Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVII, No. 9

SYNTHESIS AND LABELLING WITH ^{18}F OF AN MK 801 ANALOGUE : [^{18}F]5-(β-FLUOROETHYL)-10,11-DIHYDRO-5H-DIBENZOCYCLOHEPTENE-5,10-IMINE

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ABSTRACT

The 5-(β-fluoro)ethyl analogue 1 of MK 801 2 was synthesized and labelled with ^{18}F in order to visualize the NMDA receptors by positron emission tomography. A tosyloxy precursor 3 was synthesized in 8 steps from dibromodibenzosuberone; the nucleophilic substitution of the tosyl group of 3 by the $K^{18}F$/Krytofix 2,2,2 complex in CH_3CN gave [^{18}F] 1 in 25% radiochemical yield with a specific activity of 40 GBq/μmol (1 Ci/μmol).

The major excitatory amino acids in mammalian brain CNS, L.glutamate and L. aspartate, have a neurotoxic activity mediated through excitatory synaptic receptors such as N-Methyl-D-Aspartate (NMDA) receptor subtype. These excitotoxic amino acids have been implicated in several brain damages associated with epilepsy, anoxia-ischemia and hypoglycemia (1). MK 801 [(+)5-methyl-10,11-dihydro-5H-dibenzo-cycloheptene-5,10-imine] 2 is a potent and non-competitive antagonist of NMDA (2). MK 801 was shown, in animal models, to protect against hypoxic-ischemic damage and NMDA neurotoxicity

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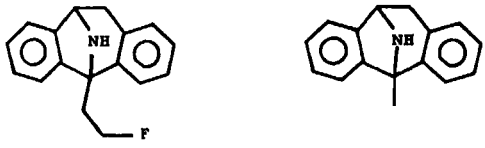
0362–4803/89/091007–07$05.00

Received November 28, 1988
Revised March 8, 1989

(3,4). These results may have important implications for the treatment in man of stroke and neurodegenerative disorders. In order to visualize the NMDA receptors by positron emission tomography, the synthesis and the labelling with fluorine 18 (β^+, $t_{1/2}$= 110 min) of the 5-(β-fluoro)ethyl 1 analogue of MK 801 2 was undertaken (Scheme I).

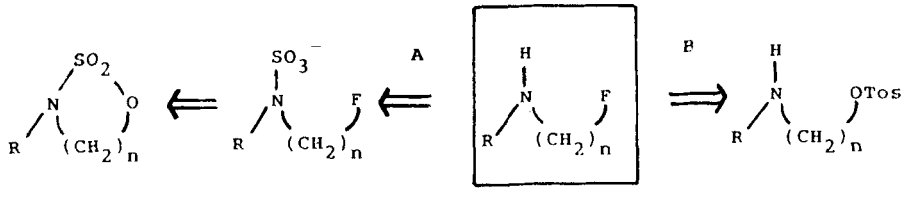
SCHEME I : 5-β-FLUOROETHYL ANALOG AND MK 801 STRUCTURES



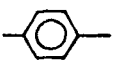
1 2 MK 801

Nucleophilic displacement of a good leaving group such as a tosylate (5) or a triflate (6) is the most efficient method to incorporate ^{18}F in organic compounds with high specific activity. In this area, a new reaction, the opening of cyclic sulfamates by fluoride (Scheme 2, path A), has been recently reported (7) and applied to the synthesis of [^{18}F] 1 (8). We have developed a different approach by using as reactive ester, a tosylate rather than a cyclic sulfamate. Indeed, the tosyl group can be introduced to the hydroxy function of an amino-alcohol without protection of the nitrogen. Therefore labelled fluoro-amines such as 1 could be synthesized in a single step, without hydrolysis of a sulfamate group (Scheme 2, path B).

SCHEME 2 : USE OF TOSYLATE OR CYCLIC SULFAMATE FOR FLUORO-AMINES SYNTHESIS

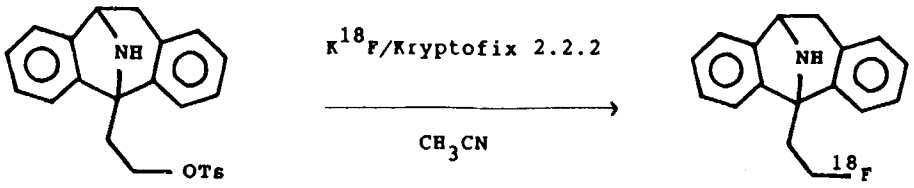


2 STEPS 1STEP

Tos = SO_2 

Here we report the procedures for the synthesis of the 5-β tosyloxyethyl derivative 3 and the radiofluorination of 3 by $K^{18}F$ in the presence of the aminopolyether Kryptofix 2,2,2 (Scheme 3).

SCHEME 3 : LABELLING OF 3 BY $K^{18}F$



3 1

MATERIAL AND METHODS

Fluorine 18 Production and resolubilization of $18F^-$

Fluorine-18 has been produced by irradiation of natural water with helium-3, 30 MeV [^{16}O (^3He, p)^{18}F] or enriched water (50% ^{18}O) with 16 MeV protons [^{18}O (p, n) ^{18}F].

Natural or enriched water was irradiated in a target similar to that described by Kilbourn (9) (Front foil : titanium or Havar foil 12 µm, rear foil : titanium 100 µm, target volume 1 ml). The target was irradiated for one hour with the 520 CGR-MeV cyclotron, with beam intensities ranging from 10 to 20 µA.

Enriched water (50%) was purchased from the ORIS-CEA Company (ref. ISO 18-2-50). Natural water was obtained from a millipore purification system (Milli Q system, water at 18 megohm x cm).

After irradiation, the water was evaporated at 120°C under a flow of nitrogen in the presence of potassium carbonate (2 mg) and kryptofix 2.2.2 (14.5 mg) in a siliconized tube (Vacutainer ^{R}, Becton Dickinson, no additive, 5ml volume).

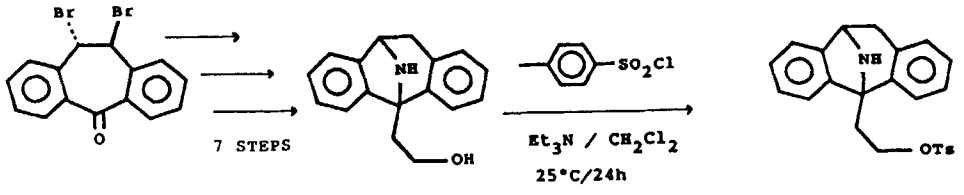
The "non carrier added" $K^{18}F$/Kryptofix 2,2,2 complex formed was recovered by addition of 0,5 ml of dry CH_3CN (previously distilled on to phosphorus pentoxide) and was then tranferred in a fitted Pyrex reactor.

Synthesis and purification

a) Synthesis of the tosyl precursor 3

The synthesis of 3 started from the commercialy available dibromodibenzosuberone (Aldrich) which was converted in 7 steps on the amino alcohol 4 (10). Treatment of 4 with p-toluenesulfonyl chloride and triethylamine in dry CH_2Cl_2 gave the tosyloxy precursor 3 in good yield (scheme 4).

SCHEME 4 : SYNTHESIS OF 3 STARTING FROM DIBROMODIBENZOSUBERONE



DIBROMO DIBENZOSUBERONE

4

3

b) Synthesis of 18F 1

The substitution reaction of the tosyl group by $^{18}F^-$ took place in a small Pyrex vial in the presence of 3 and a few mCi of the $K^{18}F$/Kryptofix 2,2,2 complex in CH_3CN . After 10 min at 90°C and purification on a silica Sep-Pak cartridge followed by two HPLC, pure [^{18}F] 1 was obtained with a 25% yield 70 min after the end of bombardment. The effect of the temperature on the yield of the reaction was also examined : the maximum yields were obtained for temperatures between 80-95°C and fell to 0-5% for temperatures above 100°C.

Starting with 6 GBq (150 mCi) of ^{18}F from enriched water, it was possible to obtain 0,8 GBq (20 mCi) of [^{18}F] 1 with a specific activity of 40 GBq/µmol (1 mCi/µmol) at the end of the synthesis. The specific activity of [^{18}F] 1 was determined from the last HPLC chromatogram (before injection of the radioactive product a standard curve was made with 20, 30, 40 nmol of cold 1).

The chemical structure of 1 has been determined chemically and radiochemically as follow :

- First, a synthesis using cold KF and Kryptofix 2,2,2 afforded 1 which was characterized physically by mass spectrometry and ^{1}H NMR (see experimental part).

- A synthesis using $^{18}F^{-}$ and cold fluoride as carrier gave a product with the same HPLC retention time as 1. The labelled product was examined after decay by mass spectrometry (chemical ionization mode) and gave the molecular ion m/e=254 (M+1) expected for the stable fluoro compound.

EXPERIMENTAL SECTION

1H NMR spectra were recorded on a Varian 80 MHz and Brucker 250 MHz spectrometers in CDCl3 with TMS as internal standard.

Infrared spectra were recorded on a Perkin-Elmer 257 spectrophotometer.

Melting points were obtained on an Reichert apparatus and are uncorrected.

5-[β-(4-methylbenzene-sulfonyl))ethyl]-10,11-dihydro-5H-dibenzo-cycloheptene-5,10-imine : 3

To a solution of 4 (0,25 g, 1 mmol) in 15 ml of dry CH_2Cl_2 and 0,2 ml of Et_3N was added 0,2g of p-toluene-sulfonyl chloride (1 mmol), and the solution was stirred 12h at room temperature under nitrogen. The solvent was removed under reduced pressure and the crude product was purified by chromatography (silicagel 230-400 mesh, CH_2Cl_2 98/EtOH 1,88/$EtNH_2$ 0,08/H_2O 0,04). Evaporation of the solvents gave 0,24g of 3. Yield= 60%. mpt = 124°C

IR (KBr) : 3250, 3000-2900, 1600, 1450, 1350, 1190 cm-1.

^{1}H NMR : δ (ppm) 2,4 (s, 1H exchanged by D_2O), 2,6 (s+m, 5H), 3,1-3,5 (m, 2H), 3,75-4,5 (m, 2H), 5,25 (d, 1H), 6,8-7,9 (m, 12H).

m/e : 406 (M+1), 234.

[18F] 5-[β-fluoroethyl]-10,11-dihydro-5H-dibenzo-cycloheptene-5,10-imine : 1

To a solution of 5 mg (1,2 µmol) of 3 in 0,3 ml of CH_3CN in a Pyrex vial was added 6 GBq (150 mCi) of n.c.a $K^{18}F$/Kryptofix 2,2,2 in 0,5 ml OF

CH_3CN ; the reactor is then fitted with a septum plug and heated at 90°C . After 10 min the reaction was diluted with CH_2Cl_2 (5 ml) and passed through a Sep-Pak silica cartridge previously activated by CH_2Cl_2. The radioactivity was eluted with 10 ml of a 9/1 CH_2Cl_2/MeOH solution. After evaporation of the solvent, the radioactivity was taken up in 1 ml of a CH_2Cl_2 + 3% B mixture (B = EtOH 98/$EtNH_2$ 4/H_2O 2) and was injected onto an HPLC columin (Lichrosorb Si 60 7µ, 250 x 9 mm), solvent CH_2Cl_2 + 0,5% B, flow rate 4 ml/min. The radioactivity as well as the U.V absorbtion at 254 nm (Waters Model 440) of the eluent was monitored. The radioactive peak with a retention time of about 14 min was collected ; the product was radiochemically pure but chemically impure ; the unknow chemical impurity was separated from [^{18}F] 1 by a second HPLC purification on an identical column with a $CHCl_3$ 920/MeOH 78/H_2O 2 mixture as solvent and a flow rate of 3 ml/min. The radioactive peak was collected at a retention time of 6 min. Evaporation of the solvent gave 0,8 GBq (20 mCi) of pure [^{18}F] 1 with a radiochemical yield (decay corrected) of 25%, 70 min after end of bombardment.

mpt = 105°C

IR (KBr) : 3200, 3000-2900, 1600 cm^{-1}.

^{1}H NMR (250 MHz) : δ (ppm) 2,6 (s, 1H, NH), 2,71 (d, 1H benzylic, J = 17 Hz), 2,74 (octuplet, 1H , $\underline{CH_2}CH_2F$), 2,93 (octuplet, 1H, $\underline{CH_2}CH_2F$) 3,42 (dd, 1H benzylic, J = 17 Hz - J' = 7 Hz), 4,72 (d, 1H bridgehead, J = 7 Hz), 4,76 (m, 2H, CH_2F), 6,9-7,25 (m, 8H aromatic).

m/e : 254 (M+1).

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