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Research Article

Synthesis of [^{18}F]-labeled adenosine analogues as potential PET imaging agents

Mian M. Alauddin, John D. Fissekis, and Peter S. Conti*
University of Southern California, PET Imaging Science Center, Los Angeles, CA 90033, USA

Summary

The syntheses of adenosine analogues, 2′-deoxy-2′-[^{18}F]fluoro-9-β-D-arabinofuranosyladenine ([^{18}F]-FAA) and 3′-deoxy-3′-[^{18}F]fluoro-9-β-D-xylofuranosyladenine ([^{18}F]-FXA) are reported. Adenosine (**1**) was converted to its methoxytrityl derivatives **2** and **3** as a mixture. After separation, these derivatives were converted to their respective triflates **4** and **5**. Each triflate was reacted with tetrabutylammonium[^{18}F]fluoride to produce **6b** or **7b**, which by acidic hydrolysis yielded compounds **8b** and **9b**. Crude preparations were purified by HPLC to obtain the desired pure products. The radiochemical yields were 10-18% decay corrected (d.c.) for **8b** and 30–40% (d.c.) for **9b** in 4 and 3 runs, respectively. Radiochemical purity was $>99\%$ and specific activity was >74 GBq/μmol at the end of synthesis (EOS). The synthesis time was 90–95 min from the end of bombardment (EOB). Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: fluorine-18; nucleoside; adenosine; PET.

Introduction

Many fluorinated analogues of adenosine nucleoside have been synthesized and studied as potential antitumor and antiviral agents.[1–11]

*Correspondence to: Peter S. Conti, USC, PET Imaging Science Center, 2250 Alcazar St., # 101, Los Angeles, CA 90033, USA. E-mail: pconti@hsc.usc.edu

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Among these, 2′-deoxy-2′-fluoro-2-chloro-9-β-D-arabinofuranosyladenine has been found to be active against human colon tumor xenografts.[6,7] The 2′-deoxy-2′-fluoro-arabino compounds gained much attention as anticancer agents, [6–8] while the 3′-deoxy-3′-fluoro-ribo compounds have shown antiviral activity.[9–11] Little information is available regarding the biological properties of the xylo-derivative, 3′-fluoro-9-β-D-xylofuranosyladenine.[1,12,13] Most recently a synthesis of [^{11}C]-adenosine monophosphate has been reported in order to investigate its potential use for imaging cancer.[14] [^{18}F]-Labeled analogues of adenosine, particularly those that are non-catabolized, also have potential for PET imaging of cancer and/or viral infection.

We have been exploring the radiofluorination of pyrimidine furanosyl derivatives as potential agents for imaging cell proliferation and gene expression using PET.[15,16] More recently, we have begun to explore radiofluorination of furanosyl purines. One synthesis of 2′-deoxy-2′-fluoro-9-β-D-arabinofuranosyladenine involves the incorporation of fluorine in the *arabino* configuration at C-2 of the sugar followed by coupling with the purine base.[2,17] An alternative approach involves the direct fluorination of an appropriate precursor of the nucleoside with various fluorinating agents followed by de-protection of the reacting intermediate.[4,8] The direct fluorination of the purine nucleoside appears to be convenient and superior to the three-step method in which the fluorinated glycosyl moiety is prepared first, converted to its 1-bromo-derivative and then coupled with the purine. This contrasts our experience within the pyrimidine series where the direct fluorination fails to provide the desired product.[4,15,16]

The high yield synthesis of 2′-deoxy-2′-fluoro-9-β-D-arabinofuranosyladenine involves treatment of N^6,3′,5′-tritrityladenosine with (diethylamino)-sulfur trifluoride (DAST).[4,13] DAST is not a suitable reagent for radiochemical synthesis of these compounds due to the unavailability of the ^{18}F-labeled reagent and the required long reaction time 6–16 h. One synthesis of [^{18}F]-labeled FAA involves the fluorination of a 2′-tosylate of a protected 9-β-D-ribofuranosyladenine.[8] However, this synthesis was reported without detailed characterization of the product. In a continuing effort towards the development of PET imaging agents we have synthesized 2′-deoxy-2′-[^{18}F]fluoro-9-β-D-arabinofuranosyladenine. Since little biological information of FXA is available, we have synthesized a new agent, 3′-deoxy-3′-[^{18}F]fluoro-9-β-D-xylofuranosyladenine ([^{18}F]-FXA), in order to study its biological properties *in vivo* with PET.

Results and Discussion

Figure 1 represents the scheme for the [18F]-labeled synthesis of the adenosine nucleoside analogues, [18F]-FAA and [18F]-FXA. Compounds **2** and **3** were prepared from adenosine following a literature method.[4] Reaction of adenosine with methoxytrityl chloride in pyridine produced a mixture of three compounds, which were separated by column chromatography. The early eluting compound (not shown), a methoxytrityl derivative of adenine as reported earlier,[4] was discarded. Compounds **2** and **3** were isolated in 28% and 50% yields, respectively. Both compounds were characterized by ^{1}H NMR spectroscopy.

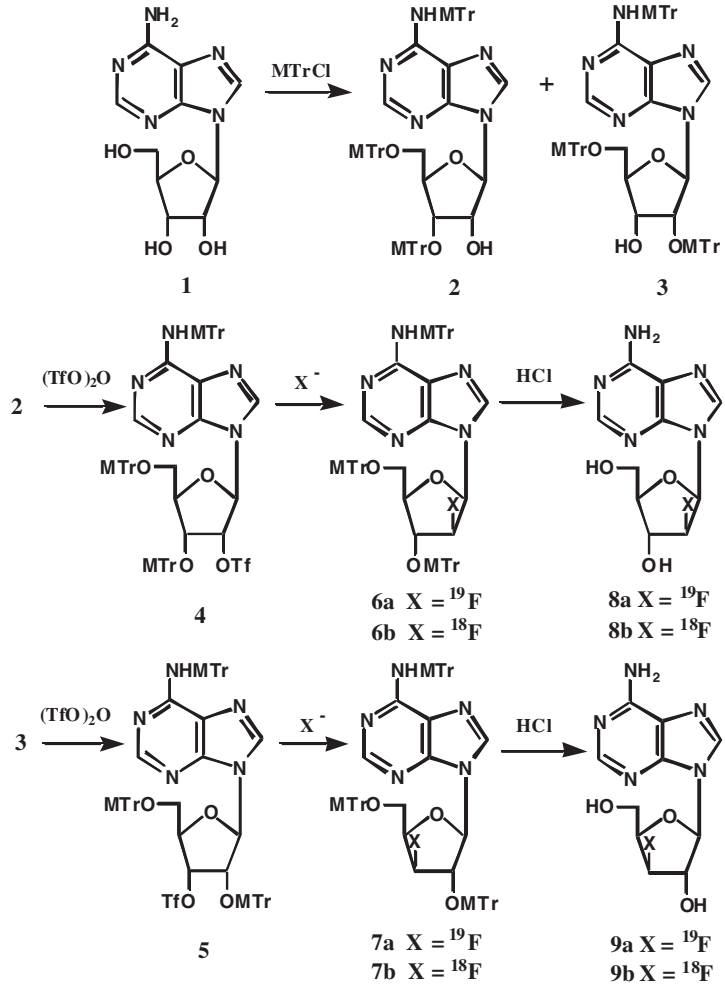

Figure 1. Synthetic scheme of [18F]-fluoro-adenosine analogues

Compounds **4** and **5** were prepared by reaction of **2** and **3** with trifluoromethanesulfonic acid anhydride in pyridine at low temperature. Yields in this step were quite high, ~90% for both compounds. These compounds were characterized by ^{1}H and ^{19}F NMR spectroscopy, and high resolution mass spectrometry. ^{19}F NMR spectra showed a singlet at –73.16 and –73.58 ppm for compound **4** and **5**, respectively.

Fluorination of the triflate precursor **4** with *n*-Bu_4NF produced the desired 2′-deoxy-2′-fluoro-9-β-D-arabinofuranosyladenine derivative **6a** in 20% yield. Reaction of the triflate precursor **5** with *n*-Bu_4NF on the other hand produced the corresponding 3′-deoxy-3′-fluoro-9-β-D-xylofuranosyladenine derivative **7a** in 50% yield. Low yield of **6a** compared **7a** may be due to steric hindrance exerted by the purine ring. Both compounds were purified, and characterized by ^{1}H and ^{19}F NMR spectroscopy and high resolution mass spectrometry. ^{19}F NMR spectrum of **6a** was more complex compared to that of **7a**, which was a clean doublet of triplet. Consistent with the earlier reports [2,5] on 2′-fluoro-arabinoadenosine there is a long range coupling between the 2′-fluorine and C_8H in compound **6a** ($J = \sim 1$ Hz). Furthermore nucleophilic substitution of the 2′-(*ribo*) triflate of adenosine with acetate or thiol is known to produce the respective *arabino* epimer.[4,8,18]

The major by-products in these fluorination reactions with *n*-Bu_4NF are the 2′-hydroxy-arabino- and 3′-hydroxy-xylo-derivatives, respectively. The Rf value (TLC) of 2′-hydroxy-arabino compound differs from that of the corresponding 2′-hydroxy-ribo compound, which further confirms the epimerisation at the 2′-position during nucleophilic substitution. Competition between hydroxide and fluoride ions has been observed in our earlier studies on fluorination of a protected sugar and acycloguanosine.[15,19,20]

Compounds **8a** and **9a** were prepared by acid hydrolysis of the protecting groups from the respective methoxytrityl derivatives **6a** and **7a** following the method reported earlier.[20] The NMR spectra of the products were compared with known compounds, reported earlier.[4,13]

Radiolabeled compounds **6b** and **7b** were prepared by fluorination of the respective triflate precursors with n-$Bu_4N^{18}F$, prepared *in situ* from *n*-Bu_4NHCO_3 and aqueous $H^{18}F$. In this reaction ~50 µl (4% soln, ~7 µmol) of *n*-Bu_4NHCO_3 was used based on our earlier experience as an optimum quantity.[15,19] As observed in the cold synthesis the radiochemical yield was much higher for compound **7b** compared to **6b**. Unreacted fluoride was removed by passing the crude reaction mixture through a Sep-Pak cartridge (silica) and the product was eluted

with 10% methanol in dichloromethane. The protected [^{18}F]-labeled compounds **6b** and **7b** were neither purified nor characterized, however, the unlabeled products **6a** and **7a** were isolated and purified. After solvent evaporation the recovered labeled compounds **6b** and **7b** could be easily hydrolyzed with acid, and the desired labeled nucleosides **8b** and **9b** isolated by high performance liquid chromatography (HPLC) purification using 9% MeCN. Figure 2 represents a typical purification trace of [^{18}F]-FAA. The uv peak at 9 min corresponds to the by-product, 2′-hydroxy-arabinoadenosine, and the radioactive peak between 15-16 min corresponding to the desired [^{18}F]-FAA. The apparent split of the radioactive peak is an artifact due to the saturation of the detector.

The radiochemical yield of this synthesis was 10-18% (d.c.) for **8b** and 30-40% for **9b**. The radiochemical purity was $>99\%$ with specific activity >74 GBq/μmol. The synthesis time was 90-95 min from the EOB. In representative syntheses, 518 MBq of labeled product **8b** was obtained starting from 6.88 GBq of [^{18}F]-fluoride, and 3.07 GBq of **9b** was obtained from 8.54 GBq of [^{18}F]-fluoride.

Analytical samples of **8b** or **9b** showed (HPLC) a single radioactive peak that *co-eluted* with an authentic sample (Figure 3).

Experimental

Reagents and Instrumentation

All reagents and solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI), and used without further purification. Solid phase

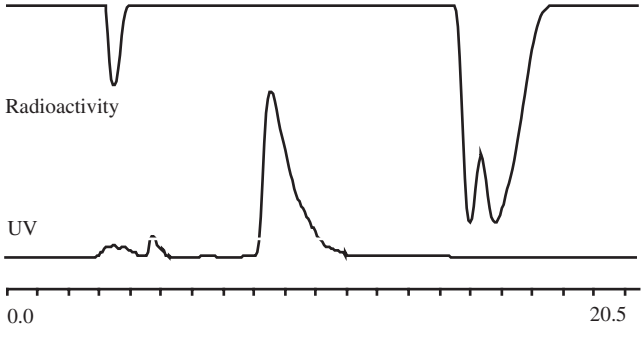

Figure 2. Purification of [^{18}F]-FAA: Semi-prep Column; 9% MeCN/H_2O; flow 3.375 ml/min. Capacity factor = 4

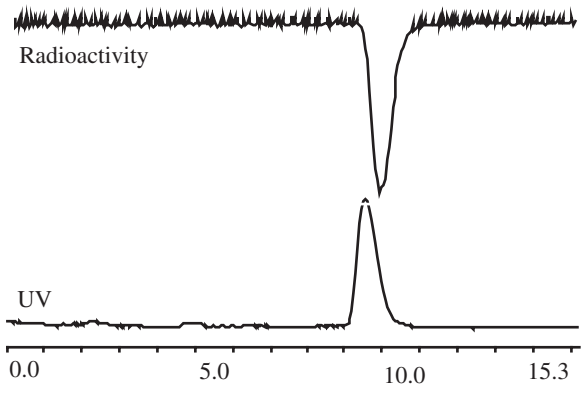

Figure 3. HPLC chromatogram of [^{18}F]-FAA, co-injected with standard FAA: Analytical C_{18} Column; 9% MeCN/H_2O; flow 1.13 ml/min

extraction cartridges (Sep-Pak) were purchased from Waters Associates (Milford, MA).

Thin layer chromatography (TLC) was performed on pre-coated Kieselgel 60 F254 (Merck) glass plates. Proton and ^{19}F NMR spectra were recorded on a Brucker 360 or 500 MHz spectrometer using tetramethylsilane as an internal reference and hexafluorobenzene as an external reference, respectively. Mass spectra were obtained on a Finnigan 400 mass spectrometer at the University of Minnesota using the ammonia chemical ionization technique.

High performance liquid chromatography (HPLC) was performed on a system using a 510 pump (Waters Associates, Milford, MA), UV detector (Isco, Lincoln, NE) operated at 254 nm, and a radioactivity detector with single-channel analyzer (Technical Associate, Woodland Hills, CA) using a semi-preparative C_{18} reverse phase column (Alltech, Econosil, 10×250 mm, Deerfield, IL) and an analytical C_{18} column (Alltech, Econosil 4.6×250 mm). A acetonitrile/water (MeCN/H_2O) solvent system (9% MeCN) was used for purification of the radiolabeled nucleosides and quality control analysis.

*N^6,3′,5′-Tri-methoxytrityl-2′-hydroxy and N^6,2′,5′-tri-methoxytrityl-3′-hydroxy-9-β-*D*-ribofuranosyladenine:* **2** *and* **3**

The title compounds were prepared following a literature method.[4] Briefly, adenosine 1.35 g (5.0 mmol), dimethylaminopyridine 0.5 g (1 equiv), and methoxytrityl chloride 5.45 g (17.6 mmol) were placed in a dry flask under argon. Pyridine (80 ml) was added and the mixture was

heated with stirring at 85–88°C for 18 h when TLC showed no significant starting material remained. Solvent was evaporated under vacuum, the residue was dissolved in 50% EtOAc/hexane and the solution washed with water. The organic phase was evaporated to dryness and purified by column chromatography using 25% ethyl acetate in hexane. Pure compound **2** and **3** were isolated in 28 and 50% yields, respectively. ^{1}H NMR (DMSO-D_6) δ: Compound **2**: 8.27 (s, 1 H, C_8H), 7.78 (s, 1 H, C_2H), 6.74–7.31 (m, 42 H, aromatic), 6.09 (d, 1 H, J = 7.25 Hz, 1′H), 5.99 (d, 1 H, J = 6.75 Hz, OH, exchangeable with D_2O), 4.88 (m, 1 H, 2′H), 4.13 (d, 1 H, J = 4.1 Hz, 3′H), 3.71 (s, 3 H, OMe), 3.70 (s, 3 H, OMe), 3.66 (s, 3 H, OMe), 3.09 (bs, 1 H, 4′H), 2.63 (bs, 2 H, 5′H).

Compound **3**: 8.06 (s, 1 H, C_8H), 7.65 (s, 1 H, C_2H), 6.60–7.32 (m, 42 H, aromatic), 5.87 (d, 1 H, J = 5.85 Hz, 1′H), 4.98 (m, 2 H, 2′H and OH, exchangeable with D_2O), 3.99 (bs, 1 H, 3′H), 3.70 (s, 3 H, OMe), 3.69 (s, 3 H, OMe), 3.63 (s, 3 H, OMe), 3.29 (bs, 1 H, 4′H), 3.08 (m, 1 H, 5′aH), 2.9 (m, 1 H, 5′bH).

*N^6,3′,5′-Tri-methoxytrityl-2′-trifluoromethanesulfonyl- and N^6,2′,5′-tri-methoxytrityl-3′-trifluoromethanesulfonyl-9-β-*D*-ribofuranosyladenine:* **4** *and* **5**

Compounds **4** and **5** were prepared by the same general method. A representative procedure is described here. Compound **2** or **3** (0.55 g, 0.5 mmol) was dissolved in pyridine (7 ml) under argon and cooled in ice bath. Trifluoromethanesulfonic acid anhydride (0.2 ml, 1.3 equiv) was added and the reaction mixture was stirred for 10 min in the cold and subsequently for 30 min at room temperature. The reaction mixture was quenched with cold water and extracted with 50% EtOAc/hexane. The organic extract was evaporated to dryness and purified by chromatography on a silica gel column using 30% ethyl acetate in hexane. Pure compound (560 mg) was obtained in 90% yield. ^{1}H NMR (DMSO-D_6) δ: Compound **4**: 8.38 (s, 1 H, C_8H), 7.62 (s, 1 H, C_2H), 6.74–7.40 (m, 42 H, aromatic), 6.62 (d, 1 H, J = 4.25 Hz, 1′H), 5.93 (t, 1 H, J = 5.0 Hz, 2′H), 4.50 (t, 1 H, J = 4.3 Hz, 3′H), 3.81 (bs, 1 H, 4′H), 3.71 (s, 3 H, OMe), 3.70 (s, 3 H, OMe), 3.65 (s, 3 H, OMe), 2.87–2.93 (m, 2 H, 5′H). ^{19}F NMR (δ): −73.16 (s). High resolution MS: M^+, Calculated 1215.4064; found 1215.4028.

Compound **5**: 8.46 (s, 1 H, C_8H), 6.64–7.40 (m, 43 H, aromatic and C_2H), 6.43 (d, 1 H, J = 8.1 Hz, 1′H), 6.04 (m, 1 H, 3′H), 4.26 (d, 1 H,

$J = 4.1$ Hz, 4′H), 4.13 (m, 1 H, 2′H), 3.72 (s, 3 H, OMe), 3.65 (s, 3 H, OMe), 3.58 (s, 3 H, OMe), 2.9 (m, 2 H, 5′H). ^{19}F NMR (δ): −73.58 (s). High resolution MS: M^+, Calculated 1215.4064; found 1215.4034.

*N^6,3′,5′-Tri-methoxytrityl-2′-fluoro-9-β-*D*-arabinofuranosyl- and N^6,2′,5′-tri-methoxytrityl-3′-fluoro-9-β-*D*-xylofuranosyladenine:* **6*a*** *and* **7*a***

Compound **4** or **5** (30 mg) was dissolved in dry MeCN (1.5 ml) in a sealed v-vial. To the above solution *n*-Bu_4NF (1 M, 30 µl) was added and the mixture was heated for 30 min at 72–74°C in a heating block. The reaction mixture was cooled to room temperature, the solvent evaporated in a stream of air and the residue purified on a short silica gel column using 30% ethyl acetate in hexane. Pure compound **6** (5 mg) or **7** (12 mg) were obtained in 20 and 50% yields, respectively. ^{1}H NMR (DMSO-D_6) δ: Compound **6a**: 7.87 (s, 1 H, C_2H), 7.83 (bs, 1 H, C_8H), 6.74–7.40 (m, 42 H, aromatic), 6.40 (dd, 1 H, $J = 24$ and 2.1 Hz, 1′H), 4.4 (m, 1 H, 4′H), 4.22–4.38 (m, 2 H, 2′H and 3′H), 3.71 (s, 3 H, OMe), 3.70 (s, 3 H, OMe), 3.65 (s, 3 H, OMe), 3.01–3.12 (m, 2 H, 5′H). ^{19}F NMR (δ): −193.95–194.18 (m). High resolution MS: M + H, Calculated 1085.45276; found 1085.45402.

Compound **7a**: 8.10 (s, 1 H, C_8H), 7.65 (s, 1 H, C_2H), 6.64–7.40 (m, 42 H, aromatic), 6.41 (s, 1 H, 1′H), 4.39, (d, $J = 14.0$ Hz, 2′H, 4.24 (dt, $J = 3.31$ and 6.0 Hz, 4′H), 3.82 (d, $J = 50.6$ Hz, 1 H, 3′H), 3.78 (s, 6 H, OMe), 3.68 (s, 3 H, OMe), 3.42–3.45 (m, 1 H, 5′aH), 3.28-3.30 (m, 1 H, 5′bH). ^{19}F NMR (δ):−202.57, (dq). High resolution MS: M^+, Calculated 1085.45276; found 1085.44915.

*2′-Fluoro-9-β-*D*-arabinofuranosyladenine and 3′-fluoro-9-β-*D*-xylofuranosyladenine:* **8*a*** *and* **9*a***

Unlabeled compounds **8a** and **9a** were prepared by acid hydrolysis of the protecting groups from the respective methoxytrityl derivatives **6a** and **7a** in 80% yields, following the method reported earlier.[20] The products were characterized by ^{1}H NMR spectroscopy, and found to be consistent with the literature.[12,17]

*2′-[^{18}F]-Fluoro-9-β-*D*-arabinofuranosyladenine and 3′-[^{18}F]-fluoro-9-β-*D*-xylofuranosyladenine:* **8*b*** *and* **9*b***

[^{18}F]-Labeled compounds were prepared as follows: Aqueous [^{18}F]fluoride (0.4 ml) was added to a solution of *n*-Bu_4NHCO_3 (50 µl, 4% by wt.)

in a v-vial and the solution evaporated azeotropically to dryness with acetonitrile (1.0 ml) at 72–74°C under a stream of argon. To the dry residue, *n*-$Bu_4N^{18}F$, a solution of either **4** or **5** (1.5–2.0 mg, 1.2–1.6 μmol) in dry acetonitrile (0.5 ml) was added, and the reaction mixture was heated at 72–74°C for 30 min. The reaction mixture was cooled to room temperature, passed through a Sep-Pak cartridge (silica gel), and eluted with 10% methanol in dichloromethane (2.5 ml). After evaporation of the solvent with a stream of argon at ~73°C, the residue was dissolved in methanol. 2N Hydrochloric acid solution in methanol (0.1 ml) was added and the mixture was refluxed for 5 min.The crude mixture was neutralized with 1N bicarbonate (0.2 ml), diluted with HPLC solvent (1 ml) and purified by HPLC. The desired product was isolated and the solvent evaporated under vacuum. The residue was re-dissolved in sterile saline and the solution filtered through a Millex-GS filter (Millipore Corp., Bedford, MA) into a sterile empty vial. The product was co-injected with an authentic unlabeled sample onto an analytical column to confirm its identity.

Conclusion

The syntheses of adenosine analogues, 2′-deoxy-2′-[^{18}F]fluoro-9-β-D-arabinofuranosyladenine ([^{18}F]-FAA) and 3′-deoxy-3′-[^{18}F]fluoro-9-β-D-xylofuranosyladenine ([^{18}F]-FXA) have been achieved. Radiochemical yield of [^{18}F]-FXA and [^{18}F]-FAA were 30–40 and 10–18% (d. c.), respectively, with specific activities greater than 74 GBq/μmol. This direct fluorination method is convenient, produces sufficient amounts of activity, and is suitable for routine production for animal and human studies with PET.

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