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# Chemo-enzymatic synthesis of 2′,3′-dideoxy-3′-fluoro-β-D-guanosine via 2,3-dideoxy-3-fluoro-α-D-ribose 1-phosphate

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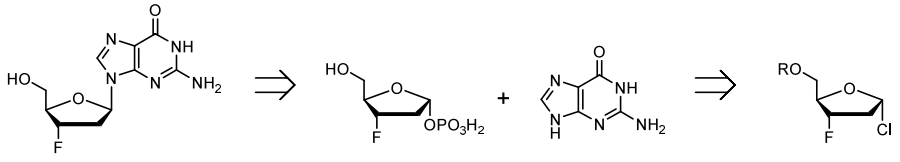
**Abstract**—2,3-Dideoxy-3-fluoro-α-D-ribose 1-phosphate **2** was stereoselectively synthesized and converted to 2′,3′-dideoxy-3-fluoro-β-D-guanosine **1** by enzymatic reaction using purine nucleoside phosphorylase. This chemo-enzymatic strategy was first applied to the synthesis of **1**. © 2003 Elsevier Science Ltd. All rights reserved.

Stereoselective glycosylation has attracted considerable attention in the field of nucleoside chemistry. For medicinal use, by-product content must be strictly controlled. Therefore, synthetic methods that do not produce isomers are preferable. A number of methods have been developed;[1] however, glycosylation with a guanine base **3** has been an obstacle for application to scalable chemical preparations. In contrast, enzymatic reaction with **3** affords β-nucleosides when using purine nucleoside phosphorylase (PNPase) as the enzyme with α-D-furanose 1-phosphate as the substrate for the reaction.[2] This strategy has not been very successful, however, due to difficulties in preparing the furanose 1-phosphate. We previously reported chemo-enzymatic synthesis of 2′-deoxy-β-D-nucleoside via the chemical synthesis of 2-deoxy-α-D-ribose 1-phosphate.[3] As shown in Scheme 1, this strategy is applicable to the synthesis of 2′,3′-dideoxy-3′-fluoro-β-D-guanosine **1** that has prominent antiviral activity.[4] Here we report chemo-enzymatic synthesis of **1**, in which 2,3-dideoxy-3-fluoro-α-D-ribose 1-phosphate **2** is first synthesized in a stereoselective manner and is then enzymatically converted to **1**.

First, chloro sugar **6** was prepared starting from methyl furanoside **4** (Scheme 2).[5] Because **6** is extremely labile under acidic conditions and prone to decomposition via furan derivatives by losing HCl and HF even at room temperature, direct conversion of **4** to **6** was avoided. Reaction of **4** with $Ac_2O$ in AcOH in the presence of $H_2SO_4$ gave an anomeric mixture of **5** (α:β = 2:1) followed by conversion to **6** (α:β = 2:1) using the stoichiometric amounts of HCl. Both of the reactions had to be maintained at 0°C to avoid decomposition of **5** and **6**.

Stereoselective phosphorylation of **6** was then investigated. Even though β-isomer of **2** was not likely to be recognized by PNPase and would not give an α-isomer of **1**, high stereoselectivity of the phosphorylation was required to increase the isolated yield of **1**. A coupling reaction of **6** with *o*-$H_3PO_4$ in the presence of $^nBu_3N$ was performed. In the first attempt, the reaction gave **7**




**Scheme 1.** Retrosynthesis of 2′,3′-dideoxy-3′-fluoro-β-D-guanosine **1**.

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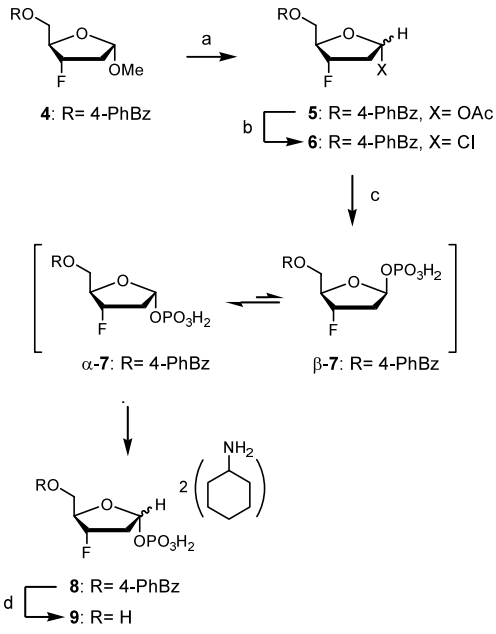
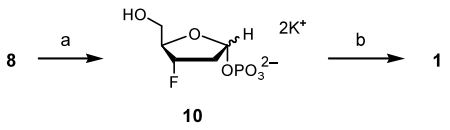



**Scheme 2.** *Reagents and conditions*: (a) $Ac_2O$, AcOH, $H_2SO_4$, 0°C, 2.5 h; (b) 4N HCl/1,4-dioxane, *c*-$C_6H_{12}$/$PhCH_3$, 0°C, 8 h; (c) *o*-$H_3PO_4$, *n*$Bu_3N$, 4 Å MS, $CH_3CN$/MIBK, 0°C, 6 h, then *c*-$C_6H_{11}NH_2$ (59% from **4**); (d) *c*-$C_6H_{11}NH_2$, MeOH, rt, 50 days (79%).

with no stereoselectivity at the anomeric C1-position (α:β = ca. 1:1). Under acidic conditions in the presence of excess amounts of *o*-$H_3PO_4$, however, equilibration between α- and β-**7** occurred and gradually shifted to the thermodynamically more stable α-**7**.

The selectivity was dependent on the solvent used for the reaction. As depicted in Table 1, a solvent with a large dielectric constant led to high stereoselectivity at the state of equilibrium saturation (entries 2–5). The reaction in propylene carbonate (PC) with the largest dielectric constant resulted in the highest stereoselectivity (α-**7**:β-**7** = 81:19) in our experiments (entry 5). To investigate the relation between the solvent effect and stereoselectivity, the heats of formation in each solvent were calculated with MOPAC/PM3[7] using the conductor-like screen model (COSMO) routine;[8] the results are shown in Table 1 as the difference ($\Delta H = H\alpha - H\beta$) between the heats of formation of α-**7** (Hα) and β-**7** (Hβ). The α-**7** was stabilized in solvents with a large dielectric constant ($CH_3CN$ and PC) and the stabilization led to the high stereoselectivity (compare entries 2–5 with entry 1). The relatively larger dipole moment of α-**7** (3.72 Debye) than of β-**7** (3.62 Debye) was attributable to the stabilization. The largest value of the dielectric constant of PC, however, did not directly affect the heat of formation energy (entry 5). The accurate ionized state of phosphate **7** in the reaction mixture should be used to calculate the heat of formation for a more precise explanation of the effect of PC.




**Scheme 3.** *Reagents and conditions*: (a) aq. KOH, 48 h, 50°C; (b) **3**, $CaCl_2$, PNPase, 40–60°C, 5 h (63% from **8**).

A synthetic sample of **7** was prepared in a $CH_3CN$/4-methyl-2-pentanone (MIBK) (10:1) mixture solvent. HPLC analysis of the reaction revealed that the solvent system affords a stereoselectivity of 78:22 (α-**7**:β-**7**). Phosphate **8** was isolated as cyclohexylammonium salt in 59% yield from **4** and the stereoselectivity was 80:20 (α:β). The structure of **8** was confirmed by spectroscopic methods ($^1H$, $^{13}C$, and $^{31}P$ NMR, and IR) and mass spectral analysis.[9] Without further purification, deprotection was performed in MeOH in the presence of cyclohexylamine. Because the reaction was too slow (50 days at room temperature for completion) to scale up this reaction condition, saponification in aqueous KOH was performed. The potassium salt of the desired compound **10** was obtained as an aqueous solution after filtration to remove potassium 4-phenylbenzoate (Scheme 3). The structure of the substrate for the key enzymatic reaction was confirmed as cyclohexylammonium salt **9** (α:β = 87:13) by spectroscopic methods ($^1H$, $^{13}C$, and $^{31}P$ NMR, and IR) and mass spectral analysis.[10]

Finally, the enzymatic glycosylation was performed by using the aqueous solution of **10** and guanine **3**. Conventional procedure[2] was modified in two ways. First, a smaller amount of **3** was used in this reaction since **3** was difficult to remove by recrystallization in the work-

**Table 1.** Solvent effect in phosphorylation

| Entry | Solvent | α-**7**:β-**7**[a] | Dielectric constant[b] | Heat of formation $\Delta H$ (kJ/mol)[c] |
|---|---|---|---|---|
| 1 | $PhCH_3$ | 49:51 | 2.4 | 1.7 |
| 2 | Acetone | 71:29 | 20.7 | −5.7 |
| 3 | 2-Butanone | 73:27 | 18.5 | −6.2 |
| 4 | $CH_3CN$ | 79:21 | 37.5 | −8.1 |
| 5 | PC[d] | 81:19 | 64.4 | −6.9 |

[a] All amomer ratios were analyzed by HPLC.
[b] All data were taken from Handbook of Organic Chemistry.[6]
[c] All values were calculated with MOPAC/PM3 using the COSMO routine and shown as $\Delta H$ ($\Delta H = H\alpha - H\beta$) where Hα and Hβ are the heats of formation of α-**7** and β-**7**, respectively.
[d] PC, propylene carbonate.

up. Second, $CaCl_2$ was added to enhance the conversion by removing $H_3PO_4$ as Ca salts from the reaction solution. Consequently, **10** was converted to **1** by using 0.84 equiv. of guanine **3** in the presence of bacterial PNPase[2,11] in 71% HPLC yield from **8**. Based on the α-isomer content, the conversion yield was 89% from **8**. The desired **1** was the only product observed in the HPLC assay and was isolated by crystallization directly from the reaction mixture in pure form. The isolated yield was 63% from **8**. The structure of **1** was confirmed by spectroscopic methods ($^1H$ and $^{13}C$ NMR, and IR) and mass spectral analysis.[12] They were identical with the reported data.[4]

In summary, stereoselective synthesis of 2,3-dideoxy-3-fluoro-α-D-ribose 1-phosphate **2** (its cyclohexylammonium salt **9** and potassium salt **10**) was achieved and its enzymatic conversion to 2′,3′-dideoxy-3′-fluoro-β-D-guanosine **1** is presented. This chemo-enzymatic strategy produced no isomers of **1**, thus providing an alternative synthetic route for the preparation of various unnatural nucleosides.

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9. Compound **8** (α:β = 80:20): mp 146–150°C; $[\alpha]_D^{28} = +2.8°$ (*c* 2.7, $CH_3OH$); $^1H$ NMR ($CD_3OD$, 400 MHz): $\delta$ 8.13 (d, $J = 8.3$ Hz, 1/5 of 2H), 8.08 (d, $J = 8.3$ Hz, 4/5 of 2H), 7.75 (d, $J = 8.3$ Hz, 2H), 7.67 (d, $J = 8.3$ Hz, 2H), 7.47 (dd, $J = 8.3$ and 7.3 Hz, 2H), 7.39 (m, 1H), 6.02 (m, 1/5H), 5.96 (dd, $J = 5.6$ and 4.6 Hz, 4/5H), 5.34 (bd, $J = 54.6$ Hz, 1/5H), 5.26 (bd, $J = 55.6$ Hz, 4/5H), 4.71 (bd, $J = 25.7$ Hz, 4/5H), 4.49 (dd, $J = 13.2$, 4.1 Hz, 1H), 4.42 (dd, $J = 13.2$, 4.1 Hz, 1H), 3.00 (m, 2H), 2.54–2.32 (m, 2H), 2.01 (m, 4H), 1.78 (m, 4H), 1.65 (d, $J = 12.4$ Hz, 2H), 1.32 (m, 8H), 1.20 (m, 2H); $^{13}C$ NMR ($CD_3OD$, 100 MHz): $\delta$ 167.58 (β), 167.53 (α), 147.42, 141.07 (α), 147.35 (β), 141.13 (β), 141.07 (α), 131.33 (β), 131.21 (α), 130.08 (α), 129.80 (β), 129.38 (α), 129.83 (β), 129.74 (α), 128.24 (α), 128.19 (α), 128.15 (β), 101.59 (β), 101.20 (d, $J = 4.1$ Hz, α), 95.89 (d, $J = 177.6$ Hz, β), 95.22 (d, $J = 179.5$ Hz, α), 83.72 (d, $J = 27.3$ Hz, α), 83.23 (d, $J = 24.8$ Hz, β), 66.04 (d, $J = 9.9$ Hz, β), 65.36 (d, $J = 9.1$ Hz, α), 51.25, 41.96 (dd, $J = 20.7$, 6.2 Hz, α), 32.84, 26.11, 25.58; $^{31}P$ NMR ($CD_3OD$, 162 MHz): $\delta$ 1.57 (α), 1.42 (β); IR ($cm^{-1}$, KBr): 3424, 2938, 2858, 1709, 1612, 1561, 1451, 1390, 1279, 1084, 980, 935, 748, 699; MS (APCI) *m*/*z* 395 $(M-H)^-$.
10. Compound **9** (α:β = 87:13): mp 170–171°C (dec.); $[\alpha]_D^{28} = +26.1°$ (*c* 5.0, $H_2O$); $^1H$ NMR ($D_2O$, 400 MHz): $\delta$ 5.64 (dd, $J = 5.6$, 5.6 Hz, 1/6H), 5.61 (dd, $J = 5.7$, 5.4 Hz, 5/6H), 5.02 (dd, $J = 54.6$, 6.1 Hz, 1/6H), 4.96 (dd, $J = 54.9$, 5.1 Hz, 5/6H), 4.30 (ddd, $J = 27.1$, 5.1, 4.4 Hz, 5/6H), 4.06 (ddd, $J = 24.2$, 5.4, 6.1 Hz, 1/6H), 3.5–3.3 (m, 2H), 2.90 (m, 2H), 2.2–2.1 (m, 2H), 1.74 (m, 4H), 1.56 (m, 4H), 1.41 (d, $J = 11.7$ Hz, 2H), 1.10 (m, 8H), 0.95 (m, 2H); $^{13}C$ NMR ($D_2O$, 100 MHz): $\delta$ 100.41 (d, $J = 4.0$ Hz, α), 95.52 (d, $J = 173.4$ Hz, α), 95.31 (d, $J = 171.4$ Hz, β), 85.83 (d, $J = 23.8$ Hz, α), 85.43 (d, $J = 23.0$ Hz, β), 62.30 (d, $J = 10.7$ Hz, β), 62.02 (d, $J = 10.7$ Hz, α), 51.03, 41.5 (dd, $J = 18$, 5.7 Hz, β), 41.13 (dd, $J = 18.9$, 4.9 Hz, α), 31.32, 25.10, 24.61; $^{31}P$ NMR ($D_2O$, 162 MHz): $\delta$ 1.63 (β), 1.57 (α); IR ($cm^{-1}$, KBr): 2936, 2858, 2560, 2209, 1625, 1561, 1453, 1392, 1243, 1066, 980, 933, 844, 732; MS (APCI) *m*/*z* 215 $(M-H)^-$.
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12. Compound **1**: mp 253–255°C (dec.); $[\alpha]_D^{23} = -32.7°$ (*c* 0.0585, $H_2O$); $^1H$ NMR (DMSO-$d_6$, 270 MHz): $\delta$ 10.6 (s, 1H), 7.92 (s, 1H), 6.44 (s, 2H), 6.15 (dd, $J = 9.2$, 5.7 Hz, 1H), 5.37 (dd, $J = 53.7$, 4.3 Hz, 1H), 5.11 (bs, 1H), 4.15 (ddd, $J = 27.0$, 5.1, 4.6 Hz, 1H), 3.56 (m, 2H), 2.79 (dddd, $J = 39.4$, 14.6, 9.2, 4.3 Hz, 1H), 2.58 (ddd, $J = 21.1$, 14.6, 5.7 Hz, 1H); $^{13}C$ NMR (DMSO-$d_6$, 67.5 MHz): $\delta$ 156.67, 153.70, 151.00, 135.31, 94.94 (d, $J = 173.8$ Hz), 85.17 (d, $J = 22.4$ Hz), 82.64, 60.98 (d, $J = 10.6$ Hz), 36.87 (d, $J = 20.7$ Hz); IR ($cm^{-1}$, KBr): 3440, 3320, 3166, 1691, 1636, 1600, 1402, 1105, 1058, 945, 780; MS (APCI) *m*/*z* 270 $(M+H)^+$.