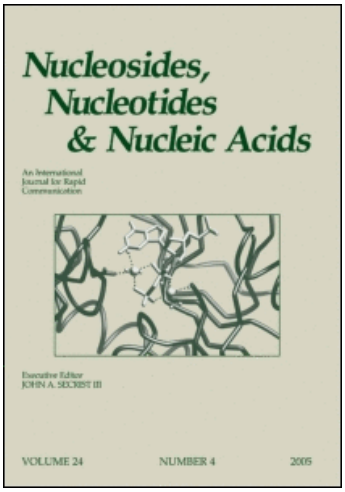

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 24 November 2009

To cite this Article Yamada, Kohei , Matsumoto, Noritake and Hayakawa, Hiroyuki(2009) 'Stereoselective Synthesis of 2-Deoxy-2-Fluoroarabinofuranosyl-α-1-Phosphate and Its Application to the Synthesis of 2'-Deoxy-2'-Fluoroarabinofuranosyl Purine Nucleosides by a Chemo-Enzymatic Method', Nucleosides, Nucleotides and Nucleic Acids, 28: 11, 1117 — 1130
To link to this Article: DOI: 10.1080/15257770903396741
URL: http://dx.doi.org/10.1080/15257770903396741

Nucleosides, Nucleotides and Nucleic Acids, 28:1117–1130, 2009

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770903396741

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STEREOSELECTIVE SYNTHESIS OF 2-DEOXY-2-FLUOROARABINOFURANOSYL-α-1-PHOSPHATE AND ITS APPLICATION TO THE SYNTHESIS OF 2′-DEOXY-2′-FLUOROARABINOFURANOSYL PURINE NUCLEOSIDES BY A CHEMO-ENZYMATIC METHOD

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□ *Stereoselective introduction of a phosphate moiety into 2-deoxy-2-fluoroarabinofuranose derivatives at the anomeric position was investigated by two methods. One involved a stereoselective hydrolysis of 1-bromo-derivative, and the consecutive phosphorylation of 2-deoxy-2-fluoro-α-D-arabinofuranose via a phosphoramidite derivative. The other method involved stereoselective α-phosphorylation of the 1-bromo-derivative at the 1-position. The resulting α-1-phosphate was utilized to prepare 2′-deoxy-2′-fluoroarabinofuranosyl purine nucleosides by an enzymatic glycosylation reaction. This chemo-enzymatic method will be applicable to the synthesis of some 2′F-araNs, and three important 2′F-araNs were actually obtained in 30–40% yields from 1,3,5-tri-O-benzoyl-2-deoxy-2-fluoro-α-D-arabinose with high purity.*

Keywords Chemo-enzymatic synthesis; α-phosphorylation; purine nucleoside phosphorylase; 2′-deoxy-2′-fluoroarabinofuranosyl nucleosides

INTRODUCTION

Arabinose-derived antisense oligonucleotides (2′-deoxy-2′-fluoro-β-D-arabinofuranose nucleic acids, 2′F-ANA) have efficient gene silencing abilities and potential for therapeutic use.[1] 2′-Deoxy-2′-fluoroarabinofuranosyl nucleosides (2′F-araNs) are key materials for 2′F-ANA. However, there are no efficient methods to make 2′F-araNs except that 2′-deoxy-2′-

Received 24 August 2009; accepted 8 October 2009.

The authors are grateful to Prof. Dr. M.J. Damha of McGill University for helpful discussions and providing us authentic samples. They are also grateful to Drs. T. Hamamoto, K. Okuyama, and T. Noguchi of Yamasa Corporation for helpful discussion and providing us with NPases; and to Dr. K. Kodama of Yamasa Corporation for helpful discussions and for supporting this work.

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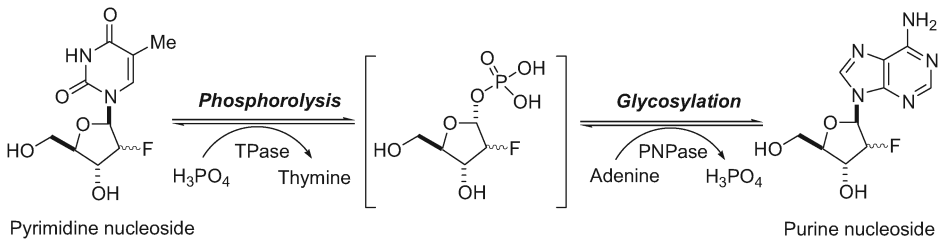
fluoroarabinofuranosyl pyrimidine nucleosides can be prepared chemically. In general, it is difficult to prepare the purine 2′F-araNs chemically from natural ribo-nucleosides, because purine-base migration to the 2′-position occurs, accompanied with an activation of the 2′-hydroxyl group during fluorination.[2] (A facile method to introduce fluorine at the up-side of the 2′-carbon of the 6-chloropurine riboside derivative was reported. See ref.[2b]) Additionally, an attempt to condense 2-deoxy-2-fluoroarabinose derivatives with purine bases failed to prevent formation of some isomers (stereo- and positional-isomer),[3,4] in contrast to pyrimidine bases,[5] and the separation of these isomers was difficult. Recently, two groups modified this coupling method and its stereoselectivity was moderately improved.[6,7] William and co-workers studied the effects of solvents and/or additives, and the high stereoselectivity at the anomeric position was achieved in the synthesis of the antineoplastic agent clofarabine.[8] These chemical methods, however, cannot suppress the production of undesired isomers, and they must be removed from the target nucleoside by various purification steps.

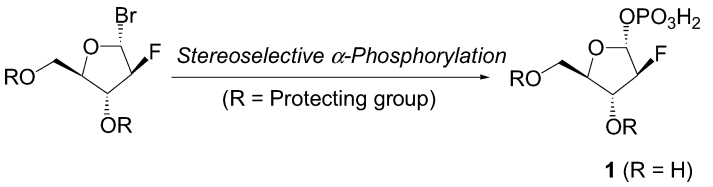
Therefore, a stereoselective and regioselective construction of a β-glycosidic linkage in the purine 2′F-araNs is a challenging assignment. We decided to investigate the application of the enzymatic glycosylation reaction to synthesize the purine 2′F-araNs. There are a few reports for the preparation of the 2′-fluoro-nucleosides by the use of enzymes. The nucleoside phosphorylase-catalyzed trans-glycosylation,[9] which includes phosphorolysis and glycosylation steps, is one of the effective methods to synthesize stereoselectively β-nucleoside analogues. Tuttle[10] had succeeded in the enzymatic trans-glycosylation to obtain an adenine derivative from 2′-deoxy-2′-fluororibosyl thymine or an arabinosyl analog (Scheme 1). However, this approach, consisting of two enzymatic reactions catalyzed by thymidine phosphorylase (TPase) and purine nucleoside phosphorylase (PNPase), would not meet our goal for a practical synthesis. Large amounts of enzyme and a longer reaction time were required, compared with the same reaction of natural nucleosides, to obtain acceptable yields. (Tuttle[10] and coworkers solved this problem by using a large excess of immobilized



SCHEME 1 The nucleoside phosphorylase-catalyzed trans-glycosylation of pyrimidine and purine nucleosides, containing a fluoro-substituent at the 2′-position.

enzymes.) This is because the enhanced stability of the glycosidic linkage by fluoro-substitution at the 2-position of the sugar[3,11] would hold up the reaction.

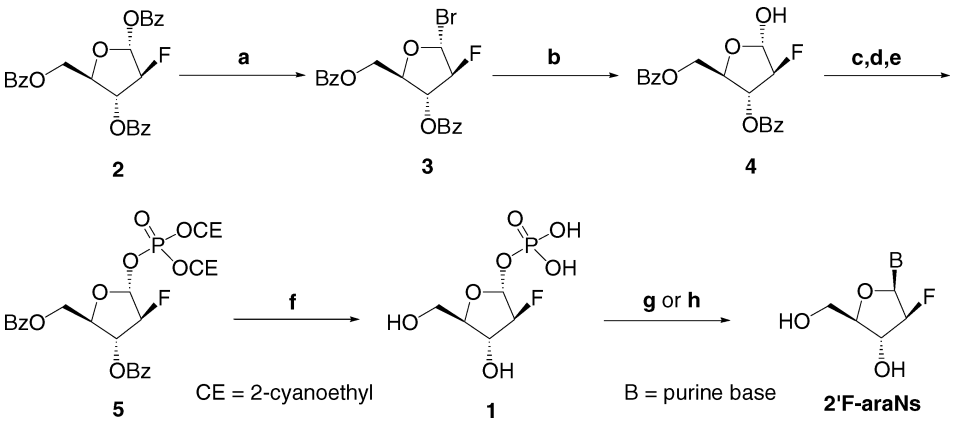
The rate-determining step in the two-step enzymatic reactions would be phosphorolysis, to give α-1-phosphate. If we can make α-1-phosphate by chemical modification in a stereoselective fashion, we are able to develop a new chemo-enzymatic approach including the chemical phosphorylation and the enzymatic glycosylation to synthesize 2′F-araNs in an efficient manner. This method features one enzymatic process, which is essential to obtain the desired β-purine-nucleoside, and the α-configuration of the 1-phosphate group in the sugar moiety is critical, because a nucleoside phosphorylase (NPase) discriminates only α-1-phosphate as a substrate. To achieve our goal, we had to explore the stereoselective synthesis of α-1-phosphorylated 2-fluoroarabinofuranose (**1**) from the corresponding 1-bromide (Scheme 2). In this article, we describe a stereoselective α-phosphorylation at the anomeric position of the 2-fluoro-sugar and its application to the synthesis of the purine 2′F-araNs.



SCHEME 2 Stereoselective α-phosphorylation at an anomeric position.

RESULTS AND DISCUSSION

Howell[5] reported that bromination of an anomeric mixture of 1-*O*-acetyl-2-deoxy-2-fluoroarabinofuranose derivative gave only the α-anomer. This indicates that the configuration of an electronegative substituent at the anomeric position of the 2-fluoroarabinofuranose derivative is greatly affected by the stereo-electronic effect and the steric effect of the β-fluoro group, in addition to an anomeric effect. Therefore, we tried to synthesize α-1-phosphate using this property. (More recently, β-selective phosphorylation of **3** was reported; see ref.[12]) Similar to the coupling reaction with a purine base,[8] direct phosphorylation of 1-bromide (**3**)[5] with phosphoric acid in polar solvents like CH_3CN or DMF did not proceed stereoselectively ($\alpha/\beta = <1$) due to the coexistence of S_N1 and S_N2 mechanisms. On the contrary, hydrolysis of **3** under mild basic conditions gave α-1-hydroxyl sugar (**4**) exclusively (α-anomer: $J_{1,2} = \sim 0$ Hz, β-anomer: $J_{1,2} = 3.4$ Hz) at an 86% yield (Scheme 3). The stereochemistry was indeed confirmed by converting **4** into the known α-1-*O*-benzoate (**2**)[5] by benzoylation of the hydroxy group at the 1-position. Unfortunately, the direct phosphorylation



SCHEME 3 Reagents and conditions: a) 30% HBr-AcOH, CH_2Cl_2; b) Et_3N, H_2O, DMF (86% from **2**); c) $(i\text{-}Pr_2N)_2PCl$, Et_3N, CH_3CN; d) 2-cyanoethanol, 1*H*-tetrazole; e) *t*-BuOOH (51% from **4**); f) NH_4OH, MeOH; g) adenine, PNPase, Tris-HCl (pH = 7.0), 50°C (50% from **5**); h) 2,6-diaminopurine, PNPase, Tris-HCl (pH = 7.0), 50°C (58% from **5**).

of **4** using $POCl_3$ was not effective because of the immediate formation of 1-chloride, even under a low temperature. Next, we tried to introduce a phosphate group at the 1-position using phosphoramidite-chemistry. The reaction of 2-cyanoethyl N,N,N′,N′-tetraisopropylphosphorodiamidite with **4** catalyzed by 1*H*-tetrazole, followed by the substitution reaction with 2-cyanoethanol, gave the corresponding bis(2-cyanoethyl) 1-phosphite, but the configuration at the 1-position was shown by ^{31}P NMR analysis to be an approx. 1:1 mixture of anomers (138.61 and 137.91 ppm). On the other hand, treatment of **4** with bis(diisopropylamino)chlorophosphine under basic conditions, followed by phosphitylation and oxidation gave protected α-1-phosphate (**5**) stereoselectively in 51% yield ($\alpha/\beta = 21$). After deprotection by NH_4OH, we could obtain the desired α-1-phosphate (**1**) in quantitative yield (Scheme 3). Thus, we could prepare a pure α-1-phosphate (**1**). We also examined the stability of the α-1-phosphate (**1**) because the enzymatic reaction of the fluorinated sugar would require a longer reaction time compared to natural substrates. The aqueous solution of α-1-phosphate (**1**) under neutral pH was allowed to stand for 1 week at 50°C, but no decomposed products were observed by ^1H NMR analysis. This result indicated that the 1-phosphate was sufficiently stable for subsequent enzymatic glycosylation.

Three 2′F-araNs containing adenine, 2,6-diaminopurine and guanine are key intermediates for the preparation of 2′F-ANA, as described in ref.[1] We examined the PNPase-catalyzed glycosylation reaction of α-1-phosphate (**1**) with adenine and 2,6-diaminopurine except guanine, because guanine was not soluble in the reaction media. 2,6-Diaminopurine is equivalent to guanine after it is deaminated. Therefore, we optimized the enzymatic

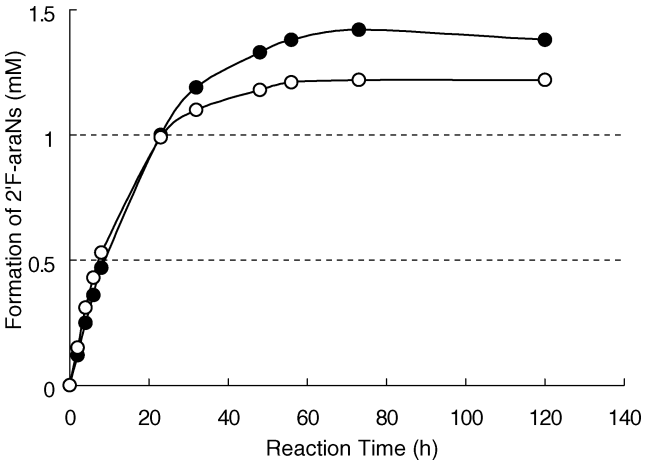
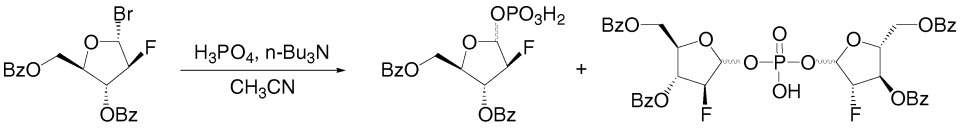


FIGURE 1 Time-course of the enzymatic glycosylation of α-1-phosphate (1) with adenine (○) or 2,6-diaminopurine (●). These reactions were performed in a mixture of α-1-phosphate (1) (2.4 mM), purine base (5.0 mM) and PNPase (15 units/ml) in Tris-HCl buffer (5.0 mM, pH 7.0) at 50°C.

reaction using adenine and 2,6-diaminopurine. The solubility of purine bases in aqueous media influences the yield and reaction time. Therefore, we chose the PNPase prepared from *Bacillus stearothermophilus*, having a higher optimal temperature[13] so that we could carry out the enzymatic reaction at 50°C. α-1-Phosphate (**1**) was treated with two purines at 50°C in the presence of the PNPase (the crude extract from the cell culture was used for the reaction without purification of the enzyme); the reactions were monitored by HPLC analysis for 120 hours. The conversions of two nucleobases to the corresponding 2′F-araNs were monitored by HPLC analyses.

As shown in Figure 1, the reaction with adenine or 2,6-diaminopurine reached to plateau after 60 hours, and the corresponding nucleosides were formed at yields of 50% to 60% from **5**. This result demonstrates the potency of the chemo-enzymatic synthesis of 2′F-araNs. (In addition to adenine and 2,6-diaminopurine, we tested the reactions with 2-aminopurine, 2-fluoroadenine, 2-amino-6-chloropurine, 6-chloropurine, and 2,6-dichloropurine. We made sure these reactions proceeded, except for 6-cholropurine and 2,6-dichloropurine.)

A short and simple introduction of the phosphate group into the 2-deoxy-2-fluoroarabinofuranose derivative at an anomeric position would be required for the practical preparation of 2′F-araNs. Therefore, we examined the direct α-phosphorylation of 1-bromide (**3**). As shown in Scheme 4, a typical phosphorylation condition in CH_3CN gave α,β-1-phosphate (**6**) at a ratio of $\alpha/\beta = 0.6$ at a 47% yield, and the dimer (**7**) was formed in 45% yield as a byproduct. The yield of α,β-1-phosphate (**6**) was improved to



3 α,β-**6** **7**

SCHEME 4 Substitution reaction of 1-bromide (**3**) with phosphoric acid.

70% as the amount of phosphoric acid increased, but the α/β-selectivity of phosphorylation was not changed.

Komatsu[14] has reported the isomerization reaction of the α,β-1-phosphorylated 2-deoxyribose derivative to give α-1-phosphate. In contrast, the isomerization reaction of α,β-1-phosphate (**6**) did not occur at all because the stability of the glycosidic bond was enhanced due to fluoro-substitution at the 2-position. (In fact, the anomeric ratio of **6** could not be changed by the treatment with an excess of phosphoric acid.) We, therefore, had to control the stereoselectivity in the substitution reaction of 1-bromide (**3**). In general, this substitution reaction proceeds through two mechanisms. One was a nucleophilic displacement of the 1-halogenated sugar at the anomeric position in an S_N2 mechanism;[12] the other was the generation of an oxonium ion that was trapped subsequently by the nucleophile.[15] In the latter reaction, it could be expected that the reaction from the α-face was favored,[5] due to the stereo-electronic effect and the steric effect of the 2-β-fluoro substituent. Therefore, we searched for reaction conditions to retain the configuration at the 1-position of α-1-bromide (**3**).

We first examined the effect of solvents on the reaction of 1-bromide (**3**). The reaction did not proceed stereoselectively in polar or nonpolar solvents (Table 1, entries 1–3). It is well known that the halide ion-catalyzed Lemieux's glycosidation method proceeds in an α-selective manner. However, it is limited to pyranosides.[15] To suppress the direct S_N2 reaction of α-1-bromide, we added various tetra-*n*-butylammonium salts to the mixture. The addition of tetra-*n*-butylammonium iodide (TBAI)[16] at room temperature gave good results. As shown in Table 1, the α/β-selectivity was raised from 0.6 to 3.2, when 5.0 equivalents of TBAI was added (entry 1 vs. 5). In this reaction condition, S_N2-type double inversion might be considered in addition to S_N1-type substitution,[5] and the phosphate anion might attack stereoselectively the C1-carbon from the α-face.

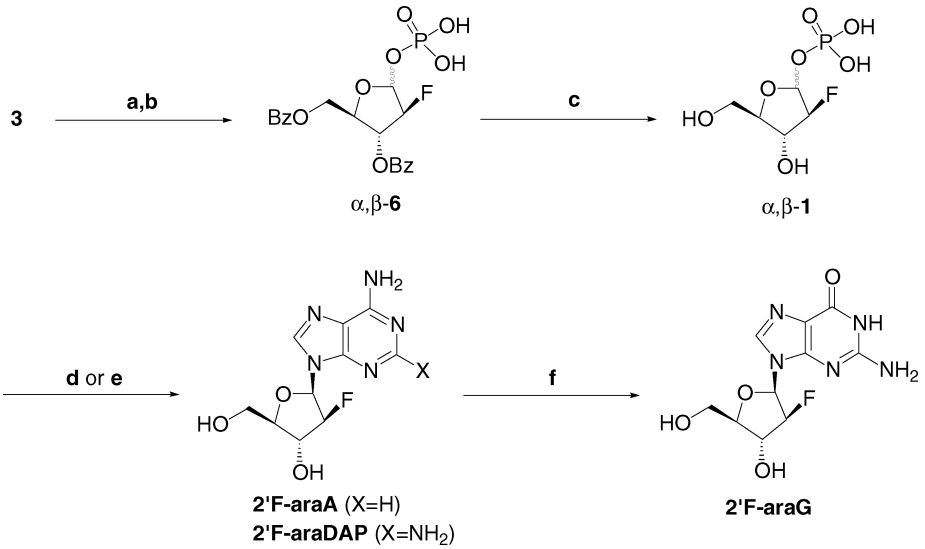
Next, the addition of nitrate and bromide showed effects comparable to the iodo anion, but triflate and sulfate were not effective at all. On the other hand, α,β-1-phosphate (**6**) was not obtained when chloride or fluoride were added because of the lower reactivity of plausible intermediates (1-halides) at room temperature.

TABLE 1 The effect of additive for steroselective phosphorylation

Entry	Solvent	Additive (eq.) n-Bu_4N·X	1-Phosphate **6** (%)	α/β-ratio[a]	Dimer **7** (%)
1	CH_3CN	-	67	0.6	9
2	CH_2Cl_2	-	77	0.7	15
3	DMF	-	49	0.8	8
4	CH_3CN	X = I (1.0)	67	1.3	N.D.
5	CH_3CN	X = I (5.0)	63	3.2	23
6	CH_3CN	X = F (5.0)	Not detected[c, d]	-	N.D.
7	CH_3CN	X = Cl (5.0)	71[c]	1.9	N.D.
8	CH_3CN	X = Br (5.0)	69	2.2	N.D.
9	CH_3CN	X = NO_3 (5.0)	65	2.6	N.D.
10	CH_3CN	X = OTf (5.0)	68	0.9	N.D.
11	CH_3CN	X = HSO_4 (5.0)	9	0.4	N.D.

[a]The ratio were estimated by HPLC analysis.
[b]Not determined.
[c]The reaction was carried out at 70°C.
[d]A β-1-fluorinated sugar was obtained (85%).

The addition of chloride worked well at an elevated temperature, but the selectivity was low. (The reaction of 1-chloride proceeded at 70°C to afford it at a selectivity of $\alpha/\beta = 1.9$.). Consequently, the iodide was the most effective additive in terms of stereoselectivity, handling, and cost. The resulting anomeric mixtures of 1-phosphate (**1**), which were obtained after the deprotection of the benzoyl groups by NH_4OH-MeOH, could be used for the enzymatic glycosylation reaction without isolating the α-anomer.



SCHEME 5 Reagents and conditions: a) H_3PO_4, *n*-Bu_3N, TBAI, CH_3CN, MS4A; b) H_3PO_4, 2-butanone, MS4A, 80°C; c) NH_4OH, MeOH; d) adenine, PNPase, phosphate buffer (pH = 7.0), 50°C (X = H, 29% from **3**); e) 2,6-diaminopurine, PNPase, phosphate buffer (pH = 7.0), 50°C (X = NH_2, 39% from **3**); f) ADase, 40°C (X = NH_2, 87%).

The chemo-enzymatic method was applied to prepare 2′F-araNs containing adenine and guanine moieties[17] (Scheme 5). 1-Bromide (**3**) was directly phosphorylated by the method mentioned above. After the treatment of 1-phosphate mixtures with phosphoric acid in butanone at 80°C, (α-**6** was recovered by the degradation of dimer (**7**) under this acidic condition, although isomerization of β to α-**6** was not observed.) α-1-phosphate (**6**) was produced at a 63% yield (α/β = 3.1), which was estimated by HPLC analysis. After the benzoyl groups were removed by NH_4OH-MeOH treatment, the crude α-1-phosphate (**1**) was used for the enzymatic reaction without separation of the β-isomer. For the large scale syntheses, the reaction conditions were slightly modified to increase the concentration of substrates (approximately 6-fold), and decrease the amount of PNPase (approximately 1/4∼1/9). About 15 mM of α-**1** was treated with 1.3∼2.0 eqivalents of adenine or 2,6-diaminopurine in 5 mM KH_2PO_4 at 50°C in the presence of the PNPase (10∼20 units/ml). In both reactions, the precipitation of the purine base was observed in each reaction mixture after 48 hours. Although these enzymatic reactions were relatively slow to primary reaction conditions (see Figure 1), the desired 2′F-araNs were obtained in similar yields after about 1 week. It should be noted that any undesired isomers were not produced in contrast to chemical methods,[6–8] and highly pure 2′F-araNs were obtained at yields of 30–40% from **3** by a simple purification procedure. Additionally, the 2,6-diaminopurine derivative was easily converted to 2′F-araG in 87% yield (more than 99% of purity by HPLC analysis) by the use of adenosine deaminase-catalyzed hydrolysis at the 6-position.[18]

CONCLUSION

The stereoselective phosphorylation at the anomeric position of the 1-bromo-2-fluoroarabinofuranose derivative (**3**) was attained chemically in two ways. Both the 1-bromo-derivative (**3**) and the 1-hydroxy derivative (**4**) were key starting materials for the preparation of α-1-phosphate (**1**), which was a good substrate for enzymatic glycosylation. We proved that α-1-phosphate (**1**) was recognized as a substrate for the PNPase we selected. Moreover, the addition of TBAI enabled us to substitute the 1-bromo-group in compound (**3**) with phosphoric acid directly and stereoselectively. The chemo-enzymatic method we developed was applied to the synthesis of the purine 2′F-araNs. The quality of the desired 2′F-araNs was excellent after simple purification when it was compared with the chemical condensation method. We believe that this chemo-enzymatic method is most useful for the synthesis of the purine 2′F-araNs and will facilitate the development of 2′F-ANA-oligonucleotides as drugs.

EXPERIMENTS

Physical data were measured as follows: Melting points were determined on a Yanagimoto MP-500D micromelting point apparatus (Yanagimoto Analytical Instruments Corp., Kyoto, Japan), and were uncorrected. ^{1}H NMR spectra were recorded at 500 MHz on a Bruker AV-500 instrument in $CDCl_3$, DMSO-d_6 or D_2O as the solvent, with tetramethylsilane or 3-(trimethylsilyl)propionic acid-d_4 sodium salts as the internal standards. ^{31}P NMR spectra were recorded at 203 MHz on a Bruker AV-500 instrument in $CDCl_3$ or D_2O as the solvent, with 85% H_3PO_4 as the external standard (Bruker, Yokohama, Japan). Ultraviolet (UV) spectra were recorded with a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Mass spectra were obtained in the fast atom bombardment (FAB) mode on a JEOL JMS-AX500 instrument. High-resolution ESI mass spectra were recorded on a JEOL JMS-T100LP instrument (JEOL, Tokyo, Japan). Thin laser chromatography (TLC) was carried out on Merck precoated plates (Kieselgel 60F254). Purine nucleoside phosphorylase was prepared in the laboratory of Yamasa Corp. (Chosi, Japan). The activity of the enzyme was measured by the method of the literature.[13] One unit of the enzyme was defined as the amount of enzyme forming a μmol of adenine per minute under the reaction condition. Adenosine deaminase (from calf intestine) was purchased from Roche Diagnostics Corp. (Manheim, Germany), and used without further purification.

2-Deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranose (4)

To a solution of 1-bromide (**3**) (387 mg, 0.91 mmol) in DMF (15 mL), Et_3N (0.76 mL, 0.71 mmol) and H_2O (0.5 mL) were added, and the mixture was stirred at room temperature for 30 minutes. Solvents were removed under reduced pressure, and the residue was purified by column chromatography over silica gel (2.3 × 14 cm, $CHCl_3$) to give **4** (282 mg, 86%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 8.08–8.01 (4H, m), 7.63–7.41 (6H, m), 5.70 (1H, dd, J = 3.6 and 10.2 Hz), 5.50 (1H, dd, J = 4.3 and 22.0 Hz), 5.18 (1H, d, J = 49.1 Hz), 4.77–4.60 (3H, m), 2.89 (1H, t, J = 3.4 Hz); FAB MS (m/z) 361 (M^++H). Anal. Calcd for $C_{19}H_{17}FO_6$: C, 63.33; H, 4.76. Found: C, 63.42; H, 4.72.

Bis(2-cyanoethyl)-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl-1-phosphate (5)

To a solution of 1-hydroxysugar (**4**) (1.01 g, 2.8 mmol) in acetonitrile (20 mL), Et_3N (1.2 mL, 8.4 mmol) and (i-Pr_2N)$_2$PCl (1.69 g, 5.6 mmol) were added. After 1 hour of stirring at room temperature, 2-cyanoethanol (1.9 mL, 28.0 mmol) and 1*H*-tetrazole (981 mg, 14.0 mmol) were added

to the mixture. After 1.5 hours of stirring at room temperature, 70% *t*-BuOOH (2.5 ml) was added to the mixture and stirred for 30 minutes. The mixture was extracted with AcOEt, and the organic phase was washed with water (twice), saturated $NaHCO_3$ and brine, and then dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was purified by column chromatography over silica gel (3.6 × 16 cm, 50–100% AcOEt in hexane) to give **5** (784 mg, 51%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 8.07–8.05 (4H, m), 7.65–7.43 (6H, m), 6.14 (1H, dd, J = 4.2 and 8.3 Hz), 5.56 (1H, dd, J = 3.9 and 20.9 Hz), 5.34 (1H, d, J = 48.4 Hz), 4.82 (1H, q, J = 3.9 Hz), 4.78 (1H, dd, J = 3.5 and 12.2 Hz), 4.68 (1H, dd, J = 4.6 and 12.2 Hz), 4.32–4.27 (4H, m), 2.78–2.68 (4H, m); ^{31}P NMR ($CDCl_3$) δ -3.73; FAB MS (m/z) 343 (M-OPO(OCE)$_2$). Anal. Calcd for $C_{25}H_{24}FN_2O_9P$: C, 54.95; H, 4.43; N, 5.13. Found: C, 54.97; H, 4.59; N, 4.98.

Disodium (2-deoxy-2-fluoro-α-D-arabinofuranosyl)-1-phosphate (1)

To a solution of 1-phosphate ester (**5**) (590 mg, 1.08 mmol) in MeOH-THF (1:1, 6 mL), 28% NH_4OH (6 mL) was added. After 1 h of stirring at room temperature, the solvent was removed under reduced pressure. Then 28% NH_4OH (10 mL) was added to the residue and the mixture was stirred at room temperature overnight. After concentration, the residue was dissolved in water (20 mL) and washed with AcOEt (20 mL). The solvent was removed under reduced pressure and the residue was dissolved in 100 mL of water. The solution was passed through an ion-exchange resin (30 mL, PK 216 Na form, Mitsubishi Chemical Corp., Tokyo, Japan) to give a sodium salt of **1** (295 mg, 99%) as an amorphous foam: ^{1}H NMR (D_2O) δ 5.70 (1H, dd, J = 6.9 and 9.8 Hz), 5.02 (1H, d, J = 50.5 Hz), 4.26–4.17 (2H, m), 3.87 (1H, dd, J = 3.2 and 12.4 Hz), 3.74 (1H, dd, J = 5.3 and 12.4 Hz); ^{13}C NMR (D_2O) δ 103.65 (dd, J = 7.1 and 180.1 Hz), 103.17 (dd, J = 3.7 and 36.7 Hz), 87.51 (d, J = 2.5 Hz), 77.73 (d, J = 27.1 Hz), 63.94; ^{31}P NMR (D_2O) δ 2.19; FAB MS (m/z) 277 (M^++H); HR-ESIMS (m/z). Calcd for $[C_5H_9FO_7P]^-$:231.0070; found: 231.0062.

2-Deoxy-3,5-di-*O*-benzoyl-2-fluoro-α(and β)-D-arabinofuranosyl-1-phosphate (6)

To a solution of 1.0 M tri-n-butylammonium phosphate in acetonitrile (0.75 mL, 0.75 mmol), MS4A(50 mg) and various tetra-n-butylammonium salts (shown in Table 1) were added. After 40 minutes of stirring at room temperature, a solution of 1-bromide **3** (50 mg, 0.12 mmol) in acetonitrile (0.5 mL) was added to the mixture at 0°C or room temperature. The α,β-ratio of reactions shown in Table 1 were determined by HPLC analysis under the following conditions: detector, UV 230 nm/column, Shiseido CAPCELL

PAK NH_2 (4.6 mm × 250 mm; temperature, 30°C; mobile phase, a mixture of 120 mM KH_2PO_4 (50%) and acetonitrile (50%); pH 4; flow rate, 1.0 mL/min). An analytical sample of $\alpha\beta$-**6** ($\alpha/\beta = 1.5$) was obtained as a 2/3 tri-n-butylammonium salt via purification by ODS column chromatography: ^{1}H NMR ($CDCl_3$) δ 8.09–7.98 (4H, m), 7.56–7.30 (6H, m), 6.03–6.00 (1H, m), 5.79 (0.4H, dt, $J = 4.8$ and 16.3 Hz), 5.47 (0.6H, dd, $J = 4.2$ and 22.1 Hz), 5.40 (0.6H, d, $J = 49.1$ Hz), 5.15 (0.4H, br d, $J = 52.1$ Hz), 4.75–4.59 (2.6H, m), 4.34 (0.4H, q, $J = 5.5$ Hz), 2.91–2.87 (6H, m), 1.68–1.62 (4H, m), 1.36–1.29 (4H, m), 0.90 (6H, t, $J = 7.3$ Hz); ^{31}P NMR ($CDCl_3$) δ 0.33, -0.35; HR-ESIMS (m/z). Calcd for $[C_{19}H_{17}FO_9P]^-$: 439.0594; found 439.0583.

9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)adenine (2′F-araA)

To the suspension of o-phosphoric acid (2.8 g, 28.3 mmol) and MS4A (2.0 g) in acetonitrile (10 mL), tri-n-butylamine (13.4 mL, 56.4 mmol) was added at 0°C and the mixture was stirred at room temperature for 1 hour. After the addition of TBAI (8.7 g, 23.5 mmol), a solution of **3** (2.0 g, 4.7 mmol) in acetonitrile (20 mL) was added dropwise to the mixture at room temperature. After 2 hours of stirring at room temperature, the filtrate was concentrated under reduced pressure. The residue was extracted with AcOEt (150 mL), and the organic phase was washed with 0.4% HCl (three times) and dried (Na_2SO_4). After the solvent was removed under reduced pressure, the residue was dissolved in 2-butanone (50 mL), and o-phosphoric acid (7.4 g) and MS4A (2.0 g) were added to the solution. After 2 hours of stirring at 80°C, the filtrate was concentrated under reduced pressure. The residue was extracted with AcOEt (150 mL), and the organic phase was washed with water and dried using Na_2SO_4. The solvent was removed under reduced pressure, the residue was dissolved in methanol (40 mL) and 28% NH_4OH (40 mL) was added to the mixture and then stirred at room temperature overnight. After concentration, the residue was dissolved in water (50 mL) and washed with AcOEt (200 mL × 2), and the water phase was concentrated to give crude **1**.

To the solution of **1** in 20 mM KH_2PO_4 (200 mL, pH 7.6), adenine (540 mg, 4.0 mmol) and PNPase (2000 units) were added. The mixture was kept at 50°C for 144 hours. After dilution, the solution was passed through a membrane filter (0.45 μm) and applied to reverse-phase ODS column chromatography (400 mL). After it was washed with 2000 mL of water, the eluate of 3% aqueous CH_3CN was collected and concentrated under reduced pressure to leave crystalline **2′F-araA** (362 mg, 29% from **3**): m.p. 232–233°C (lit.[17a] 232–234°C); UV (H_2O) λ_{max} 258.5 nm ($\varepsilon = 15\,300$);^{1}H NMR (DMSO-d_6): δ 8.26 (1H, d, $J = 1.7$ Hz), 8.17 (1H, s), 7.36 (2H, br s), 6.41 (1H, dd, $J = 4.6$ and 14.1 Hz), 6.15 (1H, br), 5.25 (1H, br), 5.22 (1H, dt, $J = 4.3$ and 52.7 Hz), 4.47 (1H, dt, $J = 4.5$ and 19.4 Hz), 3.86–3.84 (1H, m), 3.68–3.63 (2H, m); FAB MS (m/z) 270 (M^++H). Anal. Calcd for

$C_{10}H_{12}FN_5O_3 \cdot 0.3H_2O$: C, 43.73; H, 4.62; N, 25.50. Found: C, 43.63; H, 4.46; N, 25.48.

9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)guanine (2′F-araG)

To the suspension of o-phosphoric acid (12.7 g, 130 mmol) and MS4A (10 g) in acetonitrile (49 mL), tri-n-butylamine (61.8 mL, 258 mmol) was added at 0°C and the mixture was stirred at room temperature for 1 hour. After the addition of TBAI (39.9 g, 108 mmol), a solution of **3** (10.3 g, 21.5 mmol) in acetonitrile (85 mL) was added dropwise to the mixture at room temperature. After 2 hours of stirring at room temperature, the filtrate was concentrated under reduced pressure. The residue was extracted with AcOEt (400 mL), and the organic phase was washed with water (400 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the precipitated material was removed by suction. After the filtrate was concentrated, the residue was washed with hexane (15 mL × 5) and dried under reduced pressure, to give crude 1-phosphate (**5**). The crude **5** was dissolved in 2-butanone (255 mL) and o-phosphoric acid (34.0 g) and MS4A (10 g) were added to the solution. After 2 hours of stirring at 80°C, the filtrate was concentrated under reduced pressure. The residue was extracted with AcOEt (400 mL), and the organic phase was washed with water (400 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure, the residue was dissolved in methanol (200 mL) and 28% NH_4OH (200 mL) was added to the mixture and then stirred at room temperature overnight. After concentration, the residue was dissolved in water (200 mL) and washed with AcOEt (250 mL × 2), and the water phase was concentrated to give crude **1**.

To the solution of **1** in 5 mM KH_2PO_4 (1 L, pH 7.6), 2,6-diaminopurine (4.05 g, 27.0 mmol) and PNPase (23460 units) were added. The mixture was kept at 50°C for 190 hours. After dilution, the solution was passed through a membrane filter (0.45 μm) and applied to a column of adsorption resin (350 mL, Sepabeads SP 206, Mitsubishi Chemical Corp.). After it was washed with 3 L of water, the eluate of 1–15% aqueous EtOH was collected and concentrated under reduced pressure to give **2′F-araDAP**[19] as a water solution. (The analytical sample was isolated and the structure was confirmed by its spectroscopic analyses. See ref.[19]).

To the solution of **2′F-araDAP** in 10 mM KH_2PO_4 (1 L, pH 7.0), adenosine deaminase (Roche, 1500 units) were added. The mixture was kept at 40°C for 2 hours. The solution was passed through a membrane filter (0.45 μm) and applied to a column of adsorption resin (500 mL, Sepabeads SP 206, Mitsubishi Chemical Corp.). After it was washed with 3 L of water, the eluate of 1–10% aqueous EtOH was collected and concentrated under reduced pressure to leave crystalline **2′F-araG** (2.09 g, 34% from **3**): m.p. 257–259°C (lit.[17b] 250—251°C); UV (H_2O) λ_{max} 251.9 nm (ε = 13 300);

^{1}H NMR (DMSO-d_6): δ 10.50 (1H, br s), 7.80 (1H, s), 6.54 (2H, br s), 6.13 (1H, dd, J = 4.2 and 16.0 Hz), 5.94 (1H, d, J = 4.5Hz), 5.11 (1H, dt, J = 3.8 and 52.4 Hz), 5.08 (1H, t, J = 5.7 Hz), 4.37 (1H, dd, J = 3.9 and 17.8 Hz), 3.82–3.79 (1H, m), 3.66–3.57 (2H, m); FAB MS (m/z) 286 (M^++H). Anal. Calcd for $C_{10}H_{12}FN_5O_4 \cdot 0.3H_2O$: C, 41.33; H, 4.37; N, 24.10. Found: C, 41.57; H, 4.33; N, 23.69.

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