SYNTHESIS OF N^1-3-[^{18}F]FLUOROPROPYL-N^4-2-([3,4-DICHLOROPHENYL]ETHYL)PIPERAZINE, A HIGH AFFINITY LIGAND FOR SIGMA RECEPTOR

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SUMMARY

The radiochemical synthesis of the high affinity, selective sigma receptor ligand, N^1-3-[^{18}F]Fluoropropyl-N^4-2-([3,4-dichlorophenyl]ethyl)piperazine, is reported. The labeled compound is prepared by fluoride displacement on a bis-methanesulfonate salt of the propyl methanesulfonyloxy precursor. The product is isolated by first passage through a BONDELUT-SI and subsequently HPLC in high radiochemical and chemical purity with a decay corrected radiochemical yield of 49 ± 9 %.

Keywords: Sigma receptor, sigma ligand, [^{18}F]fluoride, N^1-3-[^{18}F]Fluoropropyl-N^4-2-([3,4-dichlorophenyl]ethyl)piperazine

INTRODUCTION

Sigma receptors are non-opioid, non-dopaminergic binding sites which have been implicated in psychoses, neurodegeneration, and the physiological aspects of motor disorders such as tardive dyskinesia and dystonia. The biology and function of the sigma receptors was the subject of a recent review.[1] Sigma receptors are observed to be most abundant in the cerebellum, hippocampus, cranial nerve nuclei, and red nucleus. The motor side effects of haloperidol and related neuroleptics, which bind with high affinity, may be mediated by sigma receptors.

N^1-3-Fluoropropyl-N^4-2-([3,4-dichlorophenyl]ethyl)piperazine (BD 1304) is a high affinity and specific ligand for the sigma receptor.[2] BD 1304 exhibits high affinity (K_i 0.39 nM) for the sigma receptor labelled with [^{3}H]pentazocine. At the same time it displays K_i values greater than 10,000 nM for PCP, D_2-dopamine , κ-opiate, and muscarinic binding sites. As an indication of the binding potential of this ligand, the Purkinje layer of the cerebellum exhibits [^{3}H] pentazocine binding to the extent of 515 fmol/mg tissue while the binding in the frontal cortex is 152 fmol/mg tissue.[1]

The high selectivity of BD 1304 identifies it as a candidate for further studies in positron emission tomography (PET). The Johns Hopkins Medical Institutions and collaborators have contributed studies of two sigma receptor ligands to the PET radiopharmaceutical

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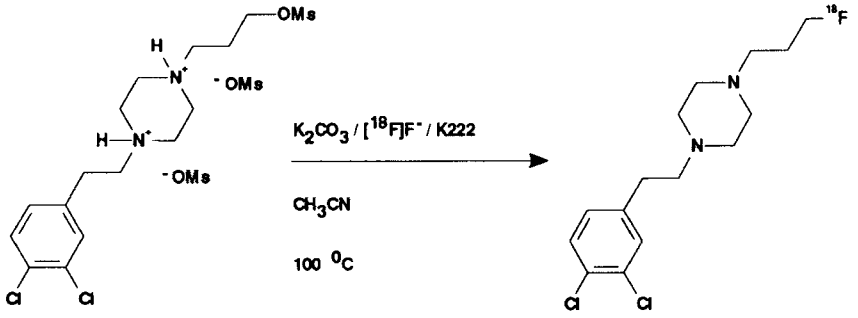
literature. The first, reported as a radiochemical synthesis, was 1-(4-[^{11}C]-methoxyphenyl)-3-(1-adamantyl) guanidine.[3] The second, 1'-[^{11}C] benzylspiro-[1,2,3,4,tetrahydronaphthylene-1,4']piperidine ([^{11}C]L-687,384[4], was reported to exhibit suitable in vivo properties for imaging.

We have prepared [^{18}F]BD 1304 in order to evaluate its potential utility as a radiopharmaceutical for *in vivo* studies of sigma receptors using PET.[5]

RESULTS AND DISCUSSION

Radiochemistry. The radiochemical synthesis was accomplished by nucleophilic displacement by fluoride on an aliphatic methanesulfonate (Scheme 1). The [^{18}F]fluoride was obtained as an aqueous solution in dilute K_2CO_3. The water was evaporated, acetonitrile and substrate added, and the reactions heated in a hot block at approximately 100 °C for five minutes.



SCHEME 1.

Since attempts to store the methanesulfonate ester as the free base resulted in rapid decomposition, the substrate was prepared and stored as its bis-methanesulfonate salt. Consequently, the displacement was attempted utilizing the bis-methanesulfonate salt as the precursor. The displacement was studied using both Bu_4NOH and K_2CO_3/Kryptofix 2.2.2. Both the salt and freshly prepared free base were tested as substrates (Table 1).

The reaction proceeded better using Kryptofix/K_2CO_3 primarily due to increased solubility of fluoride, as indicated by the column 'Soluble ^{18}F', in the reaction medium. The salt provided the same level of incorporation as freshly prepared free base.

Eleven syntheses were performed utilizing the same molar ratio of substrate:base:Kryptofix (1:3:3). The reactions were conducted in an identical fashion. The product was collected on and subsequently eluted from a BONDELUT-SI prior to final purification and collection from the HPLC column. The quantity of substrate ranged from 3.6 to 0.9 μmol; a slight decrease in yield was noted with 0.9 μmol of substrate. The eleven reactions had a average isolated radiochemical yield (corrected for decay) of 49(9) % (1 SD).

Purification. The chemical and radiochemical purity were determined by analytical HPLC following concentration and desalting of the eluate. Because of the high specific activity of the product, concentration of the semiprepartive eluate is required in order order to detect the mass peak upon reinjection onto an analytical column. In the initial

Table 1. Incorporation results. Bu_4NOH vs. K_2CO_3/Kryptofix 2.2.2.[a]

Substrate (μmol)	Base	Base (μmol)	Soluble[b] ^{18}F %	TLC[c] %	RCY[d] %
I. Substrate - - Free base					
3.4	Bu_4NOH	3.4	45	73	32
3.4	K_2CO_3 K222	3.4 6.8	69	88	61
II. Substrate - - Bis-mesylate salt					
3.7	Bu_4NOH	10.7	65	80	52
3.6	K_2CO_3 K222	10.4 20.0	82	87	71

[a] All reactions were run on the same day using the same batch of ^{18}F. Each yield is the result of only one reaction.
[b] Soluble ^{18}F was determined at the end of heating by transfer of the reaction solution.
[c] TLC (thin layer radiochromatography) indicated the percent of the soluble radioactivity that migrated with the desired product.
[d] RCY (Radiochemical yield) is calculated as the product of Soluble ^{18}F and TLC.

runs, reinjection onto an analytical HPLC system revealed a chemical impurity. A reduction in the elution strength of the semipreparative eluant by reducing the proportion of acetonitrile from 60% to 50% allowed the separation and removal of this impurity. The final two runs at the 1.7 μmol substrate level exhibited high chemical purity. Sample chromatograms are presented in Figure 1.

CONCLUSIONS

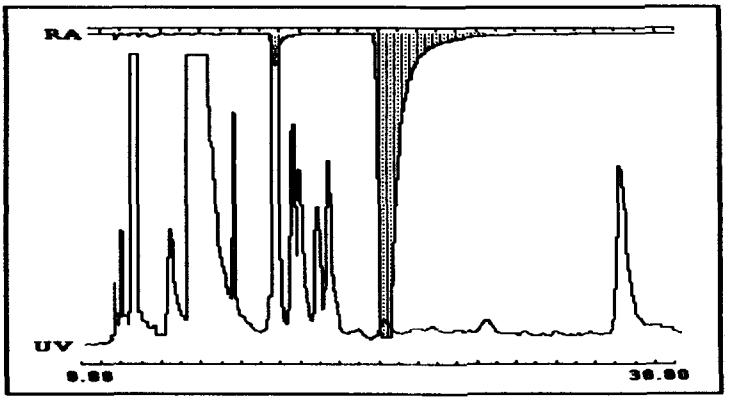
We have prepared fluorine-18 labeled BD 1304 in 49 ± 9 % radiochemical yield (corrected for decay) following HPLC purification. The synthesis is composed of three major stages. Stage 1 is the separation of $[^{18}F]$ from the $[^{18}O]H_2O$ via simple ion exchange resin[6] which is completed within 20-30 minutes of EOB. Stage 2 is the reaction and purification of the product which requires approximately 60 min. The third stage is the formulation. The HPLC purification provided a product with high radiochemical(>99%) purity. The specific activity for the product, determined in two separate preparations was 4.0 and 3.7 Ci/μmol (EOB). Thus, starting from 5.8 mCi of $[^{18}F]$fluoride, 1.8 mCi {31%; (45% corrected)} of product was collected from the HPLC column in approximately 60 min.

EXPERIMENTAL

The bis methanesulfonate salt of the N^1-[3-[(methanesulfonyl)oxy]-1-propyl]-N^4-[2-(3,4-dichorophenyl)ethyl]piperazine was prepared as previously described.[2] The free base was obtained by partitioning the salt between excess aqueous ammonia and methylene chloride. ^{18}F-fluoride was prepared in a $[^{18}O]H_2O$ liquid target on the Japanese Steel Works BC-1710 cyclotron at the National Institutes of Health using the $^{18}O(p,n)^{18}F$ nuclear reaction.

TLC was performed on silica gel with $CHCl_3$:MeOH:NH_4OH (90:9:1) as eluant. HPLC was performed utilizing a Perkin-Elmer Series 4 pump, a Perkin-Elmer Model 735 diode

Semipreparative HPLC Beckman ODS (9.4 x 250 mm)
50 % ACN / 50 % Buffer
5 mL / min



Analytical HPLC Beckman ODS (4.6 x 250 mm)
80 % ACN / 20 % Buffer
1 mL / min

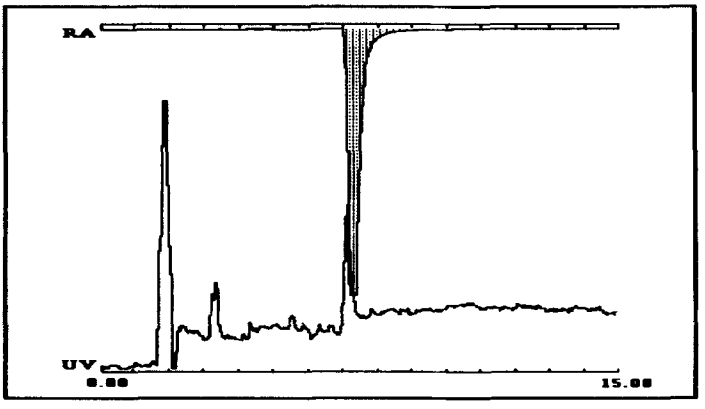


Figure 1. The large shaded peak on the RA (radioactive) trace is the product.

array ultraviolet detector, and a Beckman Model 170 radioactive detector. Analytical HPLC used a Beckman ODS (4.6 x 250 mm) column and an eluant of 80% CH_3CN and 20% buffer (5 mM Na_2PO_4, 5 mM Et_3N, pH about 8.0 and not adjusted) with a flow rate of 1 mL/min. Semipreparative HPLC employed a Beckman ODS (9.4 x 250 mm) column and an eluant of 50% CH_3CN and 50% buffer (same as above) with a flow rate of 5 mL/min.

Preparation of N^1-3-[^{18}F]fluoropropyl-N^4-2-([3,4-dichlorophenyl]ethyl)piperazine. Aqueous [^{18}F]fluoride is processed through cation and anion exchange resins and obtained in 0.5 mL of 0.05 mM K_2CO_3 solution.[6] This procedure requires 20 to 30 minutes from EOB.

A test tube is charged with the required quantity of 0.1 M K_2CO_3 solution and the required amount of Kryptofix 2.2.2 as a solution in CH_3CN. A small aliquot (10 - 30 µL) of this solution is added. The solution is evaporated under a stream of nitrogen while heating in a hot block at 95 to 100 °C. After evaporation, three portions of 100 µL of CH_3CN were added and each in turn evaporated. Finally, 200 µL of CH_3CN was added followed by the solid bis methanesulfonate precursor. The solution was heated in the hot block at 95-100 °C for five minutes, cooled, and transferred onto a 1 mL BONDELUT-SI column. The product eluted with 5 mL of $CHCl_3$: MeOH: NH_4OH (100:1:0.1). The eluate was evaporated under a stream of nitrogen. The residue was taken up in 200 µL of semipreparative HPLC eluant and injected onto the semipreparative column. The major radioactive peak eluting at approximately 15 minutes was collected in about 5 mL total volume. The isolated yields were determined from the quantity collected. The time required from the beginning of synthesis to this point was approximately 60 min.

Radiochemical Purity and Specific Activity. In order to determine specific activity and chemical purity, concentration of the sample was required. The HPLC fraction was concentrated under a nitrogen stream to approximately 2 mL. The remaining volume was loaded onto a 1 mL BONDELUT-C18 and the liquid pushed through with air. The column was washed with 1 mL water and then 1 mL hexane . Finally the product was eluted with 1 mL ethanol:CH_2Cl_2 (1:9). The organic phase was concentrated to dryness. For analysis the residue was taken up in the analytical HPLC eluant and a portion representing 300 µCi or more was injected onto the analytical column. The product elutes between 7 and 8 minutes. Only one radioactive peak was detected. Only the mass peak for the product eluted with the radioactivity. A standard mass curve for the product was used to estimate the specific activity of the product.

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