Published in final edited form as:

J Org Chem. 2007 October 26; 72(22): 8551–8554. doi:10.1021/jo7015778.

The efficient syntheses of 5'-deoxy-5'-fluoro -guanosine and – inosine

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Abstract

Electron-Withdrawing Intermediates

Substitution of oxygen with a weak hydrogen bond acceptor such as fluorine provides a single-atom modification that can have grave effects on the chemical and medicinal properties of nucleoside analogs. To that end, we present a simple and high yielding method for the novel synthesis of 5'-deoxy-5'-fluoro-guanosine and 5'-deoxy-5'-fluoro-inosine utilizing an intramolecular electron withdrawing approach. The properties of the resulting modified nucleosides, as well as the halogenated intermediates, are notable for their similarity to nucleoside analogs used in the treatment of cancer, as well as enzyme inhibitors designed to target parasitic protozoa.

As many as 20% of pharmaceuticals on the market contain fluorine including half of the top ten drugs sold in 2005.¹ Introducing fluorine into a molecule presents unique problems in terms of selectivity, facility and safety. Nucleosides are the building blocks of DNA, RNA, many essential vitamins and serve as regulatory molecules in all cells. Fluorinated nucleoside analogs have been employed to study the chemical and metabolic properties of biological systems via ¹⁹F NMR^{2–6}, as anti-sense probes for positron emission tomography studies⁷ and as inhibitors of biochemical reactions.^{8–10} Functional group transformation at the 5'-position of nucleosides has historically been an area of intense interest, due primarily to the biological importance of this position in phosphoryl transfer.^{11–15} Derivatives of adenosine such as 5'-deoxy-5'-fluoro-adenosine (5'-F-A) are rare in nature, but can be produced enzymatically with high yield and in a single step.^{16,17} Here, we describe the first synthesis of 5'-deoxy-5'-fluoro-guanosine (5'-F-G) and 5'-deoxy-5'-fluoro-inosine (5'-F-I). Alteration of the 5'-substituent to fluorine renders the nucleoside inert to enzymatic phosphorylation and unreactive as a nucleophile, while retaining a size comparable to oxygen, as well as the capacity for modest hydrogen bonding in the appropriate environment.^{23–25}

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In general, synthetic strategies used to produce nucleoside analogs must overcome unique challenges arising from the reactivity of nucleobase functional groups. Towards this end, the fluorination of nucleoside derivatives has been accomplished either by direct incorporation from a fluoride source or by attachment chemistry involving a fluorinated building block. The chemical synthesis of 5'-F-A has been achieved (i) by fluorination of a primary 5'-alcohol^{18–20}, (ii) enzymatic synthesis from S-adenosyl-L-methionine²¹ or (iii) use of Vörbruggen's conditions in combination with method (i)²². Selective fluorination at the 2'- and 3'-positions has been realized as well, but with high step count and low individual yields.^{26–29}

At present, the syntheses of 5'-F-G and 5'-F-I have not been reported, although 5'-F-I has been reported as a side product of 5'-F-A production in bacterial extracts derived from *Streptomyces cattleya*.³⁰ Here we describe the chemical syntheses of 5'-F-G and 5'-F-I utilizing an electron-withdrawing-intermediate approach first employed for 5'-FA¹⁸. Earlier attempts at 5'-carbon modification by S_N2 strategies proved unrewarding, due to intramolecular cyclization that formed a 7-membered ring between the N-3 nitrogen and the 5'-carbon.^{31–34} To decrease N-3 basicity and alleviate intramolecular cyclization, we hypothesized that a strong electron-withdrawing substituent at the C-2 position would lead to the desired displacement of the tosylated 5'-oxygen by fluoride. A precedent for this approach came from the observation that incorporation of chlorine at the C-6 position of adenosine avoided cyclization, augmented the stability of the tosylated intermediate and enhanced fluorination yields.¹⁸ This strategy appeared more attractive since the production of both C2-fluorinated and C2-chlorinated inosine have been reported with low step count and high yield. These syntheses utilized a protecting group on the O-6 oxygen, which is normally susceptible to tautomerization and hence could be vulnerable to tosylation (Figure 1). As such, 2-F-I and 2-Cl-I were both attractive starting materials because the benzyl and allyl protecting groups were shown previously to be stable under the conditions to be employed for protection and fluorination. In addition, their deprotection on similar guanosine compounds had been demonstrated to occur in high yield.^{35,36} To assess how the addition of fluorine or chlorine at the C-2 position of guanosine or inosine would effect the basicity of the N-3 nitrogen, we subjected the nucleobase of each in the absence and presence of electron-withdrawing substituents to electrostatic potential calculations using GAMESS software.³⁷ Structures were minimized with the B3LYP density function using the 6-31G(d) basis set. The results showed a significant loss in electron density at the N-3 position when accompanied by an adjacent halogen (Figure 1D versus 1E & 1F). This observation further corroborates the concept that the N-3 nitrogen was activated by the adjacent exocyclic amine in the parental guanine base. This calculation is also illustrative of the differences in amine activation of adenosine¹⁸, but is expected to be absent in inosine. Given the strategy of starting with a C-2 halogenated adduct, efforts proceeded with protection of the cis-diols of O⁶-benzyl-2-fluoro-inosine and O⁶-allyl-2-chloro-inosine with 2,2-dimethoxypropane under acidic conditions (Scheme 1). Each protected nucleoside yielded a product with a single primary 5'-alcohol for displacement (Scheme 1, compounds **3** & **4**). Previous fluorination attempts on nucleic acid derivatives employed a two-step procedure in which tosylation occurred in high yield (52–83%), but fluorination was poor (35–46%).^{19, 20,22} In contrast, we fluorinated the respective 5'-positions of guanosine and inosine with facility in a single step (Scheme 1). This was accomplished by generating the tosylated alcohol *in situ*, and then fluorinating with excess fluoride providing high yields of **5** and **6** (88% and 90%) with no intramolecular cyclization.

To create a hydrogen atom at the C-2 position of **5**, we conducted Pd-catalyzed reduction under basic conditions (Scheme 1). The allylic protecting group, as well as chlorine, were each removed in high yield (81%) giving **7**. Finally, the isopropylidene group was removed in 86% yield, generating 5'-deoxy-5'-fluoro-inosine, **8**, in 52% overall yield in 4 steps.

Efforts to produce 5'-F-G from chlorinated inosine intermediate **3**, via displacement with ammonia, proved unsuccessful; specifically, displacement succeeded using dibenzylamine, but palladium-catalyzed reduction did not. Therefore, we were pleased to observe amination of **6** in 98% yield (Scheme 1) as indicated by the presence of a broad peak at 5 ppm in crude ^1H NMR, which integrated to 2 H. Without need for chromatographic purification, the aminated product was reduced readily under catalytic conditions producing **9** (Scheme 1) in 80% yield over two steps. Thus, **10** was produced subsequently after deprotection in 52% yield from **2** in five overall steps.

Selective fluorination of purine nucleosides has become an important aspect of medicinal chemistry because the C-F bond is refractory to cellular modifications, and can enhance biostability³⁸. Purine analogs of adenine such as fludarabine and cladribine exhibit fluorine or chlorine at their base C-2 positions and have received FDA approval in the treatment of neoplasias³⁹. In contrast, the site specific modification of Immunocillin-H (i.e., an inosine derivative harboring iminoribitol) with a 5'-deoxy-5'-fluoro adduct results in a potent inhibitor of purine nucleoside phosphorylase (PNP).⁴⁰ The toxicity of this compound with respect to the malarial parasite is likely due to inhibition of PNP-mediated hypoxanthine production, which appears to serve as a primary purine source for *Plasmodium falciparum*.⁴¹ Thus, selective and high yielding fluorination of inosine (**8**) and guanosine (**10**), as well as precursors (**5** and **6**) presented here, should be of broad interest not only as agents that target cancer, but also as potential inhibitors of parasitic protozoa.

In summary, we have reported the first synthesis of 5'-deoxy-5'-fluoro-guanosine and -inosine. By use of an electron-withdrawing halogen at the C-2 base position, we developed short, inexpensive syntheses to generate high yields of 5'-fluorinated products. This method provides the first framework to introduce weak nucleophiles at the 5'-position of guanosine- and inosine-related nucleosides. The C-2 halogenated intermediates (**5** and **6**) are also attractive products because their electrophilic character facilitates the incorporation of varied functional groups at the C-2 position, which is suitable for the preparation of a 5'-fluorinated library. Such nucleosides have potential in the treatment of cancer or infection by purine-scavenging parasites.

Experimental Section

The procedures for the production of compounds (**3**) through (**10**) are as follows:

(3): O⁶-allyl-2-chloro-2',3'-O-isopropylideneguanosine

O⁶-allyl-2-chloro-guanosine (**1**) (0.550 g, 1.6 mmol) was placed in a dry 0.25 L round bottom flask. To the solution, 10.5 mL of freshly distilled acetone was added with 0.978 mL of 2,2-dimethoxypropane; 0.864 g of *p*-toluenesulfonic acid monohydrate was added and the solution was stirred 4 h at 24 °C. The solution was neutralized with concentrated sodium bicarbonate and extracted exhaustively with dichloromethane. Organic extracts were dried (sodium sulfate) and purified on silica gel (10:90 ether to dichloromethane) yielding 0.528 g of **3**, which appeared as a viscous colorless solid in 86% yield. ^1H NMR in CDCl_3 δ (ppm): 8.10 (s, 1H), 6.11-6.01 (m, 1H), 5.86 (d, 1H, $J = 4.8$), 5.44 (d, 1H, $J = 17.2$), 5.29 (d, 1H, $J = 10.4$), 5.11 (d, 1H, $J = 5.6$), 5.05 (d, 1H, $J = 5.6$), 4.44 (s, 1H), 3.94 (d, 1H, $J = 12.8$), 3.79 (d, 1H, $J = 12.8$), 1.58 (s, 3H) 1.31 (s, 3H). ^{13}C NMR in CDCl_3 δ (ppm): 160.9, 153.0, 151.9, 142.2, 131.3, 121.7, 119.6, 114.2, 93.3, 86.1, 83.2, 81.4, 68.8, 63.1, 27.4, 25.2. IR ν^{max} (cm^{-1}): 3524–3345, 2990, 1647, 1319, 1216, 1110, 1078. High resolution mass spectroscopy (H.R.M.S.) was performed for all compounds. ($\text{C}_{16}\text{H}_{19}\text{ClN}_4\text{O}_5$): calcd.: 382.1044; obsd.: 382.1047.

(4): 2-fluoro-O⁶-benzyl-2',3'-O-isopropylidene-guanosine

2-fluoro-O⁶-benzyl-guanosine (**2**) (0.860 g, 2.27 mmol) was placed in a dry round bottom flask. 15 mL of freshly distilled acetone was added with 1.5 mL of 2,2-dimethoxypropane; 0.464 g of *p*-toluenesulfonic acid monohydrate was added. The solution was stirred 4 h and then neutralized with concentrated sodium bicarbonate followed by exhaustive extraction with dichloromethane. The extracts were dried (sodium sulfate) and purified on silica gel (10:90 ether to dichloromethane) generating 0.776 g of **4** as a colorless solid in 82% yield. ¹H NMR in CDCl₃ δ (ppm): 8.11 (s, 1H), 7.46 (m, 2H), 7.29 (m, 3H), 5.93 (s, 1H), 5.69 (d, 2H, *J* = 4.0), 5.09 (d, 1H, *J* = 5.6), 5.00 (d, 1H, *J* = 6.0), 4.38 (s, 1H), 3.86 (d, 1H, *J* = 12.4), 3.67 (d, 1H, *J* = 12.4), 1.61 (s, 3H), 1.57 (s, 3H). ¹³C NMR in CDCl₃ δ (ppm): 162.6 (d, *J*_{CF} = 14), 158.5 (d, *J*_{CF} = 173), 152.2 (d, *J*_{CF} = 14), 142.0, 134.9, 128.7, 128.6, 128.5, 128.1, 120.7 (d, *J*_{CF} = 4), 114.2, 93.0, 86.2, 83.4, 81.4, 69.8, 62.9, 27.4, 25.3. ¹⁹F NMR in CDCl₃ δ (ppm): 13.4 (s). IR ν^{max} (cm⁻¹): 3628–3233, 2987, 2359, 1635, 1375, 1231, 1076. H.R.M.S. (C₂₀H₂₁FN₄O₅): calcd: 416.1496; obsd: 416.1491.

(5): O⁶-allyl-2-chloro-5'-fluoro-2',3'-O-isopropylidene-guanosine

To an oven dried 100 mL round bottom flask, 0.726 g (1.9 mmol) of **3** was added and dissolved in 28 mL of freshly distilled THF. To the flask, 0.487 g of *p*-toluenesulfonyl fluoride was added (2 molar equivalents) followed by dropwise addition of 5.7 mL of 1 M TBAF in THF (3 molar equivalents). The solution was heated to 60 °C, stirred 12 h, cooled, concentrated to dryness, and purified by silica gel chromatography using (50:50, ethyl acetate to hexanes) to yield 0.642 g of **5** as a clear oil in 88% yield. ¹H NMR in CDCl₃ δ (ppm): 8.07 (s, 1H), 6.24 (s, 1H), 6.20–6.13 (m, 1H), 5.53 (d, 1H, *J* = 14), 5.36 (d, 1H, *J* = 8.4), 5.28 (d, 1H, *J* = 04.4), 5.14 (d, 2H, *J* = 4.4), 5.12–5.10 (m, 1H), 4.76 (dd, 1H, *J* = 11.6, 8.4), 4.66 (dd, 1H, *J* = 11.6, 8.4), 4.55 (d, 1H, *J* = 20), 1.66 (s, 3H), 1.42 (s, 3H). ¹³C NMR in CDCl₃ δ (ppm): 160.7, 153.1, 152.5, 141.2, 131.5, 120.9, 119.4, 114.8, 90.5, 85.5 (d, *J*_{CF} = 15), 84.4, 83.5 (d, *J*_{CF} = 136), 80.6 (d, *J*_{CF} = 6), 68.6, 27.1, 25.3. ¹⁹F NMR in CDCl₃ δ (ppm): –168.685 (m). IR ν^{max} (cm⁻¹): 2939, 1635, 1310, 1216. H.R.M.S. (C₁₆H₁₈ClFN₄O₄): calcd.: 384.1001; obsd.: 384.1004.

(6): 2-fluoro-5'-fluoro-O⁶-benzyl-2',3'-O-isopropylidene-guanosine

To an oven dried 100 mL round bottom flask, 0.600 g (1.44 mmol) of **4** was added and dissolved in 25 mL of freshly distilled THF. To the flask 0.500 g of *p*-toluenesulfonyl fluoride was added (2 molar equivalents), followed by dropwise addition of 4.32 mL of 1M TBAF in THF (3 molar equivalents). The solution was heated to 60 °C, stirred overnight, then cooled and concentrated to dryness. Purification was by silica gel chromatography (50:50, ethyl acetate to hexanes) to produce 0.542 g of **6** as a clear oil in 90% yield. ¹H NMR in CDCl₃ δ (ppm): 7.99 (s, 1H), 7.44 (m, 2H), 7.25 (m, 3H), 6.10 (s, 1H), 5.56 (s, 2H), 5.19 (d, 1H, *J* = 5.6), 4.97 (d, 1H, *J* = 5.6), 4.64 (dd, 1H, *J* = 10.4, 6.4), 4.52 (dd, 1H, *J* = 10.4, 6.4), 4.44 (d, 1H, *J* = 23.6), 1.54 (s, 3H), 1.35 (s, 3H). ¹³C NMR in CDCl₃ δ (ppm): 162.3 (d, *J*_{CF} = 14), 158.6 (d, *J*_{CF} = 171), 152.8 (d, *J*_{CF} = 15), 142.1, 135.1, 128.5, 128.4, 128.4, 128.1, 120.2 (d, *J*_{CF} = 4), 114.6, 90.6, 85.4 (d, *J*_{CF} = 15), 84.2, 83.5 (d, *J*_{CF} = 136), 80.5 (d, *J*_{CF} = 5), 69.5, 27.0, 25.2. ¹⁹F NMR in CDCl₃ δ (ppm): 13.7 (s, 1F), –166.9 (m, 1F). IR ν^{max} (cm⁻¹): 2987, 1581, 1377, 1225, 1078, 1019. H.R.M.S. (C₂₀H₂₀F₂N₄O₄): calcd.: 418.1453, obsd.: 418.1451.

(7): 5'-fluoro-2',3'-O-isopropylidene-inosine

0.320 g of **5** (0.833 mmol) was added to a round bottom flask; 15 mL of ethanol was added, followed by 128 μL of triethylamine. The solution was transferred to a hydrogenation pressure bottle. 138 mg of 10% Pd/C was added, then shaken at 50 p.s.i. for 12 h. Catalyst was removed by filtering through celite, the solution was concentrated, and purified on silica gel (10:90, MeOH to DCM) giving 0.209 g of **7** as a colorless solid in 81% yield. ¹H NMR in MeOD δ (ppm): 8.172 (s, 1H), 8.059 (s, 1H), 6.241 (s, 1H), 5.351 (d, 1H, *J* = 6), 5.088 (d, 1H, *J* = 6),

4.688 (m, 1H), 4.552-4.512 (m, 1H), 4.501 (d, 1H, $J = 18.8$), 1.589 (s, 3H), 1.377 (s, 3H). ^{13}C NMR in MeOD δ (ppm): 157.4, 148.2, 145.5, 139.1, 124.4, 114.1, 90.6, 85.6 (d, $J_{\text{CF}} = 15$), 84.5, 83.4 (d, $J_{\text{CF}} = 135$), 80.6 (d, $J_{\text{CF}} = 5$), 26.0, 24.0. ^{19}F NMR in MeOD δ (ppm): -168.391 (m). IR ν^{max} (cm^{-1}): 2942, 1695, 1338, 1237, 1108, 1060, 1019. H.R.M.S. ($\text{C}_{13}\text{H}_{15}\text{FN}_4\text{O}_4$): calcd.: 310.1077, obsd.: 310.1075.

(8) 5'-fluoro-5'-deoxy-inosine

10 mL of 70% TFA was added to 0.162 g of **7** at 0 °C. The solution was warmed to 24 °C, stirred 2 h, evaporated to dryness and coevaporated 3x with toluene. The resulting white solid was recrystallized from methanol to yield 0.121 g of **8** (86% yield) as colorless needles. ^1H NMR in DMSO- d_6 δ (ppm): 12.47 (br s, 1H), 8.27 (s, 1H), 8.14 (s, 1H), 5.97 (s, 1H), 5.72 (s, 1H), 5.49 (s, 1H), 4.74 (s, 1H), 4.69 (s, 1H), 4.62 (s, 1H), 4.25 (s, 1H), 4.19 (app d, 1H, $J = 12.2$), 4.13 (app d, 1H, $J = 12.2$). ^{13}C NMR in DMSO- d_6 δ (ppm): 157.0, 148.7, 146.48, 138.9, 124.9, 88.13, 83.97 (d, $J = 14.5$), 83.04 (d, $J_{\text{CF}} = 134$), 73.96, 69.83 (d, $J_{\text{CF}} = 4.6$). ^{19}F NMR in DMSO- d_6 δ (ppm): -169.2 (m). IR ν^{max} (cm^{-1}): 2872, 1680, 1410, 1220, 1110, 960. H.R.M.S. ($\text{C}_{10}\text{H}_{11}\text{FN}_4\text{O}_4$): calcd. 270.0764; obsd. $[\text{M}+\text{H}^+]$ 271.0840.

(9): 5'-fluoro-2',3'-O-isopropylidene-guanosine

1.00 g (2.4 mmol) of **6** was placed in a sealed pressure tube and dissolved in 20 mL of dry tert-butyl alcohol. Anhydrous ammonia gas was bubbled through the solution at 0 °C, which was then warmed to 80 °C for 24 h. The mixture was extracted exhaustively with chloroform from water and evaporated to produce 0.971 g of a white solid in 98% yield. The solid was dissolved in 0.60 L (50:50 MeOH to THF), and 390 mg of 10% Pd/C was added. H_2 gas was bubbled through the solution, followed by stirring for 12 h under a balloon. Catalyst was removed by filtering through celite. The solution was purified by silica gel chromatography (20% MeOH to DCM) and evaporated to dryness to generate 0.620 g of **9**, a colorless solid, in 80% yield over two steps. ^1H NMR in DMSO- d_6 δ (ppm): 10.72 (br s, 1H), 7.81 (s, 1H), 6.56 (br s, 1H), 6.01 (s, 1H), 5.21 (s, 1H), 5.10 (s, 1H), 4.62 (app d, 1H, $J = 14.4$), 4.55 (app d, 1H, $J = 15.6$), 4.33 (app d, 1H, $J = 19.6$), 1.49 (s, 3H), 1.29 (s, 3H). ^{13}C NMR in DMSO- d_6 δ (ppm): 157.1, 154.1, 150.9, 136.4, 117.3, 113.8, 88.9, 85.3 (d, $J_{\text{CF}} = 15$), 84.2 (d, $J_{\text{CF}} = 133$), 84.0, 80.7 (d, $J_{\text{CF}} = 6$), 27.4, 25.6. ^{19}F NMR in DMSO- d_6 δ (ppm): -162.7 (m). IR ν^{max} (cm^{-1}): 2984, 1719, 1374, 1203, 1108, 1071, 1000. H.R.M.S. ($\text{C}_{13}\text{H}_{16}\text{F}_1\text{N}_5\text{O}_4$): calcd.: 325.1186, obsd.: 325.1187.

(10) 5'-fluoro-5'-deoxy-guanosine

A 15 mL solution of 70% TFA was added to 0.20 g of **9** at 0 °C. The solution was warmed to 24 °C, stirred 2 h, evaporated to dryness and then coevaporated 3x with toluene. The resulting white solid was recrystallized from methanol yielding 0.154 g of **10** (88% yield) as colorless needles. ^1H NMR in DMSO- d_6 δ (ppm): 10.74 (br s, 1H), 7.93 (s, 1H), 6.49 (s, 1H), 5.69 (s, 1H), 5.42 (s, 1H), 5.14 (s, 1H), 5.06 (s, 1H), 4.62 (app d, 1H, $J = 10.4$), 4.07 (s, 1H), 3.87 (s, 1H), 3.61 (app d, 1H, $J = 12$), 3.52 (app d, 1H, $J = 12$). ^{13}C NMR in DMSO- d_6 δ (ppm): 157.2, 154.2, 151.8, 135.8, 117.2, 87.2, 84.1 (d, $J_{\text{CF}} = 133$), 82.7 (d, $J_{\text{CF}} = 14.7$), 73.6, 70.0 (d, $J_{\text{CF}} = 5.7$). ^{19}F NMR in DMSO- d_6 δ (ppm): -163.0 (m). IR ν^{max} (cm^{-1}): 2950, 1726, 1598, 1367, 1210, 1108, 1071. H.R.M.S. ($\text{C}_{10}\text{H}_{12}\text{F}_1\text{N}_5\text{O}_4$): calcd. 285.0873; obsd. $[\text{M}+\text{H}^+]$ 286.0948.

Supplementary Material

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Acknowledgements

We thank Profs. Ben Miller, Bob Boeckman and Brad Nilsson for helpful discussions. We thank B. McNaughton for assistance with GAMESS software and Dr. A. Bergmann for M.S. data collection (supported in part by NSF Grant CHE0091977). This investigation was supported in part by grants NIH R01 GM63162 and ACS/PRF 45534-AC4 to J.E.W. This work is dedicated to Prof. Perry A. Frey in honor of his career as a researcher, teacher, mentor and chemist.

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Figure 1. Electron-withdrawing substrates with space-filling surface and mapped electrostatic potential. (A) guanosine, (B) O6-benzyl-2-fluoro-inosine, 2-F-I, (C) O6-allyl-2-chloro-inosine; 2-Cl-I. (D-F) GAMESS (electrostatic potential) maps calculated for nucleoside bases in parts (A-C), respectively. Key: red denotes high electron density, blue denotes low electron density.

SCHEME 1.
Synthetic Steps for 5'-F-G and 5'-F-I Production