Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVIII, No. 10

Reductive Amination of [^{18}F]Fluorobenzaldehydes: Radiosyntheses of [2-^{18}F]- and [4-^{18}F]Fluorodexetimides

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Summary

Two [^{18}F]-labelled analogues of dexetimide, a potent muscarinic cholinergic receptor ligand, have been synthesised in good yields and at high specific activities. Reductive amination of [2-^{18}F]- and [4-^{18}F]fluorobenzaldehyde with norbenzyldexetimide and sodium cyanoborohydride gave, in a one-pot reaction, good (≈20%) yields of high specific activity [2-^{18}F]- and [4-^{18}F]fluorodexetimide respectively. The labelled benzaldehydes may be useful synthetic precursors for the radiosyntheses of other complex radiotracers for use with positron emission tomography.

Key Words: fluorine-18, dexetimide, muscarinic cholinergic receptor, positron emitting radiotracer, PET, reductive amination

Introduction

Increasing evidence of the link between defects in the central cholinergic neuronal system and many forms of dementia, including Alzheimer's, Huntington's chorea, and types of Parkinson's disease, has accumulated from several sources (1-6). This has spurred interest in imaging central muscarinic cholinergic receptors (m-AChR) using positron emission tomography (PET) and single photon emission tomography (SPECT) (7), encouraging the development of new positron emitting

0362–4803/90/101189–11$05.50

Received April 23, 1990

and single photon emitting radiotracers. IodoQNB (5,8) and 4-iododexetimide (9,10), labelled with ^{123}I, have been used successfully to image m-AChR in man with SPECT and a variety of [^{11}C]-labelled compounds have been prepared as potential central muscarinic radiotracers including dexetimide (11), QNB (12), and N-methyl-scopolamine (13,14).

As a radiotracer for imaging cerebral m-AChR, [^{11}C]dexetimide enjoys many benefits including facile brain penetration, high affinity and specificity for the m-AChR, low non-specific binding, and a eudismic ratio of over a thousand (15-17). Germane to the present work, halogenated analogues of dexetimide, as exemplified by [4-^{123}I]iododexetimide (9), also possess affinity for the m-AChR and may serve as a source of new radiotracers. We report here the radiosyntheses of 2- and 4-[^{18}F]fluorodexetimides in a one-pot method via reductive amination of 2- and 4-[^{18}F]fluorobenzaldehydes, respectively, with norbenzyldexetimide and sodium cyanoborohydride.

Results

Figure 1 illustrates the method used to prepare [2-^{18}F]fluorodexetimide. Table I shows the time taken for each step and radioactivity present (uncorrected for decay) at different stages as the synthesis progresses. The first step involves the nucleophilic aromatic substitution (SNAR) of 2-trimethylammoniumbenzaldehyde triflate by Kryptofix/K_2CO_3 activated [^{18}F]fluoride anion in DMSO at 80 °C for 4 min. Radiochemical yields from this step were typically 60 - 70 %. The [2-^{18}F]fluoro-benzaldehyde produced was subsequently reductively aminated with norbenzyl-dexetimide, using sodium cyanoborohydride (18) and acetic acid, in the same reaction vessel. Reaction was complete upon heating to 120 °C for 10 min. The product was isolated by an initial C_{18} SEP-PAK clean up followed by HPLC purification and formulation for biological studies. An HPLC chromatogram of a typical purification is shown in Figure 2.

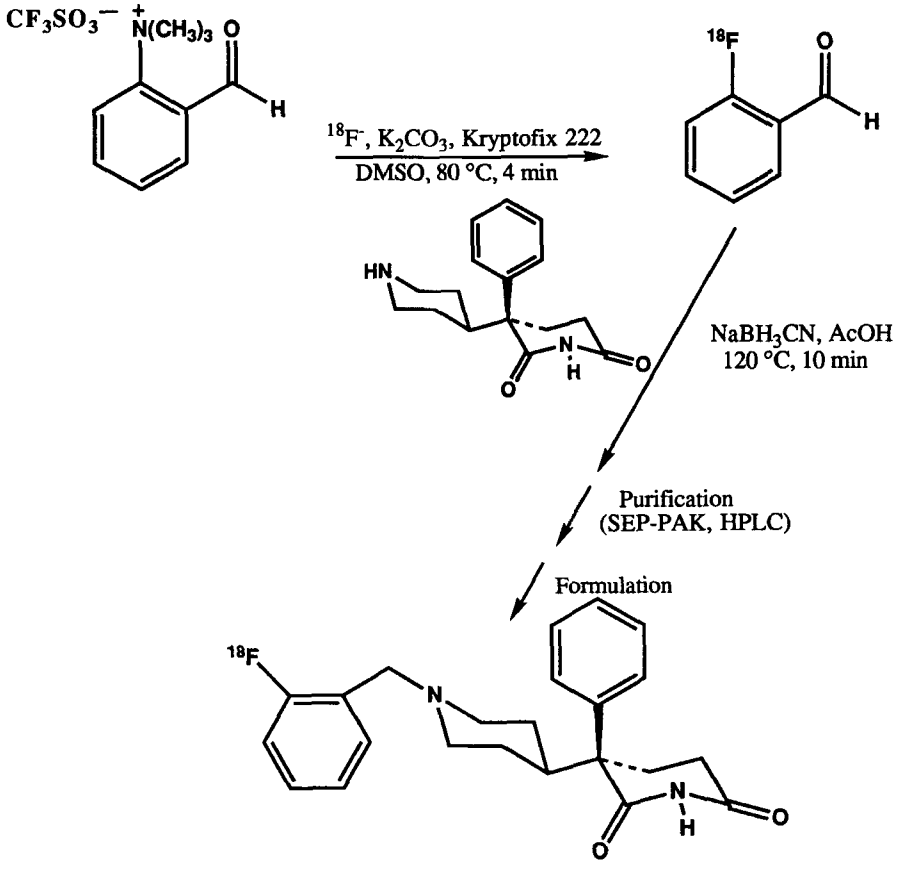

Figure 1. Radiosynthesis of [2-^{18}F]Fluorodexetimide.

The procedure for the radiosynthesis of [4-^{18}F]fluorodexetimide was identical to that described above for the 2-substituted analogue and the distribution of activity was very similar to that outlined in Table I. Isolated radiochemical yields of 15 - 23 % (2-fluoro, n = 10) and 19 - 23 % (4-fluoro, n = 3) were obtained 48 min after end-of-bombardment (EOB). Specific activities of 500 - 4000 mCi/μmol (2-fluoro) and 2400 - 8000 mCi/μmol (4-fluoro) were achieved at the end-of-synthesis. The formulated products, ready for biological studies, proved sterile and pyrogen-free in all cases.

Table I. Time Elapsed and Radioactivity Present at Various Stages in the Radiosynthesis of [2-^{18}F]Fluorodexetimide

Radioactivity (mCi)	Time from EOB (min)	Step completed
100	3	[^{18}F]Fluoride in aqueous K_2CO_3 delivered to reaction vessel containing Kryptofix.
93	10	[^{18}F]Fluoride dried at 120 °C azeotropically with CH_3CN.
89 (70% product)	16	Benzaldehyde in DMSO added to dried [^{18}F]fluoride. Heated to 80 °C for 4 min.
80 (60% product)	28	Cyanoborohydride, norbenzyldexetimide, and acetic acid added. Heated to 120 °C for 10 min.
43 (85% product)	31	SEP-PAK work up. Crude product eluted from SEP-PAK with CH_3CN onto HPLC column.
25	46	[2-^{18}F]Fluorodexetimide collected from HPLC and evaporated to dryness.
20	48	[2-^{18}F]Fluorodexetimide taken up in saline, passed through 0.2μm filter, ready for use.

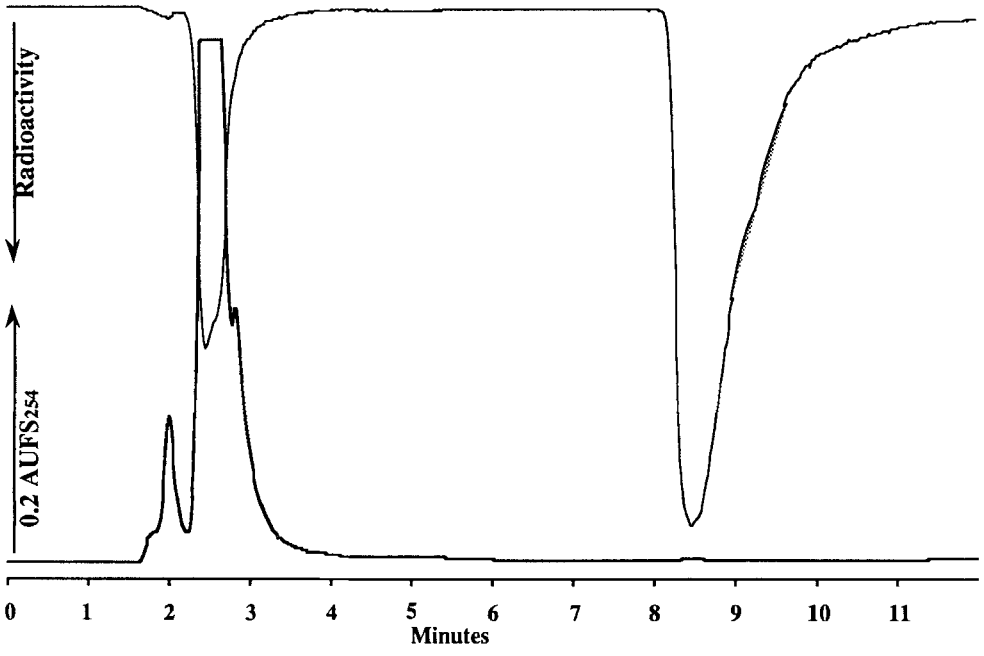

Figure 2. HPLC Purification of [2-^{18}F]Fluorodexetimide (R_T - 8.5 min)

Discussion

Radiotracers labelled with fluorine-18 have some inherent advantages over their [^{11}C]-labelled counterparts, most notably preparation and/or use remote from a cyclotron, longer imaging times, and ultimately higher resolution. These properties, plus the recent advances in introducing no-carrier-added [^{18}F]fluoride into activated aromatic rings (19-24), directed us to [^{18}F]-labelled dexetimides as potential radiotracers for the imaging of m-AChR using PET. For want of an electron withdrawing activating substituent, direct SNAR of a suitable precursor to generate [^{18}F]fluorodexetimide is not feasible, mandating a two-step procedure; the introduction of [^{18}F]fluoride into a suitable aromatic benzaldehyde and its subsequent coupling with norbenzyldexetimide.

The design of a reaction sequence to facilitate completion in one-pot is desirable for many reasons, including speed, reduced physical loss of material, and economy. In radiotracer synthesis, one-pot reactions may also result in reduced radiation exposure of personnel and prove easier to automate. The crucial factor in the present case is finding a reductive amination reaction which can occur in the presence of DMSO and Kryptofix; DMSO being the solvent of choice for the introduction of [^{18}F]fluorine into aromatic rings via nucleophilic aromatic substitution of suitable leaving groups (25).

Reductions in general have been carried out in a variety of solvents but not as a rule in DMSO, presumably because it was feared that DMSO itself would compete for the reducing agent. Indeed, recent attempts to reduce [4-^{18}F]fluoronitrobenzene in DMSO using sodium borohydride and palladium proved unsuccessful, necessitating the removal of the DMSO before reduction could proceed (22). Nevertheless, model reactions revealed that reductive alkylation of norbenzyldexetimide with 2-fluorobenzaldehyde using excess cyanoborohydride in DMSO proceeded rapidly at temperatures above 70 °C, in the presence or absence of K_2CO_3 and Kryptofix, encouraging us to pursue this approach.

Our initial precursor in the synthesis of [2-^{18}F]fluorodexetimide was 2-nitrobenzaldehyde and conditions and radiochemical yields were very similar to those outlined in Figure 1 and Table I. However, excess unreacted 2-nitro-

benzaldehyde gave, after reductive amination with cyanoborohydride, large quantities of 2-nitrodexetimide. This, plus smaller amounts of unidentified impurities, proved exceedingly difficult to separate completely from [2-^{18}F]fluorodexetimide.

Previous investigations have shown that quaternary ammonium groups are suitable leaving groups for the successful incorporation of [^{18}F]fluoride into activated aromatic rings (19,20). In addition, the spectrum of side-products produced can be easier to remove than those from nitro leaving groups (although not always (24)). As outlined in Figure 1, the use of trimethylammonium trifluoromethanesulphonate (triflate) salts in lieu of the nitro leaving group provided satisfactory yields of products and allowed a rigorous purification of the [^{18}F]-labelled dexetimides from side-products. Initial clean-up of the reaction mixtures using a C_{18} SEP-PAK before HPLC purification proved efficacious and the use of a "normal" stationary phase (silica) with a "reverse-phase" solvent (buffered aqueous acetonitrile) (26), provided more efficient separations from side products (Figure 2).

The facile production of the [^{18}F]fluorobenzaldehydes and their efficient coupling to the piperidine ring of norbenzyldexetimide under mild reducing conditions suggests that they might be useful for the introduction for the [^{18}F]fluorobenzyl group into a variety of primary and secondary amines. Benzaldehydes labelled with fluorine-18 have also been reported as intermediates in the high specific activity radiosyntheses of [4-^{18}F]fluorophenylalanine (27) and [6-^{18}F]fluorodopa (28). While the N-benzyl group is far less common than the N-methyl substituent (cf. $^{11}CH_3I$) in both naturally occurring and synthetic compounds, it is a constituent of many biologically interesting molecules. In addition, aryl substituted N-benzyls are often part of the arrays of analogues produced by pharmaceutical companies in their search for more potent drugs and structure-activity relationships.

Experimental

NMR spectra were obtained on an IBM NR/80 using $(CH_3)_4Si$ as an internal standard. DMSO was distilled from basic alumina under argon. Elemental analyses

were performed by Atlantic Microlab, Atlanta, GA; all new compounds gave satisfactory elemental analyses (C,H,N ± 0.4%). Melting points are uncorrected. HPLC purification and analyses of radioactive mixtures were performed using a previously described system (11). Both [^{18}F]fluorodexetimides and both intermediate [^{18}F]fluorobenzaldehydes were identified using analytical HPLC by co-injection with authentic standards using normal (silica) and reverse-phase (C_{18}) conditions. Radioactive and mass peak areas were measured using Hewlett-Packard 3390A recording integrators and radiochemical yields were determined with a dose-calibrator (Capintec CRC-12R). Norbenzyldexetimide was prepared by the catalytic hydrogenolysis of dexetimide as described previously (9).

2-Dimethylaminobenzaldehyde. A mixture of 2-fluorobenzaldehyde (5 mL, 47.8 mmoles), dimethylamine hydrochloride (5.0 g, 61.3 mmole), and potassium carbonate (5.0 g) in DMSO (25 mL) and water (10 mL) was stirred and heated to reflux for 6 hr. The cooled solution was diluted with aqueous potassium carbonate (1N, 100 mL) and extracted with ether (100 mL). The ethereal solution was washed with water and extracted twice with aqueous hydrochloric acid (2N, 100 mL then 50 mL). Back extraction of the neutralised (potassium carbonate) acid solution with ether, followed by filtration and evaporation, gave an orange oil. Distillation (85 - 90 °C, 0.2 mm) afforded a yellow oil (3.7 g, 52%); ^{1}H NMR($CDCl_3$) δ 2.91 (s, 6H), 6.8-7.85 (m, 4H), 10.23 (s, 1H).

2-Trimethylammoniumbenzaldehyde triflate. Methyl trifluoro-methanesulphonate (2.53 mL, 28.6 mmoles) was added slowly to a stirred solution of 2-dimethylaminobenzaldehyde (3.0 g, 20.1 mmoles) in dichloromethane (150 mL) under argon at ambient temperature. The solution was heated to reflux for 6 hr then cooled and ether (300 mL) was added. The resultant oily solid was extracted into water (250 mL) and washed with ether (200 mL) and chloroform (200 mL). Rotary evaporation of the water gave a colourless oil, which was triturated with ether to give a white solid. This solid was collected, washed with ether, and dried in vacuo. Recrystallisation from dichloromethane/ether gave white crystals (1.83 g, 29%); mp 94 - 95 °C; ^{1}H NMR (DMSO-d6) δ 3.79 (s, 9H), 7.9-8.5 (m, 4H), 10.10 (s, 1H).

4-Trimethylammoniumbenzaldehyde triflate. A white solid was obtained in 35% yield by the method outlined above which was recrystallised from

acetonitrile/ether; mp 108 - 110 °C (lit. (20) 100 - 102 °C); ^{1}H NMR (DMSO-d6) δ 3.67 (s, 9H), 8.20 (br.s, 4H), 10.13 (s, 1H).

2-Fluorodexetimide·HCl. Using norbenzyldexetimide, this compound was prepared as described for the racemate (9). mp 256 - 260 °C (dec).

4-Fluorodexetimide·HCl. Using norbenzyldexetimide, this compound was prepared as described for the racemate (9). mp 245 - 249 °C (dec.).

[2-^{18}F]Fluorodexetimide. [^{18}F]Fluoride was produced by the ^{18}O(p,n) reaction on >95% atom [^{18}O]H_2O using a Scanditronix MC-16F biomedical cyclotron. The [^{18}O]H_2O was separated from the [^{18}F]fluoride using Dowex 1-X8 anion exchange resin (29). The [^{18}F]fluoride was eluted from the column with aqueous potassium carbonate (2.3 mg / 0.2 mL) into a glass Reacti-vial (Pierce) containing Kryptofix 222 (13 mg) and evaporated to dryness at 120 °C azeotropically with acetonitrile. A solution of 2-trimethylammoniumbenzaldehyde triflate (2.0 mg) in DMSO (0.35 mL) was added and heated to 80 °C for 4 min in the sealed vial. Between 60 - 80 % incorporation of radioactivity was achieved.

The solution was then cooled, the vial opened, and norbenzyldexetimide (3 mg), sodium cyanoborohydride (4 mg), and acetic acid (8 μL) were added. The resealed vial was shaken, heated to 120 °C for 10 min, and then cooled. Water (5 mL) was added and the mixture passed through a C_{18} SEP-PAK followed by 5 mL of 4 mM diammonium hydrogen phosphate used to rinse the reaction vessel. The SEP-PAK was washed with a further 5 mL of phosphate buffer and dried by a stream of nitrogen gas for one min. The product was eluted using acetonitrile (1.5 mL) directly into the HPLC injection loop for purification. Semipreparative HPLC purification was performed on a Beckman Ultrasphere Si (5 μ, 10 mm x 25 cm) column eluted with 80:20 acetonitrile:0.004 M diammonium hydrogen phosphate buffer at a flow rate of 6 mL/min. Under these conditions, the product eluted at 8.5 min (k' = 3.7, see Figure 2).

Upon evaporation of the collected HPLC fraction, the residue was taken up in 9 mL of sterile saline and passed through a sterile 0.22 μm filter into a sterile, pyrogen free bottle when sterile aqueous sodium bicarbonate (1 mL, 8.4%) was added. The radiochemical purity and specific activity of the final solutions were determined by analytical HPLC (Waters μBondapak 25 cm x 3.9 mm; 40% CH_3CN /

60% H_2O + 0.1N NH_4HCO_3; 4 mL / min; $k'_{2\text{-fluorodexetimide}}$ = 2.4).

[4-^{18}F]Fluorodexetimide: The procedure described above was used without modifications except 4-trimethylammoniumbenzaldehyde triflate was substituted as the starting material.

Acknowledgements

The authors wish to thank Dr. J. Heykants of Janssen Pharmaceutica, Beerse, Belgium for graciously providing samples of dexetimide for these studies, and Mr. Robert Smoot for his assistance with cyclotron operations and radiochemical syntheses. This work was supported in part by grant numbers CA-32845 and NS-15080 from the U.S. Public Health Service.

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