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Technetium-99m labelled integrated tropane–BAT as a potential dopamine transporter tracer

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Abstract—To reduce the molecular weight of ^{99m}Tc-labelled tropanes with the aim to enhance the passage over the blood–brain barrier, a so-called integrated tropane–BAT construct was developed. For this purpose a mercaptoethyl substituent was attached to the amine nitrogen atom of a nortropane precursor and the methyl carboxylate in 2β-position was converted to a 2-mercaptoethylaminomethylene substituent. This integrated tropane–BAT construct could be labelled efficiently (85–90%) with technetium-99m. Results of LC–MS analysis of the tracer agent support the assumed structure. Biodistribution studies in normal rats ($n = 3$) showed a slightly higher brain uptake for the new tracer agents as compared to ^{99m}Tc-TRODAT-1. These results indicate that further biological evaluation of the integrated ^{99m}Tc-tropane–BAT is warranted.

1. Introduction

Nuclear imaging techniques are increasingly being applied in the exploration of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease (PD),[1] etc. They are of great interest to evaluate disease evolution and therapeutic effect of treatments. The availability of radiolabelled tracer agents with a high (nanomolar) affinity for one of the receptor types (dopaminergic, serotoninergic, ...) or neurotransmitter transporter proteins is a primary requirement for the progress of such scintigraphic techniques.

Dopamine is one of the key neurotransmitters that control normal brain function, including movement, emotion and other higher-level cognitive functions. PD is characterised by a degeneration of dopaminergic neurons.

The most important mechanism for regulating dopamine concentrations in the synaptic cleft is the pumping of the neurotransmitter dopamine back to the presynaptic neurons by dopamine transporters (DAT). Several ligands have been proposed for exploration of this dopaminergic transport system by positron emission tomography (PET)[2] and single photon emission computed tomography (SPECT).[3] All these radiotracers are constructed with a basic tropane structure, which is found in numerous naturally occurring alkaloids with potent biological activity.

The practical usefulness of the PET tracers [^{11}C]-cocaine and [^{11}C]- or [^{18}F]-(2β-carbomethoxy)-3β-(4-fluorophenyl)tropane ([^{18}F]CFT) is limited due to the necessity of being close to a cyclotron for production of the short-lived radionuclide. However, tracer agents labelled with the γ-emitting iodine-123 are also useful for brain receptor imaging using SPECT. ^{123}I-labelled ioflupane, also called ^{123}I-FP-CIT and commercially available from Amersham under the name DatSCAN™ is an example of such a dopamine transporter tracer agent for SPECT, but the suboptimal availability and the high cost of the iodine-123 radioisotope are drawbacks in routine clinical diagnostic procedures.

Most nuclear medicine diagnostic examinations are performed using radiopharmaceuticals labeled with technetium-99m in view of its attractive nuclear-physical properties and its continuous availability at a relatively low cost by the use of a ^{99}Mo/^{99m}Tc-generator.

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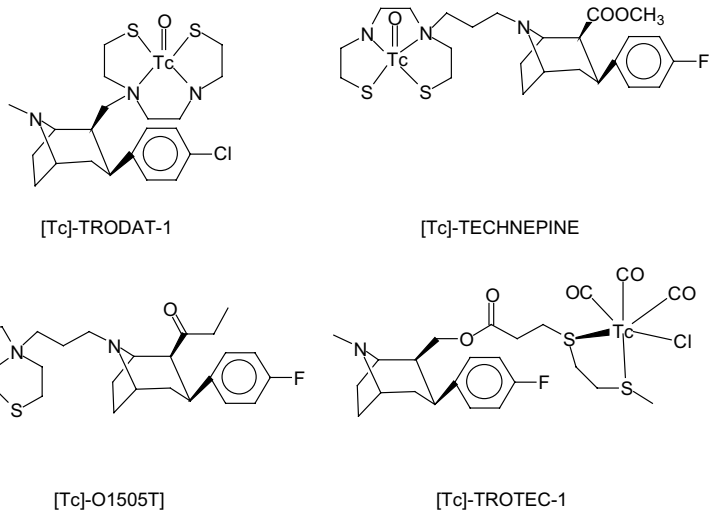


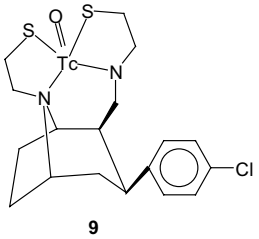
Figure 1. Structure of some technetium-99m labelled DAT tracers.

Several ^{99m}Tc-complexes have already been proposed for imaging of DAT sites such as [^{99m}Tc]-TRODAT-1,[4,5] [^{99m}Tc]-Technepine,[6] [^{99m}Tc]-O15O5T[7] and [^{99m}Tc]-TROTEC-1[8,9] (Fig. 1), but their binding and in vivo localisation properties are clearly inferior to those of the higher mentioned PET and ^{123}I labelled DAT-tracers.

Two reasons for the relatively low brain uptake of these ^{99m}Tc-tropane derivatives can be supposed to be the relatively high molecular weight of the ^{99m}Tc-chelate–tropane conjugate and the nature of the covalent bond (linker) between the ^{99m}Tc-chelate and the receptor bonding moiety,[6] Zhang et al., for instance, found a drastic reduction of brain uptake by replacing the methylene bridge of ^{99m}Tc-TRODAT-1 by an ethylene linker.[10]

In an attempt to improve the biological characteristics of such ^{99m}Tc-labelled tropanes, we have now developed a new type of derivative, in which a Tc-complexing bis-amino dithiol (BAT) moiety is partially integrated in the tropane skeleton, in this way eliminating the need for a spacer and reducing the overall size and molecular weight of the ^{99m}Tc-labelled tropane derivative (Fig. 2).

This report describes the synthesis, radiolabelling with ^{99m}Tc and preliminary biodistribution study in normal mice of a so-called 'integrated tropane–BAT radioligand'.



9

Figure 2. Assumed structure of the ^{99m}Tc-labelled integrated tropane–BAT derivative (**9**).

2. Results

2.1. Chemistry

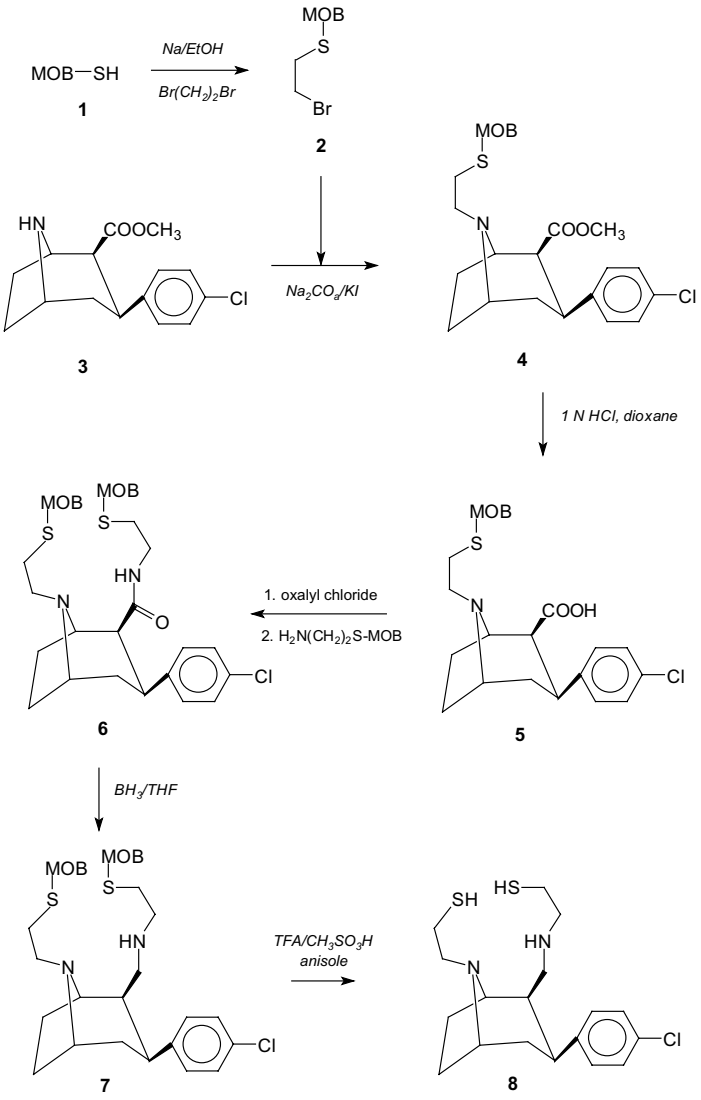
The 'integrated tropane-BAT' analog was synthesised as outlined in Scheme 1. Using *p*-methoxybenzylmercaptan[4] (**1**) instead of tritylthiol as a starting material, 1-(*p*-methoxybenzylmercapto)-2-bromoethane (**2**) was prepared following a procedure described by Meegalla et al.[11] N-Alkylation of 3β-(*p*-chlorophenyl)nortropane-2β-carboxylic acid methyl ester[12] (**3**) with **2** was achieved by refluxing in dioxane in the presence of Na_2CO_3 and KI. The resulting N-alkylated product **4** was hydrolysed to acid **5** and converted to the corresponding acid chloride by reaction with oxalyl chloride in dichloromethane. The acid chloride was reacted with S-(*p*-methoxybenzyl)-2-aminoethanethiol to give compound **6**. Reduction of the amide bond with borane, followed by deprotection to produce the bis-thiol **8** was carried out according to a described procedure.[4]

2.2. Labelling with technetium-99m

Labelling with ^{99m}Tc was carried out by heating a mixture of ^{99m}Tc-pertechnetate in 0.9% NaCl and $SnCl_2 \cdot 2H_2O$ (50 μg) in the presence of a solution containing the integrated tropane–BAT **8**, EDTA and KNa-tartrate at pH 7 in water–ethanol (1/1) at 100 °C for 10 min. The ^{99m}Tc-labelled tropane derivative was obtained in a radiochemical yield of 90%. A final purification was performed by reversed phase HPLC.

2.3. Radio-LC–MS

Radio-LC–MS analysis of the reaction mixture after labeling of **8** with ^{99m}Tc showed one major peak in the radiometric channel (Fig. 3). The background subtracted summed mass spectrum over this peak showed



Scheme 1. Pathway for the synthesis of the integrated tropane–BAT MOB = *p*-methoxybenzyl protecting group, TFA = trifluoroacetic acid.

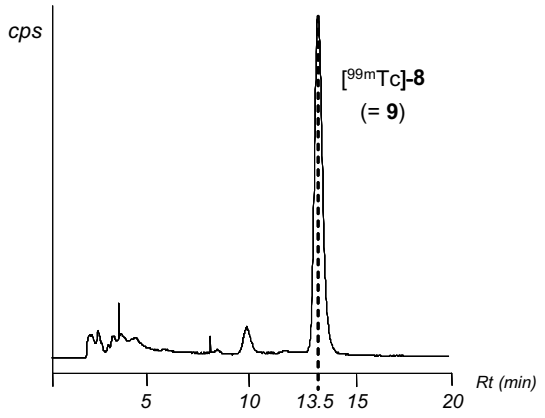


Figure 3. HPLC chromatogram of the labelling reaction mixture after labelling **8** with technetium-99m.

the presence of a molecular ion peak corresponding to the expected oxo-technetium(V)–tropane complex **9**. The found accurate mass (theoretical 483.0148 Da, found 483.0103 Da) also corresponds to the assumed structure of **9**.

2.4. Biodistribution in rats

The results of a preliminary biological evaluation of HPLC-isolated **9** in healthy rats at 60 min p.i. are shown in Table 1 together with the data obtained in the same way for ^{99m}Tc-TRODAT-1 and ^{123}I-FP-CIT. Compared to ^{99m}Tc-TRODAT-1, we found for the newly developed ^{99m}Tc-labelled integrated tropane–BAT a slightly higher liver uptake, a faster clearance from the blood and lungs and a clearly lower activity in the muscles. More interestingly, its brain activity 1 h after injection (0.24% of I.D.) is also slightly higher than that of ^{99m}Tc-TRODAT-1 (0.20% of I.D.), but the brain activity of ^{123}I-FP-CIT is 3–4 times higher than that of both ^{99m}Tc-labelled agents. Literature data for ^{99m}Tc-TRODAT-1 in rats mention a brain activity of only 0.12% of I.D. at 60 min p.i.,[13] but these data were obtained in Sprague–Dawley rats under ether anesthesia, whereas

Table 1. Biodistribution data (% of I.D.) of the ^{99m}Tc-labelled integrated tropane–BAT in rats ($n = 3$) at 60 min p.i. compared with data obtained in the same way for ^{123}I-FP-CIT and ^{99m}Tc-TRODAT-1

	^{123}I-FP-CIT	^{99m}Tc-TRODAT-1	Integrated
Kidneys	2.04 ± 0.25	3.77 ± 0.74	2.29 ± 0.16
Liver	19.21 ± 1.35	19.12 ± 2.22	24.33 ± 1.56
Lungs	1.46 ± 0.18	4.78 ± 0.51	0.47 ± 0.02
Heart	0.25 ± 0.00	0.32 ± 0.05	0.19 ± 0.03
Blood	3.10 ± 0.40	3.04 ± 0.20	1.57 ± 0.12
Muscle	14.62 ± 0.73	17.99 ± 2.30	9.94 ± 0.64
Brain	0.84 ± 0.1	0.20 ± 0.03	0.25 ± 0.04

in our study Wistar rats under Hypnorm^{R} anesthesia were used.

3. Discussion

Labelling of a tropane derivative with ^{99m}Tc requires derivatisation with a Tc-chelating ligand. Different approaches have been used by various groups in an intensive search for a ^{99m}Tc-labelled tropane with sufficient brain uptake and high affinity for the DAT. In most cases a bis-amino bis-thiol (BAT, N_2S_2) tetraligand with an alkylene chain on one of the BAT amines was coupled to the tropane at its amine nitrogen atom or attached as a 2β side chain to allow labeling with technetium-99m. The BAT ligand has also been replaced by bi- or tridentate ligands to form a ^{99m}Tc(I)-tricarbonyl complex. The most successful of these molecules so far has been ^{99m}Tc-TRODAT-1 (Fig. 1). However as it is the case with all these technetium-99m labelled tropane derivatives, ^{99m}Tc-TRODAT-1 is characterised by a relatively low brain uptake and a reduced affinity for the DAT transporter as compared to the higher mentioned PET and ^{123}I-labelled tropane derivatives. Probably, an important factor contributing to the less favourable biological properties of these ^{99m}Tc-labelled DAT binding tropanes is their relatively high molecular mass, a result of the necessity to derivatise the tropane with a Tc-binding complexing ligand. In addition, the nature of the linker between the tropane and the Tc-complexing unit has been found to have a potentially significant impact on the biological properties.[10]

Therefore, it seemed interesting to develop ^{99m}Tc-labelled tropanes with a smaller molecular weight and without a linker between the Tc-binding part and the tropane. In this study, we have developed a first so-called integrated tropane–BAT by derivatising the amine of a nortropane with a mercaptoethyl substituent and converting the methyl carboxylate into an *N*-methylene–cysteamine moiety. Although such structural change implies a significant modification of the tropane structure at the carboxylate ester and the bridged amino group, the example of ^{99m}Tc-TRODAT-1 shows that derivatisation of the tropane at the 2β-methyl carboxylate is possible with preservation of reasonable affinity for the DAT. On the other hand, several literature reports have indicated that the requirement for a basic nitrogen or even a nitrogen is not essential,[14–17] which suggests that derivatisation at this position is allowed.

Although the resulting BAT tetraligand is relatively rigid due to its partial incorporation into a tropane structure, it was found to bind reduced technetium very efficiently, as labelling yields in the order of 85–90% were obtained reproducibly. The structure of the resulting ^{99m}Tc-labelled 'N_2S_2 integrated' tropane has not yet been confirmed by X-ray crystal analysis, but the LC–MS study provided convincing data to support the formation of a Tc(V)oxo-N_2S_2–tropane complex. The molecular weight of this new ^{99m}Tc-labelled tropane is 483.01, that is, 57 Da less than the molecular weight of ^{99m}Tc-TRODAT-1 and well below 600 Da, a value generally accepted as upper limit for reasonable penetration of a compound through the blood–brain barrier.[18]

Preliminary biological evaluation of this first ^{99m}Tc-labelled integrated tropane–BAT in normal rats at 60 min p.i. shows a significant brain activity, slightly higher than that of ^{99m}Tc-TRODAT-1, and a fast blood clearance. These results demonstrate that the integrated tropane–BAT structure forms complexes with technetium that are able to pass the blood–brain barrier and are retained in some brain areas. Further studies to elucidate the exact site and characteristics of this binding to brain structures are ongoing and the results will be reported in a separate paper. As this new class of imaging agents for the dopamine transporter in the central nervous system, with an integration of the ^{99m}Tc-ligand in the structure of the biomolecule, offers many possibilities for further variation, further exploration of this new type of ^{99m}Tc-labelled tracer agents is fully justified and opens perspectives for new radiopharmaceuticals for studying brain pathologies.

4. Experimental

All reagents were obtained commercially from Acros (Geel, Belgium), Fluka (Bornem, Belgium) or Merck (Darmstadt, Germany). ^{1}H NMR spectra were obtained using a Varian 200 MHz spectrometer (Varian, Palo Alto, CA, USA). Chemical shifts are reported in ppm relative to the reference compound tetramethylsilane (TMS, $\delta = 0$). Mass spectra were acquired on a Micromass LCT mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray interface. HPLC analysis and purification was done using an Xterra RP18 5 μ column (250 mm × 4.6 mm, Waters, Milford, USA) eluted with a mixture of 0.1 M ammonium acetate–acetonitrile (35/65, V/V). Radio-LC–MS analysis

was performed on a system consisting of a Waters separation module, an Xterra MS C18 column (50 mm × 2.1 mm, Waters) and a serial 2 in NaI(Tl) radiation detector and a Micromass LCT (time of flight) mass spectrometer equipped with electrospray interface. Masslynx software (Micromass) was used for acquisition and data processing. The column was eluted at a flow rate of 300 μl/min with gradient mixtures of 0.1% ammonium formate and acetonitrile (linear gradient of 0% acetonitrile at 0 min–80% at 20 min). A solution of 0.01% Kryptofix 2.2.2 in CH_3CN–H_2O (50/50) was added to the column effluent at a flow rate of 1 μl/min and served as lock mass for accurate mass determination. An aliquot of 50 μl of the radiolabelling reaction mixture was injected on the column. 3-(*p*-Chlorophenyl)nortropane-2β-carboxylic acid methyl ester (**3**) was prepared as previously described.[19]

^{99m}Tc-TRODAT-1 was prepared following a procedure described by Kung et al.[4] ^{123}I-3-fluoropropyl-β-CIT (DatSCAN™) was obtained commercially from Amersham Health (Buckinghamshire, UK).

4.1. 1-(*p*-Methoxybenzylmercapto)-2-bromoethane (2)

Sodium (1.374 g, 60 mmol) was dissolved in 50 ml ethanol at 0 °C under a stream of N_2. *p*-Methoxybenzylmercaptan (**1**, 9.07 g, 60 mmol) was added over a period of 5 min and the resulting mixture was stirred at room temperature for 10 min. It was then added dropwise to a solution of 1,2-dibromoethane (11.27 g, 60 mmol) in 50 ml ethanol at 0 °C and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and partitioned between EtOAc (50 ml) and water (50 ml). The EtOAc layer was dried over $MgSO_4$ and concentrated in vacuo to obtain a yellow oil that solidified on standing in the refrigerator. ^{1}H NMR ($CDCl_3$, TMS) δ 2.81 (2H, t, CH_2CH_2Br, ^3J = 8.0 Hz); 3.35 (2H, t, CH_2Br, ^3J = 8.0 Hz); 3.69 (2H, s, CH_2S); 3.77 (3H, s, OCH_3); 6.85 (2H, d Ar*H*, ^3J = 8.8 Hz); 7.21 (2H, d, Ar*H*, ^3J = 8.8 Hz).

4.2. *N*-[2′-(*p*-Methoxybenzylmercapto)ethyl]-3β-(*p*-chlorophenyl)nortropane-2β-carboxylic acid methyl ester (4)

A mixture of 1,4-dioxane (125 ml), Na_2CO_3 (5.662 g, 53.18 mmol), **3** (3.18 g, 13 mmol) and **2** (2.95 g, 17.72 mmol) was heated at reflux for 36 h. The reaction mixture was cooled, concentrated and the residue was dissolved in CH_2Cl_2 (200 ml). After washing successively with water, 0.1 M citric acid, water, 10% $NaHCO_3$ and brine, the organic layer was dried over $MgSO_4$ and concentrated in vacuo to obtain a yellow oil that was purified by column chromatography on silica gel (hexane–EtOAc, 80/20). Yield: 6.93 g (82%). ^{1}H NMR ($CDCl_3$, TMS) δ 1.25 (1H, s, N–H); 1.6–1.8 (3H, m); 1.9–2.2 (2H, m, C*H* *H*-4$_{eq}$), 2.39 (1H, ddd, *H*-4$_{ax}$); 2.73 (1H, m *H*-2); 2.95 (1H, m, *H*-5); 3.20 (1H, dt, *H*-3, ^3J = 12.8 Hz, ^3J = 5.0 Hz); 3.73 (1H, m, *H*-1); 7.12 (2H, d, Ar*H*, ^3J = 8.0 Hz); 7.24 (2H, d, Ar*H*, ^3J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, TMS) δ 27.44; 28.83; 33.41; 34.93; 50.83; 51.05; 53.48; 56.18; 128.29; 128.65; 132.14; 140.73; 173.63; Exact mass (ESI–MS) for $C_{25}H_{30}ClNO_3S$ [M+H$^+$]: found 460.290, calcd 460.164.

4.3. *N*-[2′-(*p*-Methoxybenzylmercapto)ethyl]-3β-(*p*-chlorophenyl)nortropane-2β-carboxylic acid (5)

A suspension of the tropane ester **4** (3.18 g, 6.93 mmol) in a mixture of 1 M HCl (7 ml) and 1,4-dioxane (3 ml) was heated at reflux during 72 h. Next, the solution was concentrated in vacuo and the residue added to a mixture of CH_2Cl_2 (50 ml) and water (50 ml). The organic layer was separated, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2–methanol, 90:10). Yield: 2.77 g (89%); Exact mass (ESI–MS) for $C_{24}H_{28}ClNO_3S$ [M+H$^+$]: found 446.290, calcd 446.148.

4.4. *N*-[2′-(*p*-Methoxybenzylmercapto)ethyl]-3β-(*p*-chlorophenyl)-nortropane-2β-[*N*′-2′-(*p*-methoxybenzylmercapto)ethyl] carboxamide (6)

To a stirred solution of **5** (1.96 g, 4.39 mmol) in 25 ml CH_2Cl_2 was added dropwise under nitrogen oxalyl chloride (10 mmol, 5 ml of a 2 M solution in CH_2Cl_2) at room temperature. After evolution of gas had ceased, the resulting solution was stirred at room temperature for 1 h. The solvent was removed under reduced pressure at room temperature and then under high vacuum to remove residual traces of oxalyl chloride. The resulting acid chloride was suspended in CH_2Cl_2 (25 ml) under N_2 at −10 °C and treated with S-*p*-methoxybenzylcysteamine (1.005 g, 5 mmol) followed by the addition of NEt_3 (1 ml, 7.5 mmol). The mixture was stirred at room temperature overnight, washed successively with 0.1 M citric acid, 10% $NaHCO_3$ and brine and then dried over $MgSO_4$. After evaporation of the solvent, the crude reaction product was purified by column chromatography on silica gel (hexane–EtOAc–NEt_3, 90:45:10). Yield 1.73 g (63%). ^{1}H NMR ($CDCl_3$, TMS) δ 1.6–1.8 (3H, m); 1.9–2.1 (2H, m, CH *H*-4$_{eq}$), 2.35–2.65 (8H, m, NCH_2CH_2S); 3.15 (2H, m, C–*H*); 3.38 (2H, m, C–*H*); 3.50 (1H, m, C–*H*); 3.63 (2H, s, CH_2S); 3.67 (2H, s, CH_2S); 3.77 (3H, s, OCH_3); 3.78 (3H, s, OCH_3); 6.83 (2H, d, Ar*H*, ^3J = 8.8 Hz); 6.84 (2H, d, *ArH*, ^3J = 8.8 Hz), 7.19–7.25 (9H, m, Ar*H*); 9.50 (1H, t, N–*H*); ^{13}C NMR ($CDCl_3$, TMS) δ 24.83; 26.74; 30.56; 31.05; 34.69; 34.93; 35.51; 35.81; 37.72; 52.66; 54.57; 55.14; 59.09; 62.91; 113.96; 126.74; 127.71; 128.13; 129.95; 141.00; 158.60; 172.44; Exact mass (ESI–MS) for $C_{34}H_{41}ClN_2O_3S_2$ [M+H$^+$]: found 625.190, calcd 625.232.

4.5. *N*-[2′-(*p*-Methoxybenzylmercapto)ethyl]-3β-(*p*-chlorophenyl)-2β-[[*N*′-2′-*p*-methoxybenzylmercapto)ethyl]-amino]methylene]-nortropane (7)

To a solution of **6** (1.61 g, 2.6 mmol) in 10 ml THF was added 5.2 ml of a 1 M solution of BH_3/THF dropwise under N_2 and the mixture was heated at reflux under N_2 during 12 h. The reaction mixture was cooled to 0 °C and 1 M HCl (5 ml) was added dropwise. The suspension was evaporated, the residue suspended in 1 M

HCl (25 ml) and the mixture heated at 80 °C during 1 h. The colourless solution was neutralised with 2 M NH_4OH and extracted with CH_2Cl_2 (3×25 ml). The combined extracts were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (hexane–EtOAc–NEt_3, 80/15/5) to provide **7** as a yellow oil. Yield: 1.15 g (72%). ^{1}H NMR ($CDCl_3$, TMS) δ 1.26 (1H, s, N–*H*), 1.4–1.7 (3H, m); 1.8–1.9 (2H, m, CH *H*-4_{eq}); 1.97–2.18 (3H, m, C–*H*, CH_2N); 2.39–2.57 (8H, m, NCH_2CH_2S); 2.71 (1H, dt, *H*-4_{ax}); 3.00 (1H, dt, *H*-3); 3.2–3.37 (2H, m, *H*-1 *H*-5); 3.57 (2H, s, CH_2S); 3.67 (2H, s, CH_2S); 3.77 (6H, s, OCH_3); 6.81 (2H, d, Ar*H*, $^3J = 8.8$ Hz); 6.83 (2H, d, Ar*H*, $^3J = 8.8$ Hz); 7.08–7.28 (8H, m, Ar*H*). Exact mass (ESI–MS) for $C_{34}H_{43}ClN_2O_2S_2$ [M+H$^+$]: found 611.246, calcd 611.253.

4.6. *N*-[2′-Mercaptoethyl]-3β-(*p*-chlorophenyl)-2β-[*N*-(2′-mercaptoethylamino)methylene]-nortropane (8)

To a solution of **7** (611 mg, 1 mmol) in a mixture of CF_3COOH (8 ml) and anisole (0.3 ml, 2.8 mmol) at 0 °C, methanesulfonic acid (3 ml, 50 mmol) was added dropwise. The resulting mixture was stirred at room temperature under N_2 for 1 h and then concentrated in vacuo to obtain a brownish red oil that was dried under high vacuum for 1 h. Water (15 ml) was added at 0 °C and the water layer was washed with ether (3×15 ml), neutralised by the addition of solid $NaHCO_3$ and extracted with CH_2Cl_2 (3×15 ml). The CH_2Cl_2 extracts were dried ($MgSO_4$) and concentrated in vacuo to obtain a colorless oil. Anhydrous ether (60 ml) was added to the oil, and the mixture was kept under sonication for 15 min. The colorless precipitate was removed by suction filtration and the filtrate was concentrated in vacuo. The residue was treated at 0 °C with anhydrous methanol (15 ml) and a solution of 1 M HCl in ether (15 ml). The resulting mixture was stirred vigorously for 15 min, concentrated in vacuo, treated with anhydrous ether (30 ml) and stirred for an additional 30 min. The precipitate formed was collected by filtration and dried under high vacuum. Yield: 260 mg (54%), Exact mass (ESI–MS) for $C_{18}H_{27}ClN_2S_2$ [M+H$^+$]: found 371.204, calcd 371.138.

4.7. Labelling with technetium-99m

Deprotected ligand **8** (200 μg; 1 ml of a freshly prepared stock solution of 1 mg **8** in a mixture of water (2.5 ml) and ethanol (2.5 ml)) was added to 1 ml of a buffer solution containing 0.5 M phosphate buffer pH 7 (0.5 ml), 0.1 M Na_2EDTA (0.25 ml) and 0.25 ml KNa-tartrate solution (40 mg/ml). Successively were added 100 μg $SnCl_2{\cdot}2H_2O$ in 25 μl 0.05 M HCl and 1 ml ^{99m}Tc-pertechnetate solution (200 MBq ^{99m}Tc, eluate of an Ultratechnekow™ generator, Tyco Healthcare, Petten, The Netherlands). The labelling vial was heated in a boiling waterbath for 10 min, cooled and the mixture was filtered through a 0.45 μm membrane filter. The filtrate was then analysed and purified with the HPLC system described higher. The retention time of **9** was 13.5 min (Fig. 3).

4.8. Biodistribution in healthy rats

Solutions of **9** obtained after HPLC purification or of ^{99m}Tc-TRODAT-1 or ^{123}I-FP-CIT were diluted with normal saline to a concentration of 1.85 MBq/ml. Biodistribution was studied in healthy male Wistar rats (body mass 250–350 g). The animal studies were performed in accordance with the Belgian code of practice for the care and use of animals. The rats were sedated by i.m. injection of 0.1 ml of Hypnorm^R (Janssen-Cilag, Sunderton UK.). A volume of 0.5 ml of the diluted tracer solution was injected in the rats via a tail vein. The rats were sacrificed by decapitation at 60 min post injection (p.i.) ($n = 3$). Blood was collected in a tared tube and weighed. All organs and other body parts were dissected, weighed and their radioactivity was counted in a 3 in. NaI(Tl) well crystal, coupled to a multichannel analyser (Wallac, Turku, Finland). Corrections were made for background radiation and physical decay during counting. Results were expressed as percentage of injected dose (% of I.D.), equal to the sum of the net counts in all organs. For calculation of activity in total blood and in muscle, blood mass and muscle mass were assumed to be 7% and 40% of the body mass, respectively.

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