Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 4192–4195

Characterisation and biodistribution of two neutral $^{99m}Tc(CO)_3$ complexes with a tridentate ligand

Dirk Rattat, Bernard Cleynhens, Guy Bormans, Christelle Terwinghe and Alfons Verbruggen*

Laboratory for Radiopharmaceutical Chemistry and Nuclear Medicine, Catholic University of Leuven, Herestraat 49, 3000 Leuven, Belgium

Received 2 May 2005; revised 24 June 2005; accepted 27 June 2005
Available online 3 August 2005

Abstract—*N*-(2-Mercapto-propyl)-1,2-phenylenediamine (MPPDA) and *N*-β-aminoethylglycine (AEG) were labelled with $^{99m}Tc(CO)_3^+$ to form the neutral complexes [$^{99m}Tc(CO)_3$(MPPDA)] and [$^{99m}Tc(CO)_3$(AEG)]. Both complexes were formed in excellent yields and their identity was confirmed by LC–MS. In mice, none of the new tracer agents showed brain uptake. [$^{99m}Tc(CO)_3$(MPPDA)] was trapped mainly in the liver and excreted via the hepatobiliary system, whereas [$^{99m}Tc(CO)_3$(AEG)] was excreted rapidly via the kidneys to the urine.

The Tc–tricarbonyl chemistry has attracted growing interest since Alberto et al. published a convenient low pressure synthesis for the preparation of ^{99m}Tc–tricarbonyl complexes.[1] The development of novel radiopharmaceuticals for routine diagnosis in nuclear medicine based on the $^{99m}Tc(CO)_3$ chemistry is a challenge, particularly to find $^{99m}Tc(CO)_3$ complexes that can penetrate the blood–brain barrier (BBB) or show specific binding to a receptor. Despite the fact that labelling of a biomolecule with ^{99m}Tc mostly requires a significant derivatisation with a metal complexing chelator, the clinical usefulness of ^{99m}Tc–TRODAT-1 for diagnosis of Parkinson's disease has shown that the development of ^{99m}Tc-labelled receptor binding agents is feasible.[2] Efforts have been made to synthesise other tropane derivatives suitable for complexing the $^{99m}Tc(CO)_3$ moiety via a tridentate chelator or cyclopentadienyl.[3,4] Also $^{99m}Tc(CO)_3$-labelled small peptides, for example, neurotensin derivatives, were intensively studied.[5–7] For the functionalisation of biomolecules, several ligand systems have been explored systematically.[8–11] It has been reported that the nature of the chelating ligand can significantly affect the physicochemical properties of the radiolabelled peptides, including lipophilicity, and consequently change their binding properties.[5,12]

N-(2-Mercapto-propyl)-1,2-phenylenediamine (MPPDA) is a ligand with a primary amine, a secondary amine and a thiol as metal binding groups, each separated by two carbon atoms. The three heteroatoms of MPPDA in this arrangement make it a potential target molecule for labelling with a $^{99m}Tc(CO)_3$ moiety, resulting in the neutral complex [$^{99m}Tc(CO)_3$(MPPDA)]. In the literature, labelling of this compound has been reported with a ^{99m}Tc(V)O core using pertechnetate and stannous chloride. The resulting complex ^{99m}Tc(V)O MPPDA was described as lipophilic and was able to enter the brain.[13]

N-β-Aminoethyl-glycine (AEG) has also a primary and a secondary amine, although in this case both are aliphatic, but the third heteroatom for complexing the $Tc(CO)_3$ moiety is an oxygen of a carboxylic acid group. The absence of an aromatic ring makes AEG less lipophilic than MPPDA.

The aim of this study was to compare the biological characteristics of the $^{99m}Tc(CO)_3$ complexes of these two ligands, with a special focus on the extent of their brain uptake, if any. The choice for MPPDA and AEG was made because they have a low molecular weight and suitable heteroatoms (N, O, S) at the right

Keywords: Technetium; Carbonyl; LC–MS; HPLC; Biodistribution.
* Corresponding author. Tel.: +32 16 343 732; fax: +32 16 343 891; e-mail: alfons.verbruggen@uz.kuleuven.ac.be

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.06.086

distance (separated by two carbon atoms) to form neutral complexes with a $Tc(CO)_3$ moiety. MPPDA represents a more lipophilic, and AEG a more polar chelating ligand system.

N-(2-Mercapto-propyl)-1,2-phenylenediamine was prepared in a reaction of 1,2-phenylenediamine and propylene sulfide. The yield of 32% was slightly higher than the literature value of 26.7%.[8] In addition, we also characterised the side products of the reaction by reversed phase HPLC (X-Terra RP-18 column 4.6 mm × 250 mm; Waters, Brussels, Belgium; gradient elution from 0.1% trifluoroacetic acid in water to 0.1% trifluoroacetic acid in acetonitrile in 20 min at a flow rate of 1 ml/min) in combination with LC–MS (Waters separation module, XTerra MS C18 column 50 mm × 2.1 mm, 3-in. NaI(Tl) radiation detector, Micromass LCT mass spectrometer and MassLynx software, Waters-Micromass, Manchester, UK). The Main side product was the bis-adduct *N*,*N*′-bis-(2-mercapto-propyl)-1,2-phenylenediamine (BMPPDA), but also compounds from dimerisation reactions of free thiols into disulfides were found. LC–MS indicated a mass of 183.1 Da (theoretical: 182.28) for MPPDA (**1**), 257.1 Da (theoretical: 256.42) for BMPPDA (**2**) and 363.13 Da (theoretical: 362.55) for a supposed dimer (**3**) (see Fig. 1).

The Tc–tricarbonyl precursor $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was synthesised directly from a solution of $[^{99m}TcO_4]^-$ (eluate from a commercially available Ultratechnekow FM ^{99m}Tc-generator; Tyco-Mallinckrodt, Petten, The Netherlands) using a mixture of $NaBH_4$, Na_2CO_3, K–Na-tartrate and CO gas according to a published procedure.[1] Labelling of the isolated MPPDA was performed at 70 °C and pH 10 for 30 min (activities up to 500 MBq), resulting in a yield of >95%. The ^{99m}Tc complex was stable for at least 24 h. Using the unpurified reaction mixture of the organic synthesis of MPPDA under the same conditions resulted in three labelled compounds, which were separated by HPLC (water–acetonitrile 70:30 at a flow rate of 1 ml/min, no gradient) (see Fig. 2) and analysed by LC–MS. The main product was $[^{99m}Tc(CO)_3(MPPDA)]$ (t_R = 15 min, 57%), but also the labelled bis-adduct $[^{99m}Tc(CO)_3(BMPPDA)]$ (t_R = 17 min, 27%) and a labelled dimeric compound (t_R = 18.5 min, 17%) were detected.

The commercially available AEG (BACHEM, Switzerland) is known as an excellent ligand for complexation with the $^{99m}Tc(CO)_3$ moiety.[10] A labelling under the same conditions as for MPPDA (70 °C, pH 10) gave

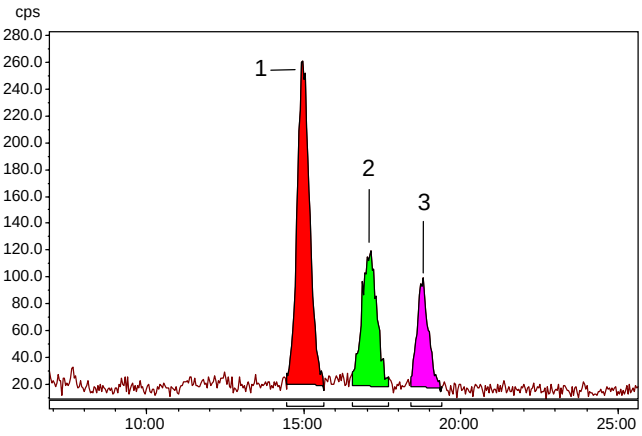

Figure 2. Reaction of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ with the crude product of the synthesis of MPPDA (see Fig. 1) resulted in a mixture of three radiolabelled products, that is, $[^{99m}Tc(CO)_3(MPPDA)]$ (**1**), $[^{99m}Tc(CO)_3(BMPPDA)]$ (**2**) and a labelled dimer (**3**).

quantitative yields within 10 min. The retention time of $[^{99m}Tc(CO)_3(AEG)]$ on RP-HPLC (t_R = 9.2 min) indicated a clearly higher polarity as compared to that of $[^{99m}Tc(CO)_3(MPPDA)]$. Mass spectrometry (LC–MS) supported the supposed structure of $[^{99m}Tc(CO)_3(AEG)]$ with AEG as tridentate ligand. The radio-HPLC trace (t_R = 2.55 min) and the single ion mass chromatogram for this complex (t_R = 2.55 min) are in accordance with each other. The detected mass of 300.99 Da also corresponds with the theoretical value of 300.96 Da for this complex. Figure 3 shows the data of the LC–MS analysis of $[^{99/99m}Tc(CO)_3(AEG)]$, to our knowledge one of the first examples where a $^{99/99m}$Tc–tricarbonyl complex was analysed by LC–MS. Generator eluate with a higher content of ^{99}Tc (0.1–0.3 ng Tc/MBq) was used for the carrier-added synthesis of the complexes to enhance the signal in mass spectrometry. The solution with the complex had to be concentrated to obtain a detectable signal, but it remained close to the detection limit of the mass spectrometer. An alternative is the addition of ^{99}Tc to the ^{99m}Tc generator eluate when the carbonylation reaction is done (200–300 µl of an aqueous ^{99}Tc solution, containing 15 µg/ml NH_4TcO_4). However, with an increasing amount of the ^{99}Tc solution the yield of the carbonylation reaction drops (using the procedure described above). It is important to find a balance between a good conversion to the Tc–carbonyl precursor and a reasonable signal in LC–MS.

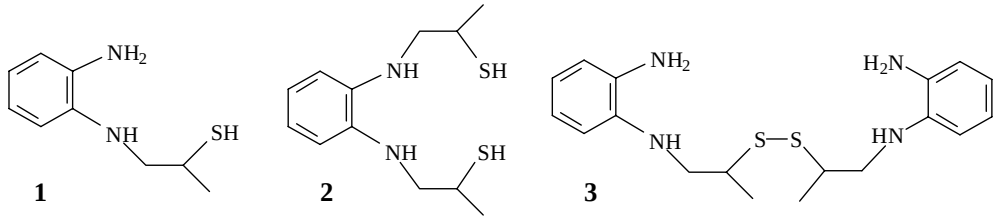

Figure 1. Structure of the main product *N*-(2-mercapto-propyl)-1,2-phenylenediamine (MPPDA) (**1**) and two side products of the synthesis, that is, *N*,*N*′-bis-(2-mercapto-propyl)-1,2-phenylenediamine (BMPPDA) (**2**) and a dimer (**3**).

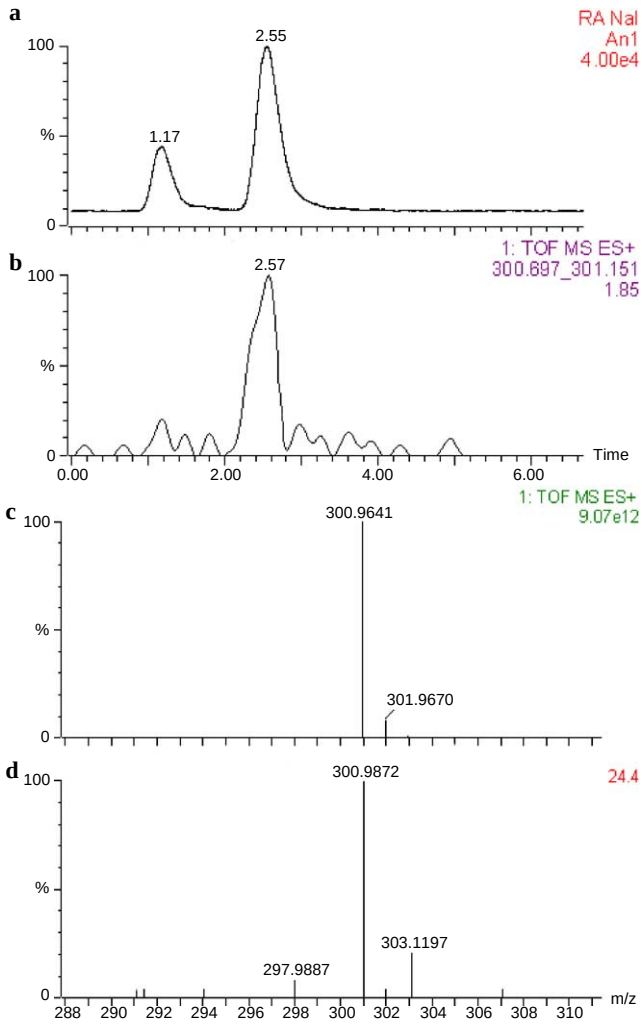

Figure 3. LC–MS analysis of [$^{99/99m}$Tc(CO)$_3$(AEG)]: (a) radiometric signal; (b) single ion mass chromatogram; (c) calculated mass; and (d) detected mass spectrum.

The octanol–water partition coefficient (P_{OW}, expressed in logarithmic form as 'log P') was determined according to a literature procedure.[14,15] It was −0.57 for [^{99m}Tc(CO)$_3$(AEG)], that is, outside the range of 1–2.5, which is believed to be ideal for a potential brain uptake.[16] On the other hand, [^{99m}Tc(CO)$_3$(MPPDA)] showed a log P of 1.35.

Biodistribution experiments were performed with the HPLC-isolated complexes [^{99m}Tc(CO)$_3$(AEG)] and [^{99m}Tc(CO)$_3$(MPPDA)], each in eight normal male NMRI mice (body mass 27–43 g). A ^{99m}Tc activity of approximately 40 kBq/mouse was injected via a tail vein. Four mice each were killed at 2, 10 and 65 min pi and the organs and body parts were dissected and weighed. The activity in the dissected organs and body parts was measured using a Wallac 1480 WIZARD 3″ automatic gamma counter (Perkin–Elmer, Boston, USA). Results are expressed as percentage of injected dose (% of ID) and percentage of injected dose per gram (% of ID/g) for selected organs (Table 1). For calculation of total blood radioactivity, blood mass was assumed to be 7% of the body mass.[17]

Table 1. Biodistribution of [^{99m}Tc(CO)$_3$(MPPDA)] and [^{99m}Tc(CO)$_3$(AEG)] (% of ID and % of ID/g) for selected organs after 2, 10 and 65 min

	MPPDA			AEG		
	2 min	10 min	65 min	2 min	10 min	65 min
% of ID						
Brain	0.01	0.06	0.03	0.10	0.08	0.05
Urine	1.51	2.99	4.89	0.12	6.88	60.77
Kidneys	5.47	1.85	0.98	13.33	8.05	1.73
Liver	42.03	57.64	64.19	14.59	15.55	7.42
Intestines	6.74	12.04	18.93	10.28	8.16	4.54
Spleen	0.91	0.29	0.09	0.89	0.88	0.65
Lungs	0.84	0.87	0.73	1.68	1.64	0.33
Heart	0.51	0.19	0.06	0.37	0.29	0.16
Stomach	1.19	0.54	2.23	1.07	0.87	0.69
Blood	7.85	5.47	3.02	21.50	19.34	8.24
% of ID/g						
Brain	0.03	0.13	0.06	0.24	0.21	0.13
Kidneys	9.19	3.17	1.63	24.72	11.01	2.56
Liver	21.13	27.49	31.35	7.66	6.63	2.94
Spleen	2.94	0.96	0.30	2.73	2.66	1.73
Lungs	3.14	3.36	2.91	6.17	5.13	1.29
Heart	2.97	1.22	0.38	2.38	1.57	0.75
Stomach	1.53	0.84	1.13	1.67	0.93	1.05
Blood	3.05	1.51	0.76	8.19	4.94	2.05

For the more lipophilic [^{99m}Tc(CO)$_3$(MPPDA)] most of the activity was located in the liver (57.6%) 10 min pi where it stayed at a high level after 65 min. The excretion via kidneys/urine was quite low (5.9% after 65 min pi). An increasing amount of the activity was excreted via the hepatobilary pathway within one hour (intestines 18.9%). Spleen, heart and lungs showed no significant uptake.

The more polar [^{99m}Tc(CO)$_3$(AEG)] was rapidly excreted via kidneys/urine (6.9% of ID in the urine after 10 min pi, 60.8% after 65 min). The initial high blood value of 21.5% after 2 min dropped to 8.2% after 65 min. At all timepoints, [^{99m}Tc(CO)$_3$(AEG)] showed a higher blood activity than [^{99m}Tc(CO)$_3$(MPPDA)]. The activity in the liver was significantly lower than for [^{99m}Tc(CO)$_3$(MPPDA)]. Spleen, heart, lungs and stomach showed no significant uptake.

Despite the fact that [^{99m}Tc(CO)$_3$(MPPDA)] and [^{99m}Tc(CO)$_3$(AEG)] are small and neutral complexes with clearly different characteristics in biodistribution experiments, none of these tracer agents showed significant brain uptake. This is especially striking for [^{99m}Tc(CO)$_3$(MPPDA)], as it is neutral, has a log P of 1.35 and a molecular weight of 361.97 Da, these characteristics in accordance with general requirements for a tracer agent able to pass the blood–brain barrier. Apparently, the ability of a compound to pass the BBB is determined by more factors than charge, lipophilicity and size. The combination of a primary and a secondary amine with a thiol or a carboxylic acid group in a tridentate ligand system was excellent for a labelling with the ^{99m}Tc(CO)$_3$ moiety. Other tridentate chelators with equal complexing properties, but a more favourable biodistribution will have to be synthesised for a

potential future application as ^{99m}Tc–tricarbonyl brain tracer agent. Information from these agents may help to unravel the requirements for passage over this intriguing biological barrier.

References and notes

1. Alberto, R.; Schibli, R.; Egli, A.; Schubiger, A. P.; Abram, U.; Kaden, T. A. *J. Am. Chem. Soc.* **1998**, *120*, 7987.
2. Kung, H. F. *Nucl. Med. Biol.* **2001**, *28*, 505.
3. Hoepping, A.; Reisgys, M.; Brust, P.; Seifert, S.; Spies, H.; Alberto, R.; Johannsen, B. *J. Med. Chem.* **1998**, *41*, 4429.
4. Cesati, R. R.; Tamagnan, G.; Baldwin, R. M.; Zoghbi, S. S.; Innis, R. B.; Kula, N. S.; Baldessarini, R. J.; Katzenellenbogen, J. A. *Bioconjug. Chem.* **2002**, *13*, 29.
5. Liu, S.; Edwards, D. S. *Chem. Rev.* **1999**, *99*, 2235.
6. Schubiger, P. A.; Allemann-Tannahill, L.; Egli, A.; Schibli, R.; Alberto, R.; Carrel-Remy, N.; Willmann, M.; Blauenstein, P.; Tourwe, D. *Q. J. Nucl. Med.* **1999**, *43*, 155.
7. García-Garayoa, E.; Allemann-Tannahill, L.; Bläuenstein, P.; Willmann, M.; Carrel-Rémy, N.; Tourwé, D.; Iterbeke, K.; Conrath, P.; Schubiger, P. A. *Nucl. Med. Biol.* **2001**, *28*, 75.
8. Schibli, R.; La Bella, R.; Alberto, R.; Garcia-Garayoa, E.; Ortner, K.; Abram, U.; Schubiger, P. A. *Bioconjugate Chem.* **2000**, *11*, 345.
9. Schibli, R.; Schubiger, P. A. *Eur. J. Nucl. Med. Mol. Imaging* **2002**, *29*, 1529.
10. Rattat, D.; Eraets, K.; Cleynhens, B.; Knight, H.; Fonge, H.; Verbruggen, A. *Tetrahedron Lett.* **2004**, *45*, 2531.
11. Saidi, M.; Seifert, S.; Kretzschmar, M.; Bergmann, R.; Pietzsch, H.-J. *J. Organomet. Chem.* **2004**, *689*, 4739.
12. Liu, S.; Edwards, D. S.; Looby, R. J.; Poirier, M. J.; Rajopadhye, M.; Bourque, J. P.; Carroll, T. R. *Bioconjugate Chem.* **1996**, *7*, 196.
13. Miao, Y.; Liu, B. In *Technetium Rhenium and Other Metals in Chemistry and Nuclear Medicine*; Nicolini, M., Mazzi, U., Eds.; S.G. Editoriali: Padova, Italy, 1999; Vol. 5.
14. IUPAC *Pure Appl. Chem.* **1996**, *68*, 1167.
15. Yamauchi, H.; Takahashi, J.; Seri, S.; Kawashima, H.; Koike, H.; Kato-Azuma, M. In *Technetium and Rhenium in Chemistry and Nuclear Medicine*; Nicolini, M., Bandoli, M., Mazzi, U., Eds.; Cortina International: Verona, Italy, 1989; Vol. 3, p 475.
16. Dischino, D. D.; Welch, M. J.; Kilbourn, M. R.; Raichle, M. E. *J. Nucl. Med.* **1983**, *24*, 1030.
17. Fritzberg, A. R.; Whitney, W. P.; Kuni, C. C.; Klingensmith, W. *Int. J. Nucl. Med. Biol.* **1982**, *9*, 79.