Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. 42, 1289-1300 (1999)

Synthesis of [^{18}F]FA-4 and [^{11}C]pipzA-4 as radioligands for the high affinity choline uptake system

C. Gilissen, G. Bormans, T. de Groot, A. Verbruggen*
Laboratory of Radiopharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University of Leuven, Herestraat 49, B-3000 Leuven, Belgium

* Author for all correspondence
Tel. ++32 16 343732 Fax. ++32 16 343759
E-mail alfons.verbruggen@uz.kuleuven.ac.be

SUMMARY

We have prepared two radiolabelled analogues of hemicholinium-3 (HC-3) as potential *in vivo* tracers of the sodium dependent high affinity choline uptake (SDHAChU) system. Thus, 4-[1-hydroxy-2-(4-[^{18}F]fluoromethylpiperidinyl)ethyl]-4'-[1-hydroxy-2-(4-methylpiperidinyl)ethyl]biphenyl ([^{18}F]FA-4) and 4-[1-hydroxy-2-(4-[^{11}C]methylpiperazinyl)ethyl]-4'-[1-hydroxy-2-(4-methylpiperidinyl)]biphenyl ([^{11}C]pipzA-4) have been synthesized. [^{18}F]FA-4 was prepared by reaction of 4-[^{18}F]fluoromethylpiperidine with 4-(α-bromoacetyl)-4'-(4-methylpiperidinylacetyl)-biphenyl followed by reduction of the keto groups to alcohols with $NaBH_4$. The total synthesis time was 300 minutes and [^{18}F]FA-4 was obtained with a specific activity of 5.6 GBq/μmol (EOS) and an overall decay corrected radiochemical yield of 3.1±0.6%. [^{11}C]pipzA-4 was prepared by reaction of [^{11}C]methyl triflate with 4-[1-hydroxy-2-piperazinyl)ethyl]-4'-[1-hydroxy-2-(4-methylpiperidinyl)ethyl]-biphenyl. The total synthesis time was 25 minutes and [^{11}C]pipzA-4 was obtained with a specific activity of 13.7 GBq/μmol (EOS) and an overall decay corrected radiochemical yield of 19.5±2.2%.

CCC 0362–4803/99/131289–12$17.50

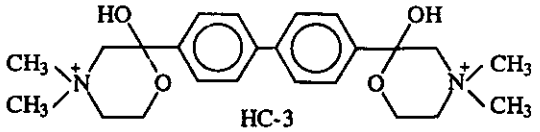
Received 28 May 1999
Accepted 24 June 1999

INTRODUCTION

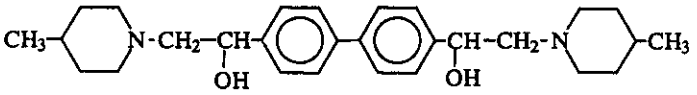
Alzheimer's disease (AD) is morphologically characterized by the presence of senile plaques and neurofibrillary tangles (1) and neurochemically by disturbances in several neurotransmitter systems (2,3). The most consistent and extensively documented neurochemical changes have been found in the cholinergic system (4). Acetylcholine synthesis proceeds via the active uptake of choline by the nerve terminal and conversion to acetylcholine by the enzyme choline acetyltransferase. Studies of choline transport have revealed the existence of a sodium dependent high affinity choline uptake (SDHAChU) system (5). The SDHAChU system, a transporter protein located on the membrane of presynaptic cholinergic terminals, was found to be the regulating and rate limiting step in the synthesis of acetylcholine (6-8). The regulation occurs by a change in the number of carrier sites in response to the demand (9). Thus, a ligand with a high affinity for the SDHAChU system would be an ideal marker for the study of the state of the cholinergic presynaptic terminals (10). The golden standard for studying the SDHAChU system *in vitro* is hemicholinium-3 (HC-3), a competitive inhibitor of this system with an affinity for the carrier which is 50 times greater than that of choline (5). Although HC-3 is very useful for *in vitro* autoradiographic analysis of the SDHAChU system it is not able to cross the blood-brain barrier (BBB) as it is a bis-quaternary ammonium salt.

We are persuing the development of a radiolabelled ligand with affinity for the SDHAChU system, which could diffuse the BBB, to enable the *in vivo* investigation of the choline uptake system. Chatterjee (11) and Sheff (12) have reported that the 4-methylpiperidine analogue of HC-3, 4,4'-bis-[1-hydroxy-2-(4-methylpiperidinyl)-ethyl]biphenyl (A-4, Fig. 1) inhibits the SDHAChU system competitively and reversibly.

A-4, a bis-tertiary amine, is potentially able to cross the BBB and a radiolabelled derivative of A-4 could therefore be a suitable tracer agent for the *in vivo* investigation of the SDHAChU system. In this paper we describe the synthesis of two radioactive derivatives of A-4, labelled with fluorine-18 and carbon-11, respectively.



HC-3



A-4

Figure 1. Structure of HC-3 and A-4

RESULTS AND DISCUSSION

Synthesis of [^{18}F]FA-4

The first approach for the synthesis of [^{18}F]FA-4 was to prepare a precursor that could provide [^{18}F]FA-4 in one step by a nucleophilic substitution reaction with [^{18}F]fluoride. Despite many attempts, we were unable to prepare such a precursor. As an example of the difficulties encountered, we were unsuccessful in transforming the hydroxyl group of 4-(4-hydroxymethylpiperidinylacetyl)-4'-(4-methylpiperidinylacetyl)biphenyl into a tosylate, a mesylate or a triflate. Attempts also failed to react 4-(*p*-toluenesulfonyloxy)methylpiperidine with 4-(α-bromoacetyl)-4'-(4-methylpiperidinylacetyl)biphenyl (**3**). As an alternative, [^{18}F]FA-4 was synthesized by reaction of 4-[^{18}F]fluoromethylpiperidine (**6***) with 4-(α-bromoacetyl)-4'-(4-methylpiperidinylacetyl)biphenyl (**3**). The bromoacetyl intermediate (**3**) was synthesized in 4 steps starting from biphenyl (Fig. 2).

The first step was a Friedel Crafts acylation with bromoacetyl bromide to yield 4,4'-bis-(α-bromoacetyl)biphenyl. One keto group was protected as a ketal followed by amination of the remaining bromoacetyl moiety with 4-methylpiperidine. In the last step, the ketal was hydrolyzed in acidic conditions to yield **3**.

4-[^{18}F]Fluoromethylpiperidine (**6***) was prepared by nucleophilic substitution with [^{18}F]fluoride on *N*-BOC-4-(*p*-toluenesulfonyloxy)methylpiperidine (**4**) (Fig. 3), which was prepared from 4-hydroxymethylpiperidine by the successive introduction of an *N*-BOC and an *O*-tosyl group. After removal of the *N*-BOC protective group, the obtained 4-[^{18}F]fluoromethylpiperidine (**6***) was reacted with **3**. The resulting

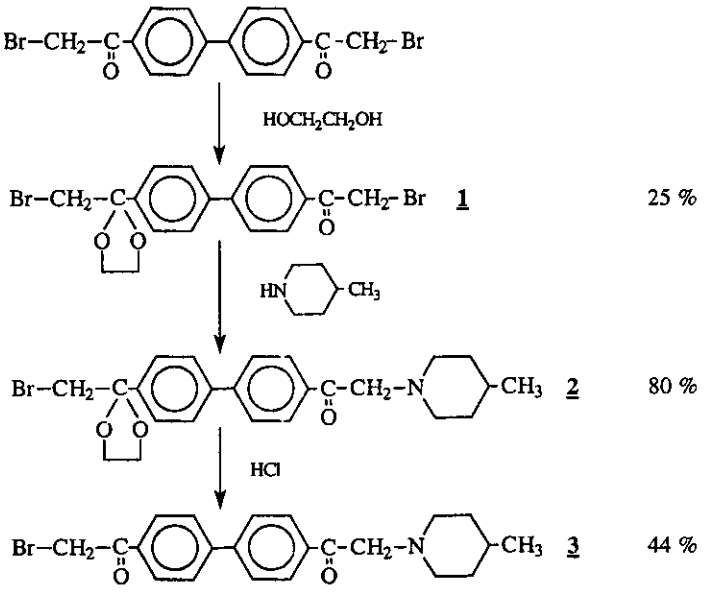


Figure 2. Synthesis of 4-(α-bromoacetyl)-4'-(4-methylpiperidinylacetyl)biphenyl

diketo intermediate (7*) was reduced to the bis-alcohol with $NaBH_4$ to yield [^{18}F]FA-4 (8*). [^{18}F]FA-4 was obtained with a specific activity of 5.6 GBq/μmol at the end of synthesis (EOS) and an overall decay corrected radiochemical yield of 3.1±0.6% in a total synthesis time of 5 hours.

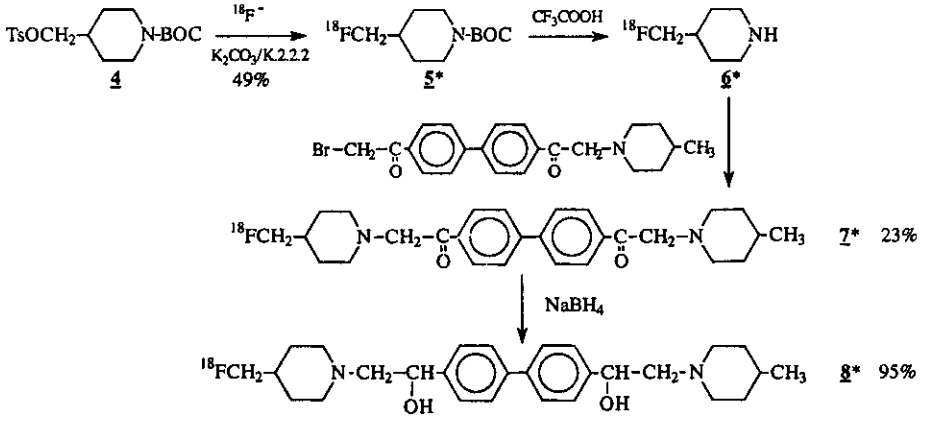


Figure 3. Synthesis of [^{18}F]FA-4

Synthesis of [^{11}C]pipzA-4

For the introduction of carbon-11, [^{11}C]methyl triflate ([^{11}C]CH_3OTf) was used. [^{11}C]CH_3OTf was trapped in THF containing the nor-compound (**12**) and was reacted for 1 minute at 70°C to yield [^{11}C]pipzA-4 that was purified by HPLC (Fig. 4). [^{11}C]pipzA-4 was obtained with a specific activity of 13.7 GBq/μmol (EOS) and an overall decay corrected radiochemical yield of 19.5±2.2% in a total synthesis time of 25 minutes.

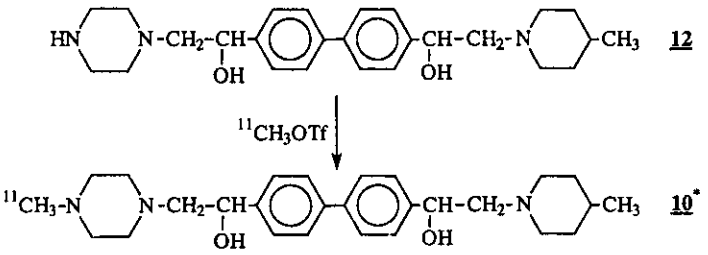


Figure 4. Synthesis of [^{11}C]pipzA-4

EXPERIMENTAL

4-Hydroxymethylpiperidine was obtained from Maybridge (Cornwall, UK) and the other chemicals were obtained either from the Aldrich Chemical Company (Milwaukee, WI) or from Acros Chimica (Geel, Belgium). Intermediates were purified by column chromatography on silica gel (Silica gel 60, Merck, Darmstadt, Germany). ^{1}H-NMR spectra were recorded on a Varian 200 MHz spectrometer (Palo Alto, CA). Chemical shifts are reported in ppm relative to TMS (δ=0). Protons of the piperazine ring of pipzA-4 are indicated with a prime. HPLC analyses and purifications of [^{18}F]FA-4 were performed on a 250 mm x 4.6 mm Hypersil BDS C18 column (Alltech, Laarne, Belgium) eluted with gradient mixtures of 0.05 M NaOAc pH=6.8 and EtOH (t=0 min: 40% EtOH, linearly increased to 70 % at t=30 min) at a flow rate of 1 ml/min. HPLC analyses and purifications of [^{11}C]pipzA-4 were performed on a 250 mm x 4.6 mm Econosphere silica column (Alltech) eluted with CH_2Cl_2/MeOH/Et_3N (95:5:0.5) at a flow rate of 1 ml/min. Melting points were determined in open capillaries immersed in an oil bath

(Electrothermal, Essex, UK) and were not corrected. [^{18}F]Fluoride was produced by irradiation of 90% enriched [^{18}O]water (Isotec, Miamisburg, OH) and [^{11}C]CO_2 was produced by irradiation of target gas (99% N_2 and 1% O_2) with 10-MeV protons using a Cyclone 10/5 cyclotron (Ion Beam Applications, Louvain-la-Neuve, Belgium).

Synthesis

4-(2-Bromomethyl-(1,3)-dioxalane)-4'-(α-bromoacetyl)biphenyl (1)

A solution of 4,4'-bis-(α-bromoacetyl)biphenyl (13) (1.0 g, 2.5 mmol), ethylene glycol (0.14 ml, 2.5 mmol) and *p*-toluenesulfonic acid hydrate (25 mg, 0.13 mmol) in 150 ml toluene was refluxed for 12 hours in a round-bottom flask connected to a Dean-Stark trap with reflux condenser. The solution was cooled to room temperature, diluted with ether and washed with saturated $NaHCO_3$ solution and water. The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography with hexane/ethyl acetate (9:1, V/V) as the eluent to yield 1 (0.28 g, 25.4 %) as a yellow powder. ^{1}H-NMR ($CDCl_3$): δ 3.7 (s, 2H, BrC*H*$_2$C(O)$_2$), 3.95 (t, 2H, OC*H*$_2$), 4.2 (t, 2H, OC*H*$_2$), 4.5 (s, 2H, BrC*H*$_2$CO), 7.6 (s, 4H, 2,6-Ar*H*), 7.7 (d, 2H, 3,5-Ar*H*), 8.1 (d, 2H, 3,5-Ar*H*).

4-(2-Bromomethyl-(1,3)-dioxalane)-4'-(4-methylpiperidinylacetyl)biphenyl (2)

To a solution of 1 (2.4 g, 5.5 mmol) in 150 ml dioxane, 4-methylpiperidine (2.6 g, 26.2 mmol) was added dropwise. The solution was refluxed for 2 hours and evaporated under reduced pressure. The residue was purified by column chromatography with CH_2Cl_2/Et_3N (99:1, V/V) as the eluent to yield 2 (2.0 g, 80%) as a yellow powder, mp 106°C. ^{1}H-NMR ($CDCl_3$): δ 0.95 (d, 3H, C*H*$_3$), 1.4 (m, 3H, 3-*H*$_{ax}$, 4-*H*$_{ax}$), 1.7 (d, 2H, 3-*H*$_{eq}$), 2.2 (t, 2H, 2-*H*$_{ax}$), 3.0 (d, 2H, 2-*H*$_{eq}$), 3.7 (s, 2H, C*H*$_2$Br), 3.8 (s, 2H, C*H*$_2$N), 3.95 (t, 2H, OC*H*$_2$), 4.2 (t, 2H, OC*H*$_2$), 7.6 (s, 4H, 2,6-Ar*H*), 7.7 (d, 2H, 3,5-Ar*H*), 8.1 (d, 2H, 3,5-Ar*H*).

4-(α-Bromoacetyl)-4'-(4-methylpiperidinylacetyl)biphenyl (3)

A solution of 2 (2.0 g, 4.4 mmol) and 6 N HCl (60 ml) in 120 ml acetone was stirred

at room temperature. After 12 hours the solution was neutralized and extracted with dichloromethane. The dichloromethane layer was washed with saturated $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography with CH_2Cl_2/MeOH/NH_4OH 2% (96:2:2, V/V) as the eluent to yield **3** (0.8 g, 44.2%) as a yellow powder, mp 128°C. ^{1}H-NMR ($CDCl_3$): δ 0.95 (d, 3H, CH_3), 1.4 (m, 3H, 3-H_{ax}, 4-H_{ax}), 1.7 (m, 2H, 3-H_{eq}), 2.2 (t, 2H, 2-H_{ax}), 3.0 (d, 2H, 2-H_{eq}), 3.8 (s, 2H, CH_2N), 4.7 (s, 2H, CH_2Br), 7.7 (2xd, 4H, 2,6-Ar*H*), 8.1 (2xd, 4H, 3,5-Ar*H*).

N-BOC-4-(p-toluenesulfonyloxy)methylpiperidine (4)

A solution of *N*-BOC-4-hydroxymethylpiperidine (14) (5.7 g, 26.5 mmol) in 30 ml dichloromethane was cooled to 0°C and triethylamine (4 ml, 29.2 mmol) was added. After addition of *p*-toluenesulfonyl chloride (5.6 g, 29.2 mmol) the solution was stirred overnight, washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography with hexane/ethyl acetate (8:2, V/V) as the eluent to yield 3.7 g (37.8%) of **4** as a white powder, mp 68°C. ^{1}H-NMR ($CDCl_3$): δ 1.1 (dq, 2H, 3-H_{ax}), 1.45 (s, 9H, 3x CCH_3), 1.65 (d, 2H, 3-H_{eq}), 1.8 (m, 1H, 4-H_{ax}), 2.4 (s, 3H, CH_3), 2.65 (t, 2H, 2-H_{ax}), 3.85 (d, 2H, CH_2OSO$_2$), 4.1 (d, 2H, 2-H_{eq}), 7.35 (d, 2H, 2,6-Ar*H*), 7.78 (d, 2H, 3,5-Ar*H*).

N-BOC-4-fluoromethylpiperidine (5)

To a solution of **4** (3.7 g, 10 mmol) in 30 ml acetonitrile was added tetraethylammonium fluoride dihydrate (2.0 g, 11 mmol), dissolved in 50 ml acetonitrile. The solution was heated at 70°C for 6 hours and concentrated under reduced pressure. The reaction product was purified by column chromatography with hexane/ethyl acetate (8:2, V/V) as the eluent to yield **5** (1.7 g, 78%) as a white powder, mp 51°C. ^{1}H-NMR ($CDCl_3$): δ 1.1 (dq, 2H, 3-H_{ax}), 1.45 (s, 9H, 3x CCH_3), 1.7 (d, 2H, 3-H_{eq}), 1.85 (m, 1H, 4-H_{ax}), 2.7 (t, 2H, 2-H_{ax}), 4.1 (d, $^3J_{HF}$=24 Hz 2H, 2-H_{eq}), 4.26 (dd, $^2J_{HF}$=48 Hz, 2H, CH_2F).

4-Fluoromethylpiperidine (6)

N-BOC-4-fluoromethylpiperidine (5, 1.7 g, 7.8 mmol) was dissolved in trifluoroacetic acid (2 ml, 25.5 mmol) and the solution was stirred at room temperature for 10 minutes. Trifluoroacetic acid was evaporated under reduced pressure to obtain a yellow oil, which was used without further purification.

4-(4-Fluoromethylpiperidinylacetyl)-4'-(4-methylpiperidinylacetyl)biphenyl (7)

A solution of 3 (600 mg, 1.45 mmol), K_2CO_3 (600 mg, 4.3 mmol) and 4-fluoromethylpiperidine (6, 500 mg, 4.3 mmol) in 100 ml dioxane was stirred overnight at 70°C. The precipitate formed was filtered off and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography with CH_2Cl_2/MeOH/NH_4OH 2% (96:2:2, V/V) as the eluent to yield 7 (300 mg, 46%) as a white solid, mp 159°C. ^{1}H-NMR ($CDCl_3$): δ 0.95 (d, 3H, C*H*$_3$), 1.4 (m, 6H, 3-*H*$_{ax}$, 4-*H*$_{ax}$), 1.7 (m, 4H, 3-*H*$_{eq}$), 2.2 (m, 4H, 2-*H*$_{ax}$), 2.95 (d, $^3J_{HF}$=24 Hz, 2H, 2-*H*$_{eq}$), 3.0 (d, $^3J_{HF}$=24 Hz, 2H, 2-*H*$_{eq}$), 3.8 (2xs, 4H, C*H*$_2$N), 4.3 (dd, $^2J_{HF}$=48 Hz, 2H, C*H*$_2$F), 7.7 (d, 4H, 2,6-Ar*H*), 8.15 (d, 4H, 3,5-Ar*H*).

4-[1-Hydroxy-2-(4-fluoromethylpiperidinyl)ethyl]-4'-[1-hydroxy-2-(4-methyl-piperidinyl)ethyl]biphenyl (FA-4; 8)

A solution of 7 (400 mg, 0.9 mmol) and $NaBH_4$ (380 mg, 10 mmol) in 35 ml ethanol was refluxed for 2 hours. The reaction mixture was neutralized with HCl 1 N and dichloromethane (50 ml) was added. The solution was extracted with saturated $NaHCO_3$ solution, the organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to yield 8 (140 mg, 35%) as a white solid, mp 156°C. ^{1}H-NMR ($CDCl_3$): δ 0.95 (d, 3H, CH_3), 1.4 (m, 6H, C3-H_{ax}, C4-H_{ax}), 1.7 (m, 4H, 3-*H*$_{eq}$), 2.05 (t, 2H, 2-*H*$_{ax}$), 2.3 (t, 2H, 2-*H*$_{ax}$), 2.5 (d, 4H, C*H*$_2$N), 2.8-3.2 (4xd, 4H, 2-*H*$_{eq}$), 4.3 (dd, 2H, $^2J_{HF}$=48 Hz, C*H*$_2$F), 4.8 (dd, 2H, C*H*$_2$OH), 7.45 (d, 4H, 2,6-Ar*H*), 7.6 (d, 4H, 3,5-Ar*H*).

4-[1-Hydroxy-2-(4-[^{18}F]fluoromethylpiperidinyl)ethyl]-4'-[1-hydroxy-2-(4-methyl-piperidinyl)ethyl]biphenyl ([^{18}F]FA-4; 8)*

After irradiation of 500 µl [^{18}O]water with 10-MeV protons for 30 minutes at a

beam current of 20 μA, the contents of the target were passed over a 4-mm diameter ion exchange membrane (AG1-X8, OH^- form, Bio-Rad, Richmond, CA) to trap [^{18}F]fluoride. After rinsing the membrane with 300 μl H_2O, the [^{18}F]fluoride was eluted with a solution of 5 mg Kryptofix 2.2.2 and 0.5 mg K_2CO_3 in 500 μl aqueous methanol (90%) (15). The solution was evaporated in a microwave oven (70°C). Next, two 200-μl aliquots of absolute ethanol were added and after each addition, the solvent was evaporated to remove traces of water. A solution of 5 mg *N*-BOC-4-(*p*-toluenesulfonyloxy)methylpiperidine (**4**) in 500 μl anhydrous acetonitrile was added to the residue containing the [^{18}F]fluoride, and the solution was heated for 5 minutes at 70°C. The reaction mixture was applied on a silica SepPak column (Waters, Milford, MA) and eluted with 6 ml dichloromethane to obtain *N*-BOC-4-[^{18}F]fluoromethylpiperidine (**5***) (yield: 48.6±8.3%). To the eluate was added 300 μl of trifluoroacetic acid and the mixture was stirred for 10 minutes at room temperature. The mixture was basified with *N,N*-diisopropylethylamine and the resulting 4-[^{18}F]fluoromethylpiperidine (**6***) was reacted with 5 mg of **3** in 0.5 ml acetonitrile at 70°C for 30 minutes, to yield fluorine-18 labelled **7** (yield: 23.2±4.2%, as determined using HPLC-analysis of the reaction mixture). The acetonitrile was evaporated and a solution of 15 mg of $NaBH_4$ in 0.5 ml ethanol was added. After stirring for 30 minutes at room temperature, 10 ml water was added and the reaction mixture was applied on a C18 light SepPak column (Waters). [^{18}F]FA-4 (**8***) was eluted with 1 ml ethanol and purified by reversed phase HPLC (overall decay corrected yield 3.1±0.6%, EOB).

*4-(1-Methylpiperazinylacetyl)-4'-(4-methylpiperidinylacetyl)biphenyl.(**9**)*

A solution of **3** (500 mg, 1.2 mmol) and 1-methylpiperazine (360 mg, 3.6 mmol) in 100 ml dioxane was refluxed for 2 hours. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography with CH_2Cl_2/MeOH/NH_4OH 2% (96:2:2, V/V) as the eluent to yield **9** (470 mg, 87%) as a yellow solid, mp 151°C. ^{1}H-NMR ($CDCl_3$): δ 0.95 (d, 3H, C*H*$_3$), 1.4 (m, 3H, 3-*H*$_{ax}$, 4-*H*$_{ax}$), 1.7 (d, 2H, 3-*H*$_{eq}$), 2.2 (t, 2H, 2-*H*$_{ax}$), 2.4 (s, 3H, C*H*$_3$'), 2.7 (m, 4H, 2-*H*'), 3.0 (d, 2H, 2-*H*$_{eq}$), 3.2 (m, 4H, 3-*H*'), 3.8 (s, 2H, C*H*$_2$N), 3.9 (s, 2H, C*H*$_2$N'), 7.7 (d, 4H, 2,6-Ar*H*), 8.15 (d, 4H, 3,5-Ar*H*).

*4-[1-Hydroxy-2-(1-methylpiperazinyl)ethyl]-4'-[1-hydroxy-2-(4-methylpiperidinyl)-ethyl]biphenyl (pipzA-4; **10**)*

A solution of **9** (400 mg, 0.9 mmol) and $NaBH_4$ (380 mg, 10 mmol) in 35 ml ethanol was refluxed for 2 hours. The reaction mixture was neutralized with HCl 1 N and dichloromethane (50 ml) was added. The solution was extracted with saturated $NaHCO_3$ solution, the organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography with CH_2Cl_2/MeOH/NH_4OH 2% (93:5:2, V/V) as the eluent to yield **10** (240 mg, 59%) as a white solid, mp 160°C. ^{1}H-NMR ($CDCl_3$): δ 0.95 (d, 3H, C*H*$_3$), 1.4 (m, 3H, 3-*H*$_{ax}$, 4-*H*$_{ax}$), 1.7 (m, 2H, 3-*H*$_{eq}$), 2.05 (t, 1H, 2-*H*$_{ax}$), 2.3 (t, 1H, 2-*H*$_{ax}$), 2.35 (s, 3H, C*H*$_3$), 2.5 (m, 10H, 2xC*H*$_2$N, 2-*H*' 4H, 3-*H*' 2H), 2.8 (d, 3H, 2-*H*$_{eq}$, 3-*H*'), 3.2 (d, 1H, 2-*H*$_{eq}$), 4.0 (br s, 2H, O*H*), 4.8 (dd, 2H, C*H*OH), 7.45 (d, 4H, 2,6-Ar*H*), 7.6 (d, 4H, 3,5-Ar*H*).

*4-(1-Piperazinylacetyl)-4'-(4-methylpiperidinylacetyl)biphenyl (**11**)*

A solution of **3** (500 mg, 1.2 mmol) and piperazine (4 g, 46.4 mmol) in 400 ml dioxane was stirred for 2 hours at room temperature. After evaporation of the solvent, the residue was purified by column chromatography with CH_2Cl_2/MeOH/NH_4OH 2% (93:5:2, V/V) as the eluent to yield **11** (250 mg, 48%) as a yellow solid. ^{1}H-NMR ($CDCl_3$): δ 0.95 (d, 3H, C*H*$_3$), 1.4 (m, 3H, 3-*H*$_{ax}$, 4-*H*$_{ax}$), 1.65 (d, 2H, 3-*H*$_{eq}$), 2.2 (t, 2H, 2-*H*$_{ax}$), 2.6 (m, 4H, 2-*H*'), 3.0 (m, 6H, 2-*H*$_{eq}$, 3-*H*'), 3.3 (s, 2H, C*H*$_2$N'), 3.8 (s, 2H, C*H*$_2$N), 7.7 (d, 4H, 2,6-Ar*H*), 8.1 (d, 4H, 3,5-Ar*H*).

*4-[1-Hydroxy-2-(1-piperazinyl)ethyl]-4'-[1-hydroxy-2-(4-methylpiperidinyl)ethyl]-biphenyl (**12**)*

A solution of **11** (250 mg, 0.6 mmol) and $NaBH_4$ (230 mg, 6.1 mmol) in 35 ml ethanol was refluxed for 2 hours. The reaction mixture was neutralized with HCl 1 N and dichloromethane (50 ml) was added. The solution was extracted with saturated $NaHCO_3$ solution, the organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography with CH_2Cl_2/MeOH/NH_4OH 2% (93:5:2, V/V) as the eluent to

yield **12** (120 mg, 46%) as a white solid, mp 147°C. ^{1}H-NMR ($CDCl_3$): δ 0.95 (d, 3H, C*H*$_3$), 1.3 (m, 3H, 3-*H*$_{ax}$, 4-*H*$_{ax}$), 1.6 (m, 2H, 3-*H*$_{eq}$), 2.05 (t, 1H, 2-*H*$_{ax}$), 2.3 (t, 1H, 2-*H*$_{ax}$), 2.5 (m, 8H, 2xC*H*$_2$N, 2-*H'*), 2.8 (m, 3H, 2-*H*$_{eq}$, 3-*H'*), 2.95 (d, 2H, 3-*H'*), 3.2 (d, 1H, 2-*H*$_{eq}$), 4.8 (dd, 2H, C*H*OH), 7.4 (d, 4H, 2,6-Ar*H*), 7.6 (d, 4H, 3,5-Ar*H*).

*4-[1-Hydroxy-2-(4-[^{11}C]methylpiperazinyl)ethyl]-4'-[1-hydroxy-2-(4-methylpiperidinyl)ethyl]biphenyl ([^{11}C]pipzA-4, **10***)*

After irradiation of the target gas (99% N_2 and 1% O_2) with 10-MeV protons for 30 minutes at a beam current of 20 μA, [^{11}C]CO_2 was reduced to [^{11}C]CH_4 with H_2 in the presence of Ni. [^{11}C]CH_4 was reacted with I_2 to yield [^{11}C]CH_3I, that was converted to [^{11}C]CH_3OTf (16). The volatile [^{11}C]CH_3OTf was trapped in 1 ml THF containing 1 mg of **12** and the solution was heated for 1 min at 70°C. The reaction mixture was purified by HPLC. The total synthesis time was 25 minutes and [^{11}C]pipzA-4 was obtained with a specific activity of 13.7 GBq/μmol (EOS) and an overall decay corrected yield of 19.5±2.2% (EOB).

CONCLUSION

[^{18}F]FA-4, a fluorine-18 labelled derivative of A-4 could not be obtained by nucleophilic substitution of a suitable precursor with [^{18}F]fluoride. The successful preparation of [^{18}F]FA-4 required the synthesis of 4-[^{18}F]fluoromethylpiperidine (**6***) as a key intermediate. Despite the long synthesis time (5 h) and the low overall radiochemical yield (3.1%, EOB), sufficient amounts could be prepared for preliminary biological evaluation.

On the other hand, [^{11}C]pipzA-4, the carbon-11 labelled derivative of A-4 could be obtained in one step by the alkylation of the nor-compound (**12**) with [^{11}C]CH_3OTf. The total synthesis was time was 25 minutes (overal yield 19.5%, EOB). The biological evaluation of [^{18}F]FA-4 and [^{11}C]pipzA-4 will be the subject of a separate paper.

REFERENCES

1. Coyle J.T., Price D.L. and DeLong M.R. *Science.* 219: 1184-1190 (1983)
2. Reisine T.D., Yamamura H.I., Bird E.D., Spokes E. and Enna S.J. *Brain Res.* 159: 477-481 (1978)
3. Engelborghs S. and De Deyn P.P. *Acta Neurol. Belg.* 97: 67-84 (1997)
4. Bartus R.T., Dean R.L., Beer B. and Lippa A.S. *Science.* 217: 408-417 (1982)
5. Sandberg K. and Coyle J.T. *Brain Res.* 348: 321-330 (1985)
6. Simon R., Atweh S. and Kuhar M.J. *J. Neurochem.* 26: 909-922 (1976)
7. Yufu F., Egashira T. and Yamanaka Y. *Jpn. J. Pharmacol.* 66: 247-255 (1994)
8. Kristofikova Z., Fales E., Majer E. and Klaschka J. *Exp. Gerontal.* 30: 125-136 (1995)
9. Happe H.K. and Murrin L.C. *J. Neurochem.* 60: 1191-1201 (1993)
10. Pascual J., Fontán A., Zarranz J., Berciano J., Flórez J. and Pazos A. *Brain Res.* 552: 170-174 (1991)
11. Chatterjee T.K., Long J.P., Cannon J.G. and Bhatnagar R.K. *Eur. J. Pharmacol.* 149: 241-248 (1988)
12. Sheff K.Y., Yorek M.A. and Long J.P. *J. Pharmacol. Exp. Ther.* 255: 357-363 (1990)
13. Long P.J.and Schueler F.W. *J. Am. Pharm. Assoc.* 43: 79-86 (1954)
14. Waterhouse R.N., Collier T.L. and O'Brien J.C. *J. Labelled Cpd. Radiopharm.* 38: 595-605 (1996)
15. Jewett D.M., Toorongian S.A., Bachelor M.A. and Kilbourn M.R. *Appl. Radiat. Isot.* 41: 583-586 (1990)
16. Jewett D.M. *Appl. Radiat Isot.* 43: 1383-1385 (1992)

