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Synthesis and biological evaluation of a technetium-99m(I)-tricarbonyl-labelled phenyltropane derivative

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Abstract—A new tropane derivative was synthesized by combining a tridentate ligand, *N*-(2-picolylamine)-*N*-acetic acid (**2-PAA**), and a phenyltropane derivative. It was labelled with a $[^{99m}Tc(CO)_3]^+$ moiety, resulting in the formation of two stable and neutral lipophilic isomers. Their identity was confirmed using radio-LC–MS. In normal mice, no brain uptake was observed for any of the isomers and in vitro autoradiography using mouse brain sections showed no specific uptake in the striatal area.

Parkinson's disease (PD) is characterized by a significant reduction in density of the presynaptic dopamine transporter (DAT) in the striatum of PD patients.[1–4]

In the past decade, several radiolabelled tropane derivatives that bind specifically to the DAT have been prepared and studied for in vivo imaging using positron emission tomography (PET)[3] or single-photon emission computed tomography (SPECT).[4]

^{123}I-labelled ioflupane, also called ^{123}I-FP-CIT and commercially available from GE Healthcare (DaTSCAN^{TM}, Little Chalfont, UK), is an example of such a dopamine transporter tracer agent for SPECT.[5] However, the suboptimal availability and the high cost of the iodine-123 radioisotope limit the application of this tracer in most nuclear medicine departments.

Therefore, great effort has been made to develop ^{99m}Tc-labelled diagnostic tropane derivatives, in view of the attractive nuclear-physical properties and continuous availability at a relatively low cost of this radionuclide. Unlike radioiodine, the transition metal ^{99m}Tc needs a chelating structure to become stably bound to an organic molecule. However, the incorporation of such a ^{99m}Tc-chelating moiety, mostly based on an amide-thiol (MAMA) or amine-thiol (BAT) tetraligand, may drastically change the biological behaviour of the resulting radiotracer as compared to the original compound.

Many ^{99m}Tc-complexes have already been proposed for imaging of DAT sites such as ^{99m}Tc-TRODAT-1,[6] ^{99m}Tc-Technepine[7] and ^{99m}Tc-Integrated-tropane-BAT[8] (Fig. 1), but images acquired using iodine-123-labelled DAT radiotracers are clearly superior.

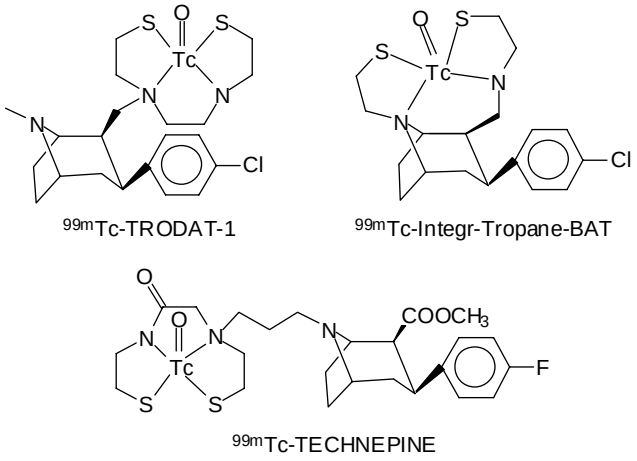

Figure 1. Structure of some ^{99m}Tc-labelled tropane derivatives.

Keywords: Technetium-99m; Tricarbonyl; Radio-LC–MS; Dopamine transporter.
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A few years ago, the organometallic aqua complex fac-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ was proposed as a versatile source for the fac-$[^{99m}Tc(CO)_3]^+$ moiety.[9] This precursor can easily form complexes with different di- or triligands by substitution of the three loosely bound water molecules. Hoepping et al.[10] described TROTEC-1 (Fig. 2), a ^{99m}Tc-tricarbonyl tropane complex in which a diligand thioether-thiol is linked through an ester bridge with a phenyltropane moiety. Unfortunally, brain uptake of the complex was limited. Recently, Zhang et al.[11] also developed a ^{99m}Tc-tricarbonyl tropane derivative, $[^{99m}Tc(CO)_3$(TROPYN)I, containing the bidentate ligand 2-(aminomethyl)pyridine at the 2β position of a phenyltropane (Fig. 2). High tracer concentration in rat striatum was reported but no characterization of the assumed complex structure was provided.

In this study, we have developed a ^{99m}Tc-labelled tropane where the phenyltropane is linked to a tridendate ligand system that is capable of forming a stable neutral complex with a fac-$[^{99m}Tc(CO)_3]^+$ moiety. Previous studies of the $[M(CO)_3]^+$ core indicated that chelating ligands incorporating one amine, an aromatic N-heterocycle and a carboxylate donor are very effective for this purpose.[12,13] Therefore, we chose 2-picolylamine-*N*-acetic acid (**2-PAA**) as the tridentate Tc-binding moiety. The new tracer agent was characterized using radio-LC–MS and its biological properties were evaluated in vivo and in vitro.

The aryltropane ligand was synthesized as outlined in Scheme 1 starting from 2β-carboxy 3β-(*p*-chlorophenyl) *N*-methyl tropane **1**.[14] This was converted to the acid chloride and reacted with 2-(aminomethyl)pyridine in the presence of Et_3N in CH_2Cl_2 at −10 °C to obtain **2**.[15] After reduction of the amide to amine **3**[16] using borane in THF, the secondary amine was alkylated with methyl bromoacetate to obtain intermediate **4**.[17] This intermediate was hydrolysed to the carboxylic acid **5** with NaOH 1 M. A reaction time of 60 min at rt was sufficient to obtain complete hydrolysis as shown by HPLC analysis and MS.[18]

For radiolabelling, the precursor fac-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ was prepared using an IsoLink™ kit and then reacted with ligand **5** or its ester precursor **4** at 70 °C in phosphate buffer 0.5 M (pH 4, 7, 9 or 11) for 20 min.[19] HPLC analysis showed in each of the tested reaction conditions the formation of two main radiochemical species with a retention time of 15.3 and

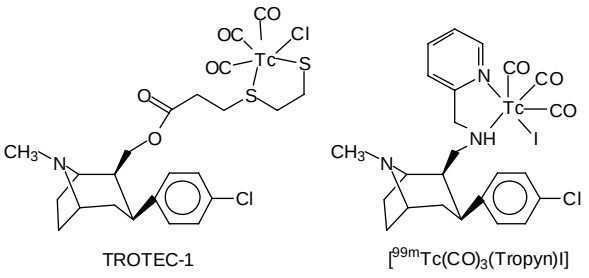
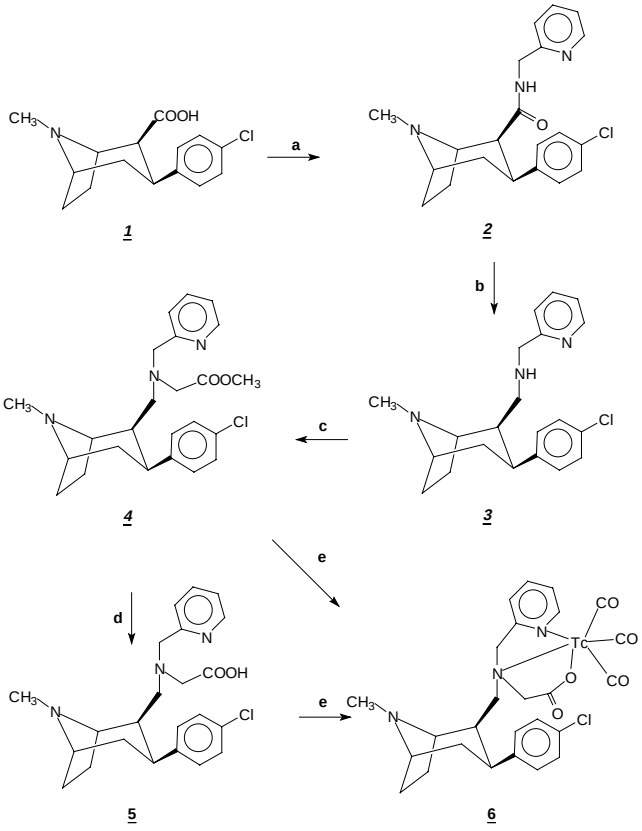


Figure 2. Structure of two bidentate $[^{99m}Tc(CO)_3]$tropane conjugates.



Scheme 1. Reagents and conditions: (a) oxalyl chloride, 2-aminopicoline, Et_3N, rt; (b) BH_3, THF 1 M, reflux; (c) methyl bromoacetate, NEt_3, MeOH rt; (d) 1 M NaOH, rt 60 min; (e) phosphate buffer 0.5 M, pH 7, $[^{99m}Tc(CO)_3(OH_2)_3]^+$, 70 °C 20 min.

16.0 min, respectively.[20] Optimal labelling yields (>85%) were obtained at pH 7, even when the ester intermediate **4** was used as starting material. The latter procedure was used for further biological evaluation experiments since more drastic hydrolysis using 1 M NaOH holds the risk of partial or complete racemisation to the α configuration.

Up to now, direct identity confirmation of a neutral ^{99m}Tc-tricarbonyl complex using radio-LC–MS analysis has, to our knowledge, not yet been described. For the purpose of such LC–MS analysis, the labelling was performed using an IsoLink™ kit, which was reconstituted with a mixture of 600 μl $^{99m}TcO_4^-$ solution and 200 μl $^{99}TcO_4^-$ solution (15 μg/ml) in order to obtain a sufficient mass of the Tc-complex. High resolution radio-LC–MS analysis of such a reaction mixture showed the expected molecular ion mass of ^{99m}Tc-**6** (596.0768 Da, Fig. 3) on the mass spectrometer channel at the time of elution of both consecutive peaks in the radiometric channel (t_R: 9.16 and 10.34 min) with a relative error of 5.5 ppm.[21] Furthermore, the single ion mass chromatogram (595.766–596.388) showed the same two peaks having an identical retention time as both peaks in the radiometric channel. These mass spectrometric data not only provide a strong support for the identity confirmation of the tracer agents formed but also indicate that these two compounds are most probably isomers. Coordination of the tricarbonyl core is

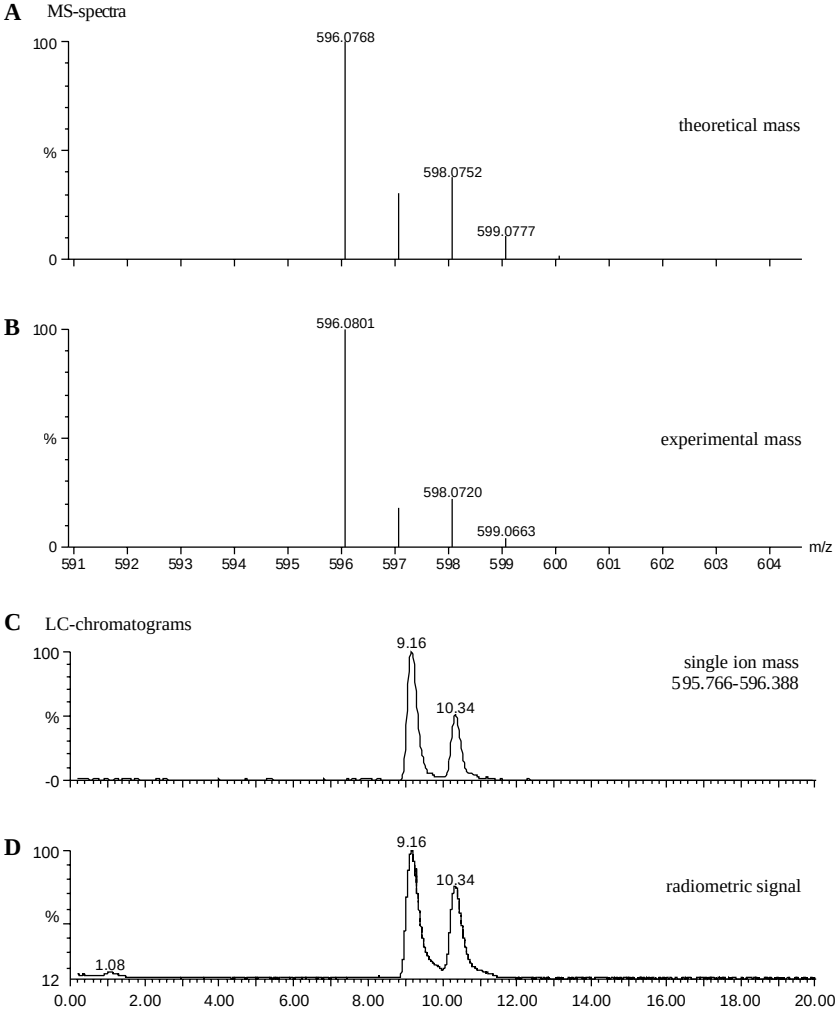


Figure 3. Radio-LC–MS analysis (ESI^+: *m/z* ($M + H^+$)) of the reaction mixture after labelling of **5** with fac-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ at pH 7. Two main peaks were observed in the radiometric signal (D) with identical retention times as two peaks in the single ion mass chromatogram (C). The experimental accurate mass spectrum (B) matches the calculated mass, 596.0768 (A).

most likely exclusively tridentate (via the carboxylate oxygen, the tertiary amine and the pyridine nitrogen). As only one of the CO-ligands binds axially to the technetium atom, either the carboxylate or the pyridine nitrogen is orientated axial to the technetium atom.

Analysis of the assumed isomers by paper electrophoresis (Whatman 4 paper, mixture of 0.025 M phosphate buffer, pH 7.4, and MeOH (50:50) v/v) as electrolyte solution, 300 V for 15 min) did not show any migration, which indicates that both tracer agents are neutral.

Table 1. Biodistribution of ^{99m}Tc-**6** in normal mice at 2 and 60 min p.i.

Organ	Isomer A		Isomer B	
	2 min p.i.	60 min p.i.	2 min p.i.	60 min p.i.
	% of injected dose ± standard deviation ($n = 4$)			
Urine	0.1 ± 0.1	1.9 ± 0.2	0.0 ± 0.0	0.4 ± 0.1
Kidneys	7.5 ± 1.5	0.4 ± 0.0	5.4 ± 0.3	1.8 ± 0.2
Liver	56.9 ± 4.8	40.4 ± 2.5	51.5 ± 1.3	67.0 ± 2.1
Lungs	0.6 ± 0.1	0.1 ± 0.0	2.7 ± 0.8	0.4 ± 0.2
Heart	0.5 ± 0.1	0.0 ± 0.0	0.5 ± 0.0	0.2 ± 0.0
Intestines	8.5 ± 0.4	63.8 ± 11.6	5.1 ± 0.4	17.4 ± 1.7
Stomach	0.9 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	0.4 ± 0.1
	% of injected dose per gram tissue ± standard deviation ($n = 4$)			
Cerebrum	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00
Cerebellum	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Blood	2.06 ± 0.59	0.06 ± 0.04	3.14 ± 0.60	0.10 ± 0.02

Log $P_{oct/buffer}$ values were determined using a published procedure[22] and found to be 2.14 ± 0.02 for isomer A and 2.12 ± 0.03 for isomer B, both compatible with reasonable brain uptake. Indeed, an optimal passage over the BBB is suggested for compounds with log $P_{oct/buffer}$ values ranging from 1 to 2.5.[23]

For determination of the binding affinity of both isomers of ^{99m}Tc-**6** for the DAT, a male NMRI mouse was sacrificed, its brain isolated and quick-frozen to −40 °C. Adjacent 20-μm sections (Bregma 0.50–1.00) were cut at −20 °C and thaw-mounted onto gelatine-coated glass slides. Tissue sections were preincubated for 10 min at rt in Tris–HCl buffer (50 mM, pH 7.4) to remove endogenous ligand. Sections were then incubated at rt with 300 μl HPLC-purified ^{99m}Tc-**6** solution (37 kBq/ml, isomer A or B) for 40 min. DaTSCAN™ was used as a reference substance. After a washing step of 60 s in a mixture of distilled water and EtOH (75:25 v/v), the sections were exposed to a high performance storage phosphor screen (Canberra, Packard) which was analyzed after 24 h using a phosphor imaging system. Under the tested conditions, no uptake in the DAT-rich striatal area was found for any of the ^{99m}Tc-**6** isomers, in contrast to the results obtained with DaTSCAN™.

Biodistribution of the two HPLC isolated isomers was studied in normal mice at 2 and 60 min p.i. The results are summarized in Table 1. Most importantly, none of the radiolabelled compounds showed significant brain uptake. Furthermore, clearance from blood by the hepatobiliary system is faster in the case of isomer A. HPLC analysis of blood and urine at 60 min p.i. revealed high stability of these compounds in vivo, as more than 95% of the activity was still in the original form.

In summary, synthesis of two isomers of the newly developed ^{99m}Tc-tricarbonyl tropane-PAA conjugate was successful and their structure was confirmed using radio-LC–MS. Although log $P_{oct/buffer}$ values of the two radiolabelled species are compatible with BBB passage, no brain uptake was found in normal mice. In addition, in vitro autoradiography using mouse brain sections showed no binding in the DAT-rich striatum. For these reasons, it may be concluded that the newly developed ^{99m}Tc-tricarbonyl tropane derivatives are not suitable as potential DAT tracer agents.

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15. Compound **2**: yellow oil; yield 90%; ^{1}H NMR (200 MHz) ($CDCl_3$) δ 1.6–1.7 (3H, m); 2.10 (1H, m); 2.20 (1H, m); 2.26 (3H, s); 2.30 (1H, dt); 2.59 (1H, dd); 3.09 (1H, dt); 3.35 (2H, m), 4.42–4.51 (2H, 2× dd); 7.00 (2H, d); 7.10 (2H, d); 7.14 (1H, d); 7.18 (1H, dd), 7.60 (1H, dd); 8.57 (1H, d); 10.24 (1H, s). MS (Micromass LCT mass spectrometer), ESI+: calcd for $C_{21}H_{24}ClN_3O$, $[M+H]^+$: *m/z* 370; found: 370.
16. Compound **3**: brown oil; yield 90%; ^{1}H NMR (200 MHz) ($CDCl_3$) δ 1.51 (1H, dt); 1.60 (1H, dd); 1.64 (1H, dd); 1.68 (1H, dd); 1.84 (1H, m); 2.05 (1H, m); 2.12 (1H, dt); 2.17 (1H, dd); 2.20 (1H, m); 2.23 (3H, s); 2.76 (1H, dd); 3.04 (1H, dt); 3.24 (1H, m); 3.34 (1H, m); 3.66 (2H, d); 7.07 (2H, m); 7.09 (2H, d); 7.21 (2H, d); 7.54 (1H, dt); 8.47 (1H, d). MS (Micromass LCT mass spectrometer), ESI+: calcd for $C_{21}H_{26}ClN_3$, $[M+H]^+$: *m/z* 356; found: 356.
17. Compound **4**: yellow oil; yield 54%; ^{1}H NMR (200 MHz) ($CDCl_3$) δ δ 1.52 (1H, dt); 1.5–1.9 (6H, m); 2.12 (2H, d); 2.25 (3H, br s); 3.05 (2H, m); 3.25 (3H, m); 3.60 (3H, s); 3.71 (2H, d); 7.04 (2H, d); 7.13 (1H, dd); 7.23 (2H, d); 7.28 (1H, d); 7.60 (1H, dt); 8.51 (1H, d). MS (Micromass LCT mass spectrometer), ESI+: calcd for $C_{24}H_{30}ClN_3O_2$, $[M+H]^+$: *m/z* 428; found: 428.
18. Compound **5**: a solution of compound **4** (10 mg) in a mixture of CH_3CN (0.5 ml) and 1 M NaOH (0.5 ml) was stirred for 60 min at rt. Purification was done with HPLC (Merck-Hitachi L-6200) on an XTerra™ RP18 column (5 μm, 4.6 mm × 250 mm) eluted at a flow rate of 1 ml/min with gradient mixtures of 0.1% trifluoroacetic acid in water (A) and 0.1% trifluoroacetic acid in CH_3CN (B) (linear gradient of 100% A to 100% B in 20 min, followed by 100% B during 5 min). The peak eluting at 13.4 min was collected and the solvent was evaporated. The identity of **5** was confirmed with MS (Micromass LCT mass spectrometer), ESI+: calcd for $C_{23}H_{28}ClN_3O_2$, $[M+H]^+$: *m/z* 413; found: 413.
19. Ligand **5** (300 μg in 30 μl CH_3CN) or its ester (**4**, 100 μg in 5 μl CH_3CN) was added to 0.5 ml of 0.5 M phosphate buffer of different pH values. One hundred microliters (37 MBq) of a freshly prepared solution of ^{99m}Tc-tricarbonyl precursor $[^{99m}Tc(CO)_3(OH_2)_3]^+$ prepared from an IsoLink™ kit (Tyco-Mallinckrodt, Petten, The Netherlands) was added and this mixture was heated at 70 °C for 20 min, cooled and filtered through a 0.45-μm membrane filter.

20. Reversed-phase radio-HPLC: Merck Hitachi L-7100 separation module connected to RP C18 column (XTerra^{TM} RP18 5 μm, 250 mm × 4.6 mm, Waters, Milford, USA). Analysis via radiometric detector (3-in NaI(Tl) detector connected to radiation analyzer module, Canberra Packard, Meriden, Connecticut). The column was eluted at a flow rate of 1 ml/min using linear gradient mixtures of CH_3CN and 0.1 M NH_4OAc (t = 0 min, 0% CH_3CN; t = 20 min, 100% CH_3CN v/v).
21. Radio-LC–MS: Waters 2690 separation module connected to RP C18 column (XTerra^{TM} MS C18 3.5 μm, 2.1 mm × 50 mm). Analysis via radiometric detector (3-in NaI(Tl) detector coupled to radiation analyzer module, The Nucleus, Oak Ridge, TN, USA). The column was eluted at a flow rate of 300 μl/min using linear gradient mixtures of CH_3CN and 0.1 M HCOOH (t = 0 min, 0% CH_3CN; t = 20 min, 80% CH_3CN v/v). Finally, the column eluate containing the unlabelled precursor **4** as an internal standard for high resolution MS was directed to a time-of-flight mass spectrometer (Micromass LCT, Waters) with an orthogonal ESI probe.
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