyanoethyl(diisopropylamino)phosphino]-5'-O-(4,4'-dimethoxytrityl)-5-[4-(4-trifluoromethylphenyl)diazenylphenyl]ethynyl-2'-deoxyuridine (**24**)*

Under an argon atmosphere, to a solution of compound **19** (98 mg, 0.122 mmol) in dry CH_2Cl_2 (2 mL) was added *N*,*N*-diisopropylamine (63 µL, 0.366 mmol) and 2-cyanoethyl-*N*,*N*'-diisopropylchlorophosphoramidite (41 µL, 0.183 mmol) at room temperature, and the reaction mixture was stirred for 3 h. The resultant mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$, 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 $CHCl_3$/MeOH (20:1 with 0.5% Et_3N) to give a 1:1 diastereomeric mixture of **24** (106 mg, 87%) as an orange foam. IR (KBr): *v* 3640 (NH), 1674 (C=O), 1251 (N=N) cm^{-1}; $[\alpha]_D^{24}$ 32.3 (c 1.00, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.39 (0.4H, s, H-6), 8.34 (0.6H, s, H-6), 8.01–7.95 (2H, m), 7.81–7.68 (4H, m), 7.51–7.45 (2H, m), 7.41–7.32 (4H, m), 7.21–7.13 (1H, m), 7.09–6.99 (2H, m), 6.85–6.75 (6H, m), 6.42–6.31 (1H, m, H-1'), 4.70–4.59 (1H, m, H-3'), 4.29–4.15 (1H, m, H-4'), 3.75–3.64 (4H, m, CH_2CH_2CN), 3.48 (6H, s, OMe), 3.43–3.18 (2H, m,

H-5'), 2.70–2.60 (2H, m, H-2'), 2.50–2.33 (2H, m, $((CH_3)_2CH)_2N$), 1.21–1.14 (7.2H, m, $((CH_3)_2CH)_2N$), 1.10-1.04 (4.8H, m, $((CH_3)_2CH)_2N$); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 161.2, 158.6, 154.3, 151.3, 149.1, 146.9, 144.4, 144.3, 142.7, 135.5, 135.4, 132.4, 132.3, 32.0, 129.97, 129.92, 129.7, 128.0, 127.9, 127.8, 127.0, 126.3, 126.2, 126.0, 125.9, 125.6, 123.0, 122.7, 120.3, 117.6, 117.4, 116.7, 113.3, 100.4, 100.2, 93.2, 87.2, 87.0, 86.3, 85.9, 85.8, 83.0, 77.4, 77.2, 77.0, 76.6, 75.0, 74.9, 73.7, 63.1, 58.4, 58.2, 58.1, 55.2, 55.4, 53.4, 50.7, 43.3, 43.2, 43.1, 43.0, 40.8, 24.8, 24.6, 24.56, 24.51, 24.47, 24.41, 22.8, 20.4, 20.3, 20.1; ^{31}P-NMR (120 MHz, $CDCl_3$): δ 148.9, 148.4; MALDI-TOF-HRMS *m/z* (MNa^+) calcd for $C_{54}H_{54}N_6O_8F_3NaP$: 1025.3585; found 1025.3596.

*2.20. Preparation of 3'-O-[2-cyanoethyl(diisopropylamino)phosphino]-5'-O-(4,4'-dimethoxytrityl)-5-[4-(4-nitrophenyl)diazenylphenyl]ethynyl-2'-deoxyuridine (**25**)*

Under an argon atmosphere, to a solution of compound **20** (111 mg, 0.142 mmol) in dry CH_2Cl_2 (2 mL) was added *N,N*-diisopropylamine (74 μL, 0.426 mmol) and 2-cyanoethyl-*N,N'*-diisopropylchlorophosphoramidite (48 μL, 0.213 mmol) at room temperature, and the reaction mixture was stirred for 3 h. The resultant mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$, 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 $CHCl_3$/MeOH (20:1 with 0.5% Et_3N) to give a 2:1 diastereomeric mixture of **25** (137 mg, 99%) as a red foam. IR (KBr): *v* 3576 (NH), 1712 (C=O), 1252 (N=N) cm^{-1}; $[\alpha]_D^{24}$ −15.0 (c 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.45 (1H, br s, NH), 8.45–8.29 (3H, m), 8.04–7.99 (2H, m), 7.76–7.70 (2H, m), 7.53–7.47 (2H, m), 7.42–7.36 (4H, m), 7.31–7.26 (2H, m), 7.20–7.14 (1H, m), 7.11–7.03 (2H, m), 6.85–6.79 (4H, m), 6.42–6.35 (1H, m, H-1'), 4.71–4.63 (1H, m, H-4'), 4.30–4.25 (0.33H, m, H-3'), 4.24–4.20 (0.67H, m, H-3'), 3.89–3.75 (4H, m, CH_2CH_2CN), 3.72–3.69 (6H, m, OMe), 3.65–3.50 (2H, m, H-5'), 2.75–2.61 (2H, m, H-2'), 2.47–2.35 (2H, m, $((CH_3)_2CH)_2N$), 1.22–1.16 (8H, m, $((CH_3)_2CH)_2N$), 1.16–1.06 (4H, m, $((CH_3)_2CH)_2N$); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 156.5, 153.3, 150.6, 146.2, 144.3, 143.7, 139.4, 137.8, 130.5, 127.5, 124.9, 123.0, 122.9, 122.0, 121.8, 119.7, 119.53, 119.52, 118.0, 112.6, 103.3, 93.2, 81.9 (d, *J* (C, P) = 3.5 Hz), 68.7, 68.4, 62.9, 62.8, 58.1, 58.0, 55.4, 54.9, 38.9 (d, *J* (C, P) = 5.0 Hz), 24.4, 24.3, 22.8, 22.7, 19.5 (d, *J* (C, P) = 7.0 Hz); ^{31}P-NMR (200 MHz, $CDCl_3$): δ 149.6, 149.3; MALDI-TOF-HRMS *m/z* (MNa^+) calcd for $C_{53}H_{54}N_7O_{10}NaP$: 1002.3567; found 1002.3570.

*2.21. Synthesis of **dU^{Az}**-Modified Oligodeoxynucleotides*

Solid-phase oligonucleotide synthesis was performed using commercially available reagents and phosphoramidites with activator 42 (Sigma-Aldrich) as the activator. *para*-Substituted-**dU^{Az}** phosphoramidites were chemically synthesized as described above. All of the reagents were assembled, and the ONs 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 ℃ for 12 h. The crude oligonucleotides were purified with Sep-Pak Plus C18 cartridges (Waters, MA, USA) followed by RP-HPLC on a XBridge™ OST C18 column, 2.5 μm, 10 × 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 1).

Table 1. Yields and MALDI-TOF MS data of **dU^{Az}**-modified ONs.

ON [a]	X	Yield/%	MALDI-TOF MS	
			Calcd. $[M-H]^-$	Found $[M-H]^-$
28	**dU^{Az}-OMe**	21	3852.6	3853.2
29	**dU^{Az}-Me**	27	3836.6	3836.2
30	**dU^{Az}-CF$_3$**	15	3890.6	3890.2
31	**dU^{Az}-NO$_2$**	22	3866.6	3866.9
32	**dU^{Az}-pyridyl**	21	3823.6	3824.2

[a] ON sequence is 5'-d(GCGTT**X**TTTGCT)-3'.

2.22. UV Melting Experiments

Equimolecular amounts of the target DNA/RNA and ONs 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 5–90 °C at a rate of 0.5 °C/min.

*2.23. Photo-Isomerization of **dU^{Az}** Analogues*

ONs were dissolved in a 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl to give a final strand concentration of 4.0 μM. The ON solution was exposed to the appropriate wavelength of monochromic light for 10 seconds and subsequently analyzed by RP-HPLC. The ratio of *cis/trans* isomers was obtained from the HPLC peak areas at 260 nm.

*2.24. Thermal Isomerization of cis-**dU^{Az}** Analogues*

ONs 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 oligonucleotide solution was exposed to the appropriate wavelength of monochromic light for 10 seconds and subsequently heated to 60 °C. The change of absorbance at 365 nm or 400 nm was monitored by a UV-Vis spectrophotometer and plotted to calculate the half-life time of *cis*-**dU^{Az}** analogues.

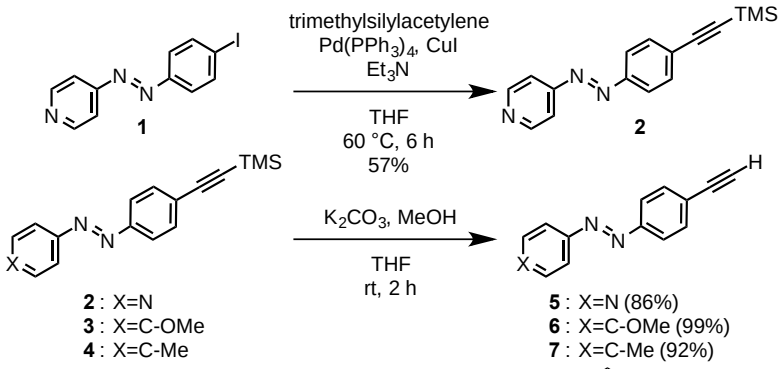
2.25. Isothermal Titration Calorimetry

Prior to experiments, all solutions (titrant and titrand) were denatured at 100 °C and annealed slowly to room temperature. Titrations were carried out by injecting 2 μL portions of the complementary RNA strand into 250 μL of the **dU^{Az}**-modified ON solution in the calorimetric cell. The **dU^{Az}**-modified ON concentrations in the cell were 0.1 μM, and the solutions in the syringe were 1 μM. The equilibration period after each injection was 360 s. The measurements were performed with and without the photo-isomerization of **dU^{Az}**-modified ONs.

3. Results

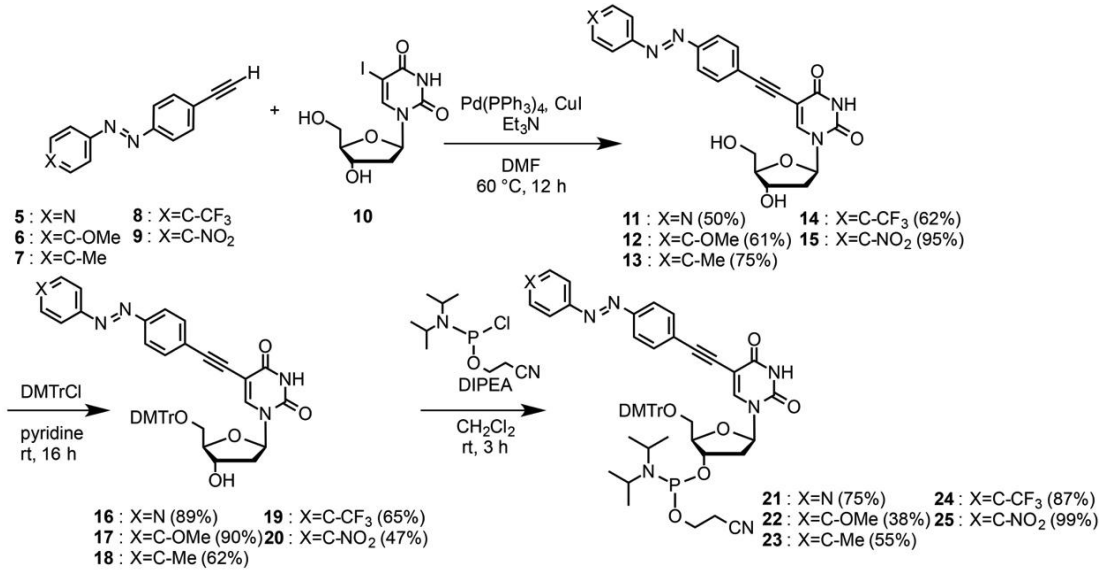
3.1. Synthesis of Para-Substituted ***dU*** ^{Az} *Phosphoramidites and Modified Oligonucleotides*

The synthetic route for *para*-substituted ethynyl-azobenzene derivatives is outlined in Scheme 1. 4-4-[(Trimethylsilyl)ethynylphenylazo]pyridine **2** was synthesized from the prepared 4-(4-iodophenylazo)pyridine **1** [18] through a palladium-catalyzed cross-coupling reaction [22] with trimethylsilylacetylene. TMS-protected ethynyl-azobenzene derivatives **2**–**4** [19] were converted to compounds **5**–**7** by removal of the silyl group. Compounds **8** and **9** were also synthesized according to the reported procedures [20,21].



Scheme 1. Route for the synthesis of *para*-substituted ethynyl-azobenzene derivatives.

The preparation of *para*-substituted **dU** ^{Az} phosphoramidites commenced with the coupling of 4-ethynyl-azobenzene derivatives **5**–**9** with 2'-deoxy-5-iodouridine **10** (Scheme 2). Tritylation at the primary hydroxyl group of **11**–**15** with DMTrCl and phosphitylation at the secondary hydroxyl group yielded phosphoramidites **21**–**25**. The amidites **21**–**25** were incorporated into ONs using conventional solid-phase phosphoramidite synthesis and purified by reverse-phase HPLC. The ON sequences used in this study are shown in Table 2.



Scheme 2. Route for the synthesis of *para*-substituted **dU** ^{Az} phosphoramidites.

Table 2. The oligonucleotides used in this study.

ON	Sequence	X
26	5'-d(GCGTTTTTTGCT)-3'	-
27	5'-d(GCGTT**X**TTTGCT)-3'	**dU^{Az}**
28	5'-d(GCGTT**X**TTTGCT)-3'	**dU^{Az}-OMe**
29	5'-d(GCGTT**X**TTTGCT)-3'	**dU^{Az}-Me**
30	5'-d(GCGTT**X**TTTGCT)-3'	**dU^{Az}-CF$_3$**
31	5'-d(GCGTT**X**TTTGCT)-3'	**dU^{Az}-NO$_2$**
32	5'-d(GCGTT**X**TTTGCT)-3'	**dU^{Az}-pyridyl**
33	5'-d(AGCAAAAAACGC)-3'	-
34	5'-r(AGCAAAAAACGC)-3'	-

*3.2. Isomerization Properties of Para-Substituted **dU^{Az}** Analogues*

The influence of *para*-substitution on the efficiency of the **dU^{Az}** *cis-trans* photo-isomerization in ONs was investigated by UV spectroscopy and HPLC analysis. The photo-isomer ratio was measured at 260 nm because ONs **27**–**32** have the same extinction coefficient before and after irradiation at this wavelength (Figure S1). The wavelengths of light used for photo-isomerization and their efficiencies are shown in Table 3. Ten seconds of irradiation was confirmed to be enough for reaching photostationary state (PSS); the *cis*/*trans* ratios of ONs **27**–**32** after longer irradiation times (30 and 120 min) were the same as that after 10 s of irradiation. Compared with ON **27** containing **dU^{Az}**, ONs **28** and **29** containing electron-donating substituted **dU^{Az}** were photo-isomerized from *trans* to *cis* more effectively. Irradiation of ON **28** (X = **dU^{Az}–OMe**) using 365 nm afforded a mixture with 79% of the *cis*-form. In contrast, ON **30** bearing the electron-withdrawing CF_3 group showed a lower *trans* to *cis* isomerization efficiency. However, the *cis* to *trans* isomerization efficiency was higher than that of ON **27** containing **dU^{Az}**. ONs **31** and **32** containing **dU^{Az}-NO$_2$** and **dU^{Az}-pyridyl** were barely photo-isomerized. The low efficiency of photo-isomerization was due to the low electron density at the azo-structure [23]. In case of **dU^{Az}-pyridyl**, electrons tend to be delocalized at the nitrogen of the pyridine ring [24]. ONs **28**–**31** were photo-isomerized *trans* to *cis* and *vice versa* for at least three cycles without any attenuation of efficiency.

Table 3. Photo-isomerization properties of *para*-substituted **dU^{Az}** [a].

ON [b]	X	Before Photo-Irradiation	*Trans* to *Cis*		*Cis* to *Trans*	
		Trans-Isomer/% [b]	Wavelength [c]/nm [a]	*cis*-isomer/% [d]	Wavelength[c] /nm [a]	*Trans*-Isomer/% [d]
27	**dU^{Az}**	82	365	58	450	82
28	**dU^{Az}-OMe**	59	365	79	450	59
29	**dU^{Az}-Me**	77	365	61	450	77
30	**dU^{Az}-CF$_3$**	91	365	28	450	91
31	**dU^{Az}-NO$_2$**	96	400	14	450	96
32	**dU^{Az}-pyridyl**	100	-	0	-	-

[a] Conditions: 10 mM Na_2HPO_4 (pH 7.0), 100 mM NaCl, 4 μM ON; [b] ON sequence is 5'-d(GCGTT**X**TTTGCT)-3'; [c] The wavelength of monochromic light corresponds to π-π * transition (*trans* to *cis*) or n-π * (*cis* to *trans*). Photo-irradiation was performed for 10 s at room temperature; [d] The ratio of isomers is calculated from the peak area of HPLC traces (260 nm).

Next, the thermo-stability of ONs with various *para*-substituted **dU^{Az}** in the *cis*-form was investigated [25]. The ONs modified with **dU^{Az}** analogues were *cis*-isomerized by the appropriate wavelength of light and subsequently heated to 60 ℃. The change in absorbance at 365 nm was monitored by a UV-Vis spectrophotometer and plotted to calculate the half-life of the *cis*-isomers (Table 4). With regard to ONs **28** and **29** containing electron-donating substituted **dU^{Az}**, thermo-stabilities of the *cis*-isomer were significantly decreased. This phenomenon could be attributed to the *para*-electron-donating substituent and ethynyl linker creating a push-pull configuration of the azobenzene moiety, which is well known in easily thermo-isomerized azobenzene derivatives [18]. On the other hand, ONs **30** and **31** containing electron-withdrawing substituted **dU^{Az}** showed long half-life times. The *cis*-isomer of ON **31** (**X** = **dU^{Az}–NO$_2$**) scarcely isomerized from *cis* to *trans* even when heated to 60 ℃ for more than 10 h. In the case of ONs **30** and **31**, both the ethynyl linker and *para*-substitution work as 4,4'-di-electron-withdrawing moieties. The 4,4'-di-electron-withdrawing substitution of azobenzene strengthens the thermal stability of the *cis*-form [26].

Table 4. Half-life time of the *cis*-form of *para*-substituted-**dU^{Az}** at 60 ℃ [a].

ON [b]	**X**	$t_{1/2}$ of *cis*-isomer/min. [c,d]
27	**dU^{Az}**	44
28	**dU^{Az}-OMe**	9
29	**dU^{Az}-Me**	24
30	**dU^{Az}-CF$_3$**	65
31	**dU^{Az}-NO$_2$**	>300
32	**dU^{Az}-pyridyl**	- [e]

[a] Conditions: 10 mM Na_2HPO_4 (pH 7.0), 100 mM NaCl, 4 μM ON; [b] ON sequence is 5'-d(GCGTT**X**TTTGCT)-3'; [c] *trans*- ONs were photo-isomerized to the *cis*-form by a 10 second irradiation of 365 nm (ONs **27**-**30**) or 400 nm (ON **31**) monochromic light; [d] Half-life times of *cis*-isomers were measured at 60 ℃; [e] $t_{1/2}$ could not be determined because **dU^{Az}-pyridyl** did not isomerize to the *cis*-form.

The *para*-substitution would sterically and electronically affect the stability of the duplexes formed by **dU^{Az}** analogue-modified ONs with complementary strands. Therefore, we investigated the T_m values of the duplexes formed between ON **28**–**32** and full match DNA or RNA (Tables 5 and 6). It was found that *para*-substitution of **dU^{Az}** slightly destabilized the DNA/DNA duplexes but had little influence on the DNA/RNA duplexes.

Table 5. T_m values of duplexes formed by **dU^{Az}** analogue-modified ONs with complementary DNA [a].

Duplex	**X**	T_m [℃]	ΔT_m [℃] [b]
27/33	**dU^{Az}**	47	-
28/33	**dU^{Az}-OMe**	44	−3
29/33	**dU^{Az}-Me**	45	−2
30/33	**dU^{Az}-CF$_3$**	45	−2
31/33	**dU^{Az}-NO$_2$**	45	−2
32/33	**dU^{Az}-pyridyl**	44	−3

[a] Conditions: 10 mM Na_2HPO_4 (pH 7.0), 100 mM NaCl, 4 μM ON. The T_m values given are the average of at least three data points. The T_m value of natural DNA **26**/DNA **33** duplex is 52 ℃; [b] The ΔT_m indicates the difference from ON **27**/DNA **33** duplex.

Table 6. T_m values of duplexes formed by **dU^{Az}** analogue-modified ONs with complementary RNA [a].

Duplex	X	T_m [℃]	ΔT_m [℃] [c]
27/34	**dU^{Az}**	42	-
28/34	**dU^{Az}-OMe**	41	−1
29/34	**dU^{Az}-Me**	43	+1
30/34	**dU^{Az}-CF$_3$**	41	−1
31/34	**dU^{Az}-NO$_2$**	41	−1
32/34	**dU^{Az}-pyridyl**	42	0

[a] Conditions: 10 mM Na_2HPO_4 (pH 7.0), 100 mM NaCl, 4 μM ON. The T_m values given are the average of at least three data points. The T_m value of natural DNA **26**/RNA **34** duplex is 47 ℃; [b] The ΔT_m indicates the difference from ON **27**/RNA **34** duplex.

The changes in T_m values of **dU^{Az}**-modified duplexes upon photo-irradiation could not be determined due to the low thermal stability of the *cis*-form itself (ONs **28** and **29**, Table 4) or low *trans* to *cis* photo-isomerization efficiency (ONs **30**–**32**, Table 3). Therefore, we investigated the changes in the thermodynamic stabilities of 12-bp DNA/RNA duplexes containing **dU^{Az}-OMe** and **dU^{Az}-Me** upon photo-irradiation by isothermal titration calorimetry (ITC) at 25 ℃ (Table 7). 1 μM of complementary RNA strand in the syringe was titrated to 0.1 μM **dU^{Az}** analogue-modified ON in the cell and the heat transfer during binding was measured to determine the thermodynamic parameters of DNA/RNA duplex formation. The experiments were conducted for **dU^{Az}** analogue-modified ONs both before and after irradiation. Duplex **28/34** showed a lower Gibbs free energy change (ΔG) after photo-irradiation than that before photo-irradiation. This trend was also observed in duplex **29/34**. These results indicate that the hybridization abilities of ONs modified with **dU^{Az}-OMe** and **dU^{Az}-Me** can be regulated by the appropriate wavelength of light, as with the case of **dU^{Az}**.

Table 7. Thermodynamic parameters of duplexes formed by **dU^{Az}** analogue-modified ONs with complementary RNA [a].

Duplex	X	$K_a \times 10^7$ [M]	ΔH [kcal/mol]	ΔS [kcal/mol/deg]	ΔG [kcal/mol]
28/34	**dU^{Az}-OMe**	2.65 ± 0.317	−54.1 ± 0.839	−0.148	−9.97
	dU^{Az}-OMe [b]	3.03 ± 0.600	−54.8 ± 1.50	−0.150	−10.1
29/34	**dU^{Az}-Me**	2.17 ± 0.414	−55.3 ± 1.60	−0.152	−9.98
	dU^{Az}-Me [b]	4.15 ± 0.798	−52.1 ± 1.20	−0.140	−10.4

[a] Conditions: 5 mM Na_2HPO_4 (pH 7.0), 50 mM NaCl, 0.1 μM **dU^{Az}** modified ON (titrand); 5 mM Na_2HPO_4 (pH 7.0), 50 mM NaCl, 1 μM complementary RNA strand (titrant). [b] After photo-irradiation at 365 nm for 10 s.

4. Discussion

As shown above, *para*-substitution strongly affected the photo-isomerization efficiency of **dU^{Az}**. It is important to modulate the electron nature of the azo structure for the optimization of **dU^{Az}** photo-isomerization properties. Notably, the electron-donating OMe group could strongly bias the equilibrium to the *cis*-isomer after irradiation at 365 nm. This would contribute to the enhancement of the properties of **dU^{Az}** in the "on" state. On the other hand, electron-withdrawing CF_3 and NO_2 groups strongly biased the equilibrium to the *trans* form before irradiation. This could suppress the undesired expression of **dU^{Az}** properties such as the hybridization ability before irradiation.

Modulating the rate of the thermal relaxation of the switched state is also very important because it realizes the spatiotemporal control of nucleic acid properties [27]. The thermal stability of the *cis*-isomer influences the method of utilization of photo-switches. *cis*-ON **28** (**X** = **dU^{Az}-OMe**) showed the shortest thermal half-life among the **dU^{Az}** analogue-modified ONs that we evaluated. This property is crucial for a photo-switch that thermally relaxes the hybridization ability. This would enable repeated activation with spatiotemporal resolution. On the other hand, ONs **30** and **31** (**X** = **dU^{Az}–CF$_3$** and **dU^{Az}–NO$_2$**) have a long half-life of the *cis*-form. In this case, it is possible to turn the hybridization property of **dU^{Az}** on and off using the appropriate wavelength of light.

The *para* substitutions decreased the affinity of ONs modified with **dU^{Az}** analogues against DNA but had little influence on that against RNA. These results suggest that *para* substitutions influence the duplex stability not electronically but sterically. The difference in the stability between DNA/DNA duplexes and DNA/RNA duplexes is attributed to the difference in the duplex structures. DNA/DNA duplexes tend to have B-form structures whereas DNA/RNA duplexes tend to have more A-form structures. Thus, the DNA/DNA duplex has a comparatively shallow major groove and *para*-substitution of the azobenzene moiety may sterically destabilize the duplex. The destabilization of duplexes in the *trans*-form may lead to an increase in the difference of hybridization ability between *trans*- and *cis*-**dU^{Az}** and improve the property of **dU^{Az}** for photo-switching hybridization.

We introduced various *para* substitutions into **dU^{Az}** and modulated the photo- and thermo-sensitivities for *trans*-*cis* isomerization. Our results represent an important strategy for optimization of the properties of photo-switches. For example, **dU^{Az}-OMe** showed better *trans* to *cis* isomerization efficiency and lower thermal stability of the *cis*-isomer than **dU^{Az}**. This analogue could be utilized as a useful photo-switch for situations in which a fast drop in activity is desired. **dU^{Az}**-modified ON shows an interesting hybridization property, namely the affinity of the *cis*-form against complementary strands is higher than that of the *trans*-form, different from most other photo-switches [16]. *Para*-substituted **dU^{Az}** analogues have the potential for unique nucleic acid probes and drugs that reversibly capture the target DNA and RNA with spatiotemporal control.

5. Conclusions

We synthesized photo-isomeric **dU^{Az}** analogues bearing various *para*-substituted azobenzene derivatives and investigated the influence of the *para* substitution on the photo-isomerization properties, thermal stabilities of the *cis*-isomer, and hybridization abilities. Electron-withdrawing substituents on **dU^{Az}** lowered the efficiency of photo-isomerization while improving the thermal stability of the *cis*-isomer. On the other hand, electron-donating substituents improved the efficiency of photo-isomerization while decreasing the thermal stability of the *cis*-isomer. According to UV-melting experiments, *para*-substitution of **dU^{Az}** tended to destabilize DNA/DNA duplexes but had little effect on the stabilities of the DNA/RNA duplexes regardless of the nature of the substituents. ITC experiments revealed that the duplex formed between **dU^{Az}-OMe**-modified ON and complementary RNA are more stable after photo-irradiation than that before photo-irradiation. This trend was observed in the duplex formed between **dU^{Az}-Me**-modified ON and complementary RNA. These results indicated that **dU^{Az}-OMe** and **dU^{Az}-Me** could regulate the stability of the DNA/RNA duplex by photo-irradiation similar to **dU^{Az}** and appropriate substitution could optimize the properties of **dU^{Az}** as light-responsive nucleic acid probes.

Acknowledgments

A part of this work was supported by KAKENHI from the Japan Society for the Promotion of Science (JSPS) and the Basic Science and Platform Technology Program for Innovative Biological Medicine from the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

Author Contributions

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

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Tashiro, R.; Sugiyama, H. Biomolecule-based switching devices that respond inversely to thermal stimuli. *J. Am. Chem. Soc.* **2005**, *127*, 2094–2097.
2. Hatano, A.; Makita, S.; Kirihara, M. Synthesis and redox-active base-pairing properties of DNA incorporating mercapto C-nucleosides. *Tetrahedron* **2005**, *61*, 1723–1730.
3. Wada, T.; Minamimoto, N.; Inaki, Y.; Inoue, Y. Peptide ribonucleic acid (PRNA). 2. A novel strategy for active control of DNA recognition though borate ester formation. *J. Am. Chem. Soc.* **2000**, *122*, 6900–6910.
4. Monroe, W.T.; McQuain, M.M.; Chang, M.S.; Alexander, J.S.; Haselton, F.R. Targeting expression with light using caged DNA. *J. Biol. Chem.* **1999**, *274*, 20895–20900.
5. 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. Engl.* **1999**, *38*, 2393–2395.
6. Szymanski, W.; Beierie, J.M.; Kistemaker, H.A.V.; Velema, W.A.; Feringa, B.L. Reversible photocontrol of biological systems by the incorporation of molecular photoswitches. *Chem. Rev.* **2013**, *113*, 6114–6178.
7. Cahova, H.; Jaschke, A. Nucleoside-based diarylethene photoswitches and their facile incorporation into photoswitchable DNA. *Angew. Chem. Int. Ed.* **2013**, *52*, 3186–3190.
8. Barrois, S.; Wagenknecht, H.A. Diarylethene-modified nucleotides for switching optical properties in DNA. *Beilstein J. Org. Chem.* **2012**, *8*, 905–914.
9. Ogasawara, S.; Maeda, M. Straightforward and reversible photoregulation of hybridization by using a photochromic nucleoside. *Angew. Chem. Int. Ed.* **2008**, *47*, 8839–8842.
10. Ogasawara, S.; Maeda, M. Photoresponsive 5'-cap for the reversible photoregulation of gene expression. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5457–5459.
11. Ogasawara, S. Control of cellular function by reversible photoregulation of translation. *Chem. Bio. Chem.* **2014**, *15*, 2652–2655.

12. Goodsell, D.S. The molecular perspective: Ultraviolet light and pyrimidine dimers. *Oncologist* **2001**, *6*, 298–299.
13. Asanuma, H.; Matsunaga, D.; Komiyama, M. Clear-cut photo-regulation of the formation and dissociation of the DNA duplex by modified oligonucleotide involving multiple azobenzenes. *Nucleic Acids Symp. Ser.* **2005**, *49*, 35–36.
14. 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**, doi:10.1039/b708952j.
15. Kamiya, Y.; Asanuma, H. Light-driven DNA nanomachine with a photoresponsive molecular engine. *Acc. Chem. Res.* **2014**, *47*, 1663–1672.
16. Mori, S.; Morihiro, K.; Obika, S. C5-azobenzene-substituted 2'-deoxyuridine-containing-oligodeoxynucleotides for photo-switching hybridization ability. *Molecules* **2014**, *19*, 5109–5118.
17. Beharry, A.A.; Woolley, G.A. Azobenzene photoswitches for biomolecules. *Chem. Soc. Rev.* **2011**, *40*, 4422–4437.
18. Zenkina, O.; Altman, M.; Leitus, G.; Shimon, L.J.W.; Cohen, R.; van Der Boom, M.E. From azobenzene coordination to aryl-halide bond activation by platinum. *Organometallics* **2007**, *26*, 4528–4534.
19. Kubischke, J.; Nather, C.; Herges, R. Synthesis of functionalized triazatriangulenes for application in photo-switchable self-assembled monolayers. *Eur. J. Org. Chem.* **2010**, *26*, 5041–5055.
20. Miao, Z.; Zhang, Y.; Zhao, Y.; Wang, Z.; Wang, D. Asymmetrical azobenzene liquid crystals with high birefringence prepared via click chemistry. *Mol. Cryst. Liq. Cryst.* **2014**, *591*, 10–18.
21. McDonagh, A.M.; Cifuentes, M.P.; Lucas, N.T.; Humphrey, M.G.; Houbrechts, S.; Persoons, A. Organometallic complexes for nonlinear optics Part 20. Syntheses and molecular quadratic hyperpolarizabilities of alkynyl complexes derived from (E)-4,4-HCCC6H4N=NC6H4NO2. *J. Organomet. Chem.* **2000**, *605*, 193–201.
22. 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.
23. Dhammika, H.M.; Burdette, S.C. Photoisomerization in different classes of azobenzene. *Chem. Soc. Rev.* **2012**, *41*, 1809–1825.
24. Nakagawa, M.; Rikukawa, M.; Watanabe, M.; Sanui, K.; Ogata, N. Electrochemical and photochromic properties of azopyridinium methylsulfates. *Chem. Lett.* **1994**, *23*, 1785–1788.
25. Takaishi, K.; Kawamoto, M. Synthesis and conformation of substituted chiral binaphthyl-azobenzene cyclic dyads with chiroptical switching capabilities. *Molecules* **2011**, *16*, 1603–1624.
26. Dokic, J.; Gothe, M.; Wirth, J.; Peters, M.V.; Schwarz, J.; Hecht, S.; Saalfrank, P. Quantum chemical investigation of thermal cis-to-trans isomerization of azobenzene derivatives: Substituent effects, solvent effects and comparison to experimental data. *J. Phys. Chem. A* **2009**, *113*, 6763–6773.
27. Velema, W.A.; Szymanski, W.; Feringa, B.L. Photopharmacology: Beyond proof of principle. *J. Am. Chem. Soc.* **2014**, *136*, 2178–2191.