No-Carrier-Added Fluorine-18-Labeled *N*-Methylspiroperidol: Synthesis and Biodistribution in Mice

Chyng-Yann Shiue, Joanna S. Fowler, Alfred P. Wolf, Daniel W. McPherson, Carroll D. Arnett, and Luigi Zecca

Chemistry Department, Brookhaven National Laboratory, Upton, New York

No-carrier-added fluorine-18- (^{18}F) labeled *N*-methylspiroperidol **(4)** was synthesized from four different substrates: *p*-nitrobenzonitrile **(1)**, cyclopropyl *p*-nitrophenyl ketone **(2A)**, *p*-cyclopropanoyl-*N,N,N*-trimethylanilinium iodide **(2B)** and *p*-cyclopropanoyl-*N,N,N*-trimethylanilinium perchlorate **(2C)** using the nucleophilic aromatic substitution reaction. Radiochemical yield, synthesis time, experimental simplicity, and specific activity were compared. In addition, factors which influence the yield of the nucleophilic aromatic substitution were studied. Based on these studies, the synthesis of **4** from **2A** maximizes product specific activity and experimental simplicity and provides **4** in 10–15% radiochemical yield [based on $[^{18}F^-]$ with a mass of <2 nmol and a specific activity of >10 Ci/μmol (EOB)]. The synthesis of **4** from 8-[4-(4-nitrophenyl)-4-oxobutyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one **(5)** and Cs[^{18}F] using the nucleophilic aromatic substitution reaction gave unacceptably low and erratic yields. The biodistribution of **4** in mice showed a maximum brain uptake of 1.1% of the administered dose at 5 min and declined to ~0.6% at 120 min.

J Nucl Med 27:226–234, 1986

We recently developed a general synthetic strategy for preparing no-carrier-added (NCA) fluorine-18-(^{18}F) labeled butyrophenones (*1*) using the nucleophilic aromatic substitution reaction (*2–6*). Four NCA [^{18}F]butyrophenone neuroleptics—haloperidol, benperidol, spiroperidol, and pipamperone—were synthesized using this method and the kinetic behavior of three of these compounds in baboon brain was compared using positron emission tomography (PET) (*7*). The in vivo behavior of [^{18}F]haloperidol was found to be significantly different from [^{18}F]benperidol and [^{18}F]spiroperidol, characterized by rapid egress from receptor rich areas and significant nonspecific binding. However, both [^{18}F]spiroperidol and [^{18}F]benperidol cross the blood-brain barrier to a lesser extent than [^{18}F]haloperidol but are retained by dopamine rich areas of the brain. No observable clearance from the striatal area was observed in the case of [^{18}F]spiroperidol for up to 8 hr postinjection. These results demonstrate the power of this experimental approach in evaluating a series of structurally similar radiotracers and have been presented as experimental evidence that [^{18}F]spiroperidol satisfies the minimum criteria for PET studies of the dopamine receptor in vivo.

Despite these positive results, one potential problem with human PET studies using [^{18}F]spiroperidol is the relatively low uptake in the brain (0.5–1.0% of the injected dose in baboon) and consequently the potentially high radiation burden which is required to obtain sufficient counting statistics for PET. To continue the pursuit of a radioligand which satisfies the minimum criteria as a dopamine receptor specific ligand while having a greater uptake into the brain's dopamine receptor rich areas, we recently used the general [^{18}F]butyrophenone synthesis to prepare ^{18}F-labeled *N*-methylspiroperidol (*8*).

N-Methylspiroperidol is more lipophilic than spiroperidol (*8, 9*) and the carbon-11- (^{11}C) labeled compound has been synthesized (*9*) and used in PET studies of the dopamine receptor in human brain (*10*). We found that ^{18}F-labeled *N*-methylspiroperidol, like [^{18}F]spiroperidol, satisfies the above criteria for receptor binding while surpassing [^{18}F]spiroperidol in its uptake into dopamine receptor rich areas.

Received Apr. 23, 1985; revision accepted Aug. 30, 1985.

For reprints contact: C.-Y. Shiue, PhD, Chemistry Dept., Brookhaven National Laboratory, Upton, NY 11973.

The potentially widespread utility of ^{18}F-labeled *N*-methylspiroperidol (**4**) as a tracer for mapping dopamine receptors in normal and disease states in the living brain has lead to the exploration of new routes for its synthesis. We report here the comparison of yield, synthesis time, experimental simplicity, and specific activity using the nucleophilic aromatic substitution reaction and four different substrates (Fig. 1) as well as some general observations on the nucleophilic aromatic substitution reaction. We also report the tissue distribution of **4** in mice.

MATERIALS AND METHODS

Cesium carbonate was purchased commercially*. Dimethylsulfoxide (DMSO) was a Gold Label reagent† and was dried over 4 Å molecular sieves. *p*-Nitrobenzonitrile† was used without further purification. 3-Methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one hydrochloride† was converted to the free base before use. Cyclopropyl *p*-fluorophenyl ketone was purchased commercially‡ and cyclopropyl lithium and cyclopropyl *p*-nitrophenyl ketone (**2A**) were synthesized by the known methods (*11, 12*). Elemental analyses were performed§ and melting points determined and left uncorrected with a Fischer-Johns melting point apparatus. Nuclear magnetic resonance (NMR) spectra were recorded with a Varian CFT-20 spectrometer with TMS as an internal standard. Infrared (IR) spectra were recorded with a Perkin-Elmer Model 337 spectrometer. The mass spectra were measured with a Finnigan MAT 5100 GC/MS/DS spectrometer.

Thin layer chromatographic (TLC) analyses were performed on plastic-backed TLC plates (Merck) with either CH_3CN:CH_3OH (4:1) or CH_2Cl_2:CH_3OH (9:1) as solvent. High performance liquid chromatography (HPLC) analyses were carried out with a Perkin-Elmer Series 3B liquid chromatograph equipped with a radioactivity monitor (Berthold Model LB503). An analytic reversed phase C18 column (4.5 × 250 mm) was used with either CH_3OH:0.01*M* $(NH_4)_2HPO_4$ (70:30) or CH_3OH:0.01*M* NH_4HCO_2 (65:35) as the solvent with a flow rate of 2 ml/min. For the preparative separations a semipreparative C_{18} column (10 × 250 mm) was used with CH_3OH:0.01*M* NH_4HCO_2 (65:35) as the solvent with a flow rate of 6 ml/min. The C18 SEP-PAK cartridges were obtained commercially¶.

In a number of syntheses, an aliquot of the ^{18}F-labeled fluoride solution obtained from the target was analyzed for fluoride content as described previously (*1*).

Synthesis of *p*-*N*,*N*-Dimethylaminophenyl Cyclopropyl Ketone

A modification of the method of Freed and Hertz was used (*13*). Cyclopropyl *p*-fluorophenyl ketone (1.12 g, 6.9 mmol) was added to 3 ml of DMSO. The flask was sealed with a septum and the solution stirred and cooled to 0°C in an ice bath. Anhydrous dimethylamine (3.4 g, 75.5 mmol) was added by syringe and the solution was gradually warmed to room temperature and allowed to stir at room temperature for 72 hr. The precipitate was filtered and washed with DMSO and ether. Recrystallization from acetone-pentane (1:1) yielded *p*-*N*,*N*-dimethylaminophenyl cyclopropyl ketone as a white crystalline solid (1.22 g, 92.8%); mp 139–141°C (138–140°C) (*13*); IR (KBr):1580 cm^{-1} (C═O); NMR ($CDCl_3$) δ:0.99 (m, 2H); 1.16 (m, 2H) 2.60 (m, 1H); 3.05 (s, 6H); 6.67 (d, 2H); 7.96 (d, 2H).

Synthesis of *p*-Cyclopropanoyl *N*,*N*,*N*-Trimethylanilinium Iodide (2B)

p-*N*,*N*-Dimethylaminophenyl cyclopropyl ketone (186.50 mg, 0.99 mmol) was added to 2 ml of dimethylformamide (DMF). Methyliodide (0.24 g, 7.4 mmol) was added to the solution, the flask stoppered and solution stirred for 18 hr. Ethyl acetate was added to the flask to precipitate the product and the solution was filtered. The precipitate was washed with ethyl acetate, ether, and chloroform. The precipitate was dissolved in a minimal amount of methanol and filtered. Ether was added to the filtrate to precipitate the product. The product was filtered and washed with ether and dried. This yielded **2B** as a white crystalline solid (56.25 mg, 17.2% yield); mp >280°C; IR (KBr):1660 cm^{-1} (C═O); NMR (CD_3CN) δ:1.15 (d, 4H); 2.60 (m, 1H); 3.61 (s, 9H); 7.93 (d, 2H); 8.24 (d, 2H); mass spectrum m/e = 189 ($M-CH_3I$). Calculated for $C_{13}H_{18}ION$: C, 47.14; H, 5.49; I, 38.31; N, 4.23. Found: C, 45.60; H, 5.49; I 38.91; N, 4.50. The product was unstable in solution; therefore, attempts to recrystallize the product proved unsuccessful. HPLC analysis of the product showed that no cyclopropyl *p*-fluorophenyl ketone was present in the final product.

Synthesis of *p*-Cyclopropanoyl-*N*,*N*,*N*-Trimethylanilinium Perchlorate (2C)

Compound **2C** was prepared by the method of Kevill and Shen (*14*). Anhydrous silver perchlorate (116.9 mg, 0.56 mmol) was placed in a three-neck, round-bottom flask with a condensor and an addition funnel and the apparatus was purged with dry nitrogen. Benzene (2 ml) was added to the flask and the solution was stirred. Methyliodide (79.57 mg, 0.56 mmol in 1 ml benzene) was added dropwise over a period of 15 min and the solution was stirred at room temperature for 4 hr. The mixture was filtered and passed through a short column of 4 Å molecular sieves. The filter was washed with 2 ml of benzene and the filtrate and washings were collected in a flask which contained *p*-*N*,*N*-dimethylaminophenyl cyclopropyl ketone (113.48 mg, 0.6 mmol). The flask was purged with nitrogen, sealed and

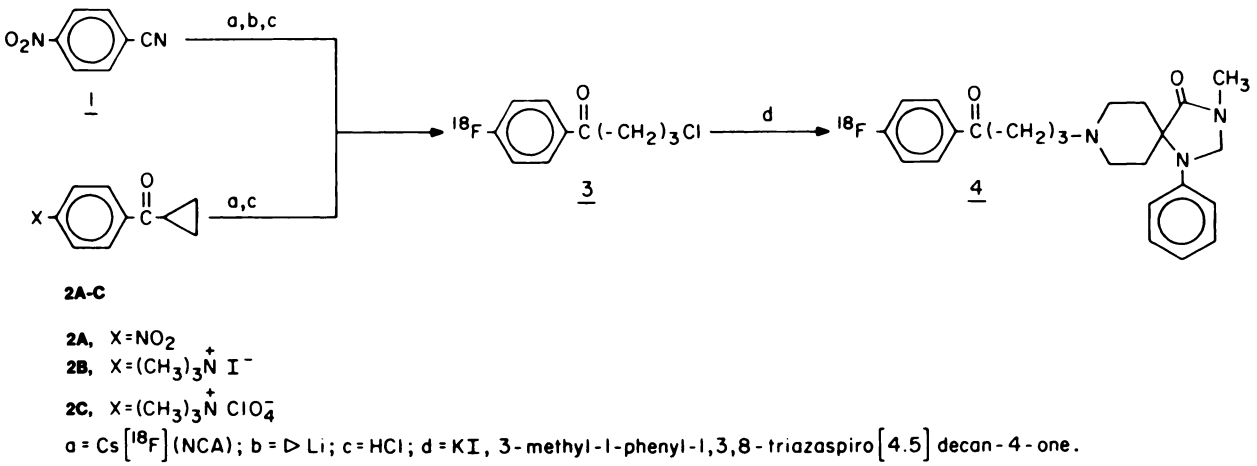


FIGURE 1
Synthesis of NCA ^{18}F-labeled *N*-methylspiroperidol

stirred at room temperature for 2 wk. The mixture was then filtered and the residue was washed with benzene and chloroform. The residue was dissolved in acetonitrile and the solution was filtered. Ether was added to the filtrate to precipitate the product. The product was filtered, washed with ether, and dried in a vacuum oven. This method afforded 11.94 mg (7.1%) of compound **2C** as a white crystal; mp 174–175°C; IR (KBr): 1655 cm^{-1} (C=O); 1095 cm^{-1} (Cl-O); NMR (CD_3CN) δ: 1.14 (d, 4H); 2.84 (m, 1H); 3.60 (s, 9H); 7.92 (d, 2H); 8.22 (d, 2H); mass spectrum m/e = 189 ($M-CH_3ClO_4$). Calculated for $C_{13}H_{18}ClO_5N$: C, 51.40; H, 5.98; N, 4.61. Found: C, 50.09; H, 6.38; N, 4.67. Sodium fusion of a small sample of the product and analysis for fluoride showed that there was no fluorine contamination in the product. Compound **2C** was also prepared by stirring **2B** with anhydrous silver perchlorate in acetonitrile, benzene, or toluene. This method afforded **2C** in yields of > 50%, however, the product could not be sufficiently purified.

Synthesis of 8-[4-(4-Nitrophenyl)-4-Oxobutyl]-3-Methyl-1-Phenyl-1,3,8-Triazaspiro[4.5]Decan-4-One (4-Nitro-*N*-Methylspiroperidol) (5)

The method (*9*) used for the synthesis of *N*-methylspiroperidol was adapted for the synthesis of 4-nitro-*N*-methylspiroperidol. 4-Nitrospiroperidol (*12*) (90 mg, 0.21 mmol) was dissolved in 30 ml of anhydrous tetrahydrofuran (THF) and then NaH (15 mg, 0.63 mmol) and methyliodide (50 μl, 0.81 mmol) were added. The mixture was vigorously stirred at room temperature for 150 min and evaporated to dryness. The residue was dissolved in CH_2Cl_2, and extracted with H_2O. The organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was dissolved in MeOH (6 ml) and injected into preparative HPLC (C18 column, 10 × 250 mm; MeOH-H_2O-diisopropylamine 70:30:0.1 as the solvent with a flow rate of 4.5 ml/min). The fractions containing 4-nitro-*N*-methylspiroperidol (retention time = 12.5 min) were collected and evaporated to dryness to give 32 mg (35%) of the product, mp 145–147°C. NMR ($CDCl_3$) δ: 1.5–3.06 (series of overlapping multiplets, 17H, incorporating 3H singlet at 2.98); 4.64 (s, 2H); 6.78–7.25 (m, 5H); 8.20 (q, J = 8Hz, 4H); mass spectrum m/e = 418 ($M-H_2O$). Calcd. for $C_{24}H_{28}N_4O_4$: C, 66.04; H, 6.47; N, 12.84. Found: C, 65.96; H, 6.66; N, 12.65.

Synthesis of *N*-Methylspiroperidol (6)

The authentic sample of *N*-methylspiroperidol was synthesized by the method of Burns et al. (*9*) and purified by preparative HPLC, mp 103–104°C (135–137°C) (*9*). NMR ($CDCl_3$) δ: 1.54–2.98 (series of overlapping multiplets, 17H, incorporating 3H singlet at 2.98); 4.65 (s, 2H); 6.81–7.26 (m, 7H); 8.0 (q, 2H); mass spectrum m/e = 391 ($M-H_2O$). Calcd. for $C_{24}H_{28}FN_3O_2$: C, 70.40; H, 6.89; N, 10.26. Found: C, 70.39; H, 6.96; N, 10.20.

Optimization of ^{18}F Substitution in 1 and 2A-C

Cs[^{18}F] was prepared as described below (Method A) and the yield of the displacement reaction was measured by carrying out the reaction through the first step of the reaction and working up the reaction mixture using SEP-PAK extraction. The identities of the products were confirmed by comparison of their HPLC retention times with authentic samples. Variables were temperature, vessel material, substrate concentration, length of irradiation, and amount of cesium carbonate.

Optimization of Hydrolysis of Cyclopropyl *p*-Fluorophenyl Ketone to γ-Chloro-*p*-Fluorobutyrophenone (3)

Cyclopropyl *p*-fluorophenyl ketone (2 mg) was heated with HCl:CH_3OH. Temperature, time, and acid concentration were varied. Reaction mixtures were

analyzed by HPLC using analytical reversed phase C_{18} column and eluted with CH_3OH–H_2O (7:3) with a flow rate of 1 ml/min. Relative amounts of the cyclopropyl *p*-fluorophenyl ketone and γ-chloro-*p*-fluorobutyrophenone were determined by comparing integrated areas using a calibration curve obtained from solutions of known concentration. Retention times were 5.8 min and 7.4 min for cyclopropyl *p*-fluorophenyl ketone and **3**, respectively.

Synthesis of NCA ^{18}F-Labeled *N*-Methylspiroperidol (4)

NCA ^{18}F-labeled *N*-methylspiroperidol was synthesized from four different substrates: **1**, **2A**, **2B**, and **2C**. The synthetic procedure using substrate **1** (Method A) differs considerably from the synthetic procedure which uses **2A**, **2B**, or **2C** (Method B).

Method A (Synthesis of 4 from 1)

This procedure is a slight modification of the procedure used for the general synthesis of ^{18}F-labeled butyrophenones (*1*). No-carrier-added aqueous [^{18}F]fluoride (0.5 ml) prepared by the ^{18}O(p, n)^{18}F reaction (*15*) on a small volume enriched water (95–99% ^{18}O) target (*16, 17*) was added to a solution of 1.8 mg of Cs_2CO_3 in 0.1 ml of water in an open Pyrex vessel. The water was removed using a stream of nitrogen at 160° and coevaporated to dryness after adding CH_3CN (2 × 0.5 ml). Two milligrams of **1** in 0.2 ml of DMSO were added to the dried Cs[^{18}F] and the procedure described previously was followed to obtain a solution of **3** in pentane. The alkylation step was carried out using 3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (4 mg) and KI (8 mg) as described previously. After alkylation, 0.5 ml of methanol and 4 ml of 2 *N* HCl were added to the crude reaction mixture and the solution passed through a C18 SEP-PAK cartridge. The cartridge was washed with water (5 ml) and pentane (5 ml), and the washes were discarded. The crude product was eluted with 4 ml of CH_2Cl_2, which was filtered through a K_2CO_3 drying tube. The solvent was evaporated and the residue was dissolved in 0.5 ml of CH_3OH and 0.5 ml of H_2O for preparative HPLC purification. The radiochemical yield of **4** synthesized by this method was 10–15% (based on total [^{18}F]fluoride delivered from the target) in a synthesis time of 120 min from EOB. The total mass of the product was 2–5 nmol as determined by the uv absorbance of the radioactive peak compared with a standard solution of *N*-methylspiroperidol. Thus, from 600 mCi of ^{18}F, 60–90 mCi of **4** is obtained with the specific activity of 12–30 Ci/μmol at EOB, representing a ^{19}F:^{18}F ratio in the range of 57–143 at EOB.

Method B (Synthesis of 4 from 2A, 2B or 2C)

A solution of 1–2 mg of **2A**, **2B**, or **2C** in 0.2 ml DMSO was added to the dried Cs [^{18}F] prepared as described above in a platinum vessel and the vessel covered. This solution was heated at 160° for **2A** (and 140° for **2B** and **2C**) for 10 min and cooled to room temperature. Two milliliters of a CH_3OH:HCl solution (CH_3OH:conc. HCl, 1:1) were then added. The mixture was heated at 110° for 5 min. Three milliliters of water were added and the mixture transferred onto a C_{18} SEP-PAK cartridge which had been prewashed with 3 ml of methanol followed by 4 ml of water. The SEP-PAK cartridge was washed with 4 ml of water and 0.5 ml of pentane, and the washing discarded. The product (γ-chloro-*p*-[^{18}F]fluorobutyrophenone) was eluted with 5 ml of pentane which was filtered through anhydrous K_2CO_3. The amine (3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, 3 mg) and KI (5–10 mg) were added to the dry pentane. A heating bath (140°) was applied and when the volume of the pentane was reduced to ~0.2 ml, 0.5 ml of a 1:10 solution of DMF:THF was added and the mixture was heated for 10 min after THF had evaporated. Methanol (0.5 ml) was added and the alkylation mixture was worked up as described in Method A and purified by preparative HPLC. Specific activity was determined as described above. In the synthesis of **4** from substrate **2A**, 4-nitro-*N*-methylspiroperidol (**5**) is produced and well separated from **4** using the HPLC system described (retention times are 16 and 24 min, respectively). Radiochemical yield of **4** using **2A**, **2B**, and **2C** are 10–15% at EOB. Synthesis times are 90 min. The use of substrate **2A** gives a mass of <2 nmol and a specific activity of >10 Ci/μmol (EOB), while the use of substrates **2B** and **2C** gives a mass of 20–70 nmol and a specific activity of ~1 Ci/μmol (EOB). Radiochemical purity was >98% as determined by radio TLC in two solvent systems and by

TABLE 1
Effect of Amount of Cs_2CO_3, Length of Irradiation, and Vessel Material on Yield of *p*-[^{18}F]Fluorobenzonitrile from 1

Run	Length of irradiation (15 μA × min)	Vessel material	Cs_2CO_3 (mg)	Yield*
1	30	Pt	2	63
2	60	Pt	2	57
3	90	Pt	2	62
4	72†	Pt	1	31
5	72†	Pt	2	48
6	3‡	Vitreous C	1	34
7	3‡	Quartz	1	49
8	3‡	Platinum	1	41
9	3‡	Pyrex	1	49

* Each value represents average of more than three experiments.

† ^{18}F-labeled fluoride solution was divided into two portions so that direct comparison of influence of amount of Cs_2CO_3 could be made.

‡ ^{18}F-labeled fluoride solution was divided into four portions for direct yield comparison.

TABLE 2
Radiochemical Yields of Cyclopropyl *p*-[^{18}F]Fluorophenyl Ketone from NO_2 Compared with $(CH_3)_3N^+$ as Leaving Groups at Different Temperatures and Vessel Composition*

Run	Substrate	Vessel composition	Temperature (°C)	Yield (%)†
1	**2A**	Pt	130	17
2	**2A**	Pt	140	30
3	**2A**	Pt	150	60
4	**2A**	Pt	160	65
5	**2A**	Pyrex	160	34
6	**2B**	Pyrex	110	29
7	**2B**	Pyrex	130	26
8	**2B**	Pyrex	140	29
9	**2B**	Pyrex	150	21
10	**2C**	Pyrex	80	2
11	**2C**	Pyrex	100	12
12	**2C**	Pyrex	110	36
13	**2C**	Pyrex	120	41
14	**2C**	Pyrex	130	58
15	**2C**	Pyrex	140	51

* Reactions were run in DMSO. Reaction time: 10 min. Substrate concentration: 4.6×10^{-2} mol l^{-1}.
† Percentage of activity isolated in product, corrected for decay. Each value represents average of more than three experiments.

HPLC using both a normal phase silica gel column eluting with CH_2Cl_2:CH_3OH (80:20) and a reversed phase C_{18} column eluting either with CH_3OH:0.01*M* $(NH_4)_2HPO_4$ (70:30) or with CH_3OH:0.01*M* NH_4HCO_2 (65:35). No other radioactive peaks were observed on TLC or HPLC and all of the radioactivity was observed to co-elute with authentic compound **4** which were co-injected (or co-spotted) with samples of the ^{18}F-labeled product.

Synthesis of 4 from 5

A solution of 1–2 mg of **5** in 0.2 ml of DMSO was added to the dried Cs [^{18}F] prepared in a platinum vessel as described above and the vessel covered. The solution was heated at 140–150° for 20 min, cooled to room temperature and then 3 ml of water was added. The mixture was transferred onto a C_{18} SEP-PAK cartridge, washed with 2 ml of water and 4 ml of pentane and the washing was discarded. The crude product was eluted with 4 ml of CH_2Cl_2. The solvent was evaporated and the residue was dissolved in 0.5 ml of CH_3OH and 0.5 ml of H_2O for preparative HPLC purification. The radiochemical yield of **4** from **5** is ~0.5% (EOB). The carrier-added **4** can be synthesized from **6** by ^{19}F-^{18}F exchange method in ~8% radiochemical yield (EOB).

Tissue Distribution of ^{18}F-Labeled *N*-Methylspiroperidol (4) in Mice

Female albino mice (BNL strain), 21–30 g, were injected in a lateral tail vein with 49–63 μCi of ^{18}F-labeled *N*-methylspiroperidol dissolved in 100 μl of a solution which contained 0.2% $(HN_4)_2HPO_4$ made isotonic with NaCl. The mice were killed at 5, 60, or 120 min after injection. The dissected tissues were blotted to remove adhering blood and placed in tared counting vials. A sample of blood was obtained from the trunk immediately after killing. Small intestines were stripped of their contents before counting. The entire tail was counted to verify the patency of the tail vein injection. All samples were counted and weighed, and the activity expressed as % injected dose per organ or % injected dose per g of tissue.

RESULTS AND DISCUSSION

Factors Influencing Yield of Nucleophilic Aromatic Substitution Reaction

During the initial phases of studying the nucleophilic aromatic substitution reaction, lack of reproducibility in the yield of displacement step was observed with lower yields being associated with longer irradiation times. While the factors responsible for this reduction of yield were not determined, the problem could be circumvented by increasing the amount of Cs_2CO_3 used in the displacement step. This is shown in Table 1 where a single batch of [$^{18}F^-$]/$H_2{}^{18}O$ (produced using a 72-min cyclotron irradiation) was divided into two portions (runs 4 and 5) and the displacement yield determined using two different amounts of Cs_2CO_3. Here a twofold increase in Cs_2CO_3 increased the yield by >50%. As can be seen by runs 1, 2, and 3, a consistently high displacement yield is obtained for long irradiation times using this amount of Cs_2CO_3.

In another series of experiments (runs 6–9) from the same batch of [$^{18}F^-$]/$H_2{}^{18}O$, it was found that the displacement yield with substrate **1** is slightly higher using a Pyrex or quartz vessel relative to a platinum vessel and very poor using a vitreous carbon vessel. This is contrasted to the displacement yield with substrate **2A** which is consistently 50% lower in a Pyrex vessel compared to a platinum vessel (Table 2, runs 4 and 5). What should be noted here is that the conditions for optimizing the displacement yield for one substrate cannot be generalized to all substrates.

TABLE 3
Influence of Cyclopropyl *p*-Nitrophenyl Ketone Concentration on Yield of Cyclopropyl *p*-[^{18}F]Fluorophenyl Ketone*

Substrate concentration (μ*M*)	Radiochemical yield (%)†
9.3	67
4.6	64
1.9	55

* All reactions were carried out at 160° in platinum crucible.
† Percentage of activity isolated in product, corrected for decay.

TABLE 4
Comparison of Mass of *N*-Methylspiroperidol with Mass of Fluoride Present in ^{18}F-Precursor, and Specific Activity of ^{18}F-Labeled *N*-Methylspiroperidol

Substrate	$^{19}F^- + {}^{18}F^-$ (nmol)*	[^{18}F] **4** (nmol)†	Specific activity of **4** (Ci/μmol) at EOB‡
1	20	2–5	12–30
2A	10	<2	30
2B	—	20–70	0.9–3.0
2C	3	20–70	0.3–3.0

* Determined by ion chromatography.
† Determined by comparison of uv absorbance of material eluting from HPLC with same retention time as authentic sample of *N*-methylspiroperidol of known concentration.
‡ Based on 600 mCi of ^{18}F produced at EOB.

In addition to the influence of Cs_2CO_3 and vessel material, the temperature for the nucleophilic substitution reactions has significant effect on the radiochemical yield (see Table 2). The optimal temperatures for the displacement on compounds **2A**, **2B**, and **2C** were 160°C, 110°C, and 130°C, respectively. Yields of ~60% of cyclopropyl *p*-[^{18}F]fluorophenyl ketone were obtained from **2A** and **2C** whereas yields of <30% were obtained from **2B**. This supports our previous observation that the trimethylammonium perchlorate group is a better leaving group than NO_2 in the nucleophilic aromatic substitution reaction (*18*). On the other hand, in substrate **2B**, where the leaving group is trimethylammonium iodide, yields are lower. With **2A**, a fourfold decrease in the concentration of the substrate only slightly lowered the radiochemical yields of the nucleophilic aromatic substitution step (Table 3). This makes it possible to use less mass in the synthesis and results in a more easily purified product mixture.

Comparison of Synthesis of ^{18}F-Labeled *N*-Methylspiroperidol (4) from 1, 2A, 2B and 2C

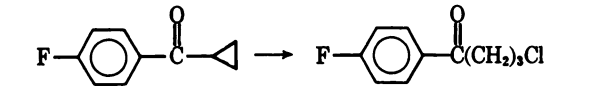
Four criteria—radiochemical yield, experimental simplicity, synthesis time and specific activity—were used in comparing the synthesis of **4** from substrates **1**, **2A**, **2B**, and **2C**. With respect to radiochemical yield, comparable yields (10–15%) were obtained from each substrate. On the other hand, the experimental procedure using **2A–C** was far simpler than that for **1** and the relative synthesis times (90 min compared with 120 min) were different. By far, the most striking difference in the syntheses was the specific activity of the products. Here, the fluoride content of the irradiated $H_2{}^{18}O$ target used in each synthesis was determined using ion chromatography and this was compared to the amount of *N*-methylspiroperidol (or a compound(s) which is chromatographically indistinguishable from *N*-methylspiroperidol) in the final product as determined by HPLC (Table 4). While substrates **1** and **2A** gave 2–5 nmol of ^{18}F-labeled **4**, substrates **2B** and **2C** gave 20–70 nmol of a compound having an HPLC retention time identical to **4**. The identity of this material is not known since the substrate used in the displacement reaction did not contain appreciable $^{19}F^-$ or cyclopropyl *p*-fluorophenyl ketone (a precursor in the substrate synthesis); it is probably not *N*-methylspiroperidol. The production of this unidentified material was not predicted. In fact, the original reason for using the trimethylammonium group, in addition to its superiority as a leaving group at low temperatures (Table 2), was the prediction that the removal of substrate and purification of product would be facilitated. Irrespective of the identity of this contaminating material, the use of this substrate gave an unacceptably high amount of mass associated with the product and did not offer any advantage in terms of product yield.

The effects of temperature, reaction times, and acid concentration on the conversion of cyclopropyl *p*-[^{18}F]fluorophenyl ketone to γ-chloro-*p*-[^{18}F]fluorobutyrophenone (**3**) were also investigated (Table 5). The optimal conditions for this reaction were 110°C for 3–5 min which gave compound **3** in ~85% radiochemical yield.

The alkylation reaction remains the weakest step in terms of yield (30%). As reported previously (*1*), one factor responsible for the lower yield is cyclization of **3** to cyclopropyl *p*-[^{18}F]fluorophenyl ketone. Although formation of the ethylene ketal derivative of **3** has been used to increase the yield of the alkylation reaction (*19*), the implementation of such a strategy with ^{18}F would add considerable time to the synthesis. Although no systematic investigation directed toward optimizing this step has been undertaken, the possibility of raising the yield of this step certainly merits further experimental effort.

Based on the above observations, the use of **2A** opti-

TABLE 5
Effects of Temperature, Reaction Time, and Acid Concentration on Conversion of Cyclopropyl *p*-Fluorophenyl Ketone to γ-Chloro-*p*-Fluorobutyrophenone



Temperature (°C)	Time (min)	Conc. HCl–MeOH	Yield (%)
80	5	1:1	65.2
80	10	1:1	75.2
80	20	1:1	84.0
100	5	1:1	83.2
100	10	1:1	82.2
110	3	1:1	84.9
110	5	1:1	83.1
110	10	1:1	77.9
110	20	1:1	65.4
110	3	1:3	21.2

TABLE 6
Tissue Distribution of [^{18}F]-*N*-Methylspiroperidol in Mice
[Time After Injection (min)]

Tissue	5*		60*		120†	
	% Dose/g	% Dose/organ	% Dose/g	% Dose/organ	% Dose/g	% Dose/organ
Brain	2.3 (2.2–2.4)	1.1 (1.0–1.1)	1.5 (1.3–1.8)	0.68 (0.60–0.74)	1.3 (1.1–1.6)	0.57 (0.43–0.68)
Blood	1.2 (1.0–1.5)		0.45 (0.40–0.50)		0.19 (0.15–0.27)	
Heart	3.3 (3.2–3.4)	0.37 (0.35–0.42)	0.61 (0.59–0.65)	0.072 (0.064–0.076)	0.24 (0.18–0.35)	0.026 (0.020–0.030)
Lungs	13 (11–14)	2.0 (2.0–2.1)	1.9 (1.6–2.3)	0.27 (0.24–0.29)	0.95 (0.70–1.50)	0.12 (0.09–0.16)
Liver	5.4 (5.2–5.7)	8.2 (8.2–8.3)	2.9 (2.4–3.4)	4.0 (3.8–4.1)	1.4 (1.2–1.9)	1.6 (1.5–1.8)
Spleen	5.8 (4.7–6.5)	0.95 (0.82–1.05)	1.6 (1.1–1.9)	0.26 (0.25–0.28)	0.57 (0.40–0.73)	0.062 (0.043–0.073)
Kidneys	12 (11–13)	4.5 (4.2–4.8)	3.4 (3.0–4.0)	1.2 (1.1–1.3)	1.5 (1.2–1.8)	0.46 (0.40–0.57)
Small intestine	4.3 (4.0–4.8)	5.3 (3.2–6.8)	2.5 (1.7–3.4)	2.9 (1.6–3.6)	1.3 (0.8–1.7)	1.3 (0.7–1.9)
Ovaries		0.12‡ (0.11–0.12)		0.040 (0.028–0.057)		0.014 (0.009–0.022)

* Mean (range) of three mice.
† Mean (range) of four mice.
‡ Mean (individual values) of two mice.

mizes specific activity and ease of synthesis. It is effectively a one-pot synthesis for the Cs[^{18}F]displacement and hydrolysis. Two C18 SEP-PAK cartridges are used. HPLC purification is straightforward with 4-nitro-*N*-methylspiroperidol (**5**), a by-product of the reaction being well-separated from *N*-methylspiroperidol using a C18 semipreparative column.

A potential one-step synthesis of **4** from **5** was attempted and gave unacceptably low yields and yielded a complicated reaction mixture. Work is in progress to explore a variety of experimental conditions in an attempt to cleanly effect the synthesis of **4** from **5**.

Tissue Distribution

Table 6 shows the distribution of radioactivity in various mouse tissues at 5, 60, and 120 min after injecting ^{18}F-labeled *N*-methylspiroperidol (**4**). As reported previously for the rat (*7, 8*), the mouse brain uptake of radioactivity was higher for radiolabeled *N*-methylspiroperidol (1.1% of the administered dose) than for radiolabeled spiroperidol (0.5% of the administered dose) (*20*). A comparison of the mouse tissue distribution of radioactivity following injection of ^{18}F-labeled *N*-methylspiroperidol (**4**) (Table 6) with the radioactivity tissue distribution reported for *N*-[^{11}C]methylspiroperidol (*9*) shows very little difference. This is an interesting observation, since Soudijn et al. (*21*) have shown for spiroperidol and several related butyrophenones that the initial step in the metabolic transformation of these compounds is the oxidative *N*-dealkylation of the parent butyrophenone, yielding the acidic β-(4-fluorobenzoyl)propionic acid and the basic amine portion of the parent structure. If this route of metabolism is correct for *N*-methylspiroperidol, then *N*-[^{11}C]methyl-

spiroperidol, with the radiolabel on the amine portion of the molecule, might be expected to show a radioactivity distribution in vivo which is markedly different from that of ^{18}F-labeled *N*-methylspiroperidol, which is radiolabeled on the β-(4-fluorobenzoyl)propionic acid portion. In support of this metabolic pathway, a comparative study of this compound radiolabeled with ^{18}F or ^{11}C in a baboon has demonstrated a significantly greater in vivo production of radioactive acidic metabolites in plasma, following administration of the ^{18}F-labeled compound (Wolf AP, unpublished data). The fact that there is no significant difference in mouse tissue distributions suggests that differences in biodistribution of radioactive acidic or basic metabolites do not have a predominant effect in the overall radioactivity biodistribution profile.

To summarize, NCA ^{18}F-labeled *N*-methylspiroperidol (**4**) has been prepared from four different substrates: *p*-nitrobenzonitrile (**1**), cyclopropyl *p*-nitrophenyl ketone (**2A**), *p*-cyclopropanoyl-*N*,*N*,*N*-trimethylanilinium iodide (**2B**), and *p*-cyclopropanoyl-*N*,*N*,*N*-trimethylanilinium perchlorate (**2C**) using the nucleophilic aromatic substitution reaction. The synthesis of **4** from **2A** maximizes the product specific activity and experimental simplicity and provides **4** in 10–15% radiochemical yield (based on [^{18}F$^-$]) with a mass of <2 nmol and a specific activity of >10 Ci/μmol (EOB). This further illustrates the generality of this synthetic method to provide access into a series of NCA ^{18}F-labeled butyrophenone neuroleptics (*1*). Since compound **4** is well separated from its nitro derivative in preparative HPLC, a far simpler synthesis of NCA ^{18}F-labeled *N*-methylspiroperidol (**4**) would be a direct substitution reaction on its nitro derivative as reported on the synthesis of NCA [^{18}F]spiroperidol (*6, 12*). The results of baboon studies (*8*) and the human studies of 3-N[^{11}C]methylspiroperidol (*10*) and compound **4** (*22*) have indicated that compound **4** may be the radioligand of choice for PET studies of the dopamine receptor in human brain.

FOOTNOTES

* Johnson, Matthey, Inc., Malvern, PA.
† Aldrich Chemical Co., Milwaukee, WI.
‡ Trans World Chemical Co., U.S.
§ Schwarzkopf Microanalytical Lab., Woodside, NY.
¶ Waters Chromatography Div., Millipor, Milford, MA.

ACKNOWLEDGMENTS

This research was carried out at Brookhaven National Laboratory under Contract DE-AC02-76CH00016 with the U.S. Department of Energy and supported by its Office of Health and Environmental Research and also supported by the National Institutes of Health Grant NS-15638. The authors thank Elinor Norton for performing the fluoride analyses and Robert MacGregor and Bruce Wieland for advice and assistance.

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