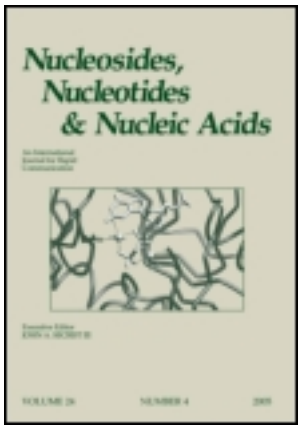

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Convenient Synthesis of 2′-Deoxy-2-fluoroadenosine from 2-Fluoroadenine

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Convenient Synthesis of 2′-Deoxy-2-fluoroadenosine from 2-Fluoroadenine

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ABSTRACT

A convenient synthesis of 2′-deoxy-2-fluoroadenosine from commercially available 2-fluoroadenine is described. The coupling reaction of silylated 2-fluoroadenine with phenyl 3,5-bis[*O*-(*t*-butyldimethylsilyl)]-2-deoxy-1-thio-D-*erythro*-pentofuranoside gave the corresponding 2-fluoro-2′-deoxyadenosine derivative ($\alpha/\beta = 1:1$) in good yield. The α- and β-anomers were separated by chromatography, and then desilylated to give compounds **1a** and **1b**.

Key Words: Fluorinated nucleosides; Thioglycoside coupling; Glycosylation; Anomer separation.

INTRODUCTION

2′-Deoxy-2-fluoroadenosine (F-dAdo) (**1b**) has received recent attention as a prodrug for the targeted delivery of highly toxic 2-fluoroadenine (2F-Ade) to tumor cells.[1] The strategy relies on the use of the selective expression of the *E. coli* purine

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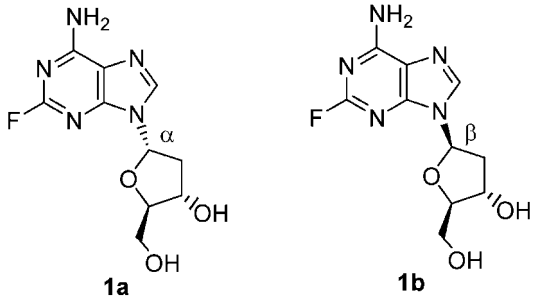
nucleoside phosphorylase (PNP) gene in tumor cells, and on the high substrate activity of F-dAdo for *E. coli* PNP. The inactivity of this and other adenosine analogues for mammalian PNP is the basis for tumor selectivity. Adding value to this approach is the fact that *E. coli* PNP-produced 2-fluoroadenine can rapidly diffuse across cell membranes and thus has high bystander activity, i.e., it is toxic to tumor cells that are not proliferating or which do not have the expressed *E. coli* PNP gene. In particular, the potential of increased responsiveness of solid tumors to this approach compared to more traditional chemotherapies that are more dependent on active proliferation provides a major impetus to this research. These issues are described in greater detail in recent publications.[1,2]

Montgomery and Hewson initially prepared F-dAdo by a multi step sequence that included fluorination of a 2,6-diaminopurine nucleoside precursor as a key step.[3] Owing to the low yield of this procedure, Secrist and co-workers recently developed a more convenient procedure based on free radical removal of the 2′-OH group from the more readily available 2-fluoroadenosine.[4] We are preparing a series of 2-fluoro-2′-deoxyadenosines to be used in biochemical studies of adenosine processing enzymes. The commercial availability of 2-fluoroadenine prompted us to explore direct glycosylation of this base with appropriate 2-deoxy-ribose partners as an efficient route to these nucleoside derivatives. Because of the current interest in F-dAdo as an anticancer prodrug, we report herein the successful application of this approach to this analogue. The synthesis is short and readily scaled up.



1a **1b**

CHEMISTRY

In preliminary experiments, using modifications of a published procedure,[5] we found conditions for coupling thiogylcoside **2**, readily prepared from 2-deoxyribose, with 2-fluoroadenine that affords products **3** in about 59% combined yield with 1:1 α/β selectivity. Attempts to separate the anomers by chromatography were unsuccessful. A sample of the mixture of compounds **3** was deacetylated using 5% triethylamine in methanol to give a mixture of **1a** and **1b.** Unfortunately we were also unable to separate anomers **1a** and **1b** by chromatography on silica gel despite repeated efforts using a variety of solvent schemes. In an effort to accentuate steric differences imposed by the different orientation of the glycosidic bond, the mixture of anomers **3** was deprotected and silylated in situ with TBSCl to give **4a** and **4b**. We were pleased to find that the silylated derivatives indeed were readily

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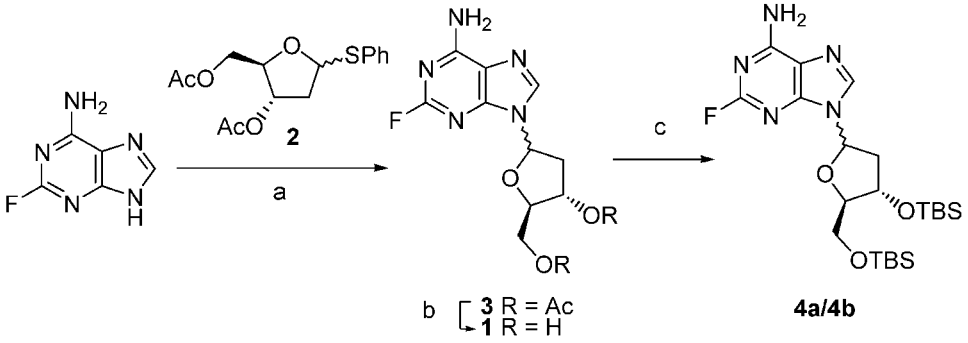
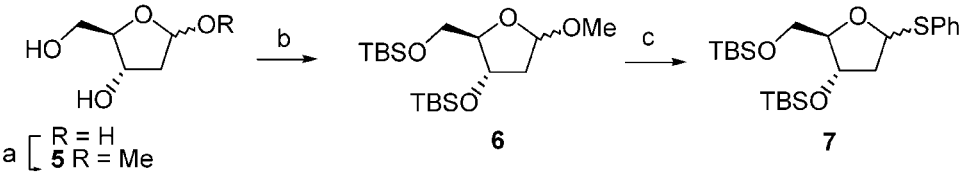


Scheme 1. Reagents and conditions: a) (i) HMDS, $(NH_4)_2SO_4$, reflux; (ii) thioglycoside **2**, NBS, MS 4D, toluene; b) Et_3N/MeOH; c) TBSCl, imidazole, DMF.



Scheme 2. Reagents and conditions: a) DowexH^+, MeOH, rt, 94%; b) TBSCl, DMAP, NEt_3, DCM/DMF, 0°C to rt, 78%; c) PhSH, ZnI_2, *n*-Bu_4NI, DCM, −15°C, 74%.

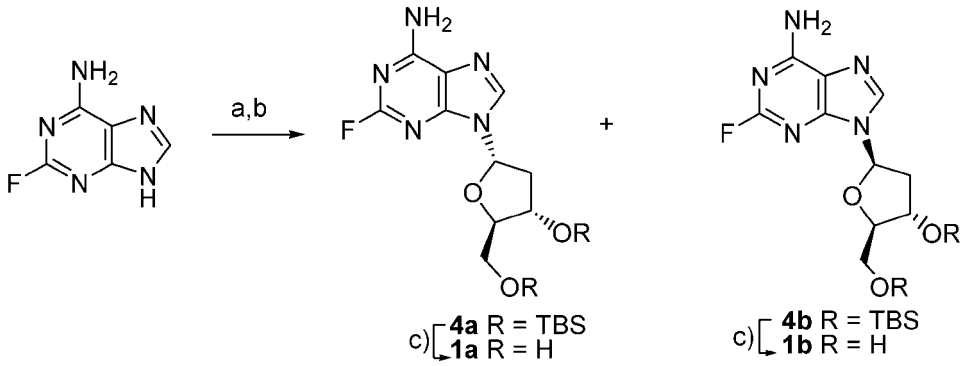
separated by chromatography, but the overall conversion from **3** was only 6% (Sch. 1).

Having determined on a small scale the effectiveness of the TBS group in facilitating anomer separation, we saw the advantage of installing this group at the beginning of the sequence. Thus, the TBS protected thioglycoside **7** was prepared from the known glycoside **6**[6] (Sch. 2), and this was used in the glycosylation reaction. This reaction proceeded smoothly to give a 1:1 mixture of anomers **4a,b** in 62% overall yield. The mixture was separated by silica gel chromatography and removal of the TBS group with tetraethylammonium fluoride completed the synthesis of compounds **1a** and **1b** (Sch. 3). The spectral and physical data were in complete agreement with the published data.[3,4]

EXPERIMENTAL

General Procedures

Melting points are uncorrected. ^1H, ^{13}C and ^{19}F NMR spectra were recorded at 300.1, 75.5 and 282.2 MHz, respectively. Chemical shifts (δ, ppm) of protons and carbons are relative to TMS (0 ppm), and the fluorine shifts are relative to trifluoroacetic acid (−76.55 ppm). Interaction constants are presented in Hz. The solvent is $CDCl_3$ unless otherwise noted. Low resolution MS (LRMS) was performed with



Scheme 3. Reagents and conditions: a) HMDS, $(NH_4)_2SO_4$, reflux; b) thiogylcoside **7**, NBS, MS 4 Å, toluene; 30% (**4a**) and 32% (**4b**) c) $Et_4NF{\cdot}H_2O$, acetone, rt; 95% (**1a**), 87% (**1b**).

chemical ionization under ammonia gas. High resolution MS (HRMS) was done with FAB ionization under xenon gas. Silica gel (0.040–0.063 mm) was used for column chromatography. All reagents and dry solvents were purchased if not otherwise indicated and used without further purification or drying. 2-Fluoroadenine was purchased from Aldrich.

Methyl 2-deoxy-D-*erythro*-pentofuranoside (5). Dowex 50wx-100 ion-exchange resin (100 mg) was added to a solution of 2-deoxy-D-ribose (10 g, 74.6 mmol) in methanol (200 mL). After being stirred at room temperature for 17 h, the Dowex resin was filtered and 2 mL of triethylamine was added to the filtrate. The solvent was removed under reduced pressure and the residue was purified by chromatography (EtOAc) to give a mixture of α and β anomers of **5** (10.4 g, 94%).

Methyl 3,5-Bis[*O*-(*t*-butyldimethylsilyl)]-2-deoxy-D-*erythro*-pentofuranoside (6). This was prepared from **5** as described in reference.[6]

Phenyl 3,5-Bis[*O*-(*t*-butyldimethylsilyl)]-2-deoxy-1-thio-D-*erythro*-pentofuranoside (7). To a solution of **6** (3.76 g, 10 mmol) in dry dichloromethane (80 mL) were added benzenethiol (2.05 mL, 20 mmol), zinc iodide (6.38 g, 20 mmol) and tetrabutylammonium iodide (739 mg, 2 mmol). This suspension was stirred under nitrogen at −15°C for 1 h. The reaction mixture was poured into cold pH 7 phosphate buffer and the organic layer was extracted with dichloromethane. The extract was washed with saturated sodium chloride and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane/ethyl acetate, 40:1) to give 3.35 g (74%) of a mixture of α and β anomers of **7** as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.6–7.4 (4H, m, 2′,6′-SPh α and β), 7.3–7.2 (6H, m, 3′,4′,5′-SPh α and β) 5.7–5.6 (2H, m, H-1α and β), 4.4–4.3 (2H, m, H-4α and β), 4.1–4.0 (1H, m, H-3α or β), 4.0–3.9 (1H, m, H-3α or β), 3.8–3.3 (4H, m, H-5α and β), 2.7–1.9 (4H, m, H-2α and β), 1.0–0.8 (36H, m, Si(C(CH_3)$_3$ α and β), 0.1–0.0 (24H, m, Si(CH_3)$_2$ α and β). ^{13}C NMR ($CDCl_3$, 75 Hz) δ: 137.2 and 135.2 (1′-Ph) 131.5 and 130.4 (2′, 6′-Ph), 128.93 and

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128.86 (3′, 4′-Ph), 127.1 and 126.6 (5′-Ph), 89.0 and 86.9 (C1), 85.8 and 85.7 (C4), 73.4 and 71.5 (C3), 63.8 and 62.5 (C5), 42.3 and 41.6 (C2), 26.15 and 26.09 ($Si(C(CH_3)_3)$), 25.98 and 25.96 ($Si(C(CH_3)_3)$), 18.5 and 18.2 ($Si(C(CH_3)_3)$), −4.5 and −5.2 ($Si(CH_3)_2$). Anal. Calcd for $C_{23}H_{42}O_3SSi_2$: C, 60.74; H, 9.31. Found: C, 61.25; H, 9.58.

2-Fluoro-9-(3,5-bis[*O*-(*t*-butyldimethylsilyl)]-2-deoxy-D-*erythro*-pentofuranosyl)-adenine (4). A suspension of 2-fluoroadenine (1.53 g, 10.0 mmol) and ammonium sulfate (1.32 g, 10.0 mmol) in hexamethyldisilazane (20 mL) was heated at vigorous reflux under N_2 until the solution became clear (5–10 h) The excess of hexamethyldisilazane was removed under reduced pressure. The residue and thioglycoside **7** (4.54 g, 10.0 mmol) were dissolved in 100 mL of dry toluene under N_2, and then 5 g of powdered molecular sieves 4 Å was added. After being stirred at room temperature for 30 min, the reaction mixture was cooled to 0°C and NBS (1.96 g, 11.0 mmol) was added. The resulting mixture was allowed to warm to room temperature and stirred for 15 h. A small amount of silica gel was added to quench the reaction. The solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel (dichloromethane/methanol, 40:1) to give 3.4 g (68%) of a mixture of α,β anomers (1:1) as a white solid. This was rechromatographed on silica gel (hexane/ethyl acetate, 7:3) to give 1.5 g (30%) of α-anomer **4a**, and 1.6 g (32%) of β-anomer **4b**. **4a:** Mp 180–181°C;^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.31 (1H, s, H-8), 6.40 (1H, dd, $J = 7.5$, 1.5 Hz, H-1′), 5.68 (2H, br, NH_2), 4.6–4.5 (1H, m, H-4′), 4.4–4.3 (1H, m, H-3′), 3.73 (1H, dd, $J = 10.8$, 3.3 Hz, H-5′), 3.60 (1H, dd, $J = 11.1$, 5.4 Hz, H-5′), 2.8–2.7 (1H, m, H-2′), 2.4–2.3 (1H, m, H-2′), 0.93 (9H, s, ($Si(C(CH_3)_3)$), 0.85 (9H, s, ($Si(C(CH_3)_3)$), 0.10 (6H, s, ($Si(CH_3)_2$)), 0.01(6H, s, ($Si(CH_3)_2$)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 159.3 (d, $^1J_{CF} = 208$ Hz, C2), 157.3 (d, $^3J_{CF} = 20.5$ Hz, C4), 151.1 (d, $^3J_{CF} = 19.4$ Hz, C6), 140.2 (C8), 117.9 (C5), 90.34 (C1′), 85.32 (C4′), 73.31 (C3′), 63.56, (C5′), 41.76, (C2′), 26.09 ($Si(C(CH_3)_3)$), 25.84 ($Si(C(CH_3)_3)$), 18.51 ($Si(C(CH_3)_3)$), 18.06 ($Si(C(CH_3)_3)$), −4.68 ($Si(CH_3)_2$), −4.77 ($Si(CH_3)_2$), −5.19 ($Si(CH_3)_2$), −5.32 ($Si(CH_3)_2$) FAB-MS m/z 498.3 [MH^+]. Anal. Calcd for $C_{22}H_{40}FN_5O_3Si_2$: C, 53.09; H, 8.10; N, 14.07. Found: C, 53.18; H, 8.24; N, 13.79. **4b**: Mp 188–190°C;^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.31 (1H, s, H-8), 6.36 (1H, t, $J = 6.3$ Hz, H-1′), 5.69 (2H, br, NH_2), 4.6–4.5 (1H, m, H-4′), 4.1–4.0 (1H, m, H-3′), 3.89 (1H, dd, $J = 11.4$, 4.2 Hz, H-5′), 3.78 (1H, $J =$ dd, 11.4, 3.3 Hz, H-5′), 2.7–2.5 (1H, m, H-2′), 2.5–2.4 (1H, m, H-2′), 0.923 (9H, s, ($Si(C(CH_3)_3)$), 0.921 (9H, s, ($Si(C(CH_3)_3)$), 0.12 (6H, s, ($Si(CH_3)_2$)), 0.01(6H, s, ($Si(CH_3)_2$)); ^{13}C NMR ($CDCl_3$, 75 Hz) δ: 159.2 (d, $^1J_{CF} = 209$ Hz, C2), 157.3 (d, $^3J_{CF} = 20.0$ Hz, C4), 151.1 (d, $^3J_{CF} = 19.4$ Hz, C6), 139.6 (C8), 118.4 (C5), 88.16 (C1′), 84.72 (C4′), 72.00 (C3′), 62.92, (C5′), 41.43, (2′), 26.15 ($Si(C(CH_3)_3)$), 26.10 ($Si(C(CH_3)_3)$), 18.62 ($Si(C(CH_3)_3)$), 18.21 ($Si(C(CH_3)_3)$), −4.46 ($Si(CH_3)_2$), −4.62 ($Si(CH_3)_2$), −5.10 ($Si(CH_3)_2$), −5.30 ($Si(CH_3)_2$) FAB-MS m/z 498.3 [MH^+]. HRMS (FAB^+) Calcd for MH^+ $C_{22}H_{41}FN_5O_3Si_2$: 498.2732; Found: 498.2723. Anal. Calcd for $C_{22}H_{40}FN_5O_3Si_2$: C, 53.09; H, 8.10; N, 14.07. Found: C, 52.95; H, 8.10; N, 13.72.

2-Fluoro-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)adenine (1a). To a solution of **4a** (1.02 g, 2.05 mmol) in acetone (30 mL) was added tetraethylammonium fluoride

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hydrate (835 mg, 4.51 mmol) at room temperature. After the solution was stirred over night the solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel (dichloromethane/methanol, 9:1) to give 527 mg (95%) of **1a** as a white solid. Mp 230–232°C, Lit (3) 232°C ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.35 (1H, s, H-8), 7.82 (2H, br, NH_2), 6.22 (1H, dd, $J = 7.8$, 3.0 Hz, H-1′), 5.50 (1H, dd, $J = 7.8$, 3.0 Hz, 3′-O*H*), 4.84 (1H, t, $J = 5.75$ Hz, Hz, 5′-OH), 4.4–4.2 (1H, m, H-4′), 4.2–4.1 (1H, m, H-3′), 3.5–3.4 (2H, m, H-5′), 2.8–2.6 (1H, m, H-2′), 2.31 (1H, dt, $J = 14.1$, 3.0 Hz, H-2′) ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 158.7 (d, $^1J_{CF} = 202.6$ Hz, C2); 157.7 (d, $^3J_{CF} = 21.1$ Hz, C4); 150.5 (d, $^3J_{CF} = 20.5$, C6); 140.0 (C8); 117.3 (d, $^4J_{CF} = 3.98$ Hz, C5); 88. 73 (C1′); 83.68 (C4′); 70.77 (C3′), 61.74 (C5′), 39.91 (C2′) FAB-MS m/z 270.2 [MH$^+$], HRMS (FAB$^+$) Calcd for MH$^+$ $C_{10}H_{13}FN_5O_3$: 270.1002; Found: 270.0999. Anal. Calcd for $C_{10}H_{12}FN_5O_5$: C, 44.61; H, 4.49; N 26.01. Found: C, 44.31; H, 4.51, N 26.14.

2-Fluoro-9-(2′-deoxy-β-D-*erythro*-pentofuranosyl)adenine (1b). The same procedure as above using **4b** instead of **4a** to give **1b** as a white solid. Yield: 87%. Mp 210°C (decomp), Lit (3), >210°C (indefinite) ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.32 (1H, s, H-8), 7.84 (2H, br, NH_2), 6.23 (1H, t, $J = 6.6$ Hz, H-1′), 5.31 (1H, d, $J = 4.2$ Hz, 3′-O*H*), 4.95 (1H, t, $J = 5.7$ Hz, 5′-OH), 4.4–4.3 (1H, m, H-4′), 3.9–3.8 (1H, m, H-3′), 3.7–3.4 (2H, m, H-5′), 2.7–2.6 (1H, m, H-2′), 2.3–2.2 (1H, m, H-2′); ^{13}C NMR (DMSO-d_6, 75 Hz) δ: 158.6 (d, $^1J_{CF} = 179$ Hz, C2), 157.7 (d, $^3J_{CF} =$ 21.0 Hz, C4), 150.4 (d, $^3J_{CF} = 20.0$ Hz, C6), 139.8 (C8), 117.6 (d, $^4J_{CF} = 3.98$ Hz, C5), 87.95 (C1′), 83.67 (C4′), 70.77 (C3′), 61.73, (C5′), 39.31, (C2′). FAB-MS, m/z 270 [MH$^+$]. HRMS (FAB$^+$) Calcd for MH$^+$ $C_{10}H_{13}FN_5O_3$: 270.1002; Found: 270.1000.

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