JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 799–804.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.720

Research Article

Regioselective F-18 radiolabeling of AM694, a CB_1 Cannabinoid Receptor Ligand

Peter G. Willis, Reeti Katoch-Rouse and Andrew G. Horti*
Neuroimaging Research Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224, USA

Summary

[^{18}F]AM694, [1-(5-[^{18}F]fluoropentyl)-1H-indol-3-yl]-(2-iodophenyl)methanone, **1b**, a potential radiotracer for imaging of cerebral cannabinoid receptor (CB_1), has been synthesized by no-carrier-added regioselective radiofluorination of the corresponding tosylate. [^{18}F]AM694 was obtained in 20% radiochemical yield (non-decay-corrected) with a specific activity of 14 500 mCi/μmol, a radiochemical purity of >99%, and a chemical purity of 95.5%. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: positron emission tomography; radiotracer; cannabinoid; ^{18}F; regioselective fluorination; AM694

Introduction

The cerebral cannabinoid receptor (CB_1) belongs to a superfamily of G-protein coupled receptors.[1] The human CB_1 was first identified 20 years ago.[2] Cannabinoid receptor ligands have been shown to possess beneficial effects in the attenuation of pain and nausea,[3] the tremors associated with Parkinsons disease,[4] and the relief of eye pressure from glaucoma.[5] The cannabinoid agonist, delta-9-tetrahydrocannabinol, the

*Correspondence to: A. Horti, Neuroimaging Research Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224, USA. E-mail: ahorti@intra.nida.nih.gov

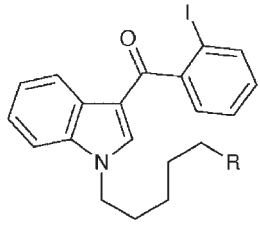
Received 30 January 2003
Revised 19 March 2003
Accepted 27 March 2003

active component of marijuana, is known to induce the 'high' associated with smoking marijuana, and CB_1 antagonists, such as SR141716, might be of interest as a potential medication for the treatment of marijuana dependence.[6] Non-invasive *in vivo* imaging of cerebral cannabinoid receptors with positron emission tomography should allow for the analysis of the biological role played by the receptors in natural and disease states. The current literature has not revealed any suitable radiotracers for the imaging of CB_1 receptors in human subjects. This absence hinders progress in the study of the CB_1 receptor.

The target molecule [1-(5-[^{18}F]fluoropentyl)-1H-indol-3-yl]-(2-iodophenyl)methanone ([^{18}F]AM694) (Figure 1, **1a**) was chosen for synthesis as a potential PET radiotracer because the unlabeled compound **1b** (AM694) displays desirably high binding affinity at the CB_1 receptor (K_i = 0.08 nM).[7]

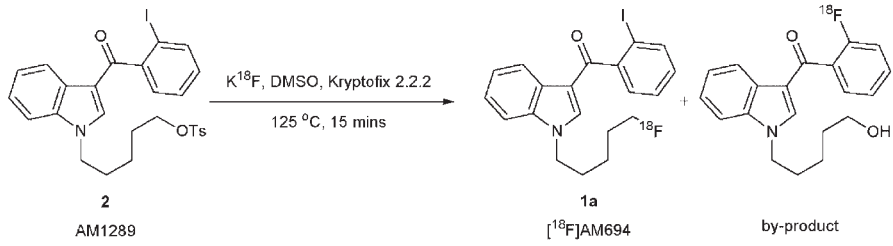
Results and discussion

[^{18}F]AM694 **1a** was prepared by the no-carrier-added nucleophilic substitution reaction of [^{18}F]fluoride with [1-(5-tosylpentyl)-1H-indol-3-yl]-(2-iodophenyl)methanone (AM1289) **2** as shown in Scheme 1. Radiofluorination precursor **2** has two electrophilic reaction sites. Both the aromatic iodo group with the activating *ortho*-carbonyl functional group and the aliphatic tosylate are potentially good leaving groups, therefore, they are susceptible to nucleophilic substitution. Yields of a nucleophilic fluorination with no-carrier-added (n.c.a.) [^{18}F]fluoride on



[^{18}F]AM694, **1a**: R = [^{18}F]
AM694, **1b**: R = F
AM1289, **2**: R = OTs

Figure 1.



Scheme 1.

a primary tosylate tend to be high under relatively mild conditions. Keeping this in mind, it is expected that a nucleophilic substitution of the tosylate can be achieved in good yield at a relatively low temperature (about 100°C)[8,9] as compared to the optimal temperature required (about 180°C) for the nucleophilic substitution of an activated aryl-iodide.[10] In the past, large alkali metal cations (Cs^+ or Rb^+)[11] were used to improve the solubility of fluoride ion in organic solvents such as DMSO or acetonitrile for the nucleophilic radiofluorination reaction, and thereby increase the yield. More recently, the tetrabutylammonium cation or phase transfer catalyst Kryptofix 2.2.2 have gained popularity as phase transfer catalysts for nucleophilic radiofluorination.[12] Therefore, we chose to radiofluorinate the precursor **2** at 125°C in DMSO in the presence of Kryptofix 2.2.2.

As expected, the reaction of tosylate **2** with [^{18}F]fluoride under the above mentioned conditions provided the radiolabeled **1a** as the major product. A small amount of the hypothesized radiolabeled by-product, shown in Scheme 1, was also found in the reaction mixture by HPLC analysis. The structure of the by-product has not been elucidated. However, the low retention time of the by-product on the reverse-phase HPLC compared to the desired product ($k' = 7.6$ versus 10.1, respectively) suggests the less lipophilic nature of the by-product with a possible structure of [1-(5-hydroxypentyl)-1H-indol-3-yl]-[^{18}F](2-fluorophenyl)methanone. The radiolabeled product **1a** was separated from the reaction mixture by a semi-preparative HPLC with overall radiochemical yield of 20% and specific radioactivity of 14 500 mCi/μmol when the reaction was done with more than 450 mCi of the radiofluoride. The conditions were optimized to achieve high and reproducible specific activity and radiochemical yield and are presented in the following experimental section. The average synthesis time for this radiofluorination was 76 min.

Conclusion

In summary, a simple one-step radiosynthesis of a high specific activity radioligand for studying the cerebral cannabinoid receptors by PET has been developed. This nucleophilic radiofluorination procedure may prove useful for the preparation of various derivatives of **1** as potential radiotracers.

Experimental

Materials and methods

All reagents were ACS or HPLC grade and were purchased from Aldrich. HPLC analyses and purification were performed with two Waters 600/610 HPLC pumps, an inline UV-detector (Waters, 254 nm), and a flow-count radioactivity detector (Bioscan 3200). HPLC chromatograms were recorded on a Rainin Dynamax dual channel control/interface module connected to a Macintosh computer with Dynamax v.1.4.2 software. A dose calibrator (Capintec CRC-35R) was used for all radioactivity measurements. [^{18}F]Fluoride was prepared using an RDS111 cyclotron (CTI, Knoxville, TN). The radiofluorination was performed with the CPCU (CTI, Knoxville, TN) automated radiochemistry module. All precursors and standards were prepared in our laboratory following previously published procedures.[7] Non-decay-corrected radiochemical yields were calculated using a comparison of radioactivity measurements taken at end-of-bombardment and end-of-synthesis.

Radiochemistry

[1-*(5-[^{18}F]fluoropentyl)-1H-indol-3-yl]-(2-iodophenyl)methanone (***1b***)*.
[1-(5-Tosylpentyl)-1H-indol-3-yl]-(2-iodophenyl)methanone (AM1289)[1,†] **2** (2.6 mg, 4.4 μmol) was dissolved in anhydrous DMSO (0.9 ml) and transferred to a reaction vessel containing K[^{18}F]F/Kryptofix 2.2.2/K_2CO_3 complex (465 mCi) prepared by the Hamacher method[12] with

† Precursor **2** (compound AMI1289) was prepared by a short synthesis described in Reference [1]. Indole is reacted with 2-iodobenzoyl chloride in the presence of CH_3MgBr. This product is then *N*-alkylated with 5-bromopentyl acetate in the presence of sodium hydride. The *N*-pentylacetate derivative was deprotected with KOH/methanol followed by a reaction with tosyl chloride.

45 mg Kryptofix®222 and 3.0 mg K_2CO_3. The reaction mixture was heated at 125°C for 15 min, cooled, diluted with 0.5 ml water, and injected onto the Waters PrepPak NOVA C18 cartridge ($25 \times 100\,mm^2$). The reaction mixture was eluted with a mixture of acetonitrile : 0.1 M ammonium formate buffer (50 : 50) at a flow rate of 7 ml/min. The radioactive peak (95.3 mCi) was collected with a retention time of 18.1 min ($k' = 10.14$), corresponding to the AM694 standard.

The precursor's retention time was significantly longer than that of AM694, so it was not collected. The radioactive peak of the by-product was also collected with a retention time of 13.7 min ($k' = 7.45$). This peak was measured at 0.3% of the total radiation measured at end-of-bombardment. There were three more substantial unidentified non-radioactive peaks in the reaction mixture with the retention times of 4.5, 5.0 and 5.6 min. The average radiosynthesis time was 76 min.

An aliquot of the final solution of known volume and radioactivity determined at end-of-synthesis was applied to an analytical HPLC column (Waters Symmetry C18 column, $4.6 \times 250\,mm^2$). A mobile phase of acetonitrile: 0.1 M ammonium formate buffer (60:40) at a flow rate of 2.5 ml/min was used to elute the radioligand, which had a retention time of 7.6 min. The area of the UV absorbance peak measured at 254 nm corresponding to the carrier product was measured and compared to the authentic unlabeled standard for specific radioactivity calculation. The radiochemical product coeluted with a sample of authentic AM694.

Acknowledgements

The authors thank Dr Elliot Stein for helpful discussion and Ms Mary Pfeiffer for assistance with manuscript preparation.

References

1. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TH. *Nature* 1990; **346**: 561.
2. Gerard CM, Mollereau C, Vassart G, Parmentier M. *Biochem J* 1991; **279**:129.
3. Walker JM, Hohmann AG, Martin WJ, Strangman NM, Huang SM, Tsou K. *Life Sciences* 1999; **65**: 665–673.

4. Sieradzan KA, Fox SH, Hill M, Dick JPR, Crossman AR, Brotchie JM. *Neurology* 2001; **57**: 2108–2111.
5. Barth F. *Exp Opin Ther Patents* 1998; **8**: 301–313.
6. Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET, Frank RA. *Arch Gen Psychiatry* 2001; **58**: 322–328.
7. Makriyannis A, Deng H. *PCT International Publication* 2001; WO 01/28557.
8. Holschbach MH, Olsson RA, Bier D, Wutz W, Sihver W, Schüller M, Palm B, and Coenen HH. *J Med Chem* 2002; **45**: 5150 –5156.
9. Yamamoto F, Aoki M, Furusawa Y, Ando K, Kuwabara Y, Masuda K, Sasaki S, Maeda M. *Biol Pharmaceut Bull* 2002; **25**: 616–621.
10. Berridge MS, Crouzel C, Comar D. *J Label Compd Radiopharm* 1984; **22**: 687–691.
11. Alauddin MM, Conti PS, Mathew T, Fissekis JD, Prakash GS, Watanabe KA. *J Fluorine Chem* 2000; **106**: 87–91.
12. Hamacher K, Coenen HH, Stocklin G. *J Nuc Med* 1986; **27**: 235–238.

