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Photoisomerization of 2′-deoxyribofuranosyl and ribofuranosyl 2-phenylazoimidazole

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Abstract—Novel photo responsive nucleosides, 1-*N*-(2′-deoxyribofuranosyl) 2-phenylazoimidazole and 1-*N*-ribofuranosyl 2-phenylazoimidazole have been designed and synthesized. *trans–cis* Photoisomerizations of the nucleosides with irradiation at a specific wavelength have been observed and the isomerizations are perfectly reversible.

Photochemical control of biomolecules both in vitro and in vivo is of increasing interest in molecular and cellular biology.[1] For application to living systems, photochemical reactions are advantageous because the reactions can be controlled from outside without addition of chemicals for initiation of biomolecular's functions. Caged compounds which can be removed by photo irradiation have been attached to DNA, RNA, and proteins for initiation of biological phenomena.[1] Many cellular processes including gene expression and signal transduction need regulations in both positive and negative ways. Biomolecules possessing a photochemically controllable residue just like a switch are required for precise control of target biological processes. Azobenzene derivatives are the most commonly used photochemical switches for functionalization of the polymers and biomolecules.[2–4] For nucleic acids, azobenzene derivatives have been introduced to DNA strands using methylene chain units analogous to a ribose for control of double and triple helix forming activities.[5–8]

Since nucleic acid bases are packed into a double helix DNA by base stacking to maintain a canonical DNA backbone structure, an azobenzene derivative attached to C1′ position of a ribose is preferable for fixing a base to the original position on the ribose. Based on this notion, we designed and synthesized photo functionalized nucleosides 1-*N*-(2′-deoxyribofuranosyl) 2-phenylazoimidazole (**3**) and 1-*N*-ribofuranosyl 2-phenylazoimidazole (**5**) (Fig. 1).

Unnatural base 2-phenylazoimidazole (**1**) was prepared according to the previously reported method.[9] We tried two coupling procedures. The first procedure we used was the standard coupling method in which the hydrogen atom of N^1 on 2-phenylazoimidazole (**1**) was activated by sodium hydride in acetonitrile and then 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-D-ribofuranosyl chloride[10] was added for base–sugar coupling. α- and β-anomers were separated by a silica gel column chromatography and stereochemistry of the anomers was determined by differential nuclear Overhauser enhancement (NOE) spectroscopy.[11] Using this procedure, the isolated yield of the protected nucleosides was 48% and undesirable α-anomer was a major product. The ratio of the α- and β-anomer was 67:33. In the second procedure, 2-phenylazoimidazole was treated with potassium hydroxide in methylenechloride in the presence of tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) and then 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-D-ribofuranosyl chloride was added. We successfully obtained the β-anomer (**2**) in 53% yield and the production of the α-anomer was almost suppressed. Deprotection of **2** by saturated

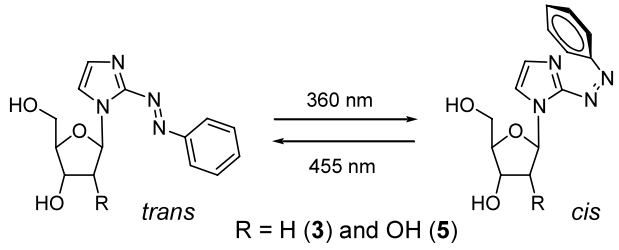

Figure 1. Schematic representation of *trans–cis* photoisomerization of the photo responsive nucleosides (**3** and **5**).

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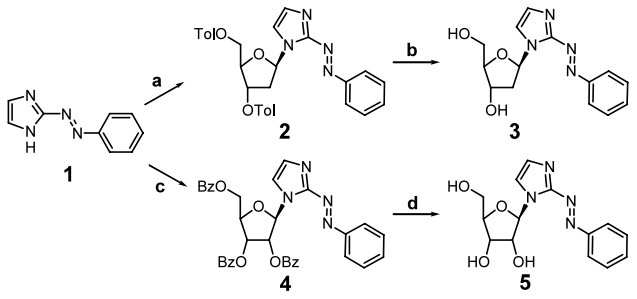

Figure 2. *Reagents*: (a) i. KOH, TDA-1, CH_2Cl_2, ii. 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-D-ribofuranosyl chloride. (b) NH_3/CH_3OH. (c) i. HMDS, TMS-Cl, ii. 1-*O*-acetyl 2,3,5-tri-*O*-benzoyl-D-ribofuranose, TMSOTf. (d) $NaOCH_3$/CH_3OH.

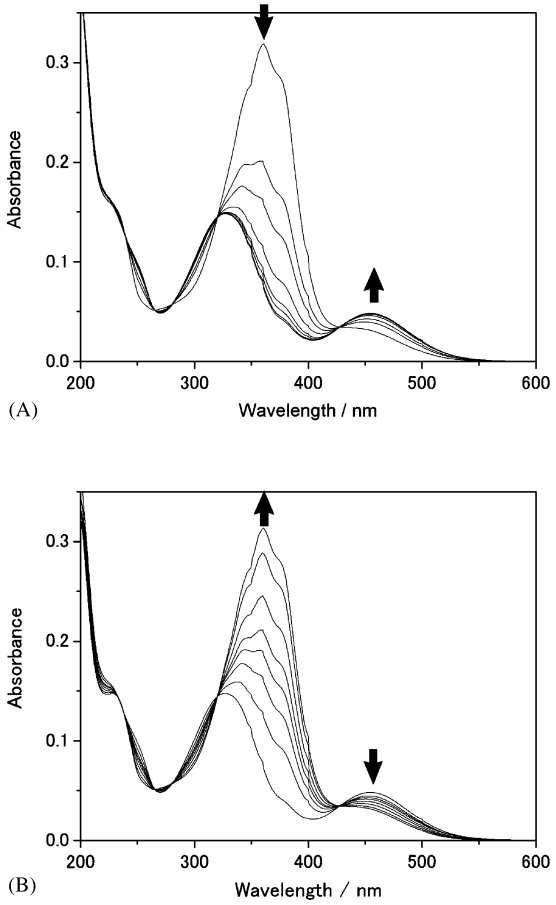

Figure 3. UV–vis spectra of photoisomerization of nucleoside **3**. (A) *trans* to *cis* isomerization with irradiation at 360 nm for 0, 5, 10, 20, 30, 40, 60, and 120 s. (B) *cis* to *trans* isomerization with irradiation at 455 nm for 0, 10, 20, 30, 40, 60, 120, and 300 s. Arrows indicate increase and decrease of absorption peaks.

methanoic ammonia at rt for 8 h gave nucleoside **3** in 78% yield (Fig. 2).[12]

For synthesis of ribonucleoside **4**, 2-phenylazoimidazole was refluxed in hexamethyldisilazane (HMDS) and small amount of trimethylsilyl chloride (TMS-Cl) for 1.5 h for silylation. After concentration, coupling of the silylated nucleoside and 1-*O*-acetyl 2,3,5-tri-*O*-bezoyl-β-D-ribofuranose was accomplished in acetonitrile with trimethylsilyl triflate (TMSOTf) at rt for 1 h. Purification by a silica gel column chromatography gave nucleoside **4** in 67% yield. Deprotection of **4** was carried out by sodium methylate at rt for 20 min. The reaction mixture was purified by a silica gel column chromatography and nucleoside **5** was isolated in 79% yield. Stereochemistry of nucleoside **5** was determined by differential NOE spectroscopy.[13,14]

To characterize photochemical behaviors of these synthetic nucleosides, we acquired UV/vis spectra for monitoring their absorption changes by photo irradiation. A spectrum of untreated **3** showed the two major peaks at 360 and 437 nm which are identified as characteristic π–π* and n–π* absorption bands of *trans* isomer of azobenzene derivatives, respectively.[15] By photo irradiation to a methanoic solution of **3** at 360 nm using a high-pressure mercury lamp, the peak at 360 nm rapidly decreased and largely shifted to 325 nm, while the peak at 437 nm increased and shifted to 455 nm[16] (Fig. 3(A)). The reaction completed within 2 min (Fig. 4(A)). When irradiation to this product (*cis*-**3**) was

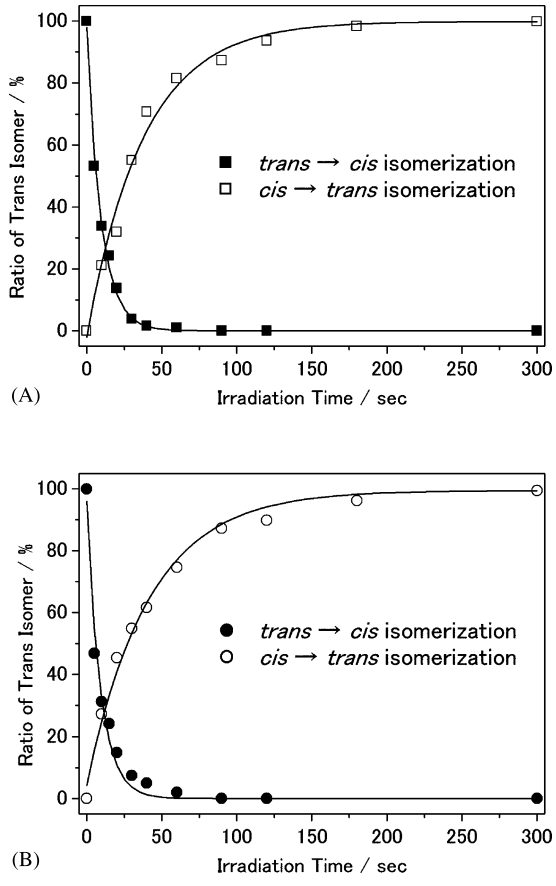

Figure 4. The ratios of *trans* isomers in *trans–cis* photoisomerization of nucleoside **3** (A) and **5** (B) under the experimental conditions in the text. The ratio was calculated from the absorption spectra.

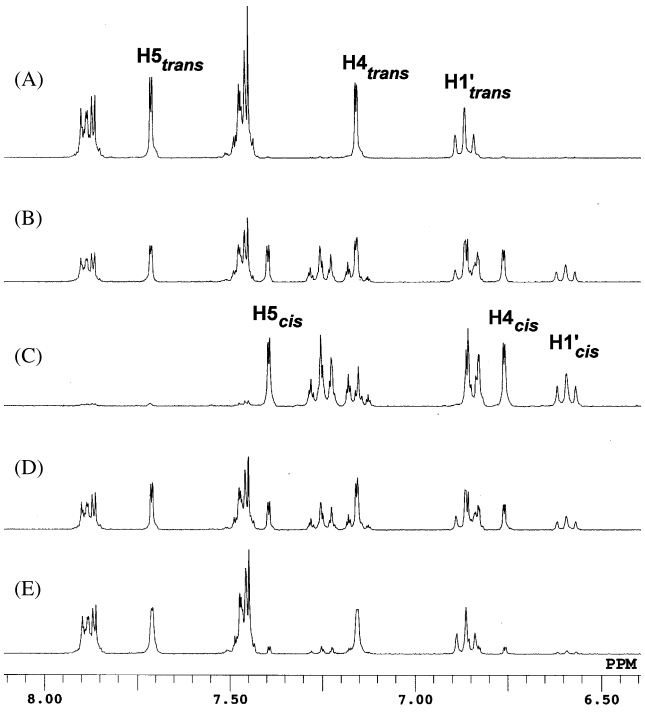

Figure 5. *cis* to *trans* photoisomerization of nucleoside **3** monitored by NMR spectroscopy. (A) Without irradiation. (B)(C) Irradiation at 365 nm for 1 and 20 min, respectively. (D)(E) Photo irradiation to the sample C using visible light for 5 and 20 min, respectively.

carried out using 455 nm light, the increasing peak at 325 nm shifted to 360 and the decreasing one at 455 nm shifted to 437 nm (Fig. 3(B)). The reaction completed within 5 min (Fig. 4(A)). This behavior is also characteristic for *cis* to *trans* isomerization of azobenzene derivatives, indicating reversible *trans–cis* photoisomerization of nucleoside **3** occurred under this condition.[15] The half-lives for *trans* to *cis* and *cis* to *trans* isomerizations of **3** are 7.1 and 35 s, respectively, under the present experimental conditions.

In the case of nucleoside **5**, a similar photoisomerization was observed by photo irradiation. The half-lives for *trans* to *cis* and *cis* to *trans* isomerizations of **5** are 9.4 and 40 s, respectively, under the present experimental conditions (Fig. 4(B)). The efficiencies of the photoisomerization were characterized by measuring quantum yields of these nucleosides. The quantum yields for *trans* to *cis* isomerizations of **3** and **5** in isopropanol were $\Phi = 0.08$ and 0.06, respectively.[17,18] Also the quantum yields for *cis* to *trans* isomerizations of **3** and **5** in isopropanol were $\Phi = 0.41$ and 0.34, respectively. These results indicate higher efficiency of the *trans–cis* isomerizations of **3** than those of **5**.[17,18] The photoisomerization efficiencies of **3** and **5** are lower than those of azobenzene, however, these nucleosides still maintain substantial potentials for photochemical switches.[19]

Further monitoring of *cis* and *trans* isomers of nucleoside **3** by photo irradiation was performed on an NMR spectroscopy (Fig. 5). The ratio of *trans* and *cis* isomers of **3** was also quantified. After irradiation at 365 nm for 20 min at 0°C,[20] the peaks identical to the *cis* isomer appeared and the ratio of *cis* and *trans* isomers of **3** was 96:4 based on the integration of the identical *trans* and *cis* peaks in the NMR spectrum (Fig. 5(C)). To investigate the behavior of *cis–trans* isomerization, the *cis* isomer of nucleoside **3** was exposed to visible light.[21] UV/vis spectra taken at several intervals showed slower spectral change than that of *trans* to *cis* isomerization. The *cis* to *trans* isomerization of **3** was observed and the ratio of the *trans* and *cis* isomers in the spectrum for 20 min irradiation was 92:8 under this experimental condition (Fig. 5(E)). No trace of degraded products was observed in the NMR spectra, indicating the *cis–trans* photoisomerization of nucleoside **3** is perfectly reversible.

In conclusion, we have synthesized the photo responsive 2′-deoxyribonucleoside and ribonucleoside possessing 2-phenylazoimidazole. These nucleosides show photoisomerization functionality similar to commonly used azobenzene derivatives and work as reversible photochemical switches.

Acknowledgements

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11. In the differential NOE spectrum of the product **2** irradiated at C1′ proton, 2″H, 4′H and 5H were enhanced, which is identical to a β-anomer. On the other hand, 2′H, 3′H and 5H were enhanced for the counterpart diastereomer of **2**, which is identical to an α-anomer.

12. ^{1}H NMR of **3** [CD_3OD (TMS)]; δ 7.98 (m, 2H, Ph), 7.80 (d, $J=1.4$ Hz, 1H, 5), 7.57 (m, 3H, Ph), 7.25 (d, $J=1.4$ Hz, 1H, 4), 6.96 (t, $J=6.5$ Hz, 1H, 1′), 4.52 (m, 1H, 3′), 4.03 (m, 1H, 4′), 3.76 (ddd, $J=4.1$, 11.9, 16.5 Hz, 2H, 5′), 2.52 (m, 2H, 2′). ESI-MS (positive) m/z calcd for $C_{14}H_{16}N_4O_3Na=311.1$; obsd 311.1 $[M+Na]^+$.
13. In the differential NOE spectrum of nucleoside **5** irradiated at C1′ proton, 2′H, 4′H, 5H and aromatic protons were enhanced, which is identical for a β-anomer.
14. ^{1}H NMR of **5** [CD_3OD (TMS)]; δ 8.00 (m, 2H, Ph), 7.85 (d, $J=1.4$ Hz, 1H, 5), 7.54 (m, 3H, Ph), 7.27 (d, $J=1.4$ Hz, 1H, 4), 6.57 (d, $J=5.0$ Hz, 1H, 1′), 4.40 (t, $J=5.0$ Hz, 1H, 2′), 4.30 (t, $J=4.3$ Hz, 1H, 3′), 4.12 (m, 1H, 4′), 3.82 (ddd, $J=3.0$, 12.2, 27.8 Hz, 1H, 5′). ESI-MS (positive) m/z calcd for $C_{14}H_{16}N_4O_4Na=327.1$; obsd 327.0 $[M+Na]^+$.
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16. Photo irradiation was carried out in a 1.0 mL of methanoic solution containing 20 μM of nucleoside **3** or **5** by using a 500 W ultra high pressure mercury lamp (Ushio USH-500D; 500 W) equipped with a monochrometor (Ritsuoyo Kogaku MC-10N) which can control specific wavelength within 4 nm full-width at half maximum. A quartz cell with 1 cm path length was used and the sample was cooled to 0°C in a cell holder equipped with a circular temperature controller. UV/vis spectra were obtained at 0°C.
17. The quantum yields for *trans* to *cis* isomerization of nucleoside **3** and **5** were measured at rt referring to the quantum yields of *trans* to *cis* and *cis* to *trans* photoisomerizations of azobenzene ($\Phi=0.14$ and 0.42, respectively) as standard.
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19. Thermal isomerizations from *cis* to *trans* isomer were observed. When *cis* isomers of nucleoside **3** and **5** were left at 20°C in methanol, the *cis* isomers slowly went back to the *trans* isomers. The rate constants for **3** and **5** are 0.18 h^{-1} ($\tau_{1/2}=4.0$ h) and 0.13 h^{-1} ($\tau_{1/2}=5.4$ h), respectively.
20. Photo irradiation to observe *trans* to *cis* isomerization was performed in a 0.6 mL of CD_3OD solution containing 20 mM of **3** in an NMR tube using a UV transilluminator (UVP LMS-20E; 40 W) at 365 nm on ice.
21. Conversion from the *cis* to *trans* isomer was carried out in the same NMR sample by irradiation using the same ultra high-pressure mercury lamp with a UV cut-off filter below 430 nm wavelength (Sigma Koki UTF-50-43Y cut-off filter).