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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.

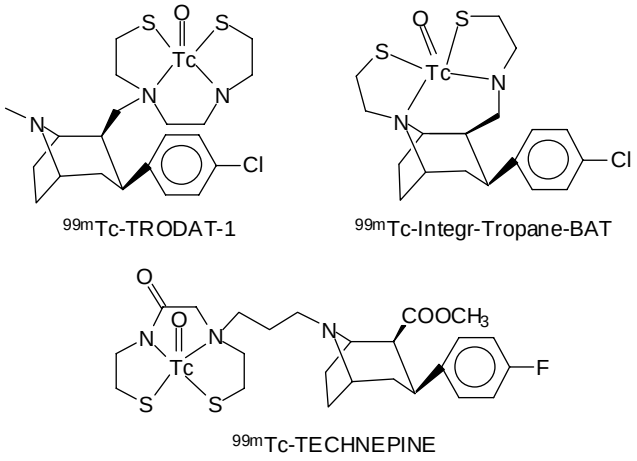

Figure 1. Structure of some ^{99m}Tc-labelled tropane derivatives.

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

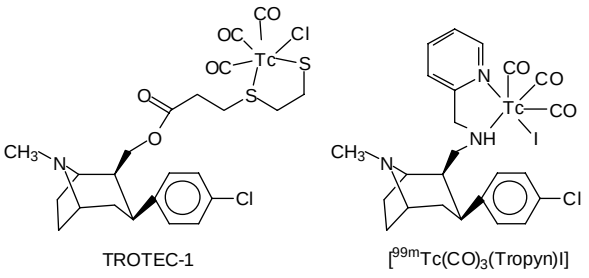
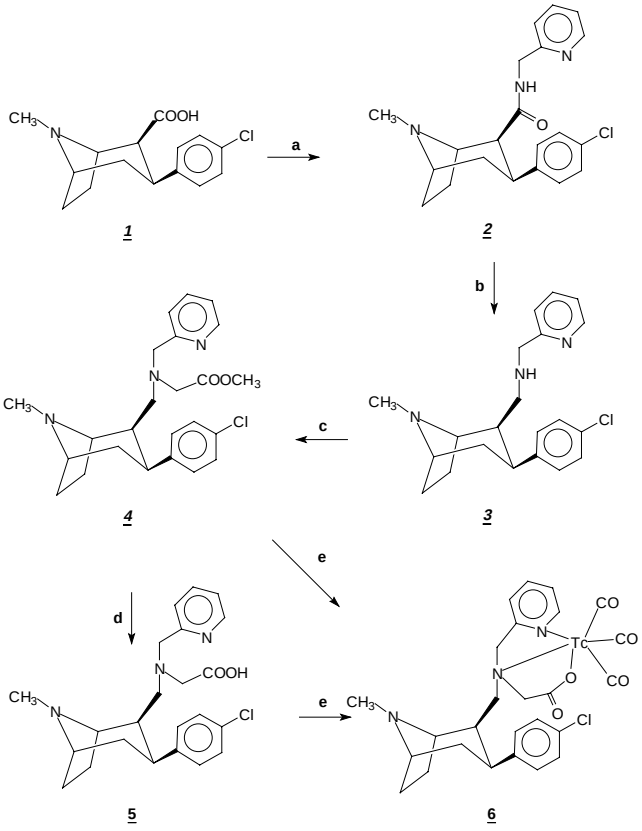


Figure 2. Structure of two bidentate $[^{99m}Tc(CO)_3]$tropane conjugates.



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