

Synthesis of 3-[1*H*-imidazol-4-yl]propyl 4-[¹⁸F]fluorobenzyl ether ([¹⁸F]fluoroproxyfan): A potential radioligand for imaging histamine H₃ receptors

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SUMMARY

3-[1*H*-Imidazol-4-yl]propyl 4-fluorobenzyl ether (fluoroproxyfan), a potential histamine H₃ receptor ligand, was labeled with ¹⁸F for clinical PET studies. The synthesis involved the *O*-alkylation of 3-(1-triphenylmethyl-1*H*-imidazol-4-yl)propanol with 4-[¹⁸F]fluorobenzyl bromide in the presence of silver triflate and a non-nucleophilic amine base. 4-[¹⁸F]Fluoroproxyfan was obtained within 100 min from the end of bombardment (EOB) in 10% radiochemical yield (decay corrected to EOB) with a radiochemical purity >99% and a specific activity >150 GBq/μmol.

Key words: fluoroproxyfan, fluorine-18, histamine H₃ receptor, radioligand, PET.

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INTRODUCTION

Histamine is found in almost every organ tissue where it plays various important roles. High concentration of histamine receptors are located in the central nervous system (CNS), and they govern several physiological and pathophysiological functions. Three subtypes of histamine receptors (H_1 , H_2 and H_3) have so far been identified. The most recently discovered histamine H_3 receptors (1) are reported to regulate the synthesis and release of histamine and also to inhibit the release of a number of different neurotransmitters. (2, 3) This suggests that their functions are implicated in different neurological diseases and disorders. (4) Accordingly, new histamine H_3 receptor antagonists have been extensively developed with the expectation that they can be used as therapeutic drugs for cerebral diseases. Among these, 3-(1*H*-imidazol-4-yl)propyl 4-iodobenzyl ether (iodoproxyfan) appears to be one of the most promising because of its high affinity ($K_i = 5 \pm 1$ nM) and high selectivity for H_3 receptors. (5) This compound was successfully labeled with ^{125}I as a radioligand for binding assays and for the detection and localization of histamine H_3 receptors. (5)

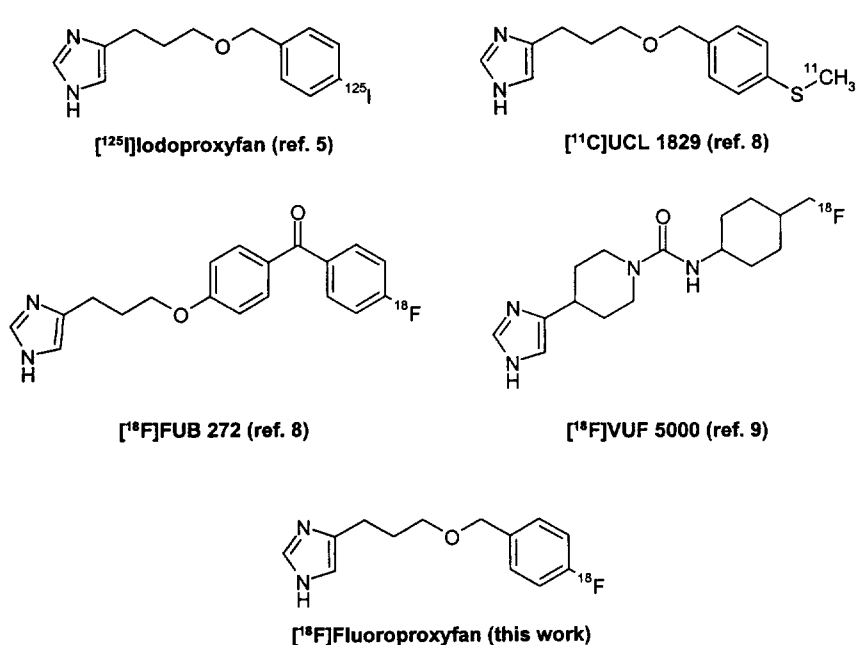


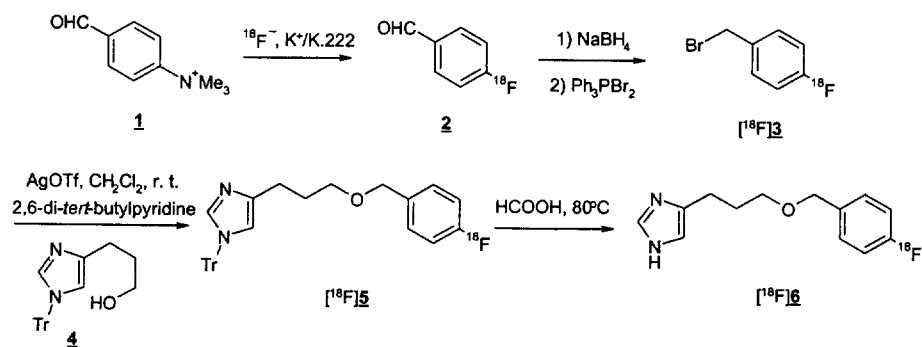
Figure 1. Chemical structures of radioligands for histamine H_3 receptors.

Measuring the distribution and density of histamine H₃ receptors in human brain is essential for characterizing their functions. From this point of view positron emission tomography (PET), in combination with suitable receptor ligands labelled with positron emitters (typically ¹¹C or ¹⁸F), is a well established and powerful tool for the quantification of receptor density in brain as well as in other regions. However, only a few radioligands for PET studies on histamine receptors have so far been developed. Histamine H₁ receptor antagonists, pyrilamine and doxepin, were labeled with ¹¹C (6, 7) and they were both used for clinical PET studies. ¹¹C-Labeled UCL 1829, ¹⁸F-labeled FUB 272, (8) and, more recently, ¹⁸F-labeled VUF 5000 (9) were prepared instead as potential PET ligands for the H₃ receptors (Figure 1).

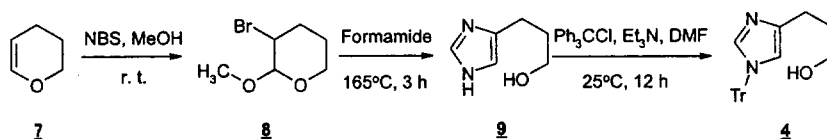
3-(1*H*-Imidazol-4-yl)propyl 4-fluorobenzyl ether (fluoroproxyfan) is an analogue of iodoproxyfan in which the iodine has been replaced by fluorine. It has been reported to possess comparable affinity and selectivity as iodoproxyfan towards histamine H₃ receptors. (10) In this paper we report a method for the radiolabelling of fluoroproxyfan with ¹⁸F.

RESULTS AND DISCUSSION

The strategy for the preparation of [¹⁸F]fluoroproxyfan [¹⁸F]**6** is shown in Scheme 1. It is based on ether bond formation between the precursor 3-(1-triphenylmethyl-1*H*-imidazol-4-yl)propanol **4** and 4-[¹⁸F]fluorobenzyl bromide **3**. The latter is a useful ¹⁸F-labeling intermediate prepared from no-carrier-added [¹⁸F]fluoride and its preparation was recently considerably simplified. (11) The



Scheme 1. Synthesis of [¹⁸F]fluoroproxyfan from [¹⁸F]fluoride.

Scheme 2. Synthesis of precursor **4**.

simplified procedure gave 4- ^{18}F fluorobenzyl bromide [^{18}F]**3** in 2 mL of CH_2Cl_2 within 30 min from the end of bombardment (EOB) with a radiochemical yield of 60% (decay corrected to EOB).

The precursor **4** was synthesized by the procedure shown in Scheme 2. Although the preparation of **4** had already been reported in the literature, (5) a different synthetic route was chosen and the precursor **4** was obtained in an overall yield of 20%.

The Williamson synthesis, the most common route to ethers by reaction of metal alkoxides with alkyl halides, was applied to the etherification of **4** with [^{18}F]**3**. However, for reasons not well understood, the reaction gave poorly reproducible results. In addition, this reaction generally requires dissolving the metal alkoxide in a more polar solvent than CH_2Cl_2 . The latter is the solvent in which [^{18}F]**3** is available at the end of its preparation. Synthesis by this approach thus requires a troublesome and time-consuming solvent exchange procedure to be carried out.

On the other hand, very conveniently, the present etherification with AgOTf and 2,6-di-*tert*-butylpyridine works in CH_2Cl_2 . One more advantage of this reaction is

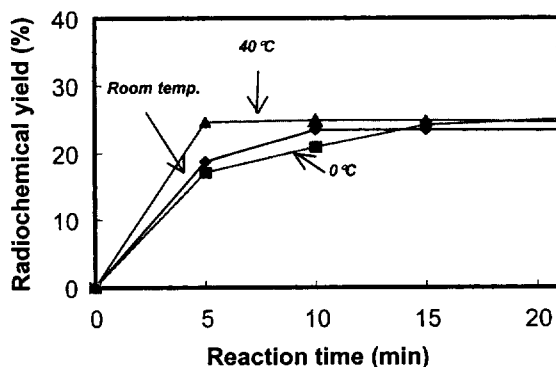


Figure 2. Influence of the temperature on the etherification.

that it rapidly proceeds under mild conditions (Figure 2). It appears that a 40 °C reaction temperature affords only a slightly higher reaction rate compared to room temperature. The convenience of operating at room temperature directed us toward this choice, since the difference in radiochemical yield could be easily compensated for by increasing the reaction time from 5 min to 10 min, an almost negligible time compared to the half-life of ^{18}F (109.8 min).

The purification of [^{18}F]**5**, dissolved in CH_2Cl_2 , was achieved by solid phase extraction using a commercially available silica Sep-Pak cartridge. Radiochemically pure tritylated product [^{18}F]**5** was thus obtained in moderate radiochemical yields of 20–25% (decay corrected to EOB).

A few volatile acids were examined for hydrolysis of [^{18}F]**5** to [^{18}F]**6**. It is apparent from Figure 3 that 50% HCO_2H showed the most efficient and rapid conversion. The hydrolysis was completed within 10 min giving over 98% radiochemical yields.

After evaporating the hydrolyzate to dryness, the desired product [^{18}F]**6** was finally purified by semi-preparative high performance liquid chromatography (HPLC). The radioactive peak eluting at 8–10 min, corresponding to [^{18}F]**6**, was collected, evaporated to dryness under reduced pressure and finally dissolved in saline. The overall synthesis time, including preparation of [^{18}F]**3**, HPLC purification and formulation, was less than 100 min from EOB.

An accurate measurement of [^{18}F]**6** specific activity was made difficult by its

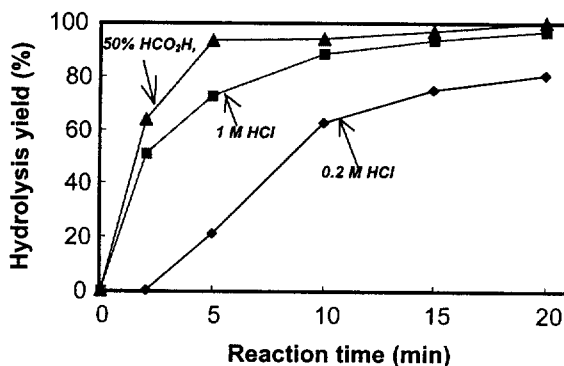


Figure 3. Acid hydrolysis of the tritylated [^{18}F]fluoroproxyfan [^{18}F]**5**.

Reaction temperature: 80°C

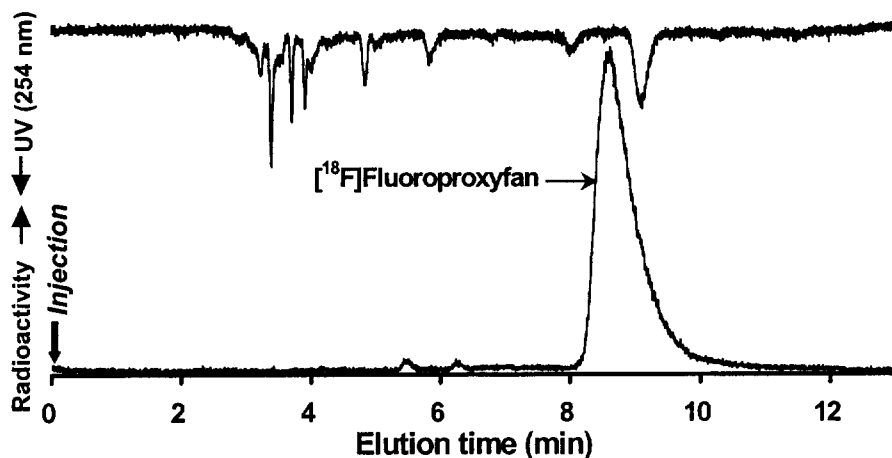


Figure 4. A typical chromatogram for the purification of [^{18}F]fluoroproxyfan by semi-preparative HPLC.

Column: YMC A-324 (ODS 10×300 mm)

Solvent: CH_3CN -0.02 M $\text{CH}_3\text{CO}_2\text{NH}_4$ (35 : 65)

Flow rate: 5.0 mL/min

very low UV absorption. That is clearly shown in Figure 4, where no UV peak can be seen associated with the radioactive signal for [^{18}F]**6**. Accordingly, it was only possible to give a quantification on the basis of the lowest concentration of cold fluoroproxyfan **6** needed for observing a UV response. The specific activity so estimated was higher than 150 GBq/ μmol (4 Ci/ μmol).

In conclusion, radiochemically pure [^{18}F]fluoroproxyfan was prepared in overall radiochemical yields of 6-10% (decay corrected at EOB) with a specific activity of >150 GBq/ μmol within 100 min.

EXPERIMENTAL

[^{18}O]Water (88-97 atom%) was purchased from Isotec Inc. Chemicals and solvents were reagent grade and used without further purification. An authentic sample of fluoroproxyfan was obtained as a generous gift from Prof. W. Schunack and Dr. H. Stark of Freie Universität Berlin.

HPLC analyses were all performed on a LiChrosorb RP-18 column (5 μ m, 4 $\times$ 250 mm, Merck) with a solvent system of CH₃CN-0.05 M CH₃CO₂NH₄ (40 : 60) for the identification of [¹⁸F]fluoroproxyfan (V_R = 11.6 mL), and a solvent system of CH₃CN-0.05 M CH₃CO₂NH₄ (70 : 30) for the identification of [¹⁸F]**5** (V_R = 22.8 mL). Semi-preparative HPLC was carried out with a YMC A-324 column (ODS 10 $\times$ 300 mm) with a solvent system of CH₃CN-0.02 M CH₃CO₂NH₄ (35 : 65). ¹H NMR spectra were recorded on a Varian XL-200 spectrometer.

3-(1-Triphenylmethyl-1*H*-imidazol-4-yl)propanol **4**

3,4-Dihydro-2*H*-pyran **7** (21 g, 0.25 mol) was added dropwise with stirring to a mixture of *N*-bromosuccinimide (NBS, 35.6 g, 0.2 mol) and methanol (100 mL). After filtering the resulting succinimide most of the excess methanol was carefully removed from the filtrate under reduced pressure. Distillation of the residue (85–88°C/12 mm Hg) gave 2-methoxy-3-bromo-tetrahydropyran **8** (25.4 g, 65%). (12)

Compound **8** (16.7 g, 83 mmol) was slowly added to stirred formamide (80 mL, 2 mol) at 165°C under He over 30 min. The mixture was heated with stirring for an additional 3 hr, after which it was cooled and diluted with water (200 mL). The solution was then passed through a Bio-Rad AG 50W-X4 resin (H⁺ form, 0.15 equivalent), and washed with water (200 mL) and methanol (100 mL). The free bases were eluted with methanolic ammonia (0.5 M), evaporated and chromatographed on silica gel (CHCl₃ : *n*-butanol saturated with NH₃; 5:1) to afford **9** as a brownish oil (5.5 g, 55%). (13)

To a stirred solution of compound **9** (3.73 g, 29.6 mmol) in DMF (30 mL) and Et₃N (10 mL) was added Ph₃CCl (8.50 g, 30.5 mmol) dissolved in DMF (110 mL). After 30 min stirring, the reaction mixture was poured over ice and the product was extracted with CH₂Cl₂ (150 mL $\times$ 3), dried over Na₂SO₄, chromatographed on silica gel (hexane : acetone; 85 : 15) and finally recrystallized from ether to give **4** (136 mg, 57%). (14) Melting point = 138–139°C. ¹H-NMR (CD₃Cl, 300 MHz): δ /ppm 1.85 (m, 2H, CH₂–CH₂–O), 2.65 (t, 2H, Im–CH₂), 3.70 (t, 2H, CH₂–O), 6.55 (s, 1H, Im-5-H), 7.00–7.45 (m, 16H, Im-2-H+Ph-H).

3-(1-Triphenylmethyl-1H-imidazol-4-yl)propyl 4-fluorobenzyl ether **5**

A solution of **4** (184 mg, 0.5 mmol), AgOTf (141.3 mg, 0.55 mmol) and 2,6-di-*tert*-butylpyridine (144 mg, 0.75 mmol) in CH₂Cl₂ (2 mL) was cooled to 0°C. 4-Fluorobenzyl bromide **3** (113.4 mg, 0.6 mmol) in CH₂Cl₂ (0.5 mL) was added and the mixture was stirred at room temperature for 1 hr. The reaction mixture was diluted with *n*-hexane and filtered through glass wool to remove a yellow precipitate. The filtrate was washed with water, saturated aqueous NaHCO₃ and brine, dried with Na₂SO₄ and evaporated to dryness. The residue was chromatographed on silica gel (hexane-acetone; 85 : 15) to afford **5** (136 mg, 57%). (15) ¹H-NMR (CD₃Cl, 300 MHz): δ/ppm 1.94 (tt, 2H, ³J_{H-H}=7.6 Hz, CH₂CH₂CH₂O), 2.64 (t, 2H, ³J_{H-H}=7.6 Hz, CH₂CH₂CH₂O), 3.48 (t, 2H, ³J_{H-H}=6.4 Hz, CH₂CH₂CH₂O), 4.41 (s, 2H, OCH₂C₆H₄F), 6.52 (s, 1H, Im-5-H), 6.94-7.35 (m, 19H, Ph-H), 7.37 (s, 1H, Im-2-H).

3-(1H-imidazol-4-yl)propyl 4-fluorobenzylether maleate, (fluoroproxyfan) **6**

Compound **5** (87 mg, 182 μmol) was dissolved in 50% formic acid (10 mL) and the reaction mixture was refluxed at 80°C for 2 hr. The solution was washed with ether and evaporated to dryness under reduced pressure. The residue was dissolved in water and neutralized with K₂CO₃. The product was extracted with CH₃Cl, evaporated and recrystallized as hydrogen maleate from ethanol-ether to give **6** (41 mg, 65%). ¹H-NMR (CD₃Cl, 300 MHz): δ/ppm 2.02 (tt, 2H, ³J_{H-H}=5.9 Hz, ³J_{H-H}=7.5 Hz, CH₂CH₂CH₂O), 2.85 (t, 2H, ³J_{H-H}=7.5 Hz, CH₂CH₂CH₂O), 3.51 (t, 2H, ³J_{H-H}=5.9 Hz, CH₂CH₂CH₂O), 4.46 (s, 2H, Im-CH₂), 6.43 (s, 2H, CH of maleic acid), 6.91 (s, 2H, Im-5-H), 7.03 (dd, 2H, ³J_{H-H}=8.7 Hz, ³J_{H-F}=8.7 Hz, 4-fluorophenyl-3,5-H), 7.29 (dd, 2H, ³J_{H-H}=8.7 Hz, ³J_{H-F}=5.5 Hz, 4-fluorophenyl-2,6-H), 9.01 (s, 1H, Im-2-H), 13.27 (br, 2H, COOH).

Radiosynthesis of [¹⁸F]fluoroproxyfan [¹⁸F]**6**

No-carrier-added [¹⁸F]fluoride was produced by 12 MeV proton irradiation of [¹⁸O]water (0.6 mL) with a Sumitomo Cypris HM12 cyclotron at CYRIC. After recovery of the enriched water by passing through anion exchange resin, the

[^{18}F]fluoride was eluted with 1 mL of aqueous K_2CO_3 solution (33 mM) and used for the preparation of 4-[^{18}F]fluorobenzyl bromide [^{18}F]**3** according to our simplified procedure. (11) In short, 4-[^{18}F]fluorobenzaldehyde [^{18}F]**2** was first synthesized by ^{18}F -substitution on 4-trimethylammoniumbenzaldehyde **1** and isolated from the reaction solution with a Sep-Pak C18 cartridge, where it was then reduced to 4-[^{18}F]fluorobenzyl alcohol by simply passing through an aqueous NaBH_4 solution. 4-[^{18}F]fluorobenzyl alcohol was then eluted from the Sep-Pak with CH_2Cl_2 , dried over MgSO_4 and added to Ph_3PBr_2 . The mixture was stirred at room temperature for 10 min and passed through a Sep-Pak silica, affording 4-[^{18}F]fluorobenzyl bromide [^{18}F]**3** in 50–60% radiochemical yields (decay corrected to EOB) within 30 min from EOB.

To a 2 mL CH_2Cl_2 solution of [^{18}F]**3** was added a mixture of the precursor **4** (8 mg), silver triflate (28 mg) and 2,6-di-*tert*-butylpyridine (15 mg) in 0.5 mL of CH_2Cl_2 . The mixture was stirred at room temperature for 5–15 min and the crude reaction products were then applied on a Sep-Pak silica cartridge. The cartridge was washed with a 10 mL mixture of CH_2Cl_2 and ethyl acetate (9 : 1) and the tritylated [^{18}F]fluoroproxyfan [^{18}F]**5** was then eluted into a flask with a mixture of CH_2Cl_2 and CH_3OH (9 : 1; 3 mL). After evaporating the solvent, 50% HCO_2H was added to the residue and the solution was heated at 80°C for 10 min. [^{18}F]Fluoroproxyfan [^{18}F]**6** was finally purified by semi-preparative HPLC on a reverse phase ODS column (YMC ODS A-324, 10×300 mm) with a solvent system of CH_3CN –0.02 M $\text{CH}_3\text{CO}_2\text{NH}_4$ (35 : 65) at a flow rate of 5 mL/min.

Acknowledgments—The authors wish to thank Dr. F. Brady (MRC Cyclotron Unit, Hammersmith Hospital, London) for helpful and critical discussion. Prof. W. Schunack and Dr. H. Stark (Freie Universität, Berlin) are greatly acknowledged for providing the authentic sample of fluoroproxyfan. This study was supported by a Grant-in-Aid (No. 09670913) for Scientific Research from the Ministry of Education, Science and Culture, Japan. The Takeda Science Foundation is also appreciated for financial support.

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