

Research Article

Synthesis of [¹⁸F]fluoroalkyl esters of carfentanil

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Summary

With the aim to develop and evaluate new ligands for depicting the μ -opioid receptor with positron emission tomography, the ¹⁸F-fluoroalkyl esters of carfentanil, 3-carboxy-(2-[¹⁸F]fluoroethyl)fentanyl, (2-[¹⁸F]fluoroethyl-carfentanil) and 3-carboxy-(3-[¹⁸F]fluoropropyl)fentanyl (3-[¹⁸F]fluoropropyl-carfentanil) were prepared by a two-step radiosynthesis. Reacting carfentanil carboxylate sodium salt, added 0.96 eqv. of tetrabutyl ammonium hydroxide (TBAH), with no-carrier-added (n.c.a.) 2-[¹⁸F]fluoroethyltosylate for 20 min at 150°C in dimethyl formamide (DMF) provided 2-[¹⁸F]fluoroethyl carfentanil in an isolated radiochemical yield (RCY) of $36 \pm 8\%$, a specific activity (S_A) of 35 ± 5 TBq/mmol ($n = 4$) within a synthesis time of ~ 100 min. Similarly, 3-[¹⁸F]fluoropropyl carfentanil could be obtained by reacting the carfentanil TBA/Na salt with 3-[¹⁸F]fluoropropyl iodide at 160°C in DMF (isolated RCY = $6 \pm 2\%$; ~ 100 min, $S_A = 27 \pm 5$ TBq/mmol, $n = 4$). The developed methods allow the production of the two ¹⁸F-labeled carfentanil derivatives in amounts and specific activities necessary and relevant for a detailed preclinical evaluation of these new potential μ -opioid receptor ligands *in vitro* and in animal models. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: fluorine-18; carfentanil; opioid receptor; fluoroalkylation

Introduction

¹¹C-labeled carfentanil ([¹¹C]CAF; $t_{1/2} = 20.3$ min) is a valuable tracer to assess the μ -opiate receptor (μ OR) system in physiologic and pathophysiologic conditions with positron emission tomography (PET).¹ Although [¹¹C]CAF has been used in various human PET-studies, its potent agonistic properties

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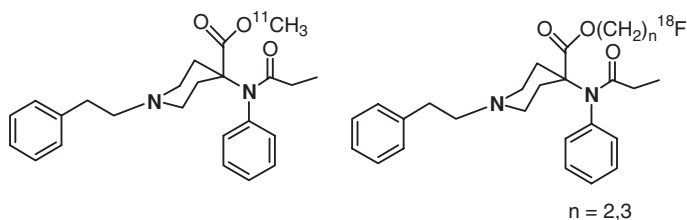


Figure 1. Structure of [^{11}C]carfentanil and the ^{18}F -labeled carfentanil derivatives of study

and pharmacological effects implies administration in amounts of less than $2.1 \mu\text{g}/70 \text{ kg}$.² For 740 MBq of [^{11}C]CAF administered as a bolus injection a specific activity at the time of injection $> 88.8 \text{ TBq}/\text{mmol}$ is indispensable. Consequently, some institutions hesitate to establish [^{11}C]CAF for clinical research. To overcome this drawback, the development of alternative μOR ligands with suitable binding characteristics and lower pharmacological potency is recommended. Furthermore, attempts to measure the central release of endogenous opioids by displacement studies *in vivo* using ^{11}C -labeled ligands are complicated by the relatively short half-life of ^{11}C and the low activity at the end of the scanning procedure with a concomitant low sensitivity for signal changes. A ^{18}F -labeled tracer (^{18}F : $t_{1/2} = 109.7 \text{ min}$) for PET could improve the signal intensity and thereby the accuracy of such protocols.

The strategy followed for the synthesis of [^{11}C]CAF,^{3,4} is based on alkylation of carfentanil carboxylic acid sodium salt. Thus, a promising route to ^{18}F -labeled carfentanil analogues may be based on alkylation of the acid by ^{18}F -labeled alkylhalides, -tosylates or -triflates. Starting with the evaluation of the analogue with the smallest structural changes, preclinical experiments with [^{18}F]fluoromethyl carfentanil revealed suitable brain uptake in mice but also low *in vivo* stability.⁵ With the aim to stabilize the ester bond we synthesized and evaluated higher ^{18}F -fluoroalkyl ester homologues. As the ethyl- and propyl ester of carfentanil (Figure 1) are less potent than carfentanil by a factor of 13 and 133, respectively,⁶ they may be used as templates for the development of radiopharmaceuticals with increased safety profile. Here, the radiosynthesis of 2- [^{18}F]fluoroethyl carfentanil ester ([^{18}F]1) and 3- [^{18}F]fluoropropyl carfentanil ester ([^{18}F]2) is described.

Results and discussion

The strategy used for preparation of the ^{18}F -alkyl esters of carfentanil [^{18}F]1 and [^{18}F]2 was based on a two-step radiosynthesis. The two syntheses consist of ^{18}F -fluorination of ethylene glycol-1,2-bistosylate and 1,3-diiodopropane,

respectively, followed by ^{18}F -fluoroalkylation of carfentanil carboxylic acid sodium salt containing 0.96 M equivalents of tetrabutyl ammonium hydroxide (TBAH). In initial experiments, the effect of temperature of the esterification was investigated using *N,N*-dimethyl formamide (DMF) or dimethyl sulfoxide (DMSO) as solvents. Alkylation of the carboxylate with no-carrier-added (n.c.a.) [^{18}F]fluoroethyltosylate at 100°C in DMF gave [^{18}F]1 in an analytical radiochemical yield (RCY) of about 7% after 30 min (Figure 2(A)). Although the conditions chosen have been reported to be suitable for the ^{18}F -fluoroethylation of hydroxyl groups⁷ and also sterically hindered 2° aliphatic hydroxyls,⁸ the low RCY obtained in this study reflects the comparatively low nucleophilicity of the carboxylate salt. Increasing the reaction temperature to 120°C increased the yield of the product to about 20% in 30 min. Compared to the use of DMF, DMSO resulted in lower yields under otherwise identical conditions, both at 100 and 120°C (Figure 2(A)). At 140°C in DMF, the product seems to decompose based on the decrease in the RCY observed after 10 min (Figure 2(A)). Based on these initial experiments, DMF was chosen for further studies on reaction optimization. Next, the influence of the addition of sodium iodide to the reaction mixture on the RCY was investigated. This strategy was chosen since direct preparation of 2- ^{18}F fluoro-1-iodo ethane, another valuable ^{18}F -alkylation reagent, has been found to result in very low yields.⁹ To obtain this intermediate *in situ*, NaI was added in final concentrations of 10–30 mM. At 140°C , an increase of the NaI concentration from 10 to 20 mM increased the yields from 25 to 55% (Figure 2(B)). Highest

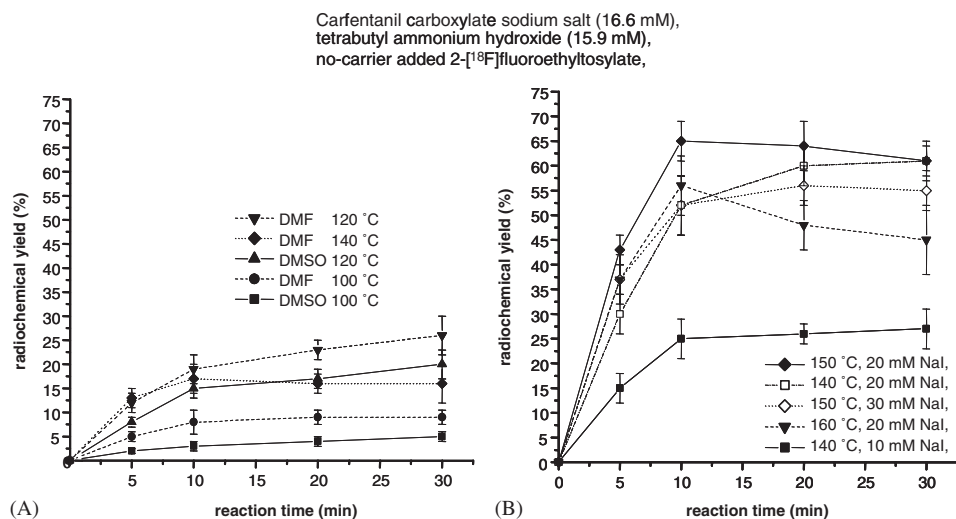


Figure 2. Analytical RCY of 2- ^{18}F fluoroethyl carfentanil as function of temperature and time without (A) or with NaI present (B)

yields were observed after 10 min at 150°C (20 mM NaI, DMF). However, for reactions conducted at 150 and 160°C (both with 20 mM NaI, DMF) the product seems to decompose after 10 min leading to a continuous decrease of the RCY during the time of observation (Figure 2(B)). Furthermore, no further improvement was observed when even higher concentrations of NaI (30 mM) were used (Figure 2(B)).

The increase in reactivity of [^{18}F]fluoroethyl bromide towards *O*- and *N*-nucleophiles by addition of sodium iodide has been demonstrated previously.¹⁰ However, our observation that ^{18}F -fluoroethylation of a carboxylate sodium salt, added TBAH, can be improved in the presence of sodium iodide seems to be contrary to the observation of Wadsak *et al.*¹¹ who observed a decrease in the formation of flumazenil carboxylic acid ester by the addition of sodium iodide when using 2- ^{18}F fluoroethyl bromide as alkylation agent. However, on the basis of the predominantly detected side product, the *N*-alkylated flumazenil carboxylic acid,¹¹ it can be speculated that these differences may be related to sodium iodide predominantly accelerating the side-reaction of the des-ethyl flumazenil radiolabeling precursor used by Wadsak *et al.*

It has been reported⁴ that the yield of [^{11}C]CAF, formed via ^{11}C -methylation of the carfentanil carboxylic acid sodium salt, added TBAH, decreases when excess TBAH is present. As a part of the present study, ^{18}F -fluoroethylation of phenyl acetic acid sodium salt was investigated as a simple model system for the reaction, as this compound possess no other reactive functional groups than the carboxylate. As can be seen from Table 1, when using a concentration of NaI of 20 mM, a TBAH/carboxylate sodium salt ratio of about unity is imperative to provide optimum yields.

Based on the results from the optimization studies, preparative runs of [^{18}F]1 were conducted under the most 'robust' conditions, i.e. by reacting 2- ^{18}F fluoroethyltosylate with a solution of carfentanil sodium salt (16.6 mM), TBAH (15.9 mM), NaI (20 mM) in DMF at 150°C for 20 min. For the preparative runs, 2- ^{18}F fluoroethyltosylate was trapped on a trace-enrichment

Table 1. ^{18}F -fluoroethylation of sodium phenyl acetate^a

Molar ratio of TBAH to sodium phenyl acetate	RCY (%) ^b
0.5	42 ± 9
0.95	70 ± 6
1.2	48 ± 5
2	35 ± 8
3	17 ± 9

^aN.c.a. [^{18}F]fluoroethyltosylate was reacted with sodium phenyl acetate (17 mM) in the presence of various amounts of TBAH, added as a 1 M solution in methanol, and NaI (20 mM) in DMF at 150°C for 20 min.

^bAs determined by analytical HPLC measurements (results are the means ± SD, *n* = 3).

cartridge after high-performance liquid chromatography (HPLC). The trace-enrichment cartridge was directly connected in-line to the HPLC outlet and subsequently eluted directly from the cartridge with DMF into the reaction vial. This ensured that the volume of DMF used for the alkylation reaction could be kept low without additional transfer and evaporation steps. At these conditions, [^{18}F]1 could be obtained in an isolated RCY of $36 \pm 8\%$ (mean \pm SD, $n=4$) in a radiochemical purity of $>97\%$ and a specific activity $S_A > 35$ TBq/mmol.

In comparison to the ^{18}F -fluoroethylation of carfentanil carboxylic acid, the corresponding ^{18}F -fluoropropylation to obtain [^{18}F]2 proceeded much less effective (Figure 3). Even at 160°C using a carfentanil sodium salt concentration of 16.6 mM, a TBAH of 15.9 mM in DMF, the analytical RCY of 3- ^{18}F fluoropropyl iodide into [^{18}F]2 was only about 12% (Figure 3). The apparent lower reactivity of 3- ^{18}F fluoropropyl iodide as compared to that of the 2- ^{18}F fluoroethyltosylate/sodium iodide system may reflect the inferior leaving-group ability of the iodo-substituent of the former compound, presumably due to less activating effects of fluorine in the 3-position than is the case for the 2-tosylate/2-iodo substituent of the latter. The preparative RCY of [^{18}F]2 was $6 \pm 2\%$, and the product was obtained in a radiochemical purity of $>97\%$ and a specific activity of $S_A = 27 \pm 5$ TBq/mmol ($n=4$).

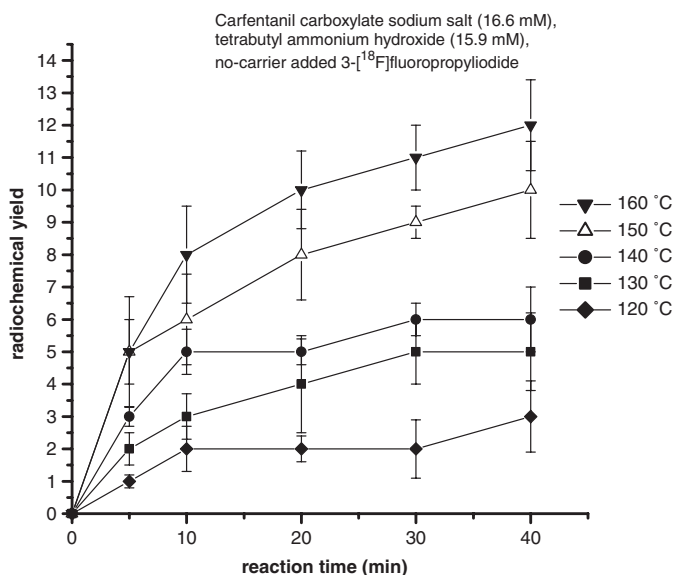


Figure 3. Analytical RCY of 3- ^{18}F fluoropropyl carfentanil at different temperatures as function of time

The two new carfentanil analogues [^{18}F]**1** and [^{18}F]**2** can be prepared in an overall synthesis time of approximately 100 min (from EOB) consisting of 45 min for the preparation of 2- [^{18}F]fluoroethyltosylate or 3- [^{18}F]fluoropropyl iodide, 20 min for the alkylation reaction and a total of about 35 min for HPLC separation and product formulation. All radioactive contaminants are eluted before the ^{18}F -labeled carfentanil analogues on a preparative reverse phase C_{18} HPLC column thus ensuring high chemical purity. A specific activity of 35 TBq/mmol for [^{18}F]**1** and 27 TBq/mmol for [^{18}F]**2** is less than that required for use of [^{11}C]CAF in the PET imaging of μOR in humans. However, based on pharmacological data of their parent non-fluorinated compounds, a lower pharmacological potency than that of carfentanil can be expected for [^{18}F]**1** and [^{18}F]**2**. In addition, the longer half-life of ^{18}F may allow a full kinetic analysis of the tracer after administration of a substantially smaller amount of activity than is the case for a ^{11}C -labeled tracer.

Experimental

Materials and equipment

Kryptofix 2.2.2. and acetonitrile were obtained from Merck Eurolab. The sodium salt of *N*-[4-(carboxy)-1-(2-phenylethyl)-4-piperidinyl]-*N*-phenylpropanamide sodium salt (carfentanil carboxylate sodium salt) was purchased from ABX Biochemicals. Other chemicals were purchased from Sigma-Aldrich and used as received. The preparative and analytical HPLC systems were from Sykam. For analytical HPLC, in-line detectors included a UV-detector (254 nm) and a well-type NaI(Tl) detector. Preparative HPLC was performed using a UV-detector (254 nm) and a Bioscan Flow-count system fitted with a PIN detector. Mass spectra (MS) were recorded on the liquid chromatography–mass spectrometry system LCQ from Finnigan using the Hewlett Packard series 1100 HPLC system.

Synthesis of 2-fluoroethyl carfentanil (1)

Five milligrams of carfentanil carboxylic acid sodium salt (12.4 μmol) was dissolved in DMF containing 13 μl of a 1 M solution of TBAH in methanol. 1.7 mg of 1-bromo-2-fluoroethane (13.4 μmol) and 2.0 mg of sodium iodide (13.4 μmol) was added to the solution and the mixture heated at 120°C for 2 h. After this the reaction was allowed to cool to room temperature and diluted with water (10 ml). The resulting solution was extracted with 10 ml dichloromethane (three times) and dried (MgSO_4), filtered and concentrated to an oil which was purified by RP-HPLC (semi-preparative Luna C_{18} column, 10 \times 250 mm; Phenomenex) using linear gradient of 15–70% B in 20 min; 0.1% trifluoro acetic acid (TFA) in water (A), 0.1% TFA in MeCN (B), flow rate of 3 ml/min. The eluent from the column was continuously monitored for UV

absorbance at 254 nm. The fraction containing the product (capacity factor, $k' = 8.6$) was evaporated to yield 1.7 mg of a yellowish oil (31%). Calculated for $\text{C}_{25}\text{H}_{31}\text{FN}_2\text{O}_3 = 426.53$; MS: 427.5 ($[\text{M} + 1]^+$).

3-fluoropropyl carfentanil (2)

3-fluoropropyl carfentanil was prepared and purified according to the synthesis of **1** using 1.9 mg of 1-bromo-3-fluoropropane (13.4 μmol) as alkylation reagent. The fraction containing the product ($k' = 8.8$) was evaporated to yield 0.6 mg of a yellowish oil (11%). Calculated for $\text{C}_{25}\text{H}_{31}\text{FN}_2\text{O}_3 = 440.56$; MS: 441.5 ($[\text{m} + \text{H}]^+$).

Radiosynthesis – general methods

^{18}F fluoride was produced through the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction. The ^{18}F fluoride was obtained in a 34 mM solution of K_2CO_3 (0.3 ml) and added to a 2 ml conical vial containing 0.5 ml dry MeCN and 15 mg (39.9 μmol) Kryptofix 2.2.2. The solvent was evaporated with heating under reduced pressure. Azeotropic drying was repeated three times with 0.5 ml portions of MeCN.

2- ^{18}F fluoroethyltosylate

2- ^{18}F fluoroethyltosylate was prepared according to the method described previously.⁷ Briefly, 5 mg (12 μmol) of ethylene glycol-1,2-ditosylate in 250 μl MeCN was added to the dried kryptate ($[\text{K} \subset 2.2.2]^+ / ^{18}\text{F}^-$), the vial was then sealed and heated at 90°C for 5 min. 2- ^{18}F fluoroethyltosylate was purified by reversed-phase HPLC (Lichrosorb C_{18} 5 μm ; 10 mm \times 250 mm (CS – chromatographie service) eluted with MeOH/ H_2O (50:50, volume/volume, flowrate 4 ml/min; $k' = 5.7$; preparative RCY $65 \pm 5\%$). After on-line fixation of the product on a Strata X cartridge (33 μm ; 30 mg/1 ml; Phenomenex) and drying of the product by argon-flush, the product was eluted with 0.15 ml DMF into a reaction vial.

3- ^{18}F fluoropropyl iodide

3.6 mg of 1,3-diiodopropane (12.1 μmol) in 250 μl MeCN was added to the dried kryptate, the vial was then sealed and heated at 80°C for 5 min. 3- ^{18}F fluoropropyl iodide was purified by reversed phase HPLC (Lichrosorb C_{18} 5 μm ; 10 mm \times 250 mm (CS) eluted with MeOH/ H_2O (60:40, volume/volume, flow rate 4 ml/min; $k' = 6.4$; preparative RCY $52 \pm 8\%$). After on-line fixation of the product on a polystyrene cartridge (LiChrolut EN) and drying of the product by argon-flush, the product was eluted with 0.15 ml DMF.

[¹⁸F]fluoroalkylesters of carfentanil – general methods

The ¹⁸F-fluoroalkylation agent solubilized in DMF and DMSO was added to 1.0 mg of carfentanil carboxylic acid sodium salt (2.5 μmol) and TBAH (2.4 μl of 1 M solution in methanol) and heated at temperatures of 100–160 °C in a sealed vial for 10–30 min and then cooled to room temperature. For the determination of the dependency of the RCY on temperature, reaction time and solvent as well as the addition of sodium iodide in the case of the ¹⁸F-fluoroethylated carfentanil analogue, aliquots were drawn for analysis by radio-HPLC.

For preparative purposes, the reaction mixture was diluted with 4 ml 0.1 M ammonium formate, loaded into a 7 ml injection loop and transferred onto a sample enrichment column (PRP-1 -polystyrene-divinylbenzene-, 10 μm; 10 mm × 50 mm, CS). The enrichment column was washed with 0.1 M ammonium formate at a flow-rate of 2 ml/min for 5 min and subsequently eluted in the reverse direction onto a semi-preparative column (μ-Bondapak C₁₈ 5 μm; 8 mm × 300 mm, CS) using a mobile phase consisting of MeCN/0.1 M ammonium formate (55:45, volume/volume) at a flow rate of 3 ml/min, continuously monitoring for radioactivity and UV absorbance at 254 nm.

Analytical HPLC was performed on a Nucleosil 100 5 μm CN-column (4.6 mm × 250 mm; CS). The *k'*-value of [¹⁸F]1 and [¹⁸F]2 under these conditions was 3.9 and 4.2, respectively, and [¹⁸F]1 and [¹⁸F]2 were co-eluted with their respective reference compounds. In addition, a C₁₈ phase was used to confirm coelution of the labeled compounds and the reference compounds (Nucleosil 100 C₁₈, 5 μm, 4.6 mm × 250 mm (CS); acetonitrile/0.1 M ammonium formate (65:45, volume/volume)). The *k'*-value of [¹⁸F]1 and [¹⁸F]2 under these conditions was 5.5 and 5.8, respectively.

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