Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **44**, 113-120 (2001)

PRECURSOR SYNTHESIS AND RADIOLABELLING OF [^{11}C]ADAM: A POTENTIAL RADIOLIGAND FOR THE SEROTONIN TRANSPORTER EXPLORATION BY PET

Johnny VERCOUILLIE [1], Jari TARKIAINEN[2], Christer HALLDIN[2], Patrick EMOND[1], Sylvie CHALON[1], Johan SANDELL[2], Oliver LANGER[2] and Denis GUILLOTEAU[1]*

[1]INSERM U316 - Laboratoire de Biophysique Médicale et Pharmaceutique - Faculté des Sciences Pharmaceutiques - Université François Rabelais - 31 avenue Monge, F-37200 Tours, France

[2]Karolinska Institutet - Department of Clinical Neuroscience - Psychiatry Section Karolinska Hospital - S-17176 Stockholm, Sweden

SUMMARY

The serotoninergic system is involved in a variety of neurological and psychiatric disorders. Exploration of the serotonin transporters (5-HTT) in living human brain by PET would be of great value for better understanding, diagnosis and therapeutic follow up of these diseases. In order to obtain a selective radioligand to explore the 5-HTT by PET we report the synthesis of [^{11}C]N,N-dimethyl-2-(2-amino-4-iodophenylthio)-benzylamine ([^{11}C]ADAM). The precursor for labelling N-demethyl ADAM, was obtained in five steps using 2,5-dibromonitrobenzene and 2-thio-N-methylbenzamide as starting material. [^{11}C]ADAM was synthesised by N-alkylation of the precursor using [^{11}C]methyl iodide in DMF. The incorporation yield of [^{11}C]methyl iodide was in the range of 50 to 70%. Finally [^{11}C]ADAM was obtained in 30 minutes synthesis time including HPLC and with a radiochemical purity better than 99%.

Key Words : N,N-dimethyl-2-(2-amino-4-iodophenylthio)benzylamine, serotonin transporter, carbon-11, PET

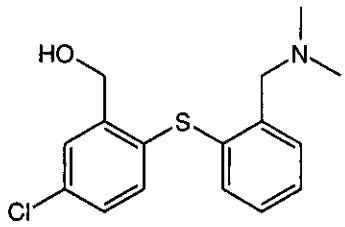
**To whom correspondence should be addressed.*

Received 16 August 2000
Revised 22 September 2000
Accepted 1 October 2000

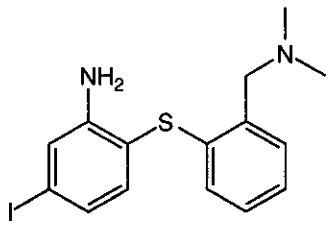
INTRODUCTION

Neuronal uptake of serotonin (5-HT) occurs via the serotonin transporter (5-HTT) which plays a major role in the regulation of synaptic 5-HT levels and is involved in neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (1, 2). However, the precise role of the 5-HTT in these neurologic disorders remains to be clarified. For this reason, *in vivo* examination of 5-HTT by scintigraphy such as positron emission tomography (PET) would be of great value for understanding mechanisms involved in neurodegenerative and mental illness and helping for the diagnosis and therapeutic follow up of these diseases. Although several compounds such as cocaine derivatives (3-6) and McN5625 (7, 8) have been synthesised for this exploration none is today optimal for imaging the 5-HTT in human brain, due to lack of specificity or unsuitable pharmacokinetic properties.

Recently, a 5-chloro-2((2-((dimethylamino)methyl)phenyl)thio)benzyl alcohol named 403U76 has been proposed as a novel antidepressant agent (9). 403U76 inhibits the monoamine uptake in rat brain synaptosomes with K_i = 2.1 and 55 nM for 5-HT and norepinephrine respectively.



403U76



ADAM

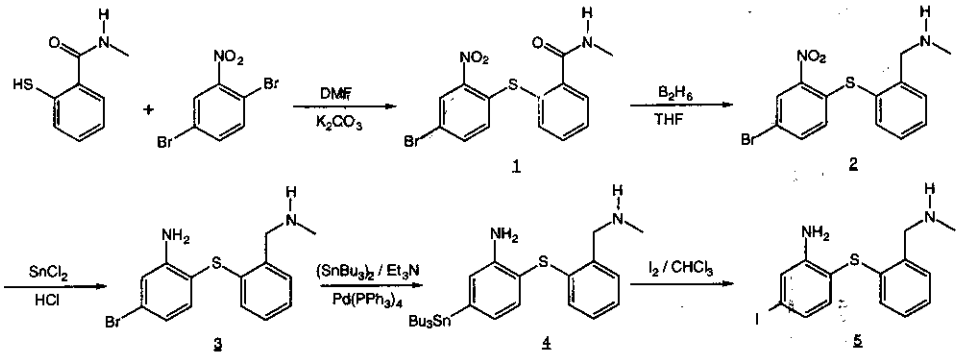
In order to develop a tracer for the SPECT exploration of the 5-HTT, Oya *et al* have described a 403U76 derivative named ADAM (10). This compound exhibited *in vitro* a high affinity and specificity for the 5-HTT (Ki = 0.013, 699 and 840 nM for 5-HTT, norepinephrine and dopamine transporter respectively). *In vivo* in rats, ADAM presented a high, rapid and specific accumulation in regions rich in 5-HTT such as hippocampus, cortex and hypothalamus. Results obtained from a preliminary study on [^{123}I]ADAM biodistribution in non human primate suggests that this compound may be useful for single photon emission computed tomography (SPECT) imaging of 5-HTT in the human brain (10). Based on these results, we could hypothesise that the chemical structure of ADAM labelled with ^{11}C would allow exploration of the 5-HTT by PET. In

order to obtain this radioligand, we report the radiosynthesis of [^{11}C]ADAM starting from its N-demethyl precursor. We prepared this precursor in a five step synthesis.

RESULTS AND DISCUSSION

Chemistry

The N-demethyl precursor of ADAM (5) was prepared as outlined in Scheme 1.

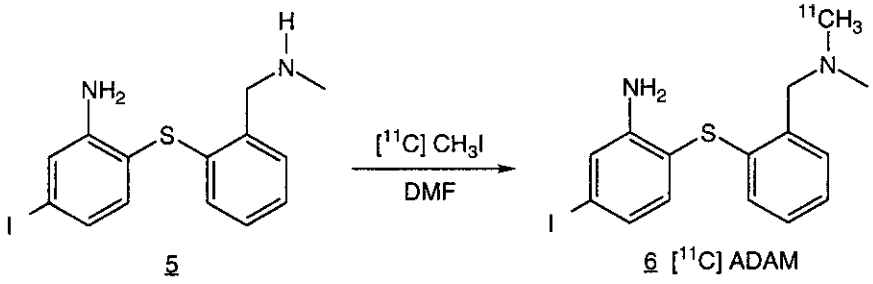


Scheme 1. Synthesis of the N-demethyl precursor of ADAM (5).

The first step involved the reaction between 2,5-dibromonitrobenzene (commercially available) and 2-thio-N-methylbenzamide prepared using literature methods (11, 12, 13). Compound 1 was obtained after purification by flash chromatography (EtOAc) in 95% yield. Reduction of the amide function of 1 was carried out using diborane in THF to afford the corresponding benzylamine 2 in 60% yield. The nitro group of compound 2 was reduced by $SnCl_2$ in hydrochloric acid affording compound 3 in 51% yield which was converted in 57% yield to its *n*-tributyltin analogue 4 using tetrakis (triphenylphosphine) palladium(0) as catalyst. The tin derivative 4 was finally converted to its iodinated analogue 5 in 78% yield. Unlaballed ADAM which was used as standard for HPLC control after radiolabelling was prepared as previously described (10).

Radiochemistry

The preparation of [^{11}C]ADAM (6) was based on an N-methylation reaction of its N-demethyl precursor (5) using [^{11}C]methyl iodide in DMF (Scheme 2). The incorporation of [^{11}C]methyl iodide was in the range of 50-70% using 0.5 mg of the N-demethyl precursor.



5 6 [^{11}C] ADAM

Scheme 2. Synthesis of [^{11}C]ADAM (6) from its N-demethyl precursor (5).

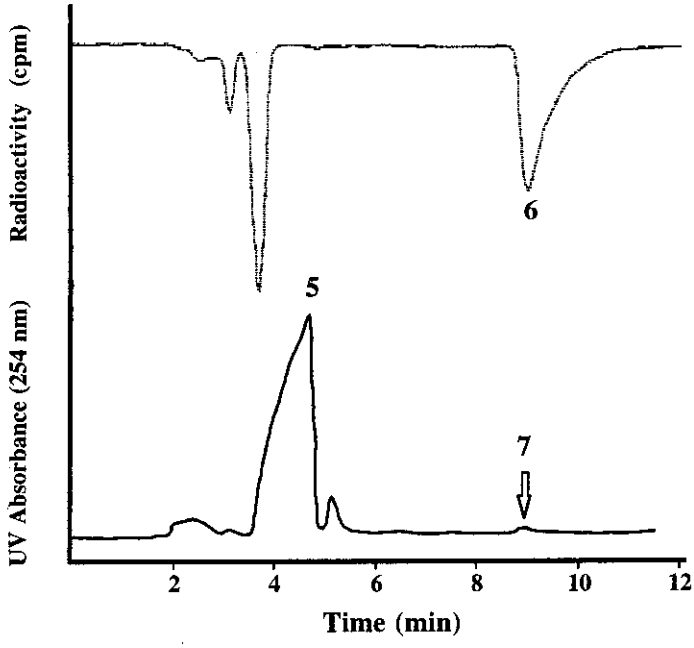


Figure 4. Semipreparative HPLC chromatogram (U.V. and radioactivity versus time) for the preparation of [^{11}C]ADAM (6). Also represented is unlabelled ADAM (7) and its demethyl precursor (5).

EXPERIMENTAL

Chemistry

NMR spectra were recorded on a Bruker DPX Avance 200 spectrometer (200 MHz for ^{1}H, 50.3 MHz for ^{13}C). $CDCl_3$ was used as solvent; chemical shifts are expressed in ppm relative to TMS as an internal standard. Mass spectra were obtained on a CG-MS Hewlett Packard 5989A spectrometer (electronic impact at 70 eV). The thin-layer chromatographic (TLC) analyses were performed using Merck 60F-254 silica gel plates. Flash chromatography was used for routine purification of reaction products using silica gel (230-400 Mesh). Visualisation was accomplished under UV or in an iodine chamber. All chemicals and solvents were of commercial quality and were purified following standard procedures.

N-methyl-2-(4-bromo-2-nitrophenylthio)benzamide (1). A mixture of 2,5-dibromonitrobenzene (1.03 g, 3.67 mmol), N-methyl-2-thiobenzamide (613 mg, 3.67 mmol) and K_2CO_3 (507 mg, 3.67 mmol) in DMF (10 mL) was stirred at 80 °C for 6 hr. The solution was then poured into cold water (20 mL) and extracted with $CHCl_3$ (2x30 mL). The combined organic fractions were dried, filtered and evaporated to give a brown oil. After flash-chromatography (EtOAc), 1.28 g of pure compound 1 was obtained (95%). ^{1}H NMR : δ = 2.82 (d, 3H, ^{3}J=4.9 Hz), 6.12 (broad s, 1H), 6.71 (d, 1H, ^{3}J=8.7 Hz), 7.39 (dd, 1H, ^{3}J=8.7 Hz, ^{4}J=2.2 Hz), 7.46-7.56 (m, 3H), 7.65 (m, 1H), 8.20 (d, 1H, ^{4}J=2.2 Hz). ^{13}C NMR : δ = 27.1, 118.9, 128.5, 128.6, 129.7, 131.1, 131.3, 131.8, 137.0, 137.5, 137.7, 142.4, 145.9, 168.7. MS (EI) : (m/z) : 368 (11); 366 (11); 338 (18); 336 (21); 292 (25); 290 (21); 183 (44); 139 (70); 137 (42); 58 (100).

N-methyl-2-(4-bromo-2-nitrophenylthio)benzylamine (2). Compound 1 (1.14 g, 3.1 mmol) was dissolved in anhydrous THF (7 mL) and a solution of 1 M borane complex in previously distilled THF (7 mL) was added at 0°C under a nitrogen atmosphere. The reaction mixture was refluxed for 5 hours and then stirred at room temperature for 17 hr. The reaction was cooled and treated with concentrated hydrochloric acid (12 mL), warmed at 70°C for 3hr and concentrated in vacuo. The residue was dissolved in CH_3Cl and the precipitate filtered. The solid was dissolved in water, basified with NaOH and extracted with $CHCl_3$. The organic phase was dried and the solvent evaporated to give pure compound 2 in 60% yield. ^{1}H NMR : δ = 1.47 (broad s, 1H), 2.33 (s, 3H), 3.74 (s,

2H), 6.49 (d, 1H, ^{3}J=8.7 Hz), 7.27-7.56 (m, 5H), 8.33 (d, 1H, ^{4}J=2.1 Hz). ^{13}C NMR : δ = 32.2, 49.3, 119.1, 129.4 (2C), 131.3, 131.8, 132.3, 132.5, 135.7, 137.0, 137.5, 138.4, 145.6. MS (EI) : (m/z) : 354 (1); 352 (1); 319 (23); 317 (22); 277 (29); 275 (27); 152 (27); 150 (33); 44 (100); 42 (71).

N-methyl-2-(2-amino-4-bromophenylthio)benzylamine (3). Compound 2 (888 mg, 2.51 mmol) was added to a cool solution of concentrated hydrochloric acid (3.7 mL) containing $SnCl_2$ (1.714g, 7.53 mmol). The reaction mixture was stirred at reflux for 2 hours and then cooled at room temperature. The resulting solution was treated with 1 M NaOH and extracted with $CHCl_3$. The organic layer was dried and the solvent evaporated off. The crude material was purified by flash chromatography (Et_2O / Et_3N / MeOH : 10/1/1) to afford pure compound 3 in 51% yield. ^{1}H NMR : δ = 1.43 (broad s, 1H), 2.44 (s, 3H), 3.94 (s, 2H), 4.46 (broad s, 2H), 6.76-6.84 (m, 3H), 6.99-7.09 (m, 2H), 7.20-7.25 (m, 2H). ^{13}C NMR : δ = 36.6, 54.5, 114.0, 118.2, 121.8, 125.3, 126.3, 127.7, 128.4, 129.9, 135.6, 138.1, 138.8, 150.4. MS (EI) : (m/z) : 324 (4); 322 (4); 212 (19); 152 (11); 151 (42); 150 (26); 120 (100); 118 (25); 44 (29); 42 (37).

N-methyl-2-(2-amino-4-tri-*n*-butyltinphenylthio)benzylamine(4). Compound 3 (549 mg, 1.7 mmol) was dissolved in triethylamine (20.3 mL) containing hexa-*n*-butylditin (6.1 mL, 11.6 mmol) and tetrakis(triphenylphosphine)palladium (0) (120 mg). The mixture was heated at 90°C in a sealed reactor for 24 hr. The solvent was removed in vacuo and the crude oil purified by flash chromatography (Et_2O / petroleum ether / Et_3N : 7/2/1). Pure compound 4 was obtained in 57% yield. ^{1}H NMR : δ = 0.87 (t, 9H, ^{3}J=7.1 Hz), 1.03 (t, 6H, ^{3}J=8.2 Hz), 1.28-1.40 (m, 7H), 1.50-1.62 (m, 6H), 2.45 (s, 3H), 3.87 (s, 2H), 4.25 (broad s, 2H), 6.75-6.85 (m, 3H), 7.01-7.08 (m, 2H), 7.23-7.34 (m, 2H). ^{13}C NMR : δ = 10.1, 14.2, 27.9, 29.6, 36.6, 54.5, 114.6, 123.6, 125.9, 127.1, 127.5, 128.3, 129.8, 136.3, 136.5, 137.9, 146.0, 148.5. MS (EI) : (m/z) : 533 (5); 477 (32); 476 (14); 214 (54); 212 (81); 180 (22); 179 (20); 151 (32); 150 (45); 120 (100).

N-methyl-2-(2-amino-4-iodophenylthio)benzylamine (5). The tin derivative 4 (102 mg, 0.19 mmol) was dissolved in $CHCl_3$ (8 mL) and a solution of iodine in chloroform (0.1 M) was added dropwise until the iodine colour persists. The solvent was evaporated off and the crude product purified by flash chromatography (EtOAc / petroleum ether / Et_3N : 8/1/1) to obtain pure compound 5 in 78% yield. ^{1}H NMR : δ = 1.65 (broad s, 1H),

2.44 (s, 3H), 3.84 (s, 2H), 4.40 (broad s, 2H), 6.79 (m, 1H), 6.96-7.10 (m, 5H), 7.20-7.25 (m, 1H). ^{13}C NMR : δ = 36.5, 54.5, 97.3, 114.9, 124.2, 126.3, 127.8, 127,9, 128.5, 130.0, 135.5, 138.0, 138.8, 150.3. MS (EI) : (m/z) : 370 (6); 212 (19); 152 (10); 151 (40); 150 (25); 120 (100); 118 (22); 44 (26); 42 (36).

Radiochemistry

All chemicals were obtained from commercial sources and were of analytical grade. $^{11}CO_2$ was produced batchwise using the Scanditronix MC 16 cyclotron at the Karolinska Hospital/Institute by bombardment of a nitrogen gas target with 16 MeV protons in the $^{14}N(p,\alpha)^{11}C$ reaction. Carbon-11 labelled methyl iodide was synthesised from $^{11}CO_2$ utilising a one-pot reaction set-up similar to that reported previously (6).

Semi-preparative reversed-phase HPLC was performed using a Waters μ-Bondapak C-18 column (300 x 7.8 mm, 10 μm) and a UV-detector (wavelength = 254 nm) in series with a GM-tube for radiation detection. [^{11}C]ADAM was purified by HPLC using acetonitrile and 0.1 M ammonium formate (60/40) as the mobile phase with a flow rate of 6 mL/min. The radiochemical purity was analysed by reversed phase HPLC with a Waters μ-Bondapak C-18 column (300 x 3.9 mm, 10 μm) and a UV-detector (wavelength = 234 nm) in series with a Beckman ß-flow radiodetector for radiation detection. Acetonitrile and 0.01 M H_3PO_4 (30/70) were used as the mobile phase with a flow rate of 3 mL/min. The chemical identity of [^{11}C]ADAM was determined by co-injection of unlabeled ADAM.

Preparation of [^{11}C]ADAM (6). [^{11}C]Methyl iodide was trapped at room temperature in a reaction vessel (1.0 mL) containing the precursor [0.5 mg of the demethylated precursor (5)] and DMF (300 μL). The vessel was sealed and heated at 90°C for 2 min. Mobile phase (600 μL) was added prior to injection into the semipreparative HPLC column. The radioactive fraction containing the methylated radioligand was collected and after evaporation of the mobile phase the residue was dissolved in 8 mL of sterile physiological phosphate buffer (pH = 7.4) solution and filtered through a Millipore filter (0.22 μm) yielding a solution which was sterile and free from pyrogens. The retention time of [^{11}C]ADAM on the preparative HPLC system was 9-10 minutes.

ACKNOWLEDGMENTS

The authors would like to thank Mr Göran Printz for assistance with the radionuclide production, Mr Arsalan Amir and Miss Vilar Marie-Paule for technical assistance. This work was supported by grants from the Swedish Medical Research Council (12983-01A), Karolinska Institutet, INSERM-MFR and the Région Centre (France). We thank SAVIT for chemical analysis.

REFERENCES

1. Tejani-Butt S. M., Yang J. and Pawlyk A. C. *NeuroReport* **6:** 1207-1210 (1995)
2. Chinaglia G., Landwehrmayer B., Probst A. and Palacios J. M. *Neuroscience* **54:** 691-699 (1993)
3. Kuikka J. T., Tiihonen J., Bergström K. A., Karhu J., Hartikainen P., Viinamäki H., Länsimies E., Lehtonen J. and Hakola P. *J. Nucl. Med.* **22:** 346-350 (1995)
4. Hiltunen J., Akerman K. K., Kuikka J. T., Bergström K. A., Halldin C., Nikula T., Rasanen P., Tiihonen J., Vauhkonen M., Karhu J., Kupila J., Länsimies E. and Farde L. *Eur. J. Nucl. Med.* **25:** 19-23 (1998)
5. Blough B.E., Abraham P., Mills A. C., Lewin A. H., Boja J. W., Scheffel U., Kuhar M. J. and Carroll F. I. *J. Med. Chem.* **40:** 3861-3864 (1997)
6. Helfenbein J., Sandell J., Halldin C., Chalon S., Emond P., Okubo Y., Chou Y-H., Frangin Y., Douziech L., Garreau L., Swahn C-G., Besnard J-C., Farde L. and Guilloteau D. *Nucl. Med. Biol.* **26:** 491-499 (1999)
7. Suehiro M., Scheffel U., Ravert H. T., Dannals R. F. and Wagner H. N. *Life Sci.* **53:** 883-892 (1993)
8. Szabo Z., Scheffel U., Suehiro M., Dannals R. F., Kim S. E., Ravert H. T., Ricaurte G. A. and Wagner H. N. *J. Cereb. Blood Flow Metab.* **15:** 798-805 (1995)
9. Ferris R. M., Brieaddy L., Mehta N., Hollingsworth E., Rigdon G., Wang C., Soroko F., Wastila W. and Cooper B. *J. Pharm. Pharmacol.* **47:** 775-781 (1995)
10. Oya S., Choi S-R., Hou C., Mu M., Kung M-P., Acton P. D., Siciliano M. and Kung H. F. *Nucl. Med. Biol.* **27:** 249-254 (2000)
11. Schindlbauer H. *Monatsh. Chem.* **99:** 1799-1807 (1968)
12. Wagner G. and Richter P. *Pharmazie* **22:** 611-620 (1967)
13. Garofalo A., Balconi G., Botta M., Corelli F., D'Incalci M., Fabrizi G., Fiorini I., Lamba D. and Nacci V. *Eur. J. Med. Chem.* **28:** 213-220 (1993)

