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Synthesis of 8-(2′-deoxy-β-D-ribofuranosyl)-imidazo[1,2-*a*]-*s*-triazin-4-one

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Abstract—We describe the synthetic strategy of a purine like nucleoside and the separation of an epimeric mixture. The nucleoside possesses two proton acceptor sites, and they are expected to provide a new base pairing pattern and new protein-DNA targeting. These isomeric structures were determined by conducting an NOE experiment.

Unnatural nucleotides are significant in biotechnology, for example as antisense sequences, to insert mutations and for increasing the variation of aptamers. Multiple hydrogen bonds, as shown in the Watson–Click hydrogen bonding, are essential for the construction of base pairs and for specific recognition. Furthermore, they play a critical role in the storing and transmitting of genetic information and for the maintenance of the double helix structures. Benner reported several unnatural base pairs that possess various hydrogen bond patterns.[1]

A base pairing pattern with all acceptor sites (A-sites) on one side and all donor sites (D-sites) on the other side was not reported. Jorgensen has described a fairly large contribution of the secondary electrostatic interactions between the adjacent sites in a complex. Because of this contribution, an AA–DD type complex for which all D-sites are located in one molecule and all A-sites in the other molecule will be more stable than a DA–AD type complex. The difference in stabilizing energy is approximately 11 kcal/mol.[2] Urea and guanidine groups are examples of DD-type moieties, and therefore AA-type nucleosides may have strong interactions with these groups. There have been reports on pyrimidine-like nucleosides, but not purine-like ones, that have all A-sites on one side.[3] Here, we present the synthesis and spectroscopical determination of a purine-like nucleoside **1** that consists of an AA-type hydrogen bonding site. We used imidazo[1,2-*a*]-*s*-triazin-4-one[4] as an AA-type base (see Fig. 1).

The route to nucleoside **1** is illustrated in Scheme 1. The heterocyclic system **2** was prepared from the reaction of chloroacetaldehyde with 5-azacytosine using the method reported by Nair et al.[4] The glycosylation of silylated **2** with 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose[5] in CH_3CN in the presence of TMS triflate as a Lewis acid catalyst resulted in a mixture of α- and β-isomers in 44% yield, specified as **3** in Scheme 1.[6] By comparing the ^{13}C NMR chemical shifts of **2** and **3** (Table 1), a large upfield shift was observed for C-7 and a small downfield shift was observed for the C-6, indicating that N-8 is the ribosylation site.[7] The ratio of this isomeric mixture was 1:1 and it was determined by the C-1′ proton (6.55

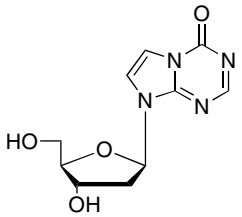

Figure 1. Nucleoside **1**.

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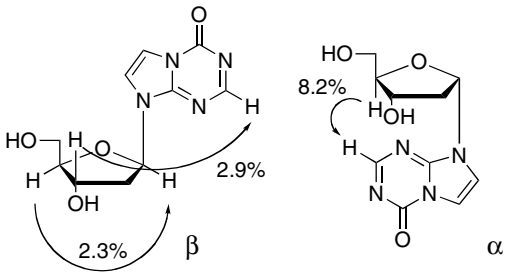

Figure 2. Correlation of NOE.

Table 1. ^{13}C NMR chemical shifts of **2** and **3**

	Chemical shift, δ, ppm				
	C2	C4	C6	C7	C9
2[a]	148.3	149.7	110.8	126.2	144.5
3[b]	151.2 150.1	148.8	109.2 108.5	115.5 115.1	148.4

[a] In DMSO-d_6.
[b] In $CDCl_3$, α and β mixture.

and 6.48 ppm) on the ribose using ^1H NMR spectroscopy. We were not able to separate this mixture.

The protecting acetyl group was then removed with a mixture of methanol and 28% NH_3 solution. However, also this isomeric mixture was difficult to separate even though we used silica gel column chromatography, reverse phase column on HPLC, as well as recrystallization. Thus, we introduced a trityl group at 5′-OH to separate the mixture.[8] The purification of the isomeric mixture was accomplished with a column chromatography on silica gel. After removing the trityl group, the stereochemistry at the nominal anomeric center was determined with ^1H NMR, with ^1H–^1H correlation methods (COSY), and with NOE experiments. In material with a higher R_f value on silica gel (Fig. 2),[9] the C-5′ protons were shifted downfield because they were deshielded by a heterocyclic base. Further, interactions between C-1′ and C-4′ protons, as well as between C-3′ and Ar-2 protons were detected in NOE experiments. We concluded that this isomer has a β form because the interactions were same as described by Nair et al.[4] On the other hand, the downfield shift depending on the deshielding effect appeared at the C-4′ proton in material with lower R_f on silica gel.[10] Furthermore, the irradiation of the C-4′ proton resulted in an enhancement at Ar-2 proton (Fig. 2). Thus, this isomer has an α-form.

In summary we have synthesized a new unnatural nucleoside **1** possessing a solely proton acceptor site. It was difficult to separate α and β anomers in the glycosylation step, but we could separate these anomers after introducing a trityl group. Nucleoside **1** is expected to be used as a new application in aptamer and molecular recognition when it is introduced in a DNA oligomer.

Acknowledgments

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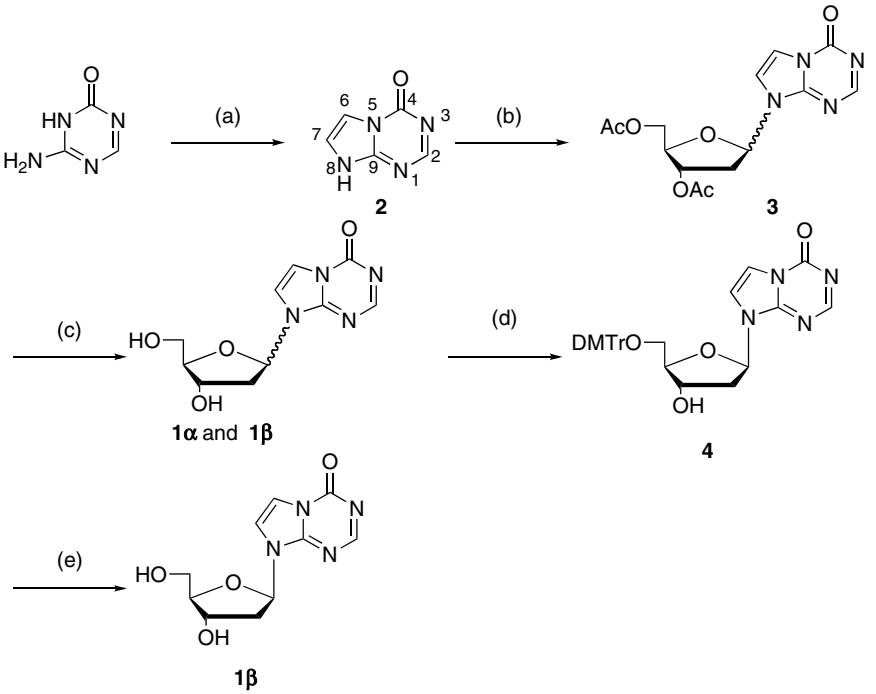

Scheme 1. Reagents and conditions: (a) Chloroacetaldehyde, H_2O, 37 °C, 5 days, 50%; (b) (i) BSA, CH_3CN, rt, 110 min; (ii) 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose, TMSOTf, rt, 18 h, 44%; (c) 28% NH_{3aq}, MeOH, rt, 1 h, 69%; (d) DMTrCl, Et_3N, pyridine, rt, 3 h, α: 42%, β: 26%; (e) Cl_3CCOOH, CH_2Cl_2, rt, 2 min, 87%.

of Education, Culture, Sports, Science and Technology of the Japanese Government to Y.H.

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6. Imidazo[1,2-*a*]-*s*-triazin-4-one (0.663 g, 4.9 mmol) was placed in 40 ml of acetonitrile. The suspension was purged with nitrogen, followed by the addition of *N*,*O*-bis(trimethylsilyl)acetamide (1.8 ml, 7.4 mmol). The resulting solution was stirred for 110 min at rt. A solution of 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose (1.277 g, 4.9 mmol) in 15 ml of acetonitrile was added to the base solution, followed by the addition of trimethylsilyl triflate (0.4 ml, 2.2 mmol). The resulting solution was stirred for 18 h at rt. 40 ml of water was added to the reaction mixture and neutralized with saturated aqueous sodium bicarbonate. The solvent was removed under reduced pressure, and the residue was partitioned between dichloromethane and saturated aqueous sodium bicarbonate. The aqueous layer was extracted with ethyl acetate (40 ml × 3). The combined organic layers were washed with water (50 ml × 3) and brine (50 ml) and dried with magnesium sulfate, concentrated, and purified by silica gel chromatography with hexane–acetone (1:2) to give 1:1 mixture of the α, β isomers of **3** (0.714 g, 44%) as an oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (m, 2H), 7.62–7.57 (m, 2H), 7.42 (d, 1H, $J = 2.8$ Hz), 7.37 (d, 1H, $J = 2.4$ Hz), 6.55 (m, 1H), 6.48 (m, 1H), 5.38–5.33 (m, 2H), 4.67–4.60 (m, 1H), 4.40–4.18 (m, 5H), 3.02–2.95 (m, 1H), 2.68–2.58 (m, 3H), 2.16–2.03 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 169.7, 150.2, 150.1, 148.8, 148.4, 115.5, 115.1, 109.2, 108.5, 85.8, 84.7, 84.6, 74.3, 74.0, 63.6, 38.1, 37.8, 37.7, 20.8.
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9. To a solution of **4β** (0.071 g, 0.13 mmol) in dichloromethane (2 ml) was added trichloroacetic acid (0.075 g, 0.46 mmol). The solution was stirred for 2 min at rt. The mixture was purified by silica gel column chromatography with dichloromethane–methanol (10:1) to give **1β** (0.028 g, 87%): ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61 (s, 1H), 7.74 (d, 1H, $J = 1.5$ Hz), 7.38 (d, 1H, $J = 1.5$ Hz), 6.29 (t, 1H, $J = 6.3$ Hz, C-1′), 5.40–5.05 (br, 2H, 3′-OH and 5′-OH), 4.35–4.28 (m, 1H, C-3′), 3.90–3.84 (m, 1H, C-4′), 3.72–3.57 (m, 2H, C-5′), 2.42–2.35 (m, 1H, C-2′), 2.34–2.26 (m, 1H, C-2′).
10. To a solution of **4α** (0.133 g, 0.24 mmol) in dichloromethane (10 ml) was added trichloroacetic acid (0.124 g, 0.76 mmol). The solution was stirred for 2 min at rt. The mixture was purified by silica gel column chromatography with dichloromethane–methanol (10:1) to give **1α** (0.060 g, 99%): ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.46 (s, 1H), 7.74 (d, 1H, $J = 1.5$ Hz), 7.38 (d, 1H, $J = 1.5$ Hz), 6.27 (d, 1H, $J = 5.9$ Hz, C-1′), 5.30 (d, 1H, $J = 2.9$ Hz, 3′-OH), 4.93–4.91 (m, 1H, 5′-OH), 4.40–4.20 (m, 2H, C-3′ and C-4′), 3.45–3.38 (2H, m, C-5′), 2.72–2.60 (m, 1H, C-2′), 2.20–2.14 (m, 1H, C-2′).