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Research Article

Synthesis of [^{18}F]FETO, a novel potential 11-β hydroxylase inhibitor

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Summary

Recent publications reported high uptake of the carbon-11 labelled 11β-hydroxylase inhibitors (R)–[*O*–methyl-^{11}C]metomidate ([^{11}C]MTO) and (R)–[*O*–ethyl-^{11}C]etomidate ([^{11}C]ETO) in adrenocortical incidentalomas with excellent selectivity for positron emission tomography (PET). In our studies [^{18}F]FETO, (the [^{18}F]fluoroethyl ester of etomidate, (R)-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylic acid, 2′-[^{18}F]fluoroethyl ester), an analogue of [^{11}C]MTO and [^{11}C]ETO was chosen due to the suspected similarity of the pharmacokinetic and pharmacodynamic properties, and was prepared in the following two step procedure: First, [^{18}F]fluoride was reacted with 2-bromoethyl triflate using the kryptofix/acetonitrile method to yield 2–bromo-[^{18}F]fluoroethane ([^{18}F]BFE). In the second step, [^{18}F]BFE was reacted with the tetrabutylammonium salt of (R)-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylic acid to yield [^{18}F]FETO, a novel inhibitor of the 11β-hydroxylase. The proposed synthesis of [^{18}F]FETO allows the production of sufficient amounts of this new PET-tracer to serve 1–2 patients with an overall synthesis time of less than 80 min. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: [^{18}F]FETO; 11β-hydroxylase; etomidate; PET; fluoroethylation

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Introduction

A major problem of magnetic resonance (MR) and computed tomography (CT) imaging with adrenal incidentalomas — accidentially detected masses at the adrenal site — is the difficulty of distinguishing between primary adrenal tumors, metastases to the adrenal and other masses of non-adrenal origin. Therefore, patients frequently have to undergo extensive clinical and laboratory examinations including analyses of the adrenal hormones. One of the key enzymes, playing a major role in the biosynthesis of these hormones in the adrenocortex is the 11β-hydroxylase (cyp11B1; $P450_{11\beta}$), forming cortisol and aldosterone in the zona fasciculata/reticularis from the corresponding deoxy-steroids.

Hence, in recent years increased interest has focused on this enzyme as a target for the imaging of adrenocortical lesions with PET.[1,2] Bergstrom *et al.*[3,4] published promising results in the diagnosis of the adrenocortical incidentalomas with the 11β-hydroxylase inhibitors [^{11}C]-metomidate and [^{11}C]-etomidate, which could be reproduced by our group.[5,6]

The intention of this work was to develop a labelling procedure for etomidate with fluorine-18 in a metabolically stable position without essential loss of the pharmacodynamic and pharmacokinetic potential. In addition, the use of fluorine-18 instead of carbon-11 reveals several improvements for the clinical routine: firstly, the examination of more patients per tracer production; secondly, the possibility of longer acquisition protocols; thirdly, the distribution to PET-centers without on-site cyclotron (satellite principle). Labelling with fluorine-18 is principally possible at two different positions of the target molecule: either on the aromatic ring or on the carboxylic group. The decision in favor of the latter was taken for various reasons: The nucleophilic substitution on the phenyl ring is rather demanding because of the complex precursor synthesis and requires elaborate purification procedures in view of the side-products formed. *In vitro* binding studies showed an increased affinity for the target enzyme ($P450_{11\beta}$) when the methyl ester was replaced by an ethyl ester ($IC_{50} = 1.1$ nM (etomidate), 2.9 nM (metomidate)) and fluoroethyl esters have been reported to show similar in vivo behavior when compared to their ethyl analogues.[7] Further, experience with the good performance of the carbon-11 labelled esters [^{11}C]ETO and [^{11}C]MTO led us to the fast forward strategy of fluoroethylations at this very position.

Table 1. Stepwise and overall reaction yields (decay corrected) and required times in the radiosynthesis of [^{18}F]FETO

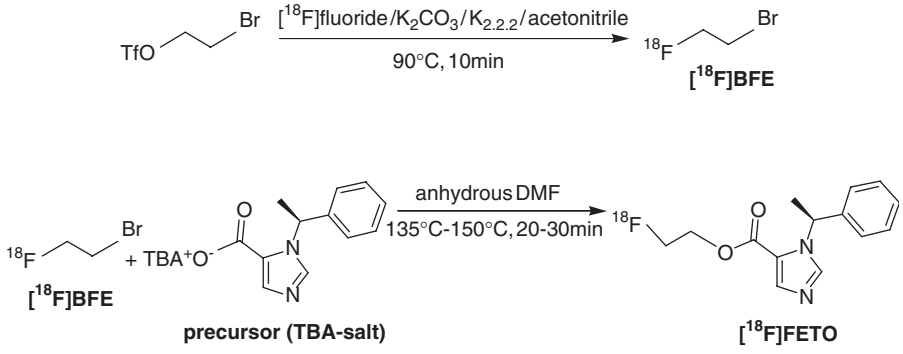
Step	Time (min)	Overall time (min)	rc. yield (%)	Overall rc. yield (%)
EOB	0	0	100.0	100.0
Azeotropic drying	18	18	98.1	98.1
Formation of [^{18}F]BFE	10	28	69.7	68.4
Distillation of [^{18}F]BFE	15	43	24.0	16.4
Formation of [^{18}F]FETO	30	73	95.0	15.6
SepPak purification	5	78	95.0	14.8
Quality control	8	86	100.0	14.8

Radiosynthesis and quality control of the fluoroethyl ester of etomidate, (R)–1–(1-phenylethyl)-1H-imidazole-5-carboxylic acid 2′-[^{18}F]fluoroethylester ([^{18}F]FETO), are discussed in the present contribution.

Results and discussion

Our synthesis route offers a highly reproducible and feasible method for the synthesis of [^{18}F]FETO in less than 80 min. So far, only 3 out of 91 (3.3%) syntheses failed. Radiochemical yields at the end of synthesis (EOS) were up to 14.8% decay corrected (d.c.). (Table 1) Radiochemical impurities of the purified product solution as detected by radio-HPLC and radio-TLC were well below our internal thresholds of 3%. Residual solvents were analyzed by GC and showed <10 ppm for DMF whereas acetonitrile and dichloromethane were below the limit of detection. No major chemical impurities were detected by HPLC (UV, 235 nm) and TLC (UV, 254 nm). Residuals of Kryptofix 2.2.2 were also below our detection limits.

The first step of the synthesis was the formation of [^{18}F]BFE (Scheme 1), followed by its distillation into a v-vial containing anhydrous DMF through a washing flask. Although [^{18}F]BFE is a well established synthon in the synthesis of fluorine-18 labelled radiotracers[8,9] and even though its distillation can be of major importance for the success of a synthesis route,[10,11] our investigation revealed that it was the critical and most challenging step in the whole radiosynthesis. Yields for this step varied between 4.1 and 28.4%. The whole distillation required 10–15 min, caused by different nitrogen flow rates. Trapping yields,



Scheme 1. Synthesis of [^{18}F]FETO

reactivity and quality of the distilled [^{18}F]BFE strongly depended on the nitrogen-flow as well as on the amount and ratio of DMSO/DMF used in the washing flask. Thus, we found out that the optimum nitrogen flow had to be approximately 5 ml/min for best purification results. This value is critical, because, on the one hand, an increased nitrogen flow results in overdistillation of by-products and expulsion of [^{18}F]BFE out of the product vial and, on the other hand, a reduced nitrogen flow leads to incomplete transfer of [^{18}F]BFE into the product trap and increased time. To complete the transfer into the product vial a total of 500 µl acetonitrile was added to the reaction vessel in 4–6 portions.

For the second reaction step, the esterification of the tetrabutylammonium salt of (R)-1-(1-phenylethyl)-1H-imidazole-5-carboxylic acid with [^{18}F]BFE, reaction kinetics regarding temperature (Figure 1) and precursor concentration (Figure 2) were investigated. As evident from the present data a continuous increase in the esterification yields is observed throughout all precursor concentrations higher than 0.05 mM within 30 mins. An abrupt drop-off in the conversion of [^{18}F]BFE into [^{18}F]FETO appears at a precursor concentration below 1.6 mM. Furthermore, our data explicitly indicate that a minimum reaction time of 20 mins is required to achieve satisfactory conversion results ($>95\%$). This fact even applies to high precursor concentrations (e.g. 3.7, 4.6 and 5.6 mM). Our experiments revealed no significant influence of the applied activity of [^{18}F]BFE (e.g. 50–3100 MBq). The temperature dependence curve shows a sigmoid trace between 20 and 180°C. Surprisingly, at temperatures above 180°C a decrease in the achieved yields was observed. A possible explanation can be given by the fact that

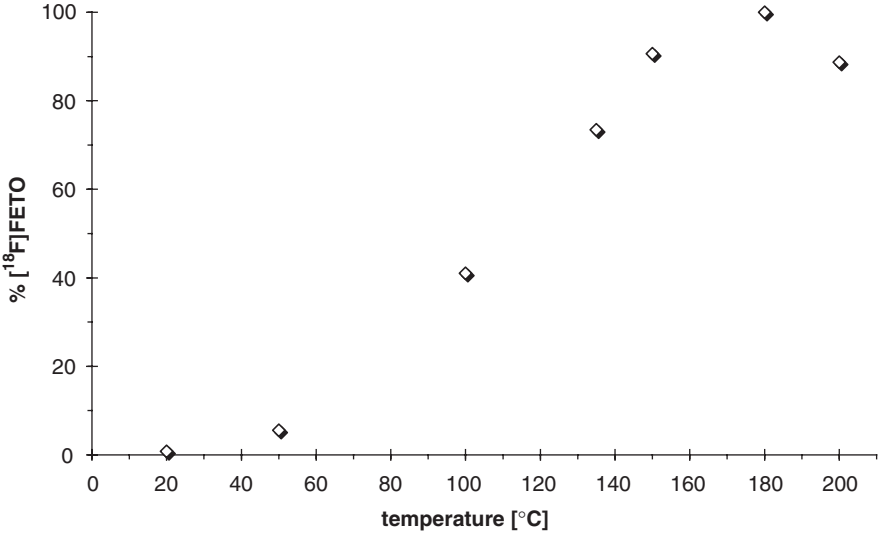


Figure 1. Temperature dependence of the conversion of [^{18}F]BFE into [^{18}F]FETO (concentration of precursor = 1.16 mM; reaction time = 30 min; n = 3–5)

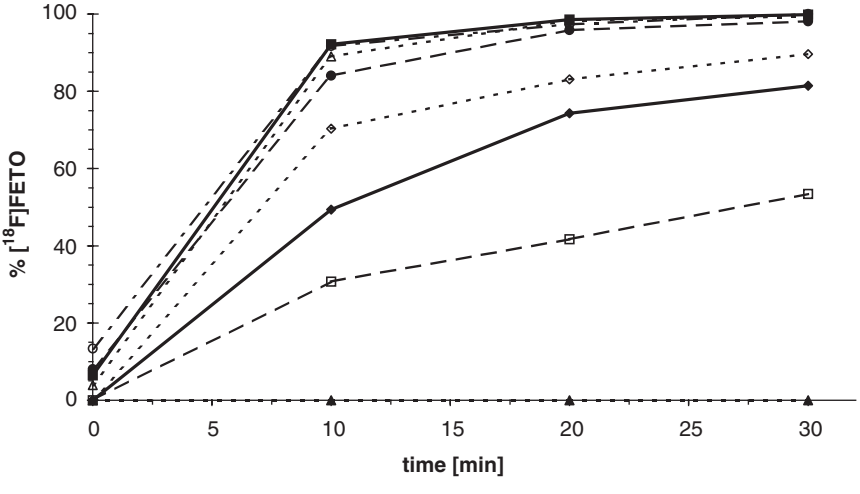


Figure 2. Kinetics of the conversion of [^{18}F]BFE into [^{18}F]FETO at different concentrations of precursor (T = 135°C; n = 3–5) (-○- 5.6 mM; -■- 3.7 mM; -△- 2.3 mM; -●- 1.6mM; -◊- 1.2 mM; -◆- 0.5mM; -□- 0.2 mM; –▲- 0.05 mM)

the reaction partners involved are volatile and therefore partially vaporize and recondense within the reaction vial during the esterification. All these results led us to the optimum reaction conditions for the

esterification of (R)-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylic acid as shown in Table 2.

After cooling the reaction mixture to ambient temperature in an ice-bath, the SepPak® purification was performed by diluting the crude reaction mixture in the reaction vial with 5 ml of water and washing the vial with a further 5 ml. These combined (aqueous) solutions of the reaction mixture were loaded onto a C18plus SepPak® cartridge. Inadequate dilution resulted in partial breakthrough of [^{18}F]FETO. The cartridge was washed with a further 10 ml of water. [^{18}F]Fluoride as well as all other hydrophilic contaminants were not retained on the matrix and therefore went through the SepPak® immediately. Consecutively, the purified product, [^{18}F]FETO, was quantitatively recovered from the cartridge by elution with 1.2 ml of absolute ethanol. Total recovery of radioactivity was $>95\%$. Since a quantification of the [^{18}F]fluoride content was not possible with the HPLC assay used for quality control, radio-TLC was performed additionally, showing that the [^{18}F]fluoride content was well below 3% throughout all measurements ($n=12$).

The used quality control systems revealed excellent separation properties: HPLC retention times were 4.5–4.7 min for [^{18}F]BFE and 7.4–7.6 for [^{18}F]FETO, respectively; TLC R_f-values were 0.0 for all potential impurities and 0.70–0.75 for [^{18}F]FETO, respectively. All values were verified by inactive reference substances.

Experimental

Materials

Precursor ((R)-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylic acid) and FETO standard ((R)-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylic acid 2′-fluoroethylester) were purchased from ABX — Advanced Biochemical Compounds (Dresden, Germany).

Solid phase extraction cartridges (SepPak® C18plus) were purchased from Waters Associates (Milford, MA). Kryptofix 2.2.2., potassium carbonate and 2-bromoethanol were purchased from Sigma-Aldrich

Table 2. Optimum reaction conditions for the conversion of [^{18}F]BFE into [^{18}F]FETO

Temperature	135–150°C
Reaction time	20–30 min
Precursor concentration	$\geqslant$2.0 mmol/l

Chemical Company (Steinheim, Germany). Acetonitrile, trifluoromethanesulfonic anhydride, tetrabutylammonium hydroxide (TBAH, 20% aqu.), dimethylsulfoxide (DMSO), dichloromethane, anhydrous *N,N*-dimethylformamide (DMF) and ammonium acetate were purchased from Merck (Darmstadt, Germany). Ethanol (100%) and acetic acid (100%) were purchased from Riedel-de Haën (Seelze, Germany). All reagents were of analytical grade and used without further purification. Analytical thin layer chromatography (TLC) was performed using Silicagel 60 F_{254} plates from Merck (Darmstadt, Germany). High performance liquid chromatography (HPLC) was performed using a LiChrospher 100 RP-18 column (5 μm, 250 mm × 4 mm) from Merck (Darmstadt, Germany). Gas chromatography (GC) was performed using an HP-innowax column (30 m × 0.32 mm × 0.25 μm).

[^{18}F]Fluoride was produced via the ^{18}O(p,n)^{18}F reaction in a GE PETtrace cyclotron (16.5 MeV protons). $H_2{}^{18}O$ was purchased from Chemotrade (Dresden, Germany).

Instruments

Analysis of radio-TLC plates was performed using an autoradiograph (Berthold). High performance liquid chromatography (HPLC) was performed with a Merck-Hitachi LaChrom L-7100 system equipped with a Merck-Hitachi LaChrom L-7400 UV detector at 235 nm and a lead-shielded NaI-radiodetector (Berthold). Gas chromatography was performed using an HP 6890 series system equipped with a flame ionization detector (FID).

2-Bromoethyl Triflate

2-Bromoethyl triflate was prepared according to a literature method[12] starting from trifluoromethanesulfonic anhydride and 2-bromoethanol. Distillation (120°C, 20 mbar) gave a colourless oil in 37% yield which was stored at −18°C.

2-Bromo-1-[^{18}F]fluoroethane ([^{18}F]BFE)

No-carrier-added (n.c.a.) aqueous [^{18}F]fluoride was prepared by the ^{18}O(p,n)^{18}F nuclear reaction on an enriched water target. 0.4–1.5 ml of the solution was added to a 2.5 ml v-vial containing Kryptofix 2.2.2. (13.3 μmol), potassium carbonate (10.0 μmol) and acetonitrile (1.0 ml,

19.1 mmol) and heated to 100°C. Azeotropic drying was performed by subsequent addition of at least four 250 µl portions of acetonitrile. To the dried complex 2-bromoethyl triflate (20 µl, 77.8 µmol) and acetonitrile (80 µl, 1.5 mmol) were added, the vial was sealed and the contents heated at 90°C for 10 min.

Distillation

Volatiles were distilled using a smooth stream of nitrogen (5 ml/min) and 1/16″ tubing with needles connecting the reaction vessel, a washing flask and the product trap. The washing flask contained 190 µl of DMSO and 10 µl of anhydrous DMF at ambient temperature, whereas the product trap contained 600 µl of anhydrous DMF at 0°C. A total of 500 µl of acetonitrile was added in small portions (appr. 100 µl) to the reaction vessel to achieve quantitative transfer of the intermediate ([^{18}F]BFE).

(R)-1-(1-phenylethyl)-1H-imidazole-5-carboxylic acid 2′-[^{18}F]fluoroethyl-ester ([^{18}F]FETO)

The precursor was activated by dissolving 3 mg (13.8 µmol) (R)-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylic acid in 300 µl dichloromethane and adding 15.6 µl tetrabutyl ammonium hydroxide (TBAH, 20%) as a phase transfer catalyst. Dichloromethane was then evaporated and the dried complex was reconstituted in 750 µl anhydrous DMF. A calculated aliquot of this solution was added to the distilled [^{18}F]BFE. The reaction was carried out for 10, 20 or 30 min for the reaction kinetics and at temperatures varying between 20 and 200°C for the temperature dependence experiments. The reaction mixture was cooled to room temperature in an ice-bath prior to the final purification step.

Product purification

A pre-conditioned (ethanol/water) C18plus SepPak® cartridge was loaded with the product solution diluted with a total of 10 ml water and washed with a further 10 ml of water. The purified [^{18}F]FETO was then quantitatively eluted with 1.2 ml of ethanol (100%).

Quality control

Chemical and radiochemical impurities were detected using radio-HPLC (mobile phase: 60% (water/ethanol/acetic acid 87.5/10/2.5

(v/v/v), 2.5 g/l ammonium acetate, pH 3.5), 40% acetonitrile) and radio-TLC (mobile phase: 30% (water/ethanol/acetic acid 87.5/10/2.5 (v/v/v), 2.5 g/l ammonium acetate, pH 3.5) 70% acetonitrile). Residual solvents were analyzed by GC (carrier gas: He; flow: 2.7 ml/min; 45°C (2.5 min)–20°C/min to 110°C–30°C/min to 200°C–200°C (10 min); FID: 270°C). Residuals of Kryptofix 2.2.2 were analyzed by TLC according to the [^{18}F]FDG monograph in the European Pharmacopoeia (1999:1325) (mobile phase: 90% methanol/10% ammonia (v/v); iodine chamber).

Conclusion

From the present investigation it is evident that the introduced synthesis allows the preparation of [^{18}F]FETO in reliable manner and excellent purity. Investigations regarding the *in vitro* and *in vivo* behavior of this promising compound are in progress.

The crucial step associated with the highest loss of activity is the purification of the intermediate via distillation. Further emphasis will be put on the improvement of this step based on recently published alternatives.[13–15] So far, the proposed synthesis offers labelling yields sufficient to serve 1–2 patients per production with high reproducibility. It may enable PET centers without on-site cyclotron to include adrenocortical imaging into patient routine.

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