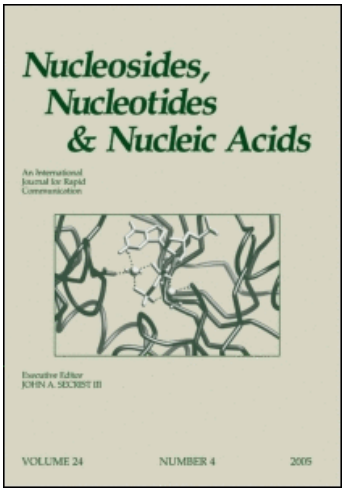

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Peter C. Ratsep[a]; Roland K. Robins[a]; Morteza M. Vaghefi[a]
[a] ICN Nucleic Acid Research Institute, Costa Mesa, California

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INTRODUCTION OF FLUORINE INTO THE C8 POSITION OF PURINE NUCLEOSIDES.

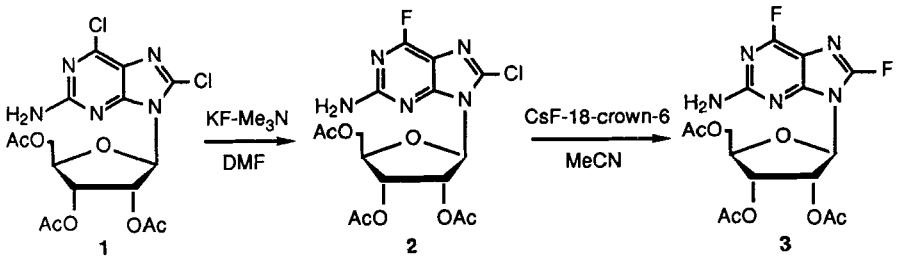
Peter C. Ratsep[1], Roland K. Robins*, and Morteza M. Vaghefi.

ICN Nucleic Acid Research Institute, 3300 Hyland Ave., Costa Mesa, California 92626.

Abstract: We have synthesized 2-amino-6,8-difluoro-9-(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)purine (3) from 2-amino-6,8-dichloro-9-(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)purine (1) in a two-step procedure. The reaction of 3 with anhydrous ammonia in dry 1,2-dimethoxyethane gave 2,8-diamino-6-fluoro-9-(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)purine (4) in 64.1% yield. Compound 4 was deaminated with t-butylnitrite in tetrahydrofuran to give 2-amino-6-fluoro-9-(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)purine (6). The ^{1}H, ^{19}F, and ^{13}C NMR spectral data were determined and evaluated for each of the compounds.

Fluorinated nucleosides[2] are of interest due to their potential antitumor and antiviral activities. A variety of methods have been employed to introduce a fluoro group into the purine ring system, e.g.: ring closures using suitably substituted fluoropyrimidines[3,4], Schiemann reactions in either aqueous[2c,5-8] or non-aqueous conditions[2d-e,9], halogen exchange reactions[4,10,11], and, nucleophilic displacements[12-15]. While the majority of the these types of reactions have been used extensively in the fluorination of either the C2 or C6 position, there have been few reports in which the C8 position has been fluorinated in purines[4,15] and purine nucleosides[8,11].

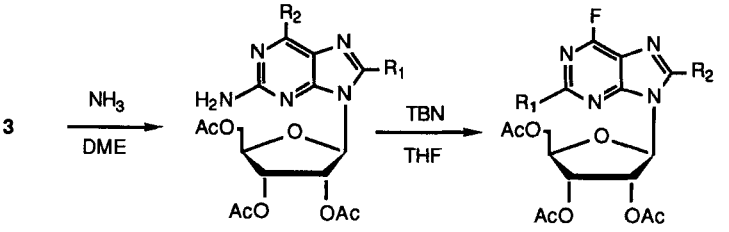
We were interested in developing a new approach for the introduction of fluorine into the C8 site of both adenosine and guanosine. Eariler methods for the synthesis of 2',3',5'-tri-O-acetyl-8-fluoroadenosine focused on the direct displacement of groups at the C8 position gave variable results[8,11]. Our aim was to first introduce the fluorine into the purine ring, then convert the intermediate to either the adenosine or guanosine derivative. In order to accomplish this, we synthesized 2-amino-6,8-difluoro-9(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)purine (3) from 2-amino-6,8-dichloro-9-(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)purine (1)[16a,b] in a two-step procedure. The first fluoro group was introduced into the C6 position of 1 using potassium fluoride with trimethylamine (Me_3N) in N,N'-dimethylformamide (DMF)[12-14] producing 2-amino-6-fluoro-8-chloro-9-(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)purine (2) in good yield. In addition to 2, this reaction typically generated approximately 10-20% of 3.



1 2 3

The conversion of 2-amino-6-fluoro-8-chloro-9-(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)purine (2) to 2-amino-6,8-difluoro-9-(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)purine (3), as well as 1 to 3, was accomplished by using either a cesium fluoride/18-crown-6[17] or a potassium fluoride/18-crown-6[18] complex in acetonitrile. It was observed that long reaction times significantly decreased the yield of 3, thus, CsF was chosen over KF as the fluoride source due to its greater nucleophilicity[17] and faster reaction rates. It should be noted that 3 could be synthesized directly from 1 using either the KF/Me_3N or CsF/18-crown-6 procedures, however, the highest yields of pure 3 were obtained by the combination of the two reactions steps.

Since it has been previously observed that nucleophilic displacements in 2-amino-6,8-dichloro-9-(ß-D-ribofuranosyl)purine and 1 are directed toward the C6 position[16a,19], we had hoped that a similar pattern would prevail in 3. When 3 was treated with anhydrous ammonia in dry 1,2-dimethoxyethane, we observed the formation of 2,8-diamino-6-fluoro-9-(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)-purine (4) and not the desired product, 2',3',5'-tri-O-acetyl-2-amino-8-fluoro-adenosine (5). It was interesting to note that, with the exception of trace amounts of 3 and deblocked 4, compound 4 was the only product. Presumably under these reaction conditions, the two amino groups sufficiently stabilize the 6-fluoro group as to prevent the formation of 2',3',5'-tri-O-acetyl-2,8-diaminoadenosine[19].



4 $R_1 = NH_2, R_2 = F$
5 $R_1 = F, R_2 = NH_2$
6 $R_1 = NH_2, R_2 = H$
7 $R_1 = H, R_2 = NH_2$

The structure of 4 was assigned on the basis of the ^{19}F and ^{13}C NMR spectra. In a comparison of the ^{19}F NMR spectra of 3 and 4, the fluorine resonance assigned to F8 (-101.90 ppm) of 3 disappeared while the F6 resonance

TABLE 1. ^{19}F-^{13}C coupling constants (Hz).

compound	substituent C6	substituent C8	$^3J_{C2F6}$	$^3J_{C4F6}$	$^2J_{C5F6}$	$^3J_{C5F8}$	$^1J_{C6F6}$	$^1J_{C8F8}$
2	F	Cl	18.0	11.1	31.8	----	209.5	-----
3	F	F	18.0	10.6	30.8	15.1	263.8	250.6
4	F	NH_2	17.8	11.2	30.8	----	268.8	-----
6	F	H	18.1	12.1	31.1	----	251.5	-----

(-72.64 ppm) was shifted upfield slightly in 4 to -79.97 ppm. Similarly in the proton-decoupled ^{13}C NMR spectra, the ^{19}F-^{13}C coupling observed for the C8 position of 3 was not observed in 4, and the ^{19}F-^{13}C coupling constants for the remaining resonances were only slightly effected (Table 1). In addition, the large increase in the intensity of the C8 resonance observed in the substitution of the fluoro group of 3 with amino is consistent with structure 4.

As an additional structural proof, 4 was deaminated using t-butylnitrite (TBN) in tetrahydrofuran (THF)[20], and the spectral properties of the product were compared with the reported values of 2-amino-6-fluoro-9-(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)purine[14] (6) and 6-fluoro-8-amino-9-(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)purine[2e] (7). The UV, ^1H NMR, and ^{19}F NMR spectra of the product matched exactly those previously reported[14] for 6. In addition, the proton-coupled and decoupled ^{13}C NMR spectra were consistent with structure 6.

The assignment of the carbon resonances in the proton-decoupled ^{13}C NMR were based on data reported for purine nucleosides[21], and remained essentially constant for the sugar carbons. The assignments of the ring carbons of each of the fluorine-containing compounds were greatly aided by characteristic ^{19}F-^{13}C coupling constants which remained fairly consistent (Table 1). Figure 1 shows the chemical-shift correlations for the purine carbon resonances. Typically, the C5 carbon has the highest electron density and its resonance is shifted well upfield from the other ring carbons. In addition, this carbon was the only resonance in 3 which exhibited ^{19}F-^{13}C coupling to both F6 and F8. The C6 carbon resonance was easily assigned by its large ^{19}F-^{13}C coupling constant. The resonances for the C8 carbon were also easily assigned since these peaks typically appear upfield from C2, C4, and C6; and, in compounds 2-4, and 6, the C8 resonances were not coupled to F6. This last observation is contrary to previous reports which demonstrated small couplings between the C8 carbon and fluorine at either the C2[22] or C6[2e] position. The C2 and C4 resonances were assigned based on their relative intensities[22] and on a comparison of their ^{19}F-^{13}C coupling constants with similar reported values[2e].

Experimental

All chemicals were purchased from Aldrich with the exception of CsF which was purchased from Fluka. Anhydrous solvents were freshly distilled from CaH_2 under

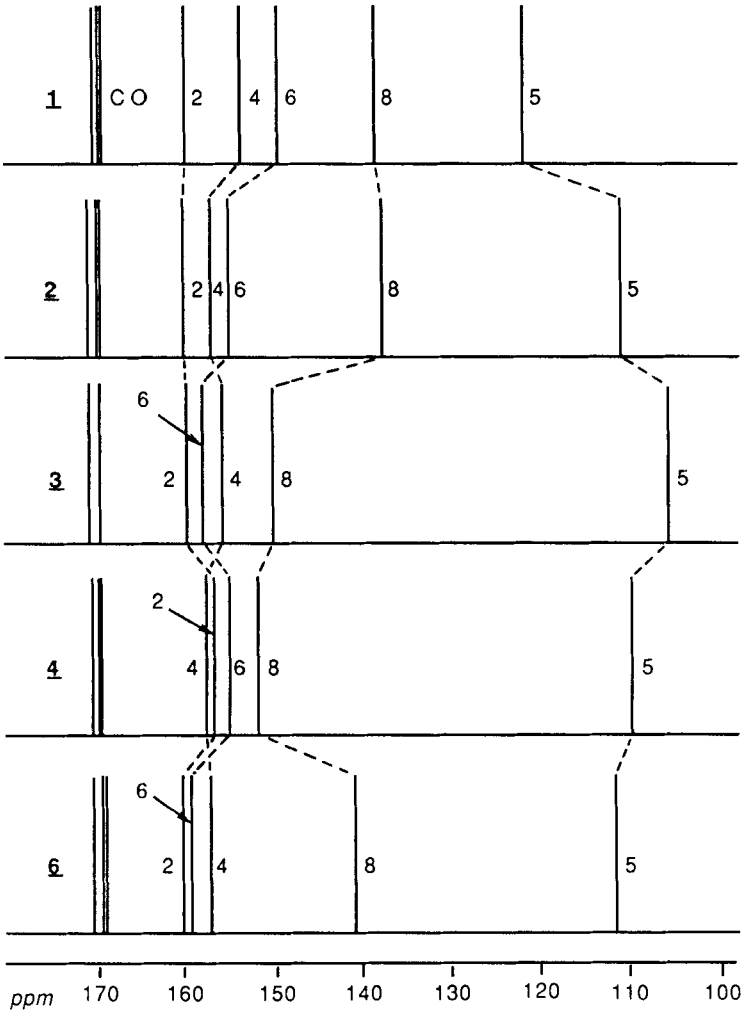


Figure 1. Chemical-shift correlations indicating assignments and observed variations.

dry argon atmosphere, with the exception of THF which was distilled from sodium. Anhydrous ammonia was prepared by distillation of liquid ammonia from sodium using a still head equipped with a dry ice/acetone condensor. Commerically available anhydrous inorganic salts were dried just prior to use by heating over a cold flame under high vacuum for 1 h unless otherwise stated. Dry 18-crown-6 was prepared according to the method in ref. 17. All compounds were dried over P_2O_5 under high vacuum at 50°C overnight. All reactions were monitored by reverse-phase HPLC using a Ranin Rabbit HPLC system equipped with MacRabbit HPLC controller and a Waters 990 Photodiode Array detector. Typical runs were carried out using an isocratic solvent system (45%

MeOH in water) with a Merck LiChroCART 125-4 (5 um) HPLC column. Rotary evaporations were carried out on an Bucchi Rotovapor R110. Melting points were determined on a HaakeBuchler melting point apparatus and were uncorrected. The ^{1}H (300 MHz), ^{19}F NMR (282.4 MHz), ^{13}C NMR (75.5 MHz) spectra were determined using an IBM NR 300 FTNMR spectrometer. Unless specified, all samples were dissolved in Me_2SO-d_6 and were referenced to Me_2SO in ^{1}H and ^{13}C spectra, and to $CFCl_3$ in ^{19}F spectra. Mass spectra were determined on a Varian MAT 731 double focusing high resolution mass spectrometer with an Ion Tech 11N FAB ion source operated at 7 keV with Xe. Elemental analysis was performed by Robertson Laboratories, Madison, N.J.

2-amino-6,8-dichloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (1). Anhydrous LiCl (9.30 g, 219 mmol) and 2',3',5'-tri-O-acetyl-8-bromoguanosine[16a] (20.00 g. 41.0 mmol) were suspended in 250 mL of anhydrous acetonitrile under a dry argon atmosphere. To this suspension was added dry N,N'-diethylaniline (6.52 mL, 41.0 mmol) and distilled $POCl_3$ (76 mL, 820 mmol). The mixture was placed into a 120°C oil bath and refluxed for 15 min. After cooling, the LiCl was filtered off and the filtrate was reduced *in vacuo* to an oil. The oil was dissolved in CH_2Cl_2 and the solution was poured onto 100 g of ice with vigorous stirring. After the layers were separated, the aqueous layer was washed with 5 x 20 mL of CH_2Cl_2. The combined organic layers were washed with 100 mL 5% $NaHCO_3$, then dried over anhydrous Na_2SO_4. The solution was concentrated under reduced pressure to app. 40 mL at which time, 100 mL of isopropanol was added. The crystals which developed upon cooling were filtered and dried *in vacuo* over P_2O_5. Yield 15.44 g (81.5%). mp 138.5°-140.5°C (lit.[16a] 138°-140°C). ^{1}H NMR, δ (ppm): 7.21 s ($2NH_2$, 2H); 6.02 m (H1' + H2', 2H); 5.67 t (H3', 1H; $J_1 = J_2 = 5.8$ Hz); 4.32 m (H4' + H5' + H5", 3H); 2.12 s (OAc, 3H); 2.06 s (OAc, 3H); 1.97 s (OAc, 3H). ^{13}C NMR, δ (ppm): 20.22, 20.36 (3(CH_3)); 62.55 (C5'); 69.75 (C2'); 71.13 (C3'); 79.13 (C4'); 86.58 (C1'); 121.62 (C5); 137.87 (C8); 148.83 (C6); 153.75 (C4); 159.59 (C2); 169.34, 169.41, 169.99 (3(CO)). Anal. Calcd for $C_{16}H_{17}Cl_2N_5O_7$: C 41.57; H 3.71; N 15.15; Cl 15.35. Found C 41.82; H 3.46; N 14.91; Cl 15.09.

2-amino-6-fluoro-8-chloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (2). Anhydrous trimethylamine (0.7 g, 12 mmol) was added to a suspension of 18.85 g (325 mmol) anhydrous KF and 10.00 g (21.6 mmol) dry 1 in 200 mL of anhydrous DMF. The reaction mixture was sealed under argon and was stirred overnight at room temperature. The reaction was filtered and the filtrate was evaporated to an oil *in vacuo*. The oil was dissolved in a minimum of ethyl acetate and was loaded onto a 4 x 30 cm Florisil (100-200 mesh, hexane) flash column. After a quick wash with 50 ml hexane, the product was eluted with 2.5 L of 3:2 hexane: ethyl acetate. The fractions containing product were pooled and evaporated to a colorless oil. The product was crystallized from app. 60 mL of isopropanol.

Yield 8.59 g (89.2%). The product was contaminated with app. 10% of 3, as determined by HPLC (245 nm), and was used without further purification. UV (45% MeOH/H_2O): 293, 251 nm. ^1H NMR, δ (ppm): 7.19 s ($2NH_2$, 2H); 6.02 m (H1' + H2', 2H); 5.68 t (H3', 1H; $J_1 = J_2 = 5.9$ Hz); 4.32 m (H4' + H5' + H5", 3H); 2.12 s (OAc, 3H); 2.07 s (OAc, 3H); 1.97 s (OAc, 3H). ^{19}F NMR, δ (ppm): -70.93 s (F6, 1F). ^{13}C NMR, δ (ppm): 20.22, 20.36 ($3(CH_3)$); 62.56 (C5'); 69.75 (C2'); 71.19 (C3'); 79.40 (C4'); 86.61 (C1'); 110.10 (C5); 137.38 (C8); 155.13 (C6); 157.02 (C4); 159.70 (C2); 169.35, 169.43, 170.01 (3(CO)).

2-amino-6,8-difluoro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (3). A mixture of anhydrous CsF (320 mg, 2.1 mmol) and dry 18-crown-6 (126 mg, 0.48 mmol) was stirred in 10 mL of anhydrous, freshly distilled acetonitrile for 15 min at room temperature. Dry, crude 2 (446 mg, 1.0 mmol) was added to the suspension, and the resultant mixture was refluxed. At 45 h, the reaction was cooled slightly and was evaporated to dryness *in vacuo*. The residue was suspended in ethyl acetate and filtered. The filtrate was concentrated to a few mL and was applied to a 2.5 x 20 cm Florisil (100-200 mesh, hexane) flash column. After a brief washing with hexane, the compound was eluted with 3:2 hexane: ethyl acetate. The appropriate fractions were pooled and were evaporated to an oil. The oil was evaporated several times from hexane until a glass formed. Yield 166 mg (38.7%). UV (45% MeOH/H_2O): 287, 239 nm. ^1H NMR, δ (ppm): 7.13 s ($2NH_2$, 2H); 6.01 s (H1', 1H; $J_1 = J_2 = 5.1$ Hz); 5.84 t (H2', 1H; $J_1 = J_2 = 5.5$ Hz); 5.57 t (H3', 1H; $J_1 = J_2 = 5.5$ Hz); 4.30 m (H4' + H5' + H5", 3H); 2.12 s (OAc, 3H); 2.05 s (OAc, 3H); 2.01 s (OAc, 3H). ^{19}F NMR, δ (ppm): -72.64 s (F6, 1F); -101.90 s (F8, 1F). ^{13}C NMR, δ (ppm): 20.19, 20.30 ($3(CH_3)$); 62.62 (C5'); 69.90 (C2'); 71.28 (C3'); 79.66 (C4'); 86.24 (C1'); 105.17 (C5); 150.47 (C8); 155.50 (C4); 158.39 (C6); 159.66 (C2); 169.39, 169.97 (3(CO)). High resolution FAB mass spectrum (MH^+), obsd 430.1178, $C_{16}H_{18}F_2N_5O_7$ requires 430.1174.

2,8-diamino-6-fluoro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (4). Dry ammonia was distilled into a solution of dry 3 (500 mg, 1.2 mmol) in 7.6 mL anhydrous 1,2-dimethoxyethane. After 16 min, the solution was evaporated under reduced pressure. The solid residue was suspended in app. 100 mL hot $CHCl_3$ and filtered. After the filtrate was evaporated, the solid was dissolved in a minimum of CH_2Cl_2 and was applied onto a silica gel flash column (CH_2Cl_2). After elution of the product with a MeOH in CH_2Cl_2 gradient, the appropriate fractions were pooled and evaporated to dryness. The residue was suspended in hexane and filtered. Yield 320 mg (64.1%). Crystals for analytical purposes were obtained by diffusing hexane into a solution of 4 in dry, distilled 1,4-dioxane. mp softens 103°C; 121.9°-123.5°C. UV (45% MeOH/H_2O): 298, 253 nm. ^1H NMR, δ (ppm): 6.77 s ($2NH_2$, 2H); 6.42 s ($8NH_2$, 2H); 6.04 t (H2', 1H; $J_1 = J_2 = 5.5$ Hz); 5.98 d (H1', 1H; $J = 4.7$ Hz); 5.65 t (H3', 1H; $J_1 = J_2 =$

5.9 Hz); 4.41 m (H2', 1H); 4.25 m (H5' + H5", 2H); 2.11 s (OAc, 3H); 2.05 s (OAc, 3H); 1.98 s (OAc, 3H). ^{19}F NMR, δ (ppm): -79.97 s (F6, 1F). ^{13}C NMR, δ (ppm): 20.25, 20.37 (3(CH_3)); 62.85 (C5'); 69.90 (C2'); 70.63 (C3'); 78.94 (C4'); 84.94 (C1'); 109.65 (C5); 152.22 (C8); 155.83 (C6); 157.36 (C4); 156.86 (C2); 169.32, 169.38, 170.01 (3(CO)). High resolution FAB mass spectrum (MH^+), obsd 427.1375, $C_{16}H_{20}FN_6O_7$ requires 427.1378.

2-amino-6-fluoro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (6). Dry 4 (100 mg, 0.24 mmol) was dissolved in 15 mL of anhydrous THF under a dry argon atmosphere; 90% t-butylnitrite (37 uL, 0.28 mmol) was added to this solution and the reaction was refluxed for 43 h. The red-brown solution was evaporated to dryness and dissolved in a minimum of ethyl acetate. This solution was applied to a 2 x 10 cm Florisil flash column (hexane), and the product was eluted with 7:3 hexane: ethyl acetate. The fractions containing pure product were pooled and evaporated under reduced pressure. UV (MeOH): 289, 245 nm. ^{1}H NMR, $CDCl_3$, δ (ppm): 7.82 s (H8, 1H); 5.99 d (H1', 1H, J = 4.9 Hz); 5.94 t (H3', 1H, J = 5.0 Hz); 5.74 t (H2', 1H, J_1 = 4.9 Hz); 5.21 s ($2NH_2$, 2H); 4.40 m (H4' + H5' + H5", 3H); 2.12 s (OAc, 3H); 2.08 s (OAc, 3H); 2.06 s (OAc, 3H). ^{19}F NMR, δ (ppm): -71.11 s (F6, 1F). ^{13}C NMR, δ (ppm): 20.16, 20.35, 20.48 (3(CH_3)); 62.94 (C5'); 70.24 (C2'); 71.91 (C3'); 79.67 (C4'); 84.97 (C1'); 111.72 (C5); 140.97 (C8, J_{CH} = 216.4 Hz); 157.10 (C4); 159.31 (C6); 159.94 (C2); 169.26, 169.41, 170.07 (3(CO)).

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