Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 3171–3175
Bioorganic & Medicinal Chemistry

Improved automated synthesis of [^{18}F]fluoroethylcholine as a radiotracer for cancer imaging

M. Piel,[a,*] A. Bauman,[a] R. P. Baum,[b] S. Höhnemann,[a] I. Klette,[b] R. Wortmann[b] and F. Rösch[a]

[a]*Institute of Nuclear Chemistry, Johannes Gutenberg-Universität Mainz, 55128 Mainz, Germany*
[b]*Department of Nuclear Medicine/PET Centre, Zentralklinik Bad Berka, 99437 Bad Berka, Germany*

Received 17 October 2006; revised 15 February 2007; accepted 20 February 2007
Available online 22 February 2007

Abstract—[^{18}F]Fluoroethylcholine has been recently introduced as a promising ^{18}F-labelled analogue of [^{11}C]choline which had been previously described as a tracer for metabolic cancer imaging with positron emission tomography (PET). Due to the practical advantages of using the longer-lived radioisotope ^{18}F ($t_{1/2}$ = 110 min), offering the opportunity of a more widespread clinical application, we established a reliable, fully automated synthesis for its production using a modified, commercially available module. [^{18}F]Fluoroethylcholine was prepared from *N*,*N*-dimethylaminoethanol by iodide catalyzed alkylation with 1-[^{18}F]fluoro-2-tosylethane as alkylating agent, resulting in a total radiochemical yield of 30 ± 6% after a synthesis time of 50 min. The specific activity of [^{18}F]fluoroethylcholine was >55 GBq/μmol and the radiochemical purity 95–99%.

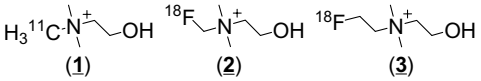
1. Introduction

Many tumours are characterized by an enhanced cell proliferation. This is usually associated with an elevated uptake and phosphorylation of choline to form choline phosphate which is used in the synthesis of membrane phospholipids.[1–3] ^{31}P magnetic resonance spectroscopy has confirmed this hypothesis by showing higher levels of choline phosphate in different tumour entities.

Therefore, [^{11}C]choline was developed and has shown its clinical potential in the evaluation of brain tumours, oesophageal carcinoma and prostate cancer[4–6] using PET. Because of the short half-life of ^{11}C ($t_{1/2}$ = 20.38 min), resulting in a limited usefulness for clinical routine, different ^{18}F-labelled ($t_{1/2}$ = 109.7 min) analogues were synthesized to overcome this problem. Shown in Figure 1 are the most prominent choline derivatives.

[^{18}F]fluorocholine ([^{18}F]FCh) (**2**), first introduced by DeGrado et al.,[7] is synthesized by reacting *N*,*N*-dimethylaminoethanol (DMAE) with [^{18}F]fluoro-bromomethane ([^{18}F]FBM). This labelling synthon has to be cleaned up via gas chromatographic purification, which is not a standard operation in radiopharmaceutical laboratories.[8] A simple and more convenient way of synthesizing choline analogue compounds is the ^{18}F-fluoroethylation of *N*,*N*-dimethylaminoethanol, leading to [^{18}F]fluoroethylcholine ([^{18}F]FECh) (**3**). The production route of ^{18}F-fluoroethylating synthons, especially1-[^{18}F]fluoro-2-tosylethane ([^{18}F]FETos), is a simple, very reliable procedure and can be established in a fully automated way without any demanding purification steps (Fig. 2).

Comparing the chemical structure differences between [^{18}F]FCh (**2**) and [^{18}F]FECh (**3**), it is obvious that [^{18}F]FCh (**2**) is more similar to [^{11}C]Cho (**1**), than [^{18}F]FECh (**3**) is. Deves and Krupka,[9] as well as Clary



(**1**) (**2**) (**3**)

Figure 1. Structure of [^{11}C]choline (1, [^{11}C]Cho) and the ^{18}F-fluorinated analogues [^{18}F]fluorocholine (2, FCh) and [^{18}F]fluoroethylcholine (3, FECh).

Keywords: [^{18}F]Fluoroethylcholine; ^{18}F-Fluoroalkylation; Positron emission tomography.
* Corresponding author. Tel.: +49 6131 3925371; fax: +49 6131 3925253; e-mail: piel@mail.uni-mainz.de

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.02.038

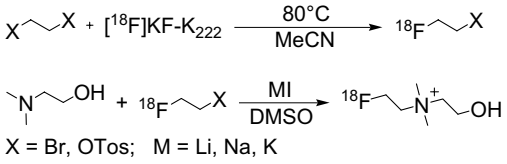


Figure 2. Synthesis of [^{18}F]fluoroethylcholine.

et al.,[10] investigated the behaviour of synthetic choline analogues. They found that 2 methyl groups are essential, while the third one can be replaced by longer alkyl chains in terms of affinity to the choline transport system and substrate specificity of choline kinase. These results were confirmed by Hara et al.[11] in vitro and in vivo.

Considering the isotope properties, complexity of synthesis and biochemical behaviour of the derivatives, [^{18}F]FECh seems to be the most promising candidate for clinical PET studies. Hence, there is a high interest in a reliable, fully automated synthesis for the production of [^{18}F]FECh. Since Hara et al. described the development and automated synthesis of [^{18}F]FECh, only minor changes in its preparation were published.[11,12] In this first publication a one-pot strategy was developed in which the secondary labelling agent [^{18}F]FETos was used without further purification for the alkylation of *N*,*N*-dimethylaminoethanol (DMAE). This may result in the formation of by-products, caused by the reaction of the ethylenglycol-1,2-ditosylate with DMAE. A purification of [^{18}F]FECh from these by-products may be problematic due to their cationic nature. Another drawback of this synthesis is that a relatively large excess of DMAE (300 μL) was used in the reaction which had to be removed afterwards by evaporation using high vacuum. These factors are all unfavourable to a reliable automated synthesis, because they may result in a high variability in the performance of the automated module.

Recently, we reported that the addition of alkali iodides to [^{18}F]FETos and 1-bromo-2-[^{18}F]fluoroethane ([^{18}F]BFE) led to drastically increased radiochemical yields, most probably due to the in situ formation of 1-iodo-2-[^{18}F]fluoroethane ([^{18}F]IFE).[13] We therefore applied this new approach to a fully automated synthesis for the production of [^{18}F]FECh, using the iodide-promoted alkylation, which circumvents the problems of the one-pot strategy.

2. Results and discussion

Due to the structure of the [^{18}F]fluoroethylcholine the fluoroethylation of *N*,*N*-dimethylaminoethanol is the most efficient way for its synthesis. Thus, [^{18}F]FECh is produced in a two-step synthesis, which consists of the formation of the ^{18}F-fluoroethylating agent, followed by the reaction with DMAE.

The most common ^{18}F-fluoroethylating agents used in the production of radiopharmaceuticals are [^{18}F]FETos and [^{18}F]BFE, resulting in various pathways for their synthesis and purification.[14–17] Hence, in a previous study, we compared these two secondary labelling precursors with regard to reaction conditions and radiochemical yields by labelling DMAE.[13] In this study, in which reaction temperatures of up to 85 °C were tested, [^{18}F]BFE showed significantly better yields than [^{18}F]FETos (Fig. 3).

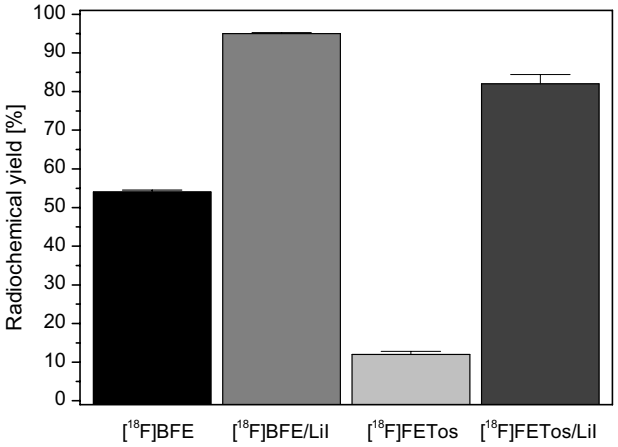


Figure 3. Radiochemical yields of the ^{18}F-fluoroethylation of DMAE using different labelling systems (DMSO; T = 85 °C; t = 20 min).

Table 1. Radiochemical yields of the ^{18}F-fluoroethylation of DMAE using higher reaction temperatures (DMSO; [^{18}F]FETos/LiI; t = 20 min)

Temperature (°C)	100	110	120
RCY (%)	86 ± 5.6	92 ± 5.8	97 ± 1.0

In the case of [^{18}F]BFE, higher reaction temperatures were avoided due to its low boiling point (bp 73 °C), as this normally results in a not negligible amount of [^{18}F]BFE in the gas phase of the reaction vial. Therefore, in further experiments higher reaction temperatures up to 120 °C were only examined for [^{18}F]FETos/LiI resulting in similar radiochemical yields (≈95%) as for [^{18}F]BFE/LiI at 85 °C (Table 1). Because of the better practical handling of [^{18}F]FETos, especially in the case of the automated modules, only this ^{18}F-fluoroethylating agent was used for further experiments.

In order to develop a new approach for a fully automated production of [^{18}F]FECh, we first established a manual synthesis, which we used to optimize the reaction conditions, regarding the use of a commercial module, in a fast and convenient manner.

2.1. Preliminary synthesis

The [^{18}F]FECh was prepared in a two-step synthesis via a ^{18}F-fluoroethylation using [^{18}F]FETos. In the first step [^{18}F]fluoride was dried and activated as [^{18}F]KF-K$_{222}$ complex and afterwards reacted with ethylenglycol-1,2-ditosylate at 80 °C for 6 min to yield [^{18}F]FETos.[18] Then the crude product was purified via HPLC, loaded on a LiChrolut EN column and eluted using DMSO. Afterwards this solution was reacted with *N*,*N*-dimethylaminoethanol for 20 minutes using alkali iodide catal-

ysis, then diluted with water and purified with a LiChrolut SCX column and HPLC to yield the [^{18}F]FECh in high radiochemical yields.

As previously reported, the highest radiochemical yields were obtained via the alkali iodide promoted ^{18}F-fluoroethylation with [^{18}F]FETos by using lithium iodide as catalyst, apparently the most potent of the iodide salts.

Due to the toxicity of the lithium, we examined the use of sodium iodide as catalyst which seems more suitable for routine production. In preliminary syntheses we therefore optimized different reaction parameters such as reaction temperature, precursor concentration and the amount of iodide, regarding the use of sodium iodide. Compared to lithium iodide this resulted in a higher reaction temperature of 120 °C, a higher precursor concentration of 250 mM, a reduced amount of sodium iodide of 7.5 mg (50 mM) and a radiochemical yield of 85–90%. Subsequently these results were adapted to the automated module (Fig. 4).

2.2. Automated radiosynthesis

The automated radiosynthesis of [^{18}F]FECh was performed by using a existing module for ^{11}C-methylation from GE Medical Systems (formerly Nuclear Interface), which is generally suitable for our purposes. Because of the different labelling techniques of ^{11}C and ^{18}F, the module had to be modified. Hence, the cooling, the heating and several valves and tubes had to be re-arranged, respectively added, resulting in the module shown in Figure 5.

Because this automated module offers one only possibility for HPLC purification per synthesis, we had to substitute the purification of the [^{18}F]FETos with a solid phase extraction. Therefore, after the reaction of the [^{18}F]fluoride with the ethyleneglycol-1,2-ditosylate the crude product was diluted with water, loaded on a LiChrolut EN cartridge and eluted with DMSO. After optimization of this purification step, which is described elsewhere,[19] the [^{18}F]FETos could be obtained in a good radiochemical and chemical purity.

The synthesis was performed as described below, resulting in a total radiochemical yield of 30 ± 6% after a synthesis time of 50 min. Due to lack of a UV-signal of the reference compound FECh, the identity was confirmed by ion exchange chromatography. The sensitivity of the established analytical system was ~10 ng/μL and the concentration of FECh in the final product was below detection limit. The radiochemical purity of the product was 95–99%, with a specific activity of >55 GBq/μmol.

3. Conclusions

The approach to generate the more reactive intermediate [^{18}F]IFE in situ significantly facilitates the nucleophilic ^{18}F-fluoroethylation of *N*,*N*-dimethylaminoethanol. Due to this new strategy we could reduce the amount of precursor drastically, whilst having the similar radiochemical yields. This lower precursor concentration allows a direct purification of the product via HPLC without a precedent evaporation of the excess of the precursor. After optimization and automation of this iodide-promoted ^{18}F-fluoroalkylation, a fast and reliable high-yield synthesis of [^{18}F]fluoroethylcholine was developed, which can be accomplished by a modified commercially available module.

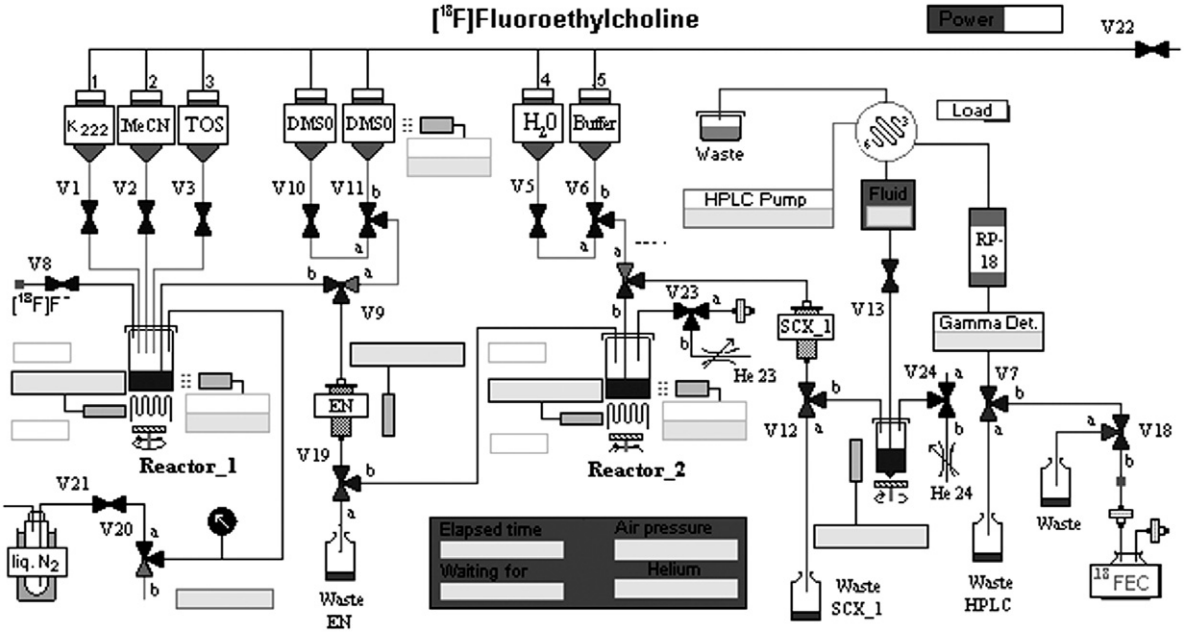


Figure 4. Schematic diagram of the automated synthesis module.

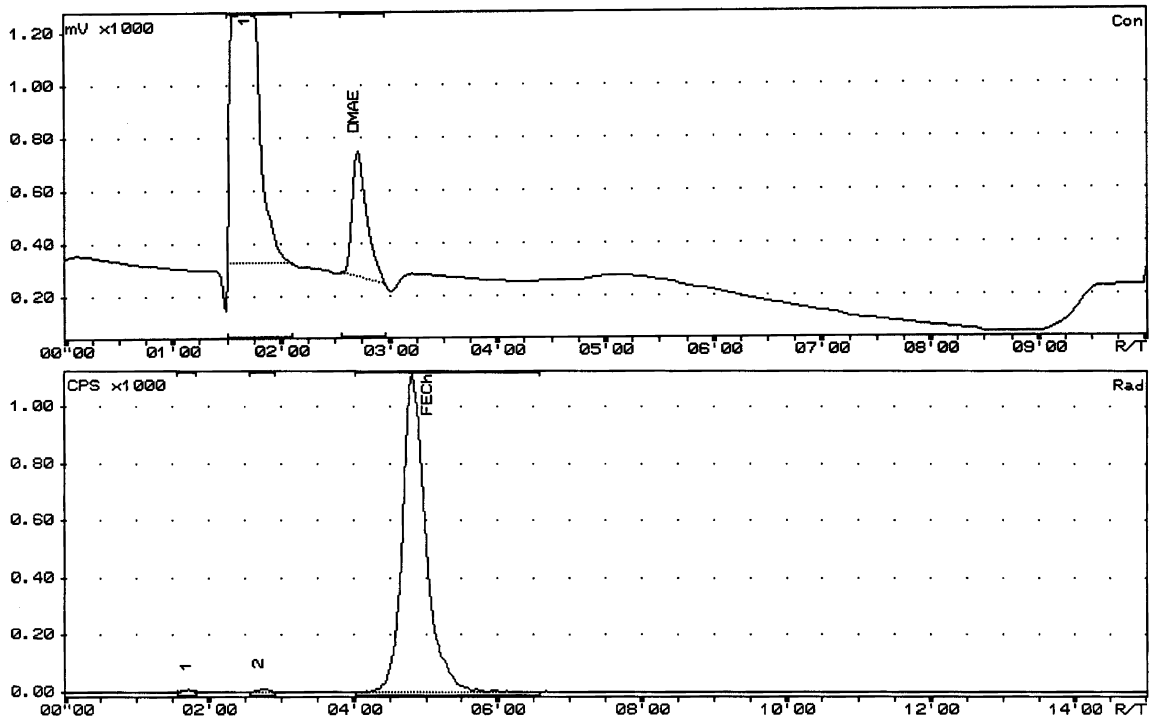


Figure 5. Typical radiochromatogram of the quality control of [^{18}F]FECh using ion exchange chromatography.

These increased yields provide sufficiently high batch productions of [^{18}F]fluoroethylcholine in a clinical environment and permit routine diagnostic applications of this radiopharmaceutical for cancer imaging.

4. Experimental

4.1. General

All reagents were purchased from commercial sources and were used without further purification. 1-Fluoro-2-tosylethane, fluoroethylcholine tosylate and [^{18}F]FE-Tos were prepared according to the literature.[11,20] Solid phase columns were purchased from Merck (LiChrolut EN, LiChrolut SCX) and the anion exchange resin from Bio-Rad (AG1X8). High performance liquid chromatography (HPLC) was performed with a Jasco PU-1588 gradient pump, a S3200 UV–Vis detector from Sycam and a NaI radioactivity detector from Canberra Packard.

[^{18}F]Fluoride was produced via the $^{18}O(p,n)^{18}F$ reaction with a CTI RDS 111 cyclotron (Zentralklinik Bad Berka, Department of Nuclear Medicine).

The synthesis of [^{18}F]fluoroethylcholine was carried out according to GMP. Therefore, the [^{18}F]FECh was produced in a hot cell equipped with the automated synthesis module under laminar flow conditions using sterile filtered argon as gas carrier. All starting materials were tested using validated methods and qualified equipment. After the synthesis of the [^{18}F]FECh, a quality control was performed in which the chemical and the radiochemical purity, the pH and other parameters were tested.

4.2. Automated radiosynthesis

The [^{18}F]fluoride (30–50 GBq) was separated from the target water using a prepared AG1X8 anion exchange column (30 mg, HCO_3^- form) and eluted into the first reactor using 200 μL of 0.1 M K_2CO_3. After addition of Kryptofix 2.2.2. (30 mg, 80 μmol) and 1 mL acetonitrile, the mixture was dried in a stream of helium at 80 °C. A solution of 15 mg (40 μmol) ethylene glycol-1,2-ditosylate in 1 mL acetonitrile was added to this dried Kryptofix 2.2.2./[^{18}F]fluoride-complex and heated at 80 °C under stirring in a sealed vial for 6 min. The mixture was diluted with 1 mL water, loaded on a LiChrolut EN column and dried with helium. The [^{18}F]FETos was eluted into the second reactor using 1.5 mL of tempered (100 °C) DMSO. Afterwards the reactor, already containing 50 μL of *N*,*N*-dimethylaminoethanol and 6.5 mg sodium iodide, was tempered for 20 min at 120 °C. The mixture was diluted with 13.5 mL of water, loaded on a LiChrolut SCX cartridge (500 mg/H^+ form) and eluted into the injection loop with 3 mL of a 0.15 M disodium hydrogen phosphate buffer in physiological saline solution. The [^{18}F]fluoroethylcholine was purified using HPLC (0.1 M H_3PO_4, flow rate: 3 mL/min) by collecting the peak between 6 and 7.5 min. This fraction was then directly sterile filtered into a vial containing 100 μL of 1 M $NaHCO_3$ to yield 7–15 GBq [^{18}F]fluoroethylcholine (pH 6–8) in a radiochemical yield of 30 ± 6% and a radiochemical purity of 95–99% after a synthesis time of about 50 min.

The [^{18}F]FECh was analysed using ion exchange chromatography (Alltech universal cation 100 × 4.6 mm, 0.0015 M methane sulfonic acid, flow rate: 1.5 mL/min).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.02.038

References and notes

1. Lanks, K.; Somers, L.; Papirmeister, B.; Yamamura, H. *Nature* **1974**, *252*, 476–478.
2. George, T. P.; Morash, S. C.; Cook, H. W.; Byers, D. M.; Palmer, F. B.; Spencer, M. *Biochim. Biophys. Acta* **1989**, *1004*, 283–291.
3. Yorek, M. A.; Dunlap, J. A.; Spector, A. A. M.; Ginsberg, B. H. *J. Lipid Res.* **1986**, *27*, 1205–1213.
4. Hara, T.; Kosaka, N.; Shinoura, N.; Kondo, T. *J. Nucl. Med.* **1997**, *38*, 842–847.
5. Larson, S. M. *J. Nucl. Med.* **1994**, *35*, 1653–1655.
6. Hara, T.; Kosaka, N.; Kishi, H. *J. Nucl. Med.* **1998**, *39*, 990–995.
7. DeGrado, T.; Coleman, R. E.; Wang, S.; Baldwin, S. W.; Orr, M. D.; Robertson, C. N.; Polascik, T. J.; Price, D. T. *Cancer Res.* **2000**, *61*, 110–117.
8. Kotzerke, J.; Gschwend, J. E.; Neumaier, B. *J. Nucl. Med.* **2002**, *43*, 200–202.
9. Deves, R.; Krupka, R. M. *Biochim. Biophys. Acta* **1979**, *557*, 469–485.
10. Clary, G. L.; Tsai, C. F.; Guynn, R. W. *Arch. Biochem. Biophys. Acta* **1987**, *254*, 214–221.
11. Hara, T.; Kosaka, N.; Kishi, H. *J. Nucl. Med.* **2002**, *43*, 187–199.
12. Solbach, C.; Neumaier, B.; Reske, S. N. *J. Nucl. Med.* **2003**(44 suppl.), 101P.
13. Bauman, A.; Piel, M.; Schirrmacher, R.; Rösch, F. *Tetrahedron Lett.* **2003**, *44*, 9165–9167.
14. Block, D.; Coenen, H. H.; Stöcklin, G. *J. Labelled Compd. Radiopharm.* **1984**, *25*, 201–216.
15. Chi, D.; Kilbourn, M.; Katzenellenbogen, J. A.; Welch, M. *J. Org. Chem.* **1987**, *52*, 658–664.
16. Mulholland, G. K.; Mock, B. H.; Zheng, Q.-H.; Vavrek, T. M. *J. Labelled Compd. Radiopharm.* **1999**, *42*, 318–320.
17. Comagic, S.; Piel, M.; Schirrmacher, R.; Höhnemann, S.; Rösch, F. *Appl. Radiat. Isot.* **2002**, *56*, 847–851.
18. Hamacher, K.; Coenen, H. H.; Stöcklin, G. *J. Nucl. Med.* **1986**, *27*, 235–238.
19. Pfütze, N.; Klette, I.; Fischer, S.; Berkholz, R.; Baum, R. P. *Nuklearmedizin* **2004**, *43*, A114.
20. Piel, M.; Schirrmacher, R.; Höhnemann, S.; Hamkens, W.; Kohl, B.; Jansen, M.; Schmitt, U.; Lüddens, H.; Dannhardt, G.; Rösch, F. *J. Labelled Compd. Radiopharm.* **2003**, *46*, 645–659.