Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 16 September 2009, Revised 11 December 2009, Accepted 14 December 2009 Published online 19 January 2010 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1742

Automated synthesis of ^{18}F-labelled analogs of metomidate, vorozole and harmine using commercial platform

Obaidur Rahman,[a]* Maria Erlandsson,[b] Elisabeth Blom,[b] and Bengt Långström[b]

Three ^{18}F-labelled PET tracers, 2-[^{18}F]fluoroethyl 1-[(1*R*)-1-phenylethyl]-1H-imidazole-5-carboxylate ([^{18}F]FETO), 6-[(*S*)-(4-chlorophenyl)-(1H)-1,2,4-triazol-1-yl)methyl]-1-(2-[^{18}F]fluoroethyl)-1H-benzotriazole ([^{18}F]FVOZ) and 7-[2-(2-[^{18}F]fluoroethoxy)ethoxy]-1-9H-β-carboline ([^{18}F]FHAR) were synthesized by a one-step nucleophilic fluorination using the automated commercial platform TRACERLab FX$_{FN}$. The labelled products were obtained with 16–20% isolated decay corrected radiochemical yields after 70–75 min synthesis time. The radiochemical and chemical purities were more than 98% in all cases. The synthesis using commercial platform may make these tracers more accessible for clinical research.

Keywords: PET; ^{18}F-labelling; [^{18}F]FETO; [^{18}F]FVOZ; [^{18}F]FHAR; automated synthesis

Introduction

Ethyl 1-[(1*R*)-1-phenylethyl]-1H-imidazole-5-carboxylate (etomidate), 6-[(*S*)-(4-chlorophenyl)-(1H)-1,2,4-triazol-1-yl)methyl]-1-methyl-1H-benzotriazole (vorozole) and 7-methoxy-1-methyl-9H-β-carboline (harmine) are inhibitors of three different enzymes 11β-hydroxylase,[1] aromatase[2] and monoamine oxidase-A,[3] respectively. ^{11}C-Labelled metomidate and etomidate have shown to be valuable for PET imaging of the adrenal cortex and its tumors[4] and especially [^{11}C]metomidate has been successfully used to visualize adrenal cortical tumors.[5,6,7,] ^{11}C-Labelled vorozole was used to study the *in vivo* bindings of this compound in whole body[8] and brain[9] of rhesus monkey. ^{11}C-Labelled analog of harmine was used to detect the high expression of MAO-A in neuroendocrine gastroenteropancreatic tumor.[10] ^{18}F-Labelled analogs of these tracers, metomidate,[11,12] vorozole[13] and harmine[14] have recently been developed and evaluated as PET tracers. The previous papers described the syntheses on the semi-automated platform Synthia[15] developed at Uppsala University PET center. Automated synthesis is important for making PET tracer clinically useful. This paper describes the fully automated synthesis of these three PET tracers by one-step nucleophilic ^{18}F-fluorination using the commercially available platform TRACERLab FX$_{FN}$.

Results and discussion

Three potential PET tracers 2-[^{18}F]fluoroethyl 1-[(1*R*)-1-phenylethyl]-1H-imidazole-5-carboxylate ([^{18}F]FETO), 6-[(*S*)-(4-chlorophenyl)-(1H)-1,2,4-triazol-1-yl)methyl]-1-(2-[^{18}F]fluoroethyl)-1H-benzotriazole ([^{18}F]FVOZ) and 7-[2-(2-[^{18}F]fluoroethoxy)-ethoxy]-1-9H-β-carboline ([^{18}F]FHAR) (Figure 1) were prepared by one-step nucleophilic ^{18}F-fluorination with radiochemical yields of 16–20% (Table 1) using an automated commercial platform TRACERLab FX$_{FN}$ (Figure 2). The radiochemical and chemical purities were over 98% in all cases. Nucleophilic fluorination of the corresponding tosylate (for [^{18}F]FETO, [^{18}F]FVOZ) or bromide (for [^{18}F]FHAR) was used to synthesize the compounds. The reaction was performed in an aprotic polar

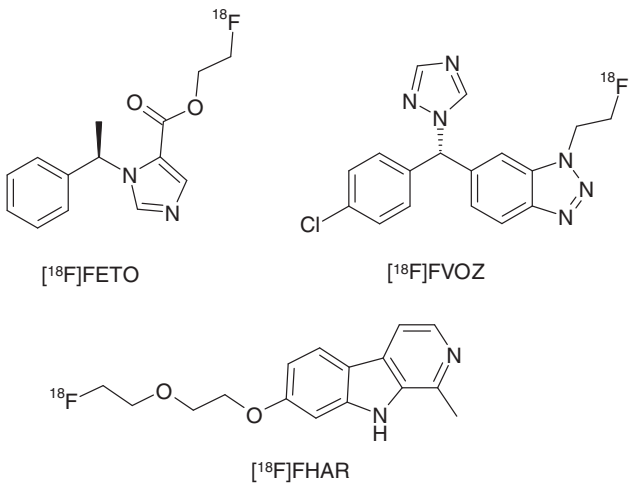

Figure 1. Structures of [18F]FETO, [18F]FVOZ and [18F]HAR.

[a]*Uppsala Applied Science Laboratory, GE Healthcare, SE-752 28 Uppsala, Sweden*

[b]*Department of Biochemistry and Organic Chemistry, Uppsala University, Box 576, Husargatan 3, BMC, S-751 23 Uppsala, Sweden*

**Correspondence to: Obaidur Rahman, Uppsala Applied Science Laboratory, GE Healthcare, SE-752 28 Uppsala, Sweden.*
E-mail: obaidur.rahman@ge.com

Table 1. Reaction conditions and radiochemical yields (RCY) of FETO, FVOZ and FHAR

Target compounds	Reaction conditions	Total synthesis time (min)	RCY* (%), (*n*)
[^{18}F]FETO	110°C for 10 min	70	20 ± 3 (7)
[^{18}F]FVOZ	140°C for 15 min	70	17 ± 3 (2)
[^{18}F]FHAR	160°C for 15 min	75	16 ± 1 (5)

*Decay corrected isolated radiochemical yield calculated from the starting radioactivity of $^{18}F^-$; *n*, number of runs.

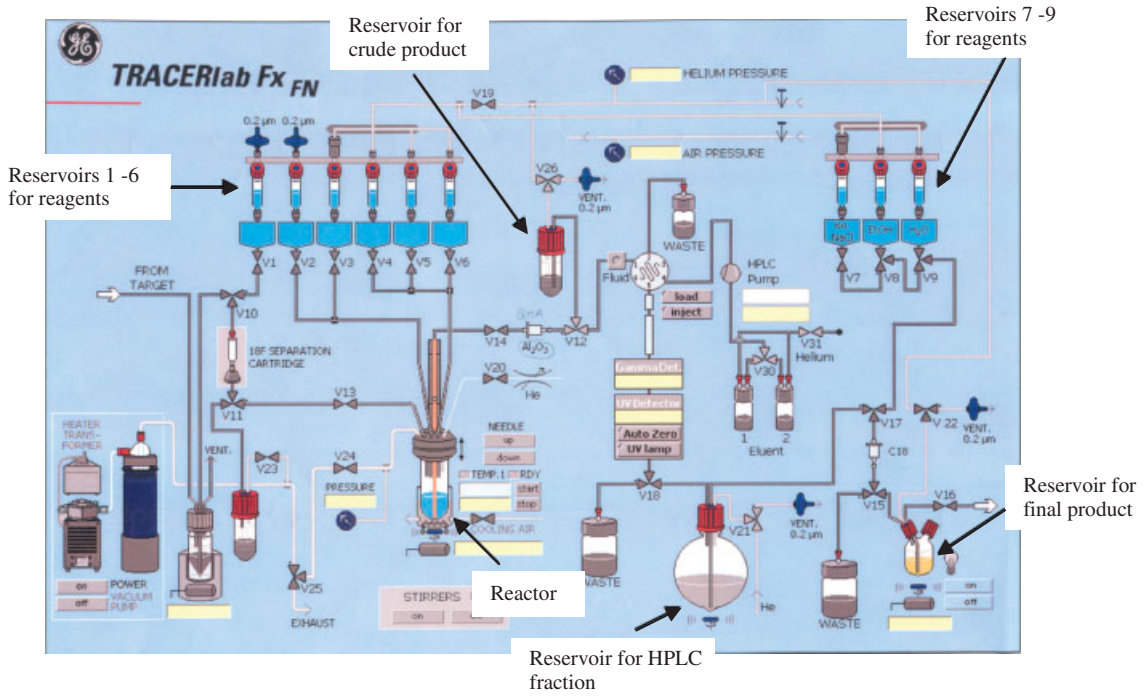

Figure 2. Graphical display of the monitor of the TRACERLab FXFN.

solvent DMF in the presence of potassium carbonate and kryptofix K2.2.2. Biological samples were prepared by dissolving the purified product in sterile phosphate buffer with 10% absolute ethanol. The final product was filtered through a DynaGard syringe filter of 0.2 μm pore size. The presence of residual solvents such as DMF and acetonitrile was investigated by GC and no trace of DMF or acetonitrile was found, i.e., the concentrations of acetonitrile and DMF in the final product were lower than 10 and 30 ppm, respectively, which were the lowest concentrations that could be detected by the instrument. The presence of kryptofix 2.2.2 was checked by LC-MS and no trace of this reagent was found, i.e., the concentration of kryptofix residue was lower than 2 μg/mL that was the lowest concentration that could be detected by the instrument. The syntheses of precursors were published previously.[11,13,14]

A simple time sequence program was applied to perform the synthesis. All valves of the TRACERLab FX_{FN} were controlled according to the preprogrammed time intervals to transfer the reagents from one part to another of the instruments. Helium pressure and vacuum pump were used to transfer the reagents. The reagents were loaded into the reservoirs 1–6 and the formulation liquids were loaded into the reservoirs 7–9 of the platform according to the graphical display showed in Figure 2. The heating and cooling of the reagents, switch on/off of helium gas and pump were also performed according to the program. The transfer of the radioactivity was traced and recorded with inbuilt radioactivity detector. After completion of the reaction the crude product was diluted with a 1:1 mixture of water and acetonitrile and the mixture was transferred into the intermediate reservoir for crude product from where it was loaded into the preparative HPLC for purification. The HPLC solvent from the collected fraction was removed using SPE technique. The final product was formulated into the reservoir of the platform (Figure 2) and finally sterile filtered and transferred into a sterile vial.

The synthesis was developed previously on different platform and some optimization was thus needed to obtain the best results using the platform. Nucleophilic fluorination using $^{18}F^-$ may be tricky and the radiochemical yield varies while running the synthesis at different times. It is thus important to clean and dry the platform just before starting the synthesis and using the same procedure every time. A time sequence program was developed to clean and dry the platform that gave reproducible results. Moreover, the preparation of reactive $^{18}F^-$ is also very important to obtain high radiochemical yield. The effect of temperatures and reaction times were investigated. The best result was obtained when $^{18}F^-$ was dried in two steps, first at 60°C for 7 min and then at 120°C for 5 min. Moreover a gentle flow of helium gas was flushing through the reactor at the same time while it was being heated at 60°C. Different reaction temperatures and times for final reaction of dried $^{18}F^-$ with

substrates were investigated and the best-obtained results are presented in Table 1.

Conclusion

The implementation of the synthesis of three potential PET tracers [^{18}F]FETO, [^{18}F]FVOZ and [^{18}F]FHAR into a commercial platform TRACERLab FX_{FN} is described. This implementation will make them more accessible for production in clinical research.

Experimental

General

All chemicals and reagents were purchased from Aldrich or Lancaster. The ^{18}F-trapping cartridge (QMA cartridge) and C18 SPE cartridge were purchased from Waters. Syringe filter DynaGard was obtained from Microgon, Inc. Laguna Hills, California. The QMA cartridge was conditioned by passing 10 mL of 0.5 M aqueous K_2CO_3 solution and 10 mL of water, respectively, through it. The noncarrier added ^{18}F was produced as $^{18}F^-$ ion by the PETtrace cyclotron from GE Medical System using the nuclear reaction $^{18}O(p,n)^{18}F$. The isotopically enriched [^{18}O]H_2O was used in the target and irradiated with a 16 MeV proton beam. The synthesis of precursors and authentic references for FETO,[11] FVOZ[13] and FHAR[14] were described previously. The identifications of the ^{18}F-labeled products were performed by analytical HPLC using authentic nonradioactive compounds as the references. The analysis was performed with a Shimadzu analytical HPLC system with variable-wavelength UV detector in series with a Bioscan $\beta+$-flow detector. The following mobile phases were used: 25 mM aqueous sodium dihydrogen phosphate (A), acetonitrile (B1) and methanol (B2). For analytical LC, an ACE reverse phase C18, 5 μm, 4.6 × 250 mm column was used with a flow of 1.0 mL/min. For preparative LC, TRACERLab's built-in HPLC system with Beckman's Ultrasphere reverse phase C18, 4 μm, 250 × 10 mm (i.d.) (for [^{18}F]FETO) or MACHEREY NAGEL's VrioPrep reverse phase C18, 250 × 21 mm (i.d.) (for [^{18}F]FVOZ and [^{18}F]FHAR) was used with a flow of 6 mL/min. An isocratic HPLC method with 50:50 mixture of mobile phases A and B1 was used for purification. For the analysis of [^{18}F]FETO and [^{18}F]FVOZ an isocratic HPLC method with 30:70 mixture of mobile phases A and B2 were used. For the analysis of [^{18}F]FHAR an isocratic HPLC method with 65:35 mixture of mobile phases A and B1 were used. Radioactivity of products was measured in an ion chamber, Veenstra Instrumenten bv, VDC-202. For coarse estimations of radioactivity during the production, a portable dose-rate meter from Alnor OY Finland was used. Nonradioactive compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy and GC-MS. NMR spectra were recorded on a Varian Unity-400 NMR spectrometer. Chloroform-d was used as the internal standard. GC-MS was performed using a Finnigan GCQ mass spectrometer coupled to a Finnigan Q-GC. The presence of solvent residue and Kryptofix residue in the final product was checked by Varian 3380 GC and Finnigan AQA LC-MS, respectively.

^{18}F-labelling synthesis

General procedure

The solutions of potassium carbonate (3.5 mg in 500 μL water), Kryptofix K2.2.2 (10.0 mg in 1 mL acetonitrile) and precursor (5.0 mg in 500 μL anhydrous DMF) were loaded into reservoirs 1, 2 and 3, respectively. Reservoirs 4, 7 and 8 were filled with a 1:1 mixture of water and acetonitrile (5 mL), water (10 mL) and absolute ethanol (1 mL), respectively. The target water containing $^{18}F^-$ was passed through a preconditioned QMA cartridge where the $^{18}F^-$ was trapped. The $^{18}F^-$ was released from QMA cartridge by passing K_2CO_3 solutions from reservoir 1 through the cartridge and allowed to enter into the reactor. Kyrptofix solution from reservoir 2 was added into the reactor and the whole mixture was dried first at 60°C for 7 min and then at 120°C for 5 min. The precursor solution from reservoir 3 was added with the dried $^{18}F^-$ ion complexed with potassium and kryptofix. The reaction mixture was heated at 110°C for 10 min ([^{18}F]FETO). In case of [^{18}F]FVOZ and [^{18}F]FHAR, the reaction was performed at 140°C for 15 min and 160°C for 15 min, respectively. The mixture was cooled to 50°C and diluted with the solvent mixture from reservoir 4 and loaded into the in-built preparative HPLC system of the platform. The appropriate fraction was collected, diluted with water (25 mL) and passed through a C18 SPE cartridge. The purified product was trapped on C18 SPE cartridge, washed with water from reservoir 7 and eluted with absolute ethanol (1 mL) from reservoir 8 and collected into the reservoir for final product containing injectionable phosphate buffer solution. Finally the product was transferred from that reservoir into a vial through the syringe filter DynaGard and the radioactivity of the final product was measured.

References

[1] I. Varga, K. Racz, R. Kiss, L. Futo, M. Toth, O. Sergev, E. Glaz, *Steroids* **1993**, *58*, 64–68.
[2] R. De Coster, W. Wouters, C. R. Bowden, H. V. Bossche, J. Bruynseels, R. W. Tuman, R. V. Ginckel, E. Snoeck, A. V. Peer, P. A. J. Janssen, *J. Steroid Biochem. Mol. Biol.* **1990**, *37*, 335–341.
[3] H. Kim, S. O. Sablin, R. R. Ramsay, *Arch. Biochem. Biophys.* **1997**, *337*, 137–142.
[4] M. Bergström, T. A. Bonasera, L. Lu, E. Bergström, C. Backlin, C. Juhlin, B. Långström, *J. Nucl. Med.* **1998**, *39*, 982–989.
[5] M. Bergström, C. Juhlin, T. A. Bonasera, A. Sundin, J. Rastad, G. Akeström, B. Långström, *J. Nucl. Med.* **2000**, *41*, 275–282.
[6] T. S. Khan, A. Sundin, C. Juhlin, B. Långström, M. Bergström, B. Eriksson, *Eur. J. Nucl. Med.* **2003**, *30*, 403–410.
[7] G. Zettining, M. Mitterhauser, W. Wadsak, A. Becherer, C. Pirich, H. Vierhapper, B. Niederle, R. Dudczak, K. Kletter, *Eur. J. Nucl. Med.* **2004**, *31*, 1224–1230.
[8] P. Lidström, T. A. Bonasera, D. Kirilovas, B. Lindblom, L. Lu, E. Bergström, M. Bergström, J.-E. Westlin, B. Långström, *Nucl. Med. Biol.* **1998**; *25*, 497–501.
[9] K. Takahashi, M. Bergström, P. Frändberg, E.-L. Vesström, Y. Watanabe, B. Långström, *Nucl. Med. Biol.* **2006**, *33*, 599–605.
[10] H. Örlefors, A. Sundin, K.-F. Fasth, K. Öberg, B. Långström, B. Eriksson, M. Bergström, *Nucl. Med. Biol.* **2003**, *30*, 669–679.
[11] M. Erlandsson, F. Karimi, B. Långström, *Nucl. Med. Biol.* **2009**, *36*, 435–445.
[12] M. Erlandsson, H. Hall, B. Långström, *J. Label. Compd. Radiopharm.* **2009**, *52*, 278–285.
[13] M. Erlandsson, F. Karimi, K. Takahashi, B. Långström, *J. Label. Compd. Radiopharm.* **2008**, *51*, 207–212.
[14] E. Blom, F. Karimi, O. Eriksson, H. Hall, B. Långström, *J. Label. Compd. Radiopharm.* **2008**, *51*, 277–282.
[15] P. Bjurling, R. Reineck, G. Westerberg, A. D. Gee, J. Sutcliffe, B. Långström, Proceedings of the VIth workshop on targetory and target chemistry, TRIUMF, Vancouver, Canada, **1995**, pp. 282–284.