

Fluorinase-Coupled Base Swaps: Synthesis of [^{18}F]-5'-Deoxy-5'-fluorouridines**

Margit Winkler,* Juozas Domarkas, Lutz F. Schweiger, and David O'Hagan*

The application of positron emission tomography (PET) for medical imaging and diagnostics^[1] is a rapidly growing area, and cyclotrons are being increasingly commissioned in major hospitals. Developing methods for the introduction of the appropriate isotopes (^{11}C , ^{13}N , ^{18}F , and ^{15}O) into organic structural motifs is a major research activity at present.^[2] The relatively long half-life of ^{18}F ($t_{1/2} = 110$ min) renders it an attractive radioisotope for PET, and synthetic protocols employing [^{18}F]fluoride ion are particularly attractive as this form of the isotope is generated in a very high specific activity in which a cold carrier ([^{19}F]fluoride) is not added.^[3] Chemical strategies for the incorporation of ^{18}F into organic compounds are being widely explored,^[4] but enzymatic approaches offer a unique, mild, and selective approach for fluorination. We have been interested in using the fluorinase enzyme (E.C. 2.5.1.63, *Streptomyces cattleya*) as a catalyst for ^{18}F -C bond formation. The enzyme catalyzes the nucleophilic attack of fluoride ion to the C5' center of (*S*)-adenosyl-L-methionine (SAM) to generate 5'-deoxy-5'-fluoroadenosine (5'-FDA) and L-methionine.^[5] Although the fluorinase is a relatively slow enzyme, it is readily available by overexpression from *E. coli*.^[6] It is easily obtained in milligram per milliliter quantities, is stable for long periods, and can be used at micromolar concentrations. [^{18}F]Fluoride is generated by the cyclotron at nanomolar concentrations; therefore the enzyme is usually in excess which can overcome sluggish reaction rates. We recently demonstrated the synthesis of 5'-[^{18}F]FDA in high radiochemical yield (RCY) using fluorinase in this

manner.^[7] More generally, radiolabeled nucleosides are being extensively studied as possible tracers for the assessment of tumor biochemistry.^[8] The presence of adenosine receptors^[9] and specific uridine receptors^[10] in the brain increases the significance of these compounds as tracers for neurological studies. Combining fluorinase with nucleoside-converting enzymes^[11] offered an attractive strategy for the preparation of radiolabeled nucleosides. Herein we report that 5'-deoxy-5'-fluoroadenosine can be prepared using fluorinase combined with appropriate nucleoside phosphorylases. The reaction of 5'-FDA with a purine nucleoside phosphorylase (PNP, E.C. 2.4.2.1) generates 5-deoxy-5-fluoro- α ,D-ribose-1-phosphate (5-FDRP) in situ. The reversibility of this reaction offers the potential for swapping adenine for another purine base. PNPs from various sources have been identified^[12] but only a few catalyze the depurination of adenosine. In this study the PNP from *S. cattleya* was used because 5'-FDA is its natural substrate. Accordingly, incubation of the fluorinase and fluoride ion with PNP and 2,6-diaminopurine resulted in the accumulation of the fluorinated nucleoside **1** (Scheme 1). The substrate specificity of this PNP is restricted to adenine analogues having an amine at C6 of the purine.^[13]

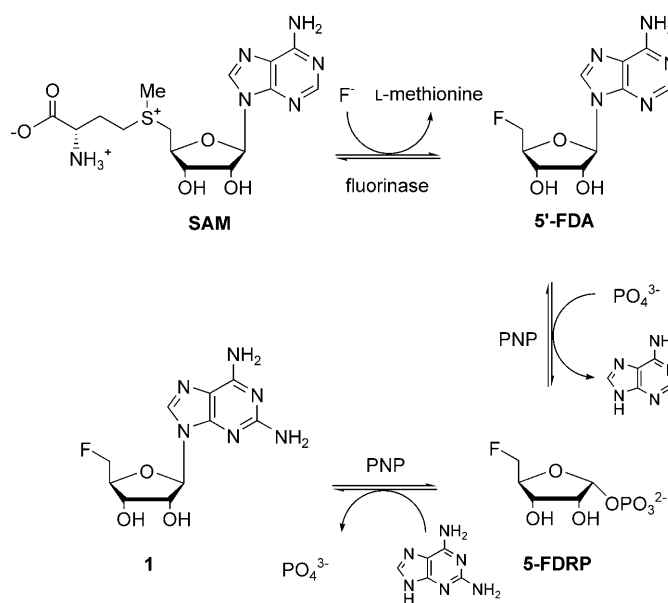
To extend the versatility of the base-swap protocol, reactions with a pyrimidine nucleoside phosphorylase (PyNP, EC 2.4.2.2 *Bacillus stearothermophilus*) were explored. This enzyme catalyses the reversible phosphorolysis of both uridine and thymidine by displacing the pyrimidine

[*] Dr. M. Winkler, Prof. Dr. D. O'Hagan
Centre for Biomolecular Sciences and School of Chemistry
University of St. Andrews
North Haugh, St. Andrews KY16 9ST (UK)
Fax: (+44) 1334-463-808
<http://chemistry.st-and.ac.uk/staff/doh/group>
E-mail: mw60@st-andrews.ac.uk
do1@st-andrews.ac.uk

Dr. J. Domarkas, Dr. L. F. Schweiger
John Mallard Scottish PET Centre, School of Medical Sciences
University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD (UK)

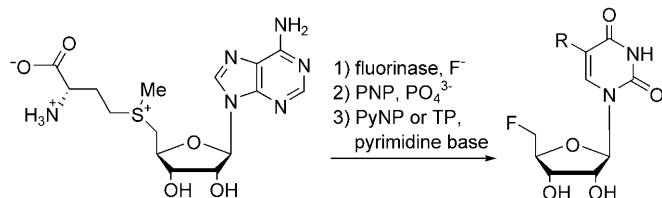
[**] We thank the Austrian Science Fund FWF for fellowship support (to M.W., Project J2694) and J.D. acknowledges the Scottish Funding Council and SINAPSE collaboration for financial support. We thank Leslie Kinsland and Steven E. Ealick (Cornell University (USA)) for providing recombinant pyrimidine nucleoside phosphorylase, and we are grateful to Louise L. Major for the expression vector for purine nucleoside phosphorylase. Caroline E. R. Horsburgh and Catherine H. Botting (MS) as well as Melanja H. Smith and Tomas Lebl (NMR) are acknowledged for analytical support and Stuart Craib for ^{18}F production.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.200804040>.



Scheme 1. Biotransformation to give 2-amino-5'-deoxy-5'-fluoroadenosine.

base in each case.^[14] Previous reports of enzymatic transglycosylation reactions have almost exclusively monitored the overall displacement of a pyrimidine by a purine base.^[11b,15] In this case, the reaction equilibria have been optimized to promote the reverse reaction and to generate pyrimidine nucleosides **2–6** (Scheme 2).



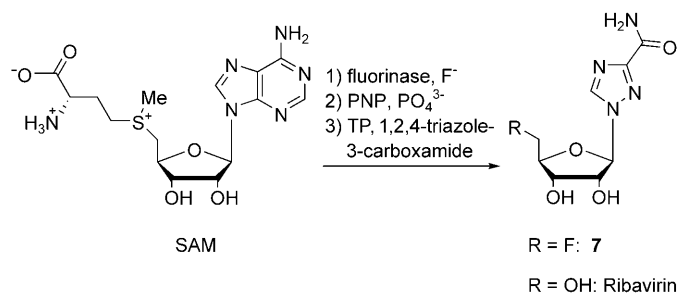
Scheme 2. One-pot fluorinase/nucleoside phosphorylase reaction to give 5'-fluorinated uridine derivatives (R = H: **2**, R = Me: **3**, R = F: **4**, R = Cl: **5**, R = Br: **6**).

Thymidine phosphorylases (TP, EC 2.4.2.4) are reported to display specificity for 2'-deoxyribonucleosides,^[11,16] and to accept a variety of pyrimidine bases, therefore emerging as an attractive prospect to add structural diversity to the fluorination/base-swap reactions. The commercial preparation of this enzyme contains 500 mM phosphate and uracil as a stabilizer, therefore the enzyme was preferably subcloned from *E. coli* (pET-28a). Unexpectedly, TP could be used in place of PyNP in the reactions shown in Scheme 2 (also see Table 1). With this observation the substrate specificity of TP was reassessed by a comparison of the rate of phosphorolysis of thymidine (2'-deoxyribose) versus 5-methyluridine (ribose). Thymidine is converted into 2-deoxy- α ,D-ribose-1-phosphate and the equilibrium is established within a few minutes; in contrast it took 2 hours to establish the equilibrium with 5-methyluridine. The K_m values reflect the selectivity of TP towards 2'-deoxy substrates (K_m 535 μ M versus 2.2 mM);

Table 1: Conversions of 5'-FDA into the nucleoside products **2–6** in PNP/PyNP or PNP/TP mediated base-swap reactions.^[a]

Entry	Base	NP ^[b]	Product	Conversion [%]
1	uracil	PyNP	2	29
2	uracil	TP	2	26
3	thymine	PyNP	3	24
4	thymine	TP	3	61
5	5-F-uracil	PyNP	4	32
6	5-F-uracil	TP	4	30
7	5-Cl-uracil	PyNP	5	19
8	5-Cl-uracil	TP	5	13
9	5-Br-uracil	PyNP	6	15
10	5-Br-uracil	TP	6	11

[a] Conditions: Tris-HCl (100 mM, pH 7.5), synthetic 5'-FDA (1 mM), PNP (0.3 mg mL⁻¹), potassium phosphate (25 mM), PyNP or TP (0.1 mg mL⁻¹), base (10 mM), 37 °C, 3 h. [b] Nucleoside phosphorylase.

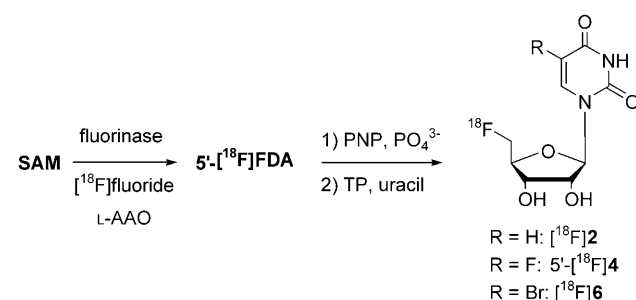


Scheme 3. Preparation of the dehydroxyfluoro analogue of antiviral ribavirin.

however, it proved to be a useful enzyme for transglycosylations to generate **2–6**.

Ribavirin is a nucleoside with broad spectrum antiviral activity.^[17,18] To additionally demonstrate the versatility of the fluorination/base-swap protocol the fluorodehydroxy analogue of ribavirin was prepared in a one-pot biotransformation (Scheme 3).

Finally, one-pot biotransformations to give radiolabeled [¹⁸F]-5'-deoxy-5'-fluorouridines **2**, **4**, and **6** were executed (Scheme 4). To improve the conversion, an additional



Scheme 4. Enzymatic radiolabeling of [¹⁸F]-5'-deoxy-5'-fluorouridines from SAM.

enzyme, L-amino acid oxidase (L-AAO, from *Crotalus adamanteus*), was added. The L-AAO oxidizes L-methionine, the byproduct of the fluorination reaction, thereby pushing the equilibrium of the reaction towards 5'-[¹⁸F]FDA production.^[7] This protocol is particularly advantageous in radiolabeling syntheses because of the reversibility of the fluorination and the low concentration of [¹⁸F]fluoride (ca. 10⁻⁹ M) used, relative to unlabeled reactions.

For the synthesis of [¹⁸F]**2**, SAM was incubated at 37 °C with four enzymes (fluorinase, PNP, TP, and L-AAO) in the presence of uracil, potassium phosphate, and [¹⁸F]fluoride (Figure 1). The radiolabeling base-swap reaction was studied with respect to the relative enzyme and substrate concentrations and the conversion of [¹⁸F]fluoride into [¹⁸F]**2** increased from less than 1 % to 33 % after a typical reaction time of 4 hours. With this optimized protocol in hand [¹⁸F]**4** and [¹⁸F]**6** were also prepared; for example, [¹⁸F]**4** was generated in 23 % (RCY) after 2 hours (see the Supporting Information). Balancing production of ¹⁸F-labeled nucleosides against radiochemical decay, the optimal reaction time

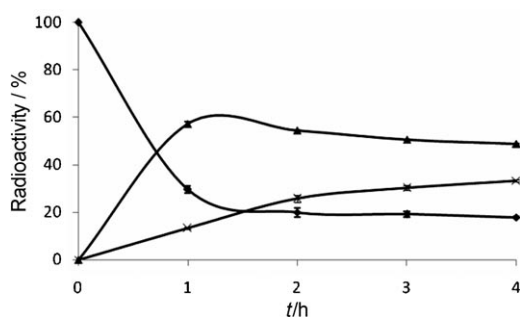


Figure 1. Time course of [¹⁸F]fluoride consumption and product formation: × [¹⁸F]2⁻; ▲ 5'-[¹⁸F]FDA; ◆ [¹⁸F]F⁻. The data was obtained by area normalization of HPLC traces (radiochemical detector), are decay corrected and represent the average of two experiments.

for these reactions was found to be between 1.5 and 3 hours, depending on the respective base (up to 13 % conversion, uncorrected for decay). This reaction time is well within two half-lives of ¹⁸F and enables the isolation of respectable amounts of these novel PET tracers for cancer cell uptake studies.

In summary, we have developed one-pot fluorination/base-swap biotransformations of fluoride ions into 5'-deoxy-5'-fluoronucleosides by using combinations of fluorinase and nucleoside phosphorylase enzymes. These biotransformations are amenable to radiolabeling syntheses starting from [¹⁸F]fluoride ion, an ideal source of isotope for PET synthesis. Studies are ongoing to explore the uptake of prepared radiolabeled compounds in various cancer cell lines.

Experimental Section

HPLC sample for compound **1**: SAM (1 mM) and KF (10 mM) were incubated overnight at 37 °C with fluorinase (40 μL, 2–6 mg mL⁻¹), PNP (40 μL, 1–13 mg mL⁻¹), potassium phosphate (5 mM), and 2,6-diaminopurine (10 mM) in Tris-HCl buffer (100 mM, pH 7.5) at 37 °C. The reaction mixture was heated at 98 °C for 3 min and then subjected to centrifugation to remove the protein. The supernatant was analyzed by HPLC-UV (260 nm) and HPLC-MS methods. HPLC samples for **2–7** were obtained using a similar protocol as that described for **1**, but the amount of PNP was decreased to 20 μL and PyNP (20 μL, 0.4–2 mg mL⁻¹) or TP (20 μL, 3.6–75 mg mL⁻¹) were added in addition to the respective nitrogenous bases (10 mM).

A semipreparative sample for **1**: Synthetic 5'-FDA (1 mM) was incubated for 18 h at 37 °C with PNP (600 μL, 5–10 mg mL⁻¹), potassium phosphate (10 mM), and 2,6-diaminopurine (10 mM) in Tris-HCl buffer (100 mM, pH 7.5) at 37 °C in a final volume of 680 μL. The reaction mixture was heated at 98 °C for 3 min and then subjected to centrifugation to remove the protein. The sample was analyzed by ¹⁹F NMR methods (376 MHz) after addition of 100 μL D₂O, and then the products were purified by preparative-HPLC. HRMS electrospray mass spectrometry data was obtained from the freeze-dried residues. Semipreparative samples of compounds **2–7** were obtained as described for **1** but the PNP aliquot was decreased (300 μL) and PyNP (300 μL, 1–2 mg mL⁻¹) or TP (300 μL, 3.6–75 mg mL⁻¹) added in addition to the respective nitrogenous base (10 mM).

Typical radiolabeling experiment: [¹⁸F]fluoride (25 μL, 180 ± 25 MBq) was added to a reaction mixture composed of SAM (15 μL, 20 mM), fluorinase (50 μL, 49 mg mL⁻¹), L-AAO (1 mg), PNP (50 μL, 20 mg mL⁻¹), TP (40 μL, 27 mg mL⁻¹), potassium phosphate (10 μL, 500 mM, pH 7.5), and uracil (9 μL, 200 mM) in Tris-HCl

buffer (100 mM, pH 7.5 at 37 °C), and the reaction was incubated at 37 °C for 4 h. Every hour an aliquot (15 μL) was withdrawn from the reaction mixture and the proteins were precipitated by the addition of acetonitrile (30 μL) and then removed by centrifugation (14 500 rpm, 2 min). Conversions were determined by HPLC, monitoring UV and radioactivity simultaneously.

Received: August 15, 2008

Published online: November 25, 2008

Keywords: enzyme catalysis · fluorides · glycosylation · nucleosides

- [1] J. Czernin, H. R. Schelbert, D. H. S. Silverman, W. P. Melega, *PET: Molecular Imaging and its Biological Applications* (Ed.: M. E. Phelps), Springer, Berlin, **2004**, pp. 321–584.
- [2] a) *Fluorine and Health—Molecular Imaging, Biomedical Materials and Pharmaceuticals* (Eds.: A. Tressaud, G. Haufe), Elsevier, Amsterdam, **2008**, pp. 3–278; b) L. Mu, A. Höhne, P. A. Schubiger, S. M. Ametamey, K. Graham, J. E. Cyr, L. Dinkelborg, T. Stellfeld, A. Srinivasan, U. Voigtmann, U. Klar, *Angew. Chem.* **2008**, *120*, 5000–5003; *Angew. Chem. Int. Ed.* **2008**, *47*, 4922–4925.
- [3] L. Cai, S. Lu, V. W. Pike, *Eur. J. Org. Chem.* **2008**, 2853–2873.
- [4] Recent examples for fluorination of nucleosides: a) H. P. Le, C. E. Muller, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6139–6142; b) R. C. Spitale, M. G. Heller, A. J. Pelly, J. E. Wedekind, *J. Org. Chem.* **2007**, *72*, 8551–8554.
- [5] a) D. O'Hagan, C. Schaffrath, S. L. Cobb, J. T. G. Hamilton, C. D. Murphy, *Nature* **2002**, *416*, 279; b) C. D. Cadicamo, J. Courtieu, H. Deng, A. Meddour, D. O'Hagan, *ChemBioChem* **2004**, *5*, 685–690.
- [6] C. Dong, F. Huang, H. Deng, C. Schaffrath, J. B. Spencer, D. O'Hagan, J. H. Naismith, *Nature* **2004**, *427*, 561–565.
- [7] H. Deng, S. L. Cobb, A. D. Gee, A. Lockhart, L. Martarello, R. P. McGlinchey, D. O'Hagan, M. Onega, *Chem. Commun.* **2006**, 652–654.
- [8] a) J. Toyohara, Y. Fujibayashi, *Nucl. Med. Biol.* **2003**, *30*, 681–685; b) L. I. Wiebe, *Braz. Arch. Biol. Technol.* **2007**, *50*, 445–459; c) M. M. Alauddin, A. Shahinian, R. Park, M. Tohme, J. D. Fissekis, P. S. Conti, *Nucl. Med. Biol.* **2007**, *34*, 267–272.
- [9] K. Ishiwata, Y. Kimura, E. F. de Vries, P. H. Elsinga, *Cent. Nerv. Syst. Agents Med. Chem.* **2007**, *7*, 57–77.
- [10] T. Kimura, I. K. Ho, I. Yamamoto, *Sleep* **2001**, *24*, 251–260.
- [11] a) M. J. Pugmire, S. E. Ealick, *Biochem. J.* **2002**, *361*, 1–25; b) E. S. Lewkowicz, A. M. Iribarren, *Curr. Org. Chem.* **2006**, *10*, 1197–1215.
- [12] A. Bzowska, E. Kulikowska, D. Shugar, *Pharmacol. Ther.* **2000**, *88*, 349–425.
- [13] Experiments using purine or its derivatives such as 6-methylpurine, 2-amino-6-chloropurine, 2-fluoro-6-chloropurine, hypoxanthine, or guanine failed to give fluorinated nucleoside analogues.
- [14] M. J. Pugmire, S. E. Ealick, *Structure* **1998**, *6*, 1467–1479.
- [15] a) A. K. Prasad, S. Tripathi, V. S. Parmar, *Bioorg. Chem.* **1999**, *27*, 135–154; b) I. A. Mikhailopulo, *Curr. Org. Chem.* **2007**, *11*, 317–335.
- [16] a) W. E. Razzell, P. Casshyap, *J. Biol. Chem.* **1964**, *239*, 1789–1793; b) N. G. Panova et al., *Biochemistry* **2007**, *46*, 21–28.
- [17] a) R. W. Sidwell, J. H. Huffman, G. P. Khare, L. B. Allen, J. T. Witkowski, R. K. Robins, *Science* **1972**, *177*, 705–706; b) W. B. Parker, *Virus Res.* **2005**, *107*, 165–171.
- [18] V. N. Barai, A. I. Zinchenko, L. A. Eroshevskaia, E. N. Kalinichenko, T. I. Kulak, I. A. Mikhailopulo, *Helv. Chim. Acta* **2002**, *85*, 1901–1908.