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SYNTHESIS OF [^{18}F]GBR 12909, A DOPAMINE REUPTAKE INHIBITOR

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SUMMARY

Preparation of no-carrier-added fluorine-18 labeled GBR 12909 (1-[2-(bis(4-fluorophenyl)methoxy)ethyl]-4-(3-phenylpropyl)piperazine), a specific and high affinity inhibitor of dopamine reuptake, is described. 4-Fluoro-4'-[^{18}F]fluorobenzophenone was prepared by [^{18}F]fluoride ion substitution of the corresponding trimethylammonium trifluoromethanesulfonate salt. The [^{18}F]benzophenone was reduced to the benzhydrol, chlorinated, then used to alkylate 1-(2-hydroxyethyl)-4-(3-phenylpropyl)piperazine to yield [^{18}F]GBR 12909 in high specific activity (>2000 Ci/mmol) and overall yields of 10-16% (corrected, 140 min synthesis).

KEY WORDS: Fluorine-18, dopamine, [^{18}F]GBR 12909, uptake

INTRODUCTION

We have recently reported the synthesis of [^{18}F]GBR 13119 (Fig. 1), a specific, high affinity inhibitor of the dopamine reuptake system (1,2). Although suitable for extensive animal use, the application of this radiotracer to human studies proved troublesome, due to the lack of pharmacological and toxicological data. A closely related compound, GBR 12909 (1-[2-(bis(4-fluorophenyl)methoxy)ethyl]-4-(3-phenylpropyl)piperazine), is in clinical trials and has been extensively studied as to its pharmacology and toxic properties in both animals and humans (3,4). We describe here the synthesis of GBR 12909 (Fig 1) in fluorine-18 form suitable for human use.

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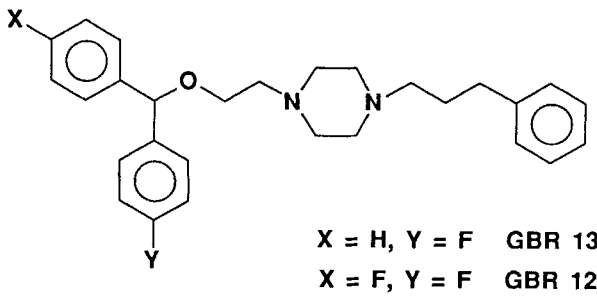

Figure. 1

EXPERIMENTAL

Materials and Methods. The following chemicals were obtained from the indicated sources and used without further purification: thionyl chloride, lithium aluminum hydride (1 M in THF), carbon disulfide, mesitylene, methyl trifluoromethanesulfonate, N,N-dimethylaniline, tin(IV) chloride, and 4-fluorobenzoyl chloride from Aldrich Chem Co. N-(2-Hydroxyethyl)-N-(3-phenylpropyl)piperazine was prepared per literature methods (5). High specific activity (approx. 50,000 Ci/mmol) [^{18}F]fluoride ion was prepared by proton bombardment of [^{18}O]water (95-99% enrichment, Isotec) as previously described (6). Thin layer chromatography was done using plastic-backed silica gel plates (Merck), and preparative layer chromatography using 2 mm silica gel plates (Merck). Melting points were determined with a Mel-Temp apparatus and are reported uncorrected. NMR spectra were done using a Bruker WM360 spectrometer, and mass spectra were determined using a VG Instruments Model 70-250S mass spectrometer utilizing fast atom bombardment with 3-nitrobenzyl alcohol as matrix.

4-Fluoro-4'-N,N-dimethylaminobenzophenone (1). A 250 ml 3-neck round bottom flask was equipped with a mechanical stirrer, a reflux condenser with $CaCl_2$ drying tube, and a dropping funnel, and an argon atmosphere introduced. To this were added 21.5 g (82.5 mmol) of $SnCl_4$ and 100 ml of dry CS_2. The mixture was cooled to 5 ^{o}C (ice-water) and vigorously stirred while N,N-dimethylaniline (5.0 g, 41.26 mmol) was added dropwise over 10 min. The dropping funnel was then washed with 10 ml of

CS_2. To the yellow slurry was then added 4-fluorobenzoyl chloride (6.54 g, 41.26 mmol) dropwise over 15 minutes. After addition was complete, the mixture was warmed to room temperature and finally refluxed for one hour. The now dark black-green mixture was cooled to 5 °C and ice (50 g) and then water (100 ml) added; a dark green oil separated at this point. Steam was then passed through the mixture to remove the carbon disulfide and unreacted N,N-dimethylaniline. The residue was cooled to 5 °C and filtered to yield a dark blue-green solid. The solid was suspended in 200 ml of water at 50 °C and then filtered, and this process was repeated twice. The remaining green solid was washed with water and cold methanol (100 ml) to yield 3.0 g of crude product.

The crude material was dissolved in 100 ml of chloroform and filtered. The solution was washed with 3 x 35 ml portions of 7.5% aqueous hydrogen peroxide containing 1.0 g NaOH per portion. Finally, the chloroform solution was washed with 100 ml water, dried ($MgSO_4$), and evaporated to give 0.5 ml of a yellow crystalline product. This was dissolved in 10 ml of acetone, heated to reflux, and filtered hot. The filtrate was then allowed to cool overnight. The yellow crystals of 4-fluoro-4'-N,N-dimethylaminobenzophenone were collected by filtration. TLC showed a small impurity, which was separated by silica gel preparative layer chromatography (2 mm silica gel, CH_2Cl_2) to afford 600 mg (6% yield) of the desired 4-fluoro-4'-N,N-dimethylaminobenzophenone **1**: m.p. 107-109 °C; ^{1}H NMR ($CDCl_3$) δ 2.97 (s, 6H, $N(CH_3)_2$), 6.57-6.59 (d, 2H, ArH ortho to amine), 7.0-7.05 (dd, 2H, ArH ortho to fluorine), 7.64-7.68 (m, 4H, ArH ortho to carbonyl).

4-Fluoro-4'-N,N,N-trimethylammoniobenzophenone (**2**). Under an Ar atmosphere, methyltrifluoromethanesulfonate (63.3 µl, 0.56 mmol) was added to a solution of dimethylaminobenzophenone **1** (127 mg, 0.523 mmol) in 10 ml of CH_2Cl_2. The yellow solution was stirred overnight, then diethyl ether added. The white precipitate which formed was collected and recrystallized to give the desired trimethylammonium salt **2**: yield 85 mg, 40%; m.p. 84-86 °C; Mass spec (FAB) m/z 258 (positive ion, calcd for $C_{16}H_{17}NFO^+$, 258); m/z 149 (negative ion, calcd for $CF_3SO_3^-$, 149). Analysis: Calcd for $C_{17}H_{17}NO_4F_4S \cdot 1/2\ H_2O$, C, 49.04; H, 4.36; N, 3.36. Found: C, 48.96; H, 4.96; N, 3.24.

4-[^{18}F]Fluoro-4'-fluorobenzophenone (3). To a solution of no-carrier-added [^{18}F]fluoride ion in DMSO (resolubilized using 1 mg K_2CO_3 and 5 mg Kryptofix-222, as per previous methods (1)) was added 1 mg of trimethylammonium salt **2**. The solution was heated at 155 °C for 20 min, then cooled and an aliquot removed. The aliquot was diluted with water and the product extracted into diethyl ether: yields 60-94%, TLC silica gel (8/2 pentane /ether) R_f = 0.57. The bulk of the reaction was not worked up but used in the next step.

4-[^{18}F]Fluoro-4'-fluorobenzhydrol (4). The solution of crude [^{18}F]benzophenone **3** in DMSO was cooled (0-5 °C, ice-water) and lithium aluminum hydride (0.1 ml, 100 μmol) added via syringe. After 1 min, the reaction was quenched by dropwise addition of 2N HCl, and then 10 ml of 2 N HCl added and the aqueous mixture passed through a C18 Sep-Pak. The Sep-Pak was washed with water (5 ml), then the product eluted with 2 ml of CH_2Cl_2 which was then dried ($MgSO_4$). Yield 55-85% (from [^{18}F]fluoride); TLC (silica gel, 7/3 hexane/ethylacetate) R_f = 0.16.

4-[^{18}F]Fluoro-4'-fluorobenzhydryl chloride (5). The solution of [^{18}F]benzhydrol **4** was evaporated (slow N_2 flow) and 300 μl of $SOCl_2$ added. The solution was heated (100 °C, closed vessel) for 20 min, cooled, and the thionyl chloride evaporated to yield crude chloride **5**; yields 80-95 % (from alcohol **4**); TLC (silica gel, 7/3 hexane/ethylacetate) R_f = 0.50.

[^{18}F]GBR 12909 ((1-[2-(bis(4-fluorophenyl)methoxy)ethyl]-4-(3-phenylpropyl)-piperazine). To the residue of [^{18}F]chloride **5** was added 10 mg of N-(2-hydroxyethyl)-N-(3-phenylpropyl)piperazine in 50 μl mesitylene, and the solution heated at 155 °C for 25 min. The vessel was cooled and the brown oil dissolved in 0.5 ml of methanol. The methanol was then diluted with 10 ml of water and passed through a C18 Sep-Pak. The Sep-Pak was washed with water, then the products eluted with 10 ml of pentane. The pentane was dried ($MgSO_4$) and then passed through a silica gel Sep-Pak. The silica gel was washed with 2 x 20 ml of 1/1 pentane/CH_2Cl_2, and the product [^{18}F]GBR 12909 eluted with 10 ml of 5% methanol/CH_2Cl_2. Yield of condensation reaction 25-60%; TLC (silica gel, 95/5 CH_2Cl_2/methanol) R_f = 0.31; HPLC, Phenomenex C18 column, 0.45 x 15

cm, 60/40 acetonitrile/0.065 M NH_4OAc (3 ml tetrahydrofuran/liter water) , 2 ml/min flow: GBR 12909, R_t = 14.0 min; piperazine, R_t = 3.5 min. Product identity was confirmed by comparison to a known standard of GBR 12909 obtained from NOVO Industri, A/S (also available from Research Biochemicals Incorporated). Overall yields of [^{18}F]GBR12909 ranged from 10-16% (corrected for decay) with a synthesis time of 120-140 min. For biological studies the product was prepared for injection by evaporation of the organic solvent and dissolution in dilute acidic saline (pH 5.5).

RESULTS AND DISCUSSION

The synthesis of [^{18}F]GBR 12909 follows the same synthetic route as we have previously developed for [^{18}F]GBR 13119 (1). The necessary precursor, 4-fluoro-4-trimethylammoniumbenzophenone triflate (2), was prepared by the method shown in Figure 2. Friedel-Crafts acylation of N,N-dimethylaniline with 4-fluorobenzoyl

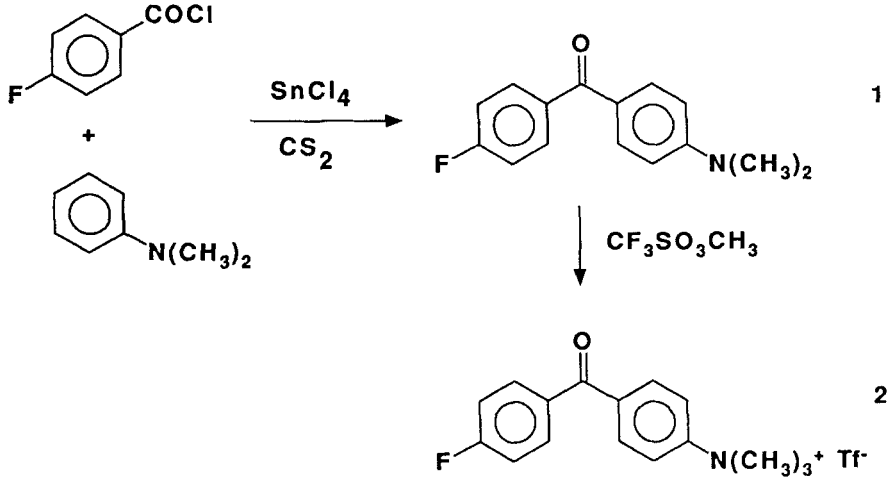

Fig. 2

chloride gave multiple acylation products, from which the desired 4-fluoro-4'-N,N-dimethylaminobenzophenone **1** was isolated by crystallization and silica gel preparative layer chromatography. Although this provided a direct route to the tertiary amine **1**, the yield was very low and alternative syntheses of **1** were examined. The use of anhydrous aluminum chloride as the Friedel-Crafts catalyst gave, instead of the

desired mono-acylation product **1**, a good yield (35%) of a product identified by NMR (^{1}H and ^{13}C) and mass spectroscopy (m/e 365, M^{+}) as a di-acylation product. This bis-acylation product was obtained even in the presence of excess N,N-dimethylaniline. At this time we are uncertain as to whether the second acyl group has added ortho- or meta- to the dimethylamine substituent; this unusually facile bis-acylation reaction is under further study. An alternative route using Friedel-Crafts acylation of acetanilide, followed by hydrolysis to the primary amine and methylation, was attempted but did not prove advantageous, due to unexpected difficulties in the hydrolysis step.

The N,N-dimethylamine **1** isolated by chromatography was then alkylated (methyl trifluoromethanesulfonate) to give the trimethylammonium salt **2**, which was separated from traces of the starting material by recrystallization.

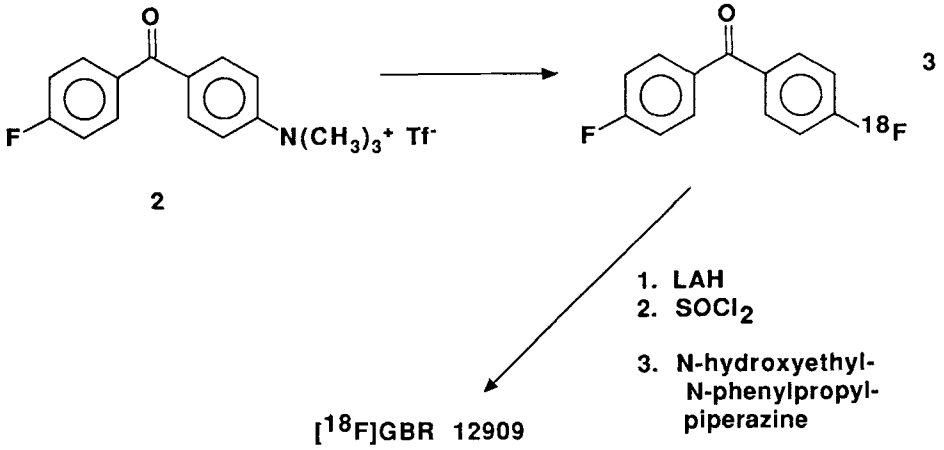

Fig 3

Reaction of the trimethylammonium salt **2** with NCA [^{18}F]fluoride ion proceeded smoothly to give the benzophenone **3** (Fig. 3) in 60-94% yields (no attempts were made to isolate the other possible product, arising from isotopic exchange of fluorines). Reduction, chlorination, and condensation with N-(2-hydroxyethyl)-N-(3-phenylpropyl)piperazine to give [^{18}F]GBR 12909 proceeded analagously to the synthesis of [^{18}F]GBR 13119 (1). The final product was purified by C-18 and silica gel SEP-PAKS, and analyzed by HPLC and TLC. Radiochemical purity was >95%, and chemical purity excellent (10-40 microgram amounts of N-(2-hydroxyethyl)-N-(3-phenyl-

propyl)piperazine were observed in some preparations). Specific activity, estimated by HPLC analysis at end of synthesis, was in excess of 2,000 Ci/mmol (limit of detection of UV detector utilized). Overall yields were 10-16% (corrected for decay) in a total synthesis time of 120-140 minutes.

Preliminary in vivo study of regional brain distribution in mice shows striatal uptake (2.4 ± 1.2 %ID/g) and striatum/cerebellum ratios (3.13 ± 1.01) determined at 1 h which are essentially identical to those obtained with [^{18}F]GBR 13119 (7). Further animal and human studies with this radiopharmaceutical will be reported in due course.

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