JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 379–393.
DOI: 10.1002/jlcr.560

Research Article

Synthesis of two novel oxocyclam-binding technetium complexes containing an analogue of cocaine

F. Turpin[1], F. Masri[2], F. Riché[2,*], E. Berthommier[1], P. Emond[3], M. Vidal[4], P. Auzeloux[5], C. Loc'h[6], N. Neuquelman[1] and L. Mauclaire[1]

[1] *CIS bio international, subsidiary of Schering, BP 32–91192 Gif-sur-Yvette, France*
[2] *Université Joseph Fourier, LEDSS–UMR CNRS 5616, BP 53–38041 Grenoble Cedex 9, France*
[3] *INSERM U 316, Laboratoire de Biophysique Médicale et Pharmaceutique, 31 avenue Monge–37200 Tours, France*
[4] *ERAS Labo–38330 St Nazaire les Eymes, France*
[5] *INSERM U484, rue Montalembert, 63005 Clermont-Ferrand Cedex 05, France*
[6] *Service Hospitalier Frédéric Joliot, DRM, CEA, 91406 Orsay, France*

Summary

In order to visualize and quantify dopamine transporters, the synthesis of two novel ligands labelled with technetium-99 m (^{99m}Tc) has been investigated. A multi-step synthesis afforded two target ligands with a tropane skeleton and a macrocyclic complexing moiety. The choice and the position of substituents are in adequation with dopamine transporter structure. The radiolabelling of these ligands with ^{99m}Tc has been studied and the results make them good candidates for SPECT imaging. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: synthesis; cocaine analogues; tropanes; dopamine transporter; SPECT; oxocyclam; technetium

*Correspondence to: Dr. F. Riche, Université Joseph Fourier, LEDSS–UMR CNRS 5616, BP 53–38041 Grenoble Cedex 9, France. E-mail: francoise.riche@ujf-grenoble.fr

Contract/grant sponsor: EUREKA-DOPIMAG program; contract/grant number: EU1836

Received 11 October 2001
Revised 20 November 2001
Accepted 21 November 2001

Introduction

The presynaptic dopamine transporter (DAT) is an essential part of dopaminergic transmission regulation.[1,2] *In vitro* and *in vivo* studies have shown that DAT is depleted in Parkinson's disease and that the extent of depletion correlates with the loss of dopamine essentially in some brain areas (striatum and substantia nigra) where dopaminergic neurons are located.[3,4] A quantitative measure of the DAT density may be achieved by selective binding of ligands containing a radioactive label. A decreased specific striatal uptake of such a radiolabelled DAT ligand may therefore indicate pathological states and allow an early diagnosis. Among drugs which bind DAT selectively, the most studied belong to the family of bicyclo[3.2.l]octanes modified at C_2, C_3 or N (WIN-analogs). All molecules with the basic *R*-cocaine skeleton are known to inhibit DAT (Figure 1).

Recently, many compounds have been proposed as PET or SPECT imaging agents. Several ligands have been reported for use in PET imaging, including [^{11}C]cocaine,[5] [^{11}C]-2β-carbomethoxy-3β-(4-iodophenyl)tropane (β-CIT[6]) or *N*-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)tropane (FPβCIT[7]). Positron-emitting nuclides have short half-lives (e.g., ^{11}C, 20 min; ^{18}F, 109 min), so they require an on-site cyclotron for their production. More recently, SPECT imaging agents have been developed, labelled with ^{123}I (γ, 160 keV, $t_{1/2} = 13$ h), like [^{123}I]-β-CIT,[8] [^{123}I]-*N*-(fluoropropyl)-β-CIT,[7] [^{123}I]-*N*-(3-iodoprop-(2E)-enyl)-2β-carbomethoxy-3β-(4-methylphenyl)nortropane (PE_2I[9]). All these derivatives showed a very good affinity for DAT but their specificity was insufficient. Other SPECT imaging agents are labelled with ^{99m}Tc. This metal is the most widely used radionuclide in diagnostic nuclear medicine. ^{99m}Tc ($t_{1/2} = 6$ h) is readily produced by ^{99}Mo/^{99m}Tc generator and its γ-ray energy emission (140 keV) is suitable for γ-camera detection. But, unlike halogenated compounds, labelling

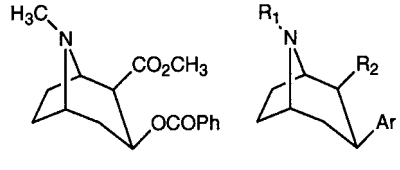


Figure 1.

with technetium needs a chelating structure of this transition metal. All the examples of Tc-99m labelled tropane derivatives have been modified at the *N*-position (R_1) or at C_2 (R_2) to introduce a technetium-carrying moiety. The best candidates are technepine,[10] "3 + 1"-mixed-ligand[11] and TRODAT-1[12] which showed a good fixation on striatum neurons. Nevertheless, they are still not perfect for the development of a SPECT imaging agent of dopaminergic neuronal function.

We propose new compounds with a WIN-analogue structure. We chose to keep the *N*-methyl group by analogy to cocaine and a tolyl group at C_3 by analogy to the good results of PE_2I. The chelating structure chosen was oxocyclam for its remarkable properties in terms of complexation of the $[TcO_2^+]$ core leading to a very stable complex, neutral at physiological pH and thus potentially able to cross the blood brain barrier.[13] This chelating structure was conjugated to the tropane ring at the C_2 position with a linker *via* an ester or an amide group as shown in Figure 2.

The choice of an ester or amide function at C_2 was controlled by SAR studies suggesting the presence in DAT of one or two hydrogen bond acceptor sites localized in the vicinity of the 2β-carbomethoxy group of cocaine.[14]

Results and discussion

Chemistry

The synthesis of ligands **9** and **17**, leading by complexation of the $[TcO_2^+]$ core to target compounds **10** and **18**, requires the building of the macrocyclic structure on one side and functionalised enantiomerically pure tropane on the other side. The key-step in this synthesis is the reaction of 8-methyl-3*S*-*p*-tolyl-8-azabicyclo[3.2.l]octane-2*S*-carboxylic acid **8** with 13-[5-hydroxypentyl]-1,4,8,11-tetraazacyclotetradecan-5-one

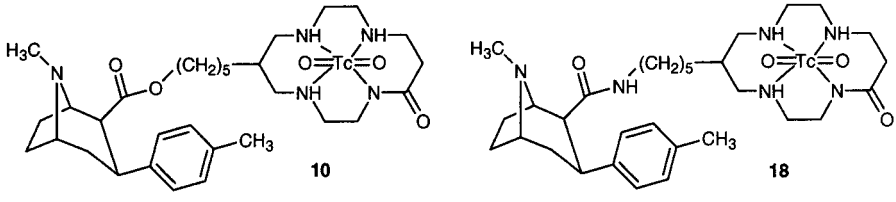
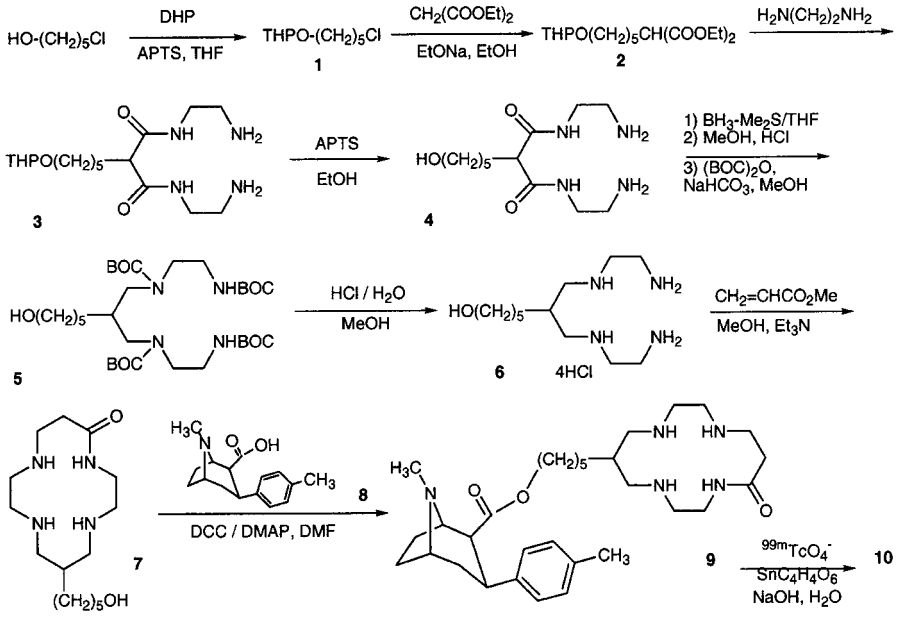


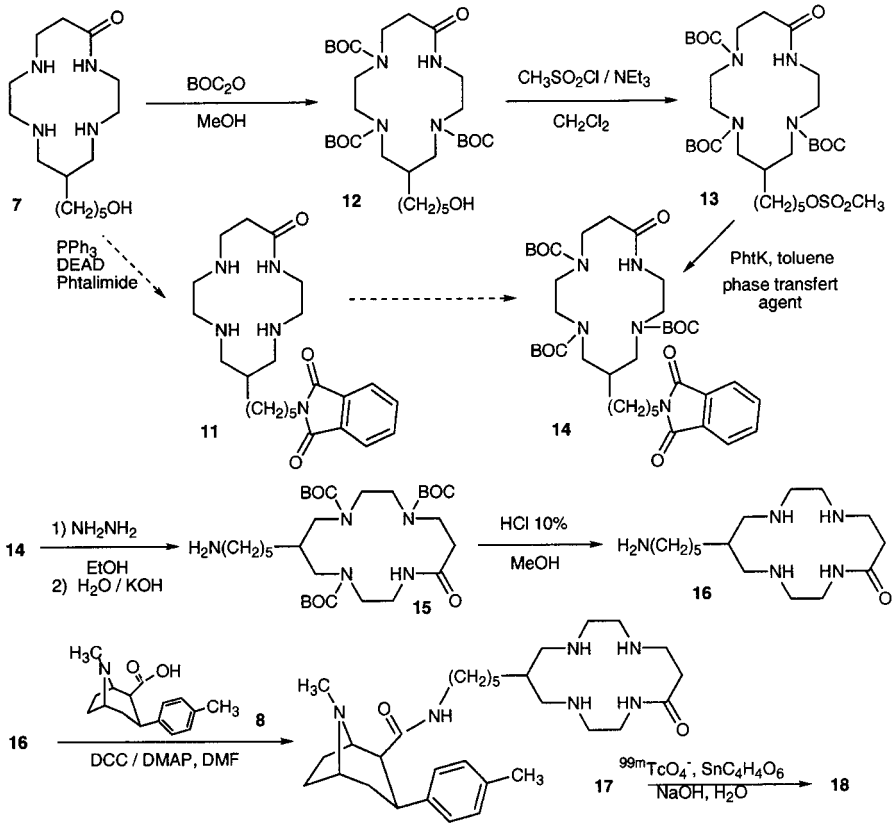
Figure 2. Target complexes



Scheme 1. Synthesis of 8-methyl-3*S*-*p*-tolyl-8-azabicyclo[3.2.1]octane-2*S*-carboxylic acid 5-(12-oxo-1,4,8,11-tetraazacyclotetradec-6-yl)pentyl ester 9

to give **9** or with 13-(5-aminopentyl)-1,4,8,11-tetraazacyclotetradecan-5-one affording **17**. The two total syntheses are shown in Schemes 1 and 2.

The starting material was 5-chloropentan-1-ol, a bifunctional compound used as a linker. After protection of the hydroxyl function by a tetrahydropyranyl group, a three-carbon bridge was introduced by a classic malonic synthesis, resulting in the condensation of monosodium salt of diethylmalonate on the chloro-compound **1** with a yield of 60%. The four nitrogens required for the complexation are easily introduced through the action of excess ethylenediamine on the diester leading to diamido/diamino compound **3** (85%). It was necessary to deprotect the alcohol before the reduction of the amide groups by BH_3–Me_2S complex because it is known that ketals undergo reductive cleavage with borane[15] affording a 4-hydroxybutyloxy group in place of tetrahydropyran-2-oxy group. The alcohol **4** was quantitatively obtained in acidic conditions. The reduction of amide groups was realised *via* Brown's method, BH_3/Me_2S complex in THF,[16] with a modification: the four amine functions resulting from reduction were protected *in situ* to make the purification easier. Thus, **5** was obtained with a global yield of 65%. Deprotection of amines was achieved with



Scheme 2. Synthesis of 8-methyl-3*S*-*p*-tolyl-8-azabicyclo[3.2.1]octane-2*S*-carboxylic acid 5-(12-oxo-1,4,8,11-tetraazacyclotetradec-6-yl)pentyl amid 17

concentrated aqueous HCl in MeOH to give **6** which was used without further purification. Cyclisation of **6** on methacrylate was realised as described[17] after the addition of 4 equiv. of Et_3N or MeONa to assure deprotonation of the amine groups, which is essential before cyclisation. Cyclisation occurred with a yield of 35%, which is a good value in the absence of a template effect or high dilution.

The synthesis of 8-methyl-3*S*-*p*-tolyl-8-azabicyclo[3.2.1]octane-2*S*-carboxylic acid **8** starting from natural R(+)-cocaine is described elsewhere.[18] The esterification reaction between **7** and **8** was carried out in the presence of dicyclohexylcarbodiimide in DMF, catalysed by dimethylaminopyridine. Compound **9** was isolated with a yield of 18% after purification. This low yield results from the low reactivity of the alcohol function but also from a very difficult purification.

The synthesis used to prepare **17** is described in Scheme 2.

The transformation of the alcohol in amine function is classically realised by a Gabriel synthesis via a phthalimide. A Mitsunobu reaction using phthalimide, DEAD and PPh_3 gave **11**. But with various conditions of temperature and solvent, we never exceeded a yield of 40%. Compound **11** was not isolated. We also chose to introduce the phthalimide group by another route which first required amine protection. Then, treatment of **12** with methanesulfonyl chloride afforded **13** which, when heated in THF containing a phase transfer catalyst and potassium phthalimide, yielded **14**. Compound **15** was obtained by hydrazinolysis (95%), then treated in acidic conditions to afford **16**. The amide **17** was synthesized under the same conditions as the esterification, leading to **9**; the yield was also relatively low (26%).

Labelling

The radiolabelling of the two ligands **9** and **17** with sodium [^{99m}Tc]pertechnetate was achieved using stannous (II) tartrate as the reducing agent as described in the literature.[13] Ligands (200 μg) were dissolved in 4 μl NaOH 0.1 M in water. Then 4 μl of a saturated aqueous solution of $SnC_4H_4O_6$ were added followed by introduction of 18 μl (0.8 MBq) TcO_4^- in physiological serum. Reaction occurred at 70–80°C over 45 min.

The labelling yields were measured by chromatography on silica gel using a ternary system, dichloromethane 3, ethanol 2 and ammonia 22% 0.5. After counting the radiochromatogram by electronic autoradiography, silica gel plates were treated with a phosphomolybdic acid ethanolic solution to visualize the ligand position. These yields varied between 46 and 72% depending on the compounds and experimental conditions. Typical chromatogram are given in Figure 3 with the free ligand encircled. Rf values were the same for **3** and **17**.

This profile leads to the following comments:

- absence of pertechnetate,
- presence of Tc-tartrate, intermediary complex which leads to Tc–L by exchange with the ligand.
- good separation of L and Tc–L, allowing a purification of Tc–L by liquid chromatography to obtain a pure complex free of ligand.

The [^{99m}Tc-oxocyclam] structure has been reported previously.[13,19] These studies, conducted on the moeity oxocyclam without

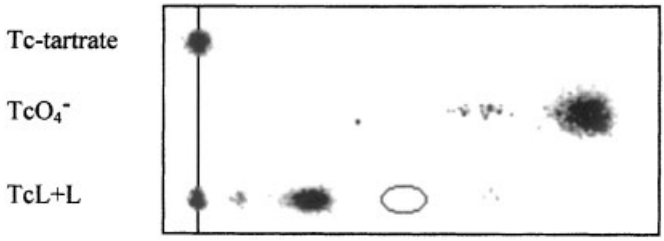
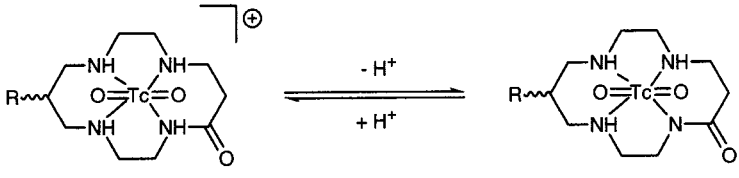


Figure 3. Chromatograms of labelling solutions of 9 or 17 (^{99m}Tc–L + L), ^{99m}Tc-tartrate and $^{99m}TcO_4^-$

any substituent at tracer level and macroscopic level, showed that the macrocyclic oxocyclam complexed with the [TcO_2^+] core and that the resulting complex established an acid–basic equilibrium in solution by protonation/deprotonation of the amide function as described in Scheme 3. The preponderant form at pH 7.4 is neutral so we hope that the target molecules will be able to cross the blood brain barrier.



Scheme 3. Structure of the complex $^{99m}TcO_2$-oxocyclam

Experimental

The compound 8-methyl-3*S*-*p*-tolyl-8-azabicyclo[3.2.l]octane-2*S*-carboxylic acid **8** was made available by the ERAS Laboratory.

^{1}H NMR spectra were run at 300 MHz and ^{13}C NMR at 75 MHz. Et_2O and THF were dried over and distilled from sodium metal with benzophenone as the indicator. DMF and CH_2Cl_2 were dried over and distilled from CaH_2. MeOH and EtOH were dried over and distilled from Mg. Analytical TLC was carried out on Merck Kieselgel 60F254-0,20 mm plates, and column chromatography on Merck Kieselgel Geduran Si 60 (0.06–0.20 mm). Radioactivity was measured by autoradiography (Instant Imager, Packard). Commercially available reagents were used without further purification.

*2-(5-chloropentyloxy)tetrahydropyran (**1**)*

5-Chloropentanol (30 g, 0.245 mol) was dissolved in 70 ml of anhydrous THF and the resulting solution was cooled to 0°C. After addition of a catalytic amount of *p*-toluenesulfonic acid, dihydropyran (20.6 g, 0.245 mol) was introduced dropwise. The resulting mixture was warmed to room temperature and stirred overnight. THF was evaporated off, then the crude mixture was dissolved in Et_2O. The ether layer was washed with $NaHCO_3$ saturated aqueous solution, then water. After evaporation *in vacuo* of ether, the crude product was purified by liquid chromatography (silica gel, Et_2O:cyclohexane, 50:50) to afford 47.51 g of **1** (94%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.5–1.8 (m, 12 H, $CH_2C\underline{H}_2CH_{(2)}$), 1.84 (qt, 2 H, $C\underline{H}_2CH_2Cl$, $^3J_{(CH_2-CH_2)} = 6.7$ Hz), 3.5–3.9 (m, 4 H, CH_2O), 3.55 (t, 2 H, CH_2Cl, $^3J_{(CH_2-CH_2)} = 6.7$ Hz), 4.6 (t, lH, $CH(O)_2$, $^3J_{(CH-CH_2)} = 3.1$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.0, 24.1, 25.8, 29.4 ($CH_2\underline{C}H_2CH_{(2)}$), 31.1 ($\underline{C}H_2CH(O)_2$), 32.8 ($\underline{C}H_2CH_2Cl$), 45.3 ($CH_2Cl$), 62.7, 67.6 ($CH_2O$), 99.3 ($CH_2\underline{C}H(O)_2$).

*2-[5-(tetrahydropyran-2-oxy)pentyl]malonic acid ethyl ester (**2**)*

An ethanolate solution was prepared by dissolving Na (6.2 g, 0.269 mol) in anhydrous ethanol (250 ml). After addition of diethylmalonate (36.9 g, 0.23 mol), the solution was heated and stirred at 60°C for 1 h. Then, 2-(5-chloropentyloxy)tetrahydropyran (47.5 g, 0.23 mol) was added and the resulting mixture was heated to 80°C and stirred for 24 h. Ethanol was evaporated off, the residue was dissolved in water and the product was extracted with ether. **2** was purified by liquid chromatography (silica gel, pentane:ether:ethyl acetate, 20:2:1) and obtained as an oil (51.74 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, 6 H, $C\underline{H}_3CH_2O$, $^3J_{(CH_3-CH_2)} = 7.2$ Hz), 1.3–2 (m, 14 H, $CH_2C\underline{H}_2CH_{(2)}$), 3.3 (t, lH, $CH_2C\underline{H}(CO)_2$), 3.3–3.9 (m, 4 H, $CH_2C\underline{H}_2O$), 4.2 (q, 4 H, $C\underline{H}_3CH_2O$, $^3J_{(CH_3-CH_2)} = 7.2$ Hz), 4.6 (m, lH, $CH(O)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8 ($\underline{C}H_3CH_2O$), 19.3, 25.2, 25.6, 26.6, 28.3, 29.1, 30.4 ($CH_2\underline{C}H_2CH_{(2)}$), 51.6 ($\underline{C}H(CO)_2$), 60.9 ($CH_3\underline{C}H_2O$), 61.9, 67.0 ($CH_2\underline{C}H_2O$), 98.4 ($CH(O)_2$), 169.1 (C=O).

*N,N′-bis(2-aminoethyl)-2-[5-(tetrahydropyran-2-yloxy)pentyl]malonamide (**3**)*

The diester **2** (51.74 g, 0.156 mol) was dissolved in ethylenediamine (93.8 g, 1.56 mol) and the resulting mixture was heated at 55°C for 2

days. Ethanol and ethylenediamine were evaporated off *in vacuo*. Purification by liquid chromatography (silica gel, $CHCl_3$:CH_3OH:NH_4OH 22%, 3:2:0.5) afforded 46.2 g of **3** (orange wax, 83%). ^{1}H NMR (300 MHz, D_2O, pH = 9–10) δ 1.5–1.8 (m, 14 H, $CH_2C\underline{H}_2CH_{(2)}$), 2.64 (t, 4 H, $C\underline{H}_2NH_2$, $^3J_{(CH_2-CH_2)} = 6.3$ Hz), 3.2 (t, 4 H, $C\underline{H}_2NH$, $^3J_{(CH_2-CH_2)} = 6.3$ Hz), 3–3.2 (m, lH, $CH_2C\underline{H}(CO)_2$), 3.4–3.85 (m, 4 H, CH_2O), 4.5 (m, 1 H, $CH(O)_2$, $^3J_{(CH-CH_2)} = 3.1$ Hz). ^{13}C NMR (75 MHz, D_2O, pH = 9–10) δ 19.7, 25, 25.4, 26.7, 28.9, 29.8, 30.6 ($CH_2\underline{C}H_2CH_{(2)}$), 40.2, 42.2 ($\underline{C}H_2NH_2$, $\underline{C}H_2NH$), 54.3 ($\underline{C}H(CO)_2$), 63.7, 68.4 ($CH_2\underline{C}H_2O$), 99.9 ($CH(O)_2$), 172.8 (C=O).

*N, N′-bis(2-aminoethyl)-2-[5-hydroxypentyl]malonamide (**4**)*

To a solution containing 28.78 g, 0.08 mol of **3** in 500 ml of absolute ethanol, *p*-toluenesulfonic acid monohydrate (38.8 g, 0.206 mol) was added and the resulting mixture was heated at 60°C for 18 h. NH_4OH 22% was added until pH = 9. A white precipitate appeared, which was removed by filtration. Ethanol was evaporated off *in vacuo* and the compound **4** was purified by liquid chromatography (silica gel, $CHCl_3$:CH_3OH:NH_4OH 22%, 3:2:0.5) to afford 21.8 g (viscous oil, 99%) of pure product. ^{1}H NMR (300 MHz, D_2O, pH = 9–10) δ 1–1.25 (m, 4 H, $CH_2C\underline{H}_2CH_{(2)}$), 1.3 (m, 2 H, $C\underline{H}_2CH_2OH$, $^3J_{(CH_2-CH_2)} = 6.5$ Hz), 1.5–1.7 (m, 2 H, $(CO)_2CHC\underline{H}_2$), 2.55 (t, 4 H, $C\underline{H}_2NH$, $^3J_{(CH_2-CH_2)} = 6.3$ Hz), 3.1 (t, 4 H, $C\underline{H}_2NH_2$, $^3J_{(CH_2\text{-}CH_2)} = 6.3$ Hz), 3.2 (s, lH, $(CO)_2C\underline{H}CH_2$), 3.4 (t, 2 H, $C\underline{H}_2OH$, $^3J_{(CH_2-CH_2)} = 6.5$ Hz). ^{13}C NMR (75 MHz, D_2O, pH = 9–10) δ 25, 26.7 ($CH_2\underline{C}H_2CH_{(2)}$), 29.7 ($(CO)_2CH\underline{C}H_2$), 31.4 ($\underline{C}H_2CH_2OH$), 40.1 ($\underline{C}H_2NH$), 42.0 ($\underline{C}H_2NH_2$), 49.2 ($\underline{C}H(CO)_2$), 61.9 ($\underline{C}H_2OH$), 172.8 ($\underline{C}$=O).

*(2-tert-butoxycarbonylaminoethyl)-(2-{[tert-butoxycarbonyl)-(2-tert-butoxycarbonylaminoethyl)-amino]methyl}-7-hydroxyheptyl)carbamic acid tert-butyl ester (**5**)*

In a Schlenk tube containing 250 ml of anhydrous THF were dissolved 13.3 g, 0.048 mol of diamide **4** and 33 ml of boran-dimethyl sulphide complex. The resulting mixture was gently refluxed for 5 days. THF was evaporated off, then 100 ml of methanol was introduced dropwise and refluxed for 3 h. A methanolic solution of HCl (30 ml aqueous HCl 32% in 250 ml MeOH) was added and then heated to reflux over 12 h. After evaporation of the solvents, 100 ml of methanol were added and

immediately evaporated to eliminate methyl borate. This last operation was repeated three times. The crude product was dissolved in 350 ml of methanol and treated with $NaHCO_3$ (60 g) and di-*tert*-butyldicarbonate (42.67 g, 0.195 mol) over 48 h at room temperature. Methanol was evaporated off, the residue dissolved in water and extracted with ether. Evaporation of ether afforded **5** which was purified by liquid chromatography (silica gel, $CHCl_3$:CH_3OH, 4:0.2) as a white wax (19.76 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.2–2 (m, 9 H, $CH_2C\underline{H}_2CH_{(2)}$, CH), 1.3 and 1.4 (s, 36 H, $(CH_3)_3CO$), 3–3.2 (m, 12 H, $NHC\underline{H}_2C\underline{H}_2N$), 3.6 (m, 2 H, $C\underline{H}_2OH$), 5 (broad s, 2 H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.0, 29.8, 32.5, 39.4 ($CH_2\underline{C}H_2CH_{(2)}$), 28.4 ($(\underline{C}H_3)_3CO$), $\approx$ 37 (broad, CH), 47.1, 49.7 (broad, $CH\underline{C}H_2N$), 63.2 (CH_2OH), 79.2, 80 ($(CH_3)_3\underline{C}O$), 156.1 (CONH).

7-(2-aminoethylamino)-6-[(2-aminoethylamino)methyl]-heptan-1-*ol* (**6**)

The compound **5** (19.76 g, 0.03 mol) was dissolved in 300 ml of methanol and 32 ml of aqueous HCl 32%. After 36 h at room temperature, the solvents were evaporated off and **6** was isolated as its tetrachlorohydrate salt (11.5 g, 97%). ^{1}H NMR (300 MHz, D_2O, pH = 1) δ 1–1.4 (m, 8 H, $CH_2C\underline{H}_2CH_{(2)}$), 2.2 (m, 1 H, CH), 3.1 (m, 4 H, $NHC\underline{H}_2CH$), 3.15–3.25 (m, 8 H, $NH_2C\underline{H}_2C\underline{H}_2NH$), 3.4 (t, 2 H, $C\underline{H}_2OH$, $^3J_{(CH_2-CH_2)} = 6.3$ Hz). ^{13}C NMR (75 MHz, D_2O, pH = 1) δ 25, 25.3, 28.9, 31.4 ($CH_2\underline{C}H_2CH_{(2)}$), 34.2 (CH), 35.9 ($NH\underline{C}H_2CH$), 45.6, 49.7 ($NH_2\underline{C}H_2\underline{C}H_2NH$), 61.9 ($CH_2OH$).

13-(5-hydroxypentyl)-1,4,8,11-tetraazacyclotetradecan-5-one (**7**)

The amine salt **6** (11.5 g, 29.3 mmol) was dissolved in dry methanol (600 ml) and triethylamine (6 equiv.) was added. Then methacrylate (2.71 ml, 29.6 mmol) was introduced and the mixture was stirred under an inert atmosphere over 2 days at room temperature and 15 days under reflux. After evaporation of the solvent, purification on silica gel ($CHCl_3$:CH_3OH:NH_4OH 22%, 10:4.5:0.75) afforded **7** (1.95 g, orange oil, 22%). ^{1}H NMR (300 MHz, D_2O, pH = 9) δ 1.05–1.25 (m, 6 H, $CH_2C\underline{H}_2CH_{(2)}$), 1.4 (qt, 2 H, $C\underline{H}_2CH_2OH$, $^3J_{(CH_2-CH_2)} = 6.6$ Hz), 1.6–1.75 (m, 1 H, CH), 2.25 (t, 2 H, CH_2CO, $^3J_{(CH_2-CH_2)} = 5.8$ Hz), 2.4–2.9 (m, 12 H, $C\underline{H}_2NHC\underline{H}_2$), 3.1–3.3 (m, 2 H, $C\underline{H}_2NHCO$), 3.4 (t, 2 H, $C\underline{H}_2OH$, $^3J_{(CH_2-CH_2)} = 6.6$ Hz). ^{13}C NMR (75 MHz, D_2O, pH = 9) δ

25.4, 26, 30.4, 31.5 ($CH_2\underline{C}H_2CH_{(2)}$), 34.0 ($\underline{C}H_2CO$), 35.1 (CH), 37.9, 43.7, 45.2, 46.9, 48.8, 54.3, 54.4 (CH_2NH), 62.1 (CH_2OH), 175.4 (C=O).

*8-methyl-3S-p-tolyl-8-azabicyclo[3.2.1] octane-2S-carboxylic acid 5-(12-oxo-1,4,8,11-tetraazacyclotetradec-6-yl)pentyl ester (**9**)*

The acid **8** (97 mg, 0.345 mmol), the alcohol **7** (210 mg, 0,69 mmol), dicyclohexylcarbodiimide (78 mg, 0.38 mmol) and dimethylaminopyridine (46 mg, 0.38 mmol) were dissolved in freshly distilled DMF (20 ml). After 3 days at room temperature, the solvent was evaporated off and the product purified by liquid chromatography (silica gel, ($CHCl_3$:CH_3OH:NH_4OH 22%, 10:4.5:0.75) to give **9** as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.9–1 (m, 2 H, $2H_{12}$), 1–1.28 (m, 4H, $2H_{11}$, $2H_{13}$), 1.3–1.5 (m, 2H, $2H_{10}$), 1.51–1.7 (m, 4H, $H_{4\alpha}$, $H_{6'}$, $H_{7'}$, H_{14}), 1.95–2.3 (m, 2H, H_6, H_7), 2.21 (s, 3H, N–CH_3), 2.28 (s, 3H, ArCH_3), 2.3–3 (m, 13H, $2H_{16}$, $2H_{20}$, $2H_{21}$, $2H_{22}$, H_3, $H_{4\beta}$, $H_{15'}$, $H_{17'}$, $H_{23'}$), 3.05–3.27 (m, 2H, H_{15}, H_{23}), 3.35 (m, 1H, H_5), 3.55 (m, 2H, H_1, H_{17}), 3.85 (dt, 1H, H_9), 4.05 (dt, 1H, H_9), 7–7.2 (m, 4H, $C_{Ar}H$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 21 (Ar$\underline{C}H_3$), 25.5 (C_6, C_7), 26.1, 26.2, 32.2 (C_{11}, C_{12}, C_{13}), 28.5 (C_{10}), 33.2 (C_3), 34.1 (C_4), 36.1, 37.7 (C_{17}, C_{19}), 39.7 (C_{14}), 42 (NCH_3), 44.4, 46.2 (C_{20}, C_{21}), 52.9 (C_2), 53.1, 53.8 (C_{16}, C_{22}), 56.7 (C_{15}, C_{23}), 62.3 (C_5), 63.5 (C_9), 65.5 (C_1), 127.1, 128.6 ($C_{5'}$, $C_{6'}$, $C_{8'}$, $C_{9'}$), 135, 140.1 ($C_{4'}$, C_7), 171.7, 172.5 (C_8, C_{18}).

*6-(5-hydroxypentyl)-12-oxo-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylic acid tri-tert-butyl ester (**12**)*

To a solution of **7** (317 mg, 1.05 mmol) and $NaHCO_3$ (500 mg) in 25 ml of methanol was added di-*tert*-butyldicarbonate (1.38 g, 6.33 mmol). The mixture was stirred at room temperature for 2 days. The solvent was evaporated off and the residue dissolved in ether. This phase was washed with water, stored over anhydrous Na_2SO_4, filtered and evaporated. A purification on silica gel (ether:methanol, 10:l) afforded **12** (595 mg, 94%) as a white oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.2–1.65 (m, 8H, $CH_2C\underline{H}_2CH_{(2)}$), 1.44, 1.48 (s, 27H, $(CH_3)_3CO$), 1.65–1.95 (m, 1H, CH), 2.2–3.9 (m, 16H, CH_2N, CH_2CO), 3.6 (t, 2H, $C\underline{H}_2OH$, $^3J_{(CH_2-CH_2)} = 6.3$ Hz), 6.85–7.15 (broad s, 1H, NHCO). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.4, 30.2, 32.7, 37.1 ($CH_2\underline{C}H_2CH_{(2)}$), 28.8 ($(\underline{C}H_3)_3CO$), 38.2 (CH), 41.1 ($\underline{C}H_2CO$), 47, 47.9, 52.7 ($\underline{C}H_2$,N), 62.7

(CH_2OH), 79.9, 80.3, 81.1 ($(CH_3)_3\underline{C}O$), 156.3 (OCONH), 172.8 ($NH\underline{C}OCH_2$).

*6-(5-methanesulfonyloxypentyl)-12-oxo-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylic acid tri-tert-butyl ester (***13***)*

The alcohol **12** (412 mg, 0.686 mmol) and triethylamine (69 mg, 0.689 mmol) were dissolved in 20 ml CH_2Cl_2. The methanesulphonylchloride (78 mg, 0.686 mmol) was added dropwise and the resulting solution was stirred at room temperature for 12 h, washed with water then brine. The dichloromethane was evaporated off and **13** (395 mg, 85%) was isolated as a white wax by liquid chromatography (silica gel, $CHCl_3$:methanol, 6:l). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1–1.6 (m, 6H, $CH_2C\underline{H}_2CH_{(2)}$), 1.45, 1.48 (s, 27H, $(CH_3)_3$,C–O), 1.65–1.8 (m, 1H, CH), 1.73 (qt, 2H, $C\underline{H}_2CH_2OH$, $^3J_{(CH_2-CH_2)} = 6.6$ Hz), 2.3–3.9 (m, 16H, CH_2N, CH_2CO), 3.0 (s, 3H, CH_3S), 4.2 (t, 2H, CH_2O, $^3J_{(CH_2-CH_2)} = 6,6$ Hz), 6.7–7 (broad s, 1H, NHCO). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.2, 29.2, 30.2, 37.2 ($CH_2\underline{C}H_2CH_{(2)}$), 28.8 ($(\underline{C}H_3)_3CO$), 37.7 ($CH_3S$), 38.8 (CH), 41.2 ($\underline{C}H_2CO$), 47.2, 49.6, 50.5, 52.9 (CH_2N), 70.2 (CH_2–O), 79.8, 80.3, 81.1 ($(CH_3)_3\underline{C}O$), 156.3 (OCONH), 172.6 ($NH\underline{C}OCH_2$).

*6-[5-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-pentyl]-12-oxo-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylic acid tri-tert-butyl ester (***14***)*

A solution of **13** (288 mg, 0.424 mmol), potassium phthalimide (116 mg, 0.636 mmol) in 30 ml of toluene was refluxed for 10 h. After evaporation *in vacuo*, the residue was dissolved in ether, washed with water, then brine, and finally evaporated. The product was purified by liquid chromatography on silica gel (ether:methanol, 10:1) to give compound **14** (263 mg, 85%) as a white wax. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1–1.55 (m, 6H, $CH_2C\underline{H}_2CH_{(2)}$), 1.43, 1.45, 1.47 (s, 27H, $(CH_3)_3CO$), 1.66 (qt, 2H, $C\underline{H}_2CH_2N(CO)_2$, $^3J_{(CH_2-CH_2)} = 7$ Hz), 1.75–1.85 (m, 1H, CH), 2.35–3.95 (m, 16H, CH_2N, CH_2CO), 3.66 (t, 2H, $CH_2N(CO)_2$, $^3J_{(CH_2-CH_2)} = 7$ Hz), 6.75–6.95 (broad s, 1H, NHCO), 7.65–7.75 (m, 2H, CH_{Ar}), 7.77–7.89 (m, 2H, CH_{Ar}). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.6, 27.6, 30.4, 37.2 ($CH_2\underline{C}H_2CH_{(2)}$), 30.2 ($(\underline{C}H_3)_3$C–O), 38.1 ($CH_2N(CO)_2$), 38.6 (CH), 41.3 ($\underline{C}H_2CO$), 47.1, 47.6, 49.7, 52.8 ($\underline{C}H_2N$), 79.8, 80.3, 81.1 ($(CH_3)_3\underline{C}O$), 123.5 ($C_{Ar}H$), 132.5 (C_{Ar}), 134.2 ($C_{Ar}H$), 156.3 (OCO–NH), 168.7($N\underline{C}OC_{Ar}$–), 172.6 ($NH\underline{C}OCH_2$).

*6-(5-aminopentyl)-12-oxo-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylic acid tri-tert-butyl ester (***15***)*

To a solution of **14** (240 mg, 0.329 mmol) in absolute ethanol (25 ml) was added a large excess of hydrazine hydrate (1 ml). The mixture was refluxed for 8 h. After cooling, a white precipitate appeared. The solvent was evaporated off and the residue dissolved in ether and aqueous KOH 30%. The organic layer was stored over anhydrous Na_2SO_4 and then evaporated. Purification on a silica gel column ($CHCl_3$:MeOH, 5:1) afforded **15** (187 mg, 95%) as an oil). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.9–1.55 (m, 8H, $CH_2C\underline{H}_2CH_{(2)}$), 1.36, 1.40 (s, 27H, $(CH_3)_3CO$), 1.6–1.8 (m, 1H, CH), 2.2–3.9 (m, 16H, CH_2N, CH_2CO), 2.64 (t, 2H, $C\underline{H}_2NH_2$, $^3J_{(CH_2-CH_2)} = 6.8$ Hz), 6.45–6.9 (broad s, 1H, NHCO). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.9, 27.6, 30.4, 37.2 ($CH_2\underline{C}H_2CH_{(2)}$), 28.8 ($(\underline{C}H_3)_3CO$), 38.6 (CH), 41.2 ($\underline{C}H_2CO$), 42.4 ($CH_2NH_2$), 47.1, 47.9, 50.0, 52.9 ($\underline{C}H_2N$), 79.8, 80.3, 81.0 ($(CH_3)_3\underline{C}O$), 156.3 (OCONH), 172.5 ($NH\underline{C}OCH_2$).

*13-(5-aminopentyl)-1,4,8,11-tetraazacyclotetradecan-5-one, tetrachlorhydrate (***16***)*

Deprotection of **15** (96 mg, 0.16 mmol) was realised in methanol (25 ml) and aqueous HCl 32% (3 ml) during 24 h at room temperature. Solvents were evaporated off and the residue was dissolved in water and washed with ether. The water was evaporated off *in vacuo* to give **16** (71 mg, 99%). ^{1}H NMR (300 MHz, D_2O, pH = l) δ 1.32–1.55 (m, 6H, $CH_2C\underline{H}_2CH_{(2)}$), 1.4 (qt, 2H, $C\underline{H}_2CH_2NH_2$, $^3J_{(CH_2-CH_2)} = 7.2$ Hz), 2.35 (m, 1H, CH), 2.97 (t, 2H, CH_2CO, $^3J_{(CH_2-CH_2)} = 5.7$ Hz), 3.02 (t, 2H, $C\underline{H}_2NH_2$, $^3J_{(CH_2-CH_2)} = 7.6$ Hz), 3.3–3.95 (m, 14H, $C\underline{H}_2NH$).^{13}C NMR (75 MHz, D_2O, pH = 1) δ 25.6, 25.7, 26.8, 29.4 (CH_2–$\underline{C}H_2$–$CH_{(2)}$), 29.9 ($\underline{C}H_2CO$), 34 (CH), 37.3, 42.2, 43.5, 44.4, 47, 48.1, 49 (CH_2–NH), 39.7 (CH_2–NH_2), 176.4 (C=O).

*8-methyl-3S-p-tolyl-8-azabicyclo[3.2.1]octane-2S-carboxylic acid [5-(12-oxo-1,4,8,11-tetraazacyclo tetradec-6-yl)-pentyl]amide (***17***)*

The work-up was the same as for the synthesis of **9**. The chromatographic conditions were silica gel, $CHCl_3$:MeOH:NH_4OH 22%, 5:1:0.2). Yield was 26% of **17**, as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.9–1 (m, 2H, $2H_{12}$), 1–1.28 (m, 4H, $2H_{11}$, $2H_{13}$), 1.3–1.5 (m, 2H, $2H_{10}$), 1.51–1.7 (m, 4H, $H_{4\alpha}$, $H_{6'}$, $H_{7'}$, H_{14}), 1.95–2.3 (m, 2H, H_6,

H_7), 2.21 (s, 3H, NCH_3), 2.28 (s, 3H, $ArCH_3$), 2.3–3 (m, 13 H, $2H_{16}$, $2H_{20}$, $2H_{21}$, $2H_{22}$, H_3, $H_{4\beta}$, $H_{15'}$, $H_{17'}$, $H_{23'}$), 3.05–3.27 (m, 2H, H_{15}, H_{23}), 3.35 (m, 1H, H_5), 3.55 (m, 2H, H_1, H_{17}), 3.85 (dt, 1H, H_9), 4.05 (dt, 1H, $H_{9'}$), 7–7.2 (m, 4H, C_{Ar}–H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 21 ($ArCH_3$), 25.5 (C_6, C_7), 26.1, 26.2, 32.2 (C_{11}, C_{12}, C_{13}), 28.5 (C_{10}), 33.2 (C_3), 34.1 (C_4), 36.1, 37.7 (C_{17}, C_{19}), 39.7 (C_{14}), 42 (NCH_3), 44.4, 46.2 (C_{20}, C_{21}), 52.9 (C_2), 53.1, 53.8 (C_{16}, C_{22}), 56.7 (C_{15}, C_{23}), 62.3 (C_5), 63.5 (C_9), 65.5 (C_1), 127.1, 128.6 ($C_{5'}$, $C_{6'}$, $C_{8'}$, $C_{9'}$), 135, 140, 1 ($C_{4'}$, C_7), 171.7, 172.5 (C_8, C_{18}).

Labelling of 9 and 17 (10 and 18)

Compounds **9** or **17** (0.2 mg) were labelled by incubating with stannous tartrate saturated solution (4 µl), NaOH 0.1 M (4 µl) and ^{99m}Tc-pertechnetate (~0.8 MBq, 18 µl) for 45 min at 80°C. The solutions were chromatographed on silica gel plates (CH_2Cl_2:EtOH:NH_4OH 22%, 3:2:0.5). The plates were autoradiographed, then a phosphomolybdic acid ethanolic solution (5%) was used to visualize the ligands.

Conclusion

In order to visualize and quantify dopamine transporters by SPECT, we synthesized two ligands with a tropane skeleton and a macrocyclic moiety, allowing the complexation of ^{99m}Tc. The radiolabelling of these ligands led to two novel complexes, good candidates for imaging the dopamine transporters. Biological studies are in progress and the results will be published in due course.

Acknowledgements

This work was post of the EUREKA-DOPIMAG program: Project EU1836.

References

1. Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. *Science* 1987; **237**: 1219–1223.

2. Koob GF, Bloom FE. *Science* 1988; **242**: 715–723.
3. Hantraye P, Brownell AL, Elmaleh D, *et al*. *NeuroReport* 1992; **3**: 265–268.
4. Kaufman MJ, Madras BK. *Synapse* 1991; **9**: 43–49.
5. Fowler JS, Volkow ND, Wolf AP, *et al*. *Synapse* 1989; **4**: 371–377.
6. Müller L, Halldin C, Farde L, Karlsson P, Hall H, Swahn CG, Neumeyer JL, Gao Y, Milius RA. *Nucl Med Biol* 1993; **20**: 249–255.
7. Neumeyer JL, Wang S, Gao Y, *et al*. *J Med Chem* 1994; **37**: 1558–1561.
8. Neumeyer JL, Wang S, Milius RA, Baldwin RM, Zea-Ponce Y, Hoffer PB, Sybirska EH, Al-Tikriti MS, Charney DS, Malison RT, Laruelle M, Innis RB. *J Med Chem* 1991; **34**: 3144–3146.
9. Emond P, Garreau L, Chalon S, Boazi M, Caillet M, Bricard J, Frangin Y, Mauclaire L, Besnard JC, Guilloteau D. *J Med Chem* 1997; **40**: 1366–1372.
10. Madras BK, Jones AG, Mahmood A, Zimmerman RE, Garada B, Holman BL, Davison A, Blundell P, Meltzer PC. *Synapse* 1996; **22**: 239–246.
11. Meegalla SK, Plossl K, Kung MP, Stevenson DA, Liable Sands LM, Rheingold AL, Kung HF. *J Am Chem Soc* 1995; **117**: 11 037–11 038.
12. Meegalla S, Plossl K, Kung MP, Chumpradit S, Stevenson DA, Kushner SA, McElgin WT, Mozley PD, Kung HF. *J Med Chem* 1997; **40**: 9–17.
13. Riché F, Trimcev I, Godard J, Benabed A, Vidal M, Pasqualini R. *Appl Radiat Isot* 1993; **44**: 907–910.
14. Carroll FI, Lewin AH, Boja JW, Kuhar MJ. *J Med Chem* 1992; **35**: 969–981.
15. Lane CF. *Chem Rev* 1976; **76**: 773–799.
16. Brown HC, Choi YM, Narasimhan S. *J Org Chem* 1982; **47**: 3153–3163.
17. Hay RW, Pujari MS, Perroti A. *Trans Met Chem* 1985; **10**: 396–399.
18. (a) Xu L, Trudell ML. *J Heterocyclic Chem* 1996; **33**: 2037–2039. (b) Carroll FI, Kotian P, Dehghani A, Gray JL, Kuzemko MA, Parkam KA, Abraham P, Lewin AH, Boja SW, Kuhar MJ. *J Med Chem* 1995; **38**; 379–388.
19. Riché F, Vidal M, Bolzati C, Uccelli L, Marchi A, Duatti A. *Inorg Chim Acta* 1995; **231**: 147–151.

