JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 73–84.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.645

Research Article

Synthesis and biological characteristics of the technetium-99m triamide derivatives of mercaptoacetyltriglycine (MAG_3)

S. M. Okarvi*, P. Adriaens, and A. M. Verbruggen
Laboratory of Radiopharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University Hospital Gasthuisberg, K.U. Leuven, B-3000 Leuven, Belgium

Summary

A number of MAG_3-derivatives, containing the three-amide functions modified by inverting the sequence –CO–NH– to –NH–CO– in the first- and/or second-amide functions, have been labelled with ^{99m}Tc in order to study the renal characteristics of the resulting MAG_3-derivatives versus the reference ^{99m}Tc-MAG_3. The ^{99m}Tc-MAG_3-derivatives displayed HPLC-profiles similar to that of ^{99m}Tc-MAG_3. Furthermore, in mice they exhibited biological behaviour comparable or even superior to ^{99m}Tc-MAG_3, which indicates that the sequence of the first- and/or second-amide bond in ^{99m}Tc-MAG_3 affects the biological behaviour only to a limited degree. However, in baboon the plasma clearance of the ^{99m}Tc-MAG_3-derivatives was relatively slower than ^{99m}Tc-MAG_3, which underlines the inter-species variability. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: Technetium-99m; MAG_3; renal agents; radiolabelling

Introduction

The introduction of the technetium-99m complex of mercaptoacetyltriglycine (^{99m}Tc-MAG_3) into clinical use as a potential replacement of

*Correspondence to: S. M. Okarvi, King Faisal Specialist Hospital & Research Centre, Cyclotron and Radiopharmaceuticals Department, MBC-03, PO Box 3354, Riyadh 11211, Saudi Arabia.
E-mail: sokarvi@kfshrc.edu.sa

Received 10 April 2002
Revised 24 June 2002
Accepted 24 July 2002

$^{123/131}$I-labelled-Hippuran (OIH) has opened up a new era in the field of renal radiopharmaceuticals.[1] ^{99m}Tc-MAG_3 has played an unchallenged role as a radiopharmaceutical of choice for dynamic kidney function studies in routine clinical use. However, the wide acceptance of ^{99m}Tc-MAG_3 in clinical nuclear medicine is mainly due to the excellent scintigraphic imaging qualities of ^{99m}Tc rather than its biological properties.[2, 3] Since, compared to ^{131}I-OIH, ^{99m}Tc-MAG_3 displays lower extraction efficiency, a significantly higher protein binding, a smaller volume of distribution and consequently slower plasma clearance (about 60–65% of OIH clearance),[2–5] efforts have continued to develop a ^{99m}Tc-labelled renal agent with a more comparable biological behaviour to ^{131}I-OIH. As a result of these efforts, many derivatives of MAG_3 with a slight structural modification have been prepared and evaluated in animals and humans.[6–12] These efforts have made clear that subtle changes in the structure of MAG_3 can have profound effects on the routes and rates of excretion of ^{99m}Tc-MAG_3-like agents.[3]

Design of an improved ^{99m}Tc-labelled renal agent requires a systematic identification of the physical properties which are directly associated with optimal tubular transport. For a long time it was believed that the carbonylglycine moiety was required for recognition by the tubular proteins.[13] However, it has now become evident that the interaction of ^{99m}Tc-MAG_3-like compounds with the tubular transport system is not determined by only one part of the molecule, but is dependent on several important factors such as the sequences of the different functionalities, the presence of a free carboxylate group and its relationship to the metal-oxo-group, the distribution of polar and less polar structural moieties and partial charges.[14–17] In view of these findings, it seemed of interest to prepare the derivatives of MAG_3 in which the three-amide functions are still present, but modified by an inversion of the –CO–NH– sequence to the –NH–CO– in order to investigate the renal characteristics of their ^{99m}Tc-labelled derivatives.

^{99m}Tc-MAG_3 is a mercaptoacetyltripeptide complex and contains three-amide bonds. However, in this study we investigated the renal characteristics of MAG_3-derivatives with modifications to the first and second amide but not the terminal amide bond (as the terminal carbonylglycine sequence is assumed to be essential for maintaining its efficient renal handling characteristics and hence is the subject of a separate study[9]). In this way, three different MAG_3-derivatives

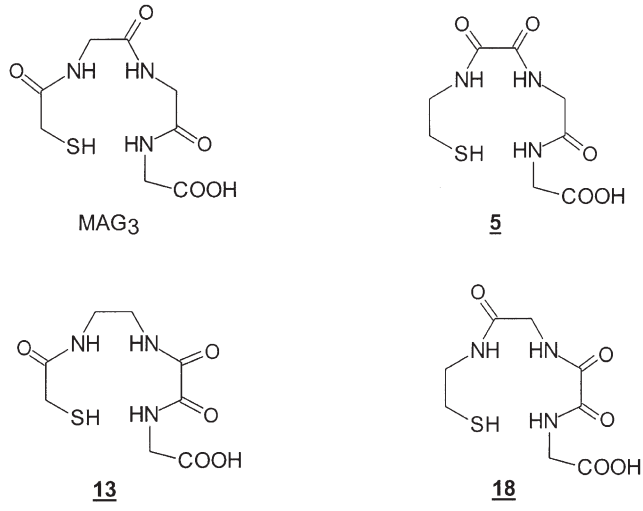


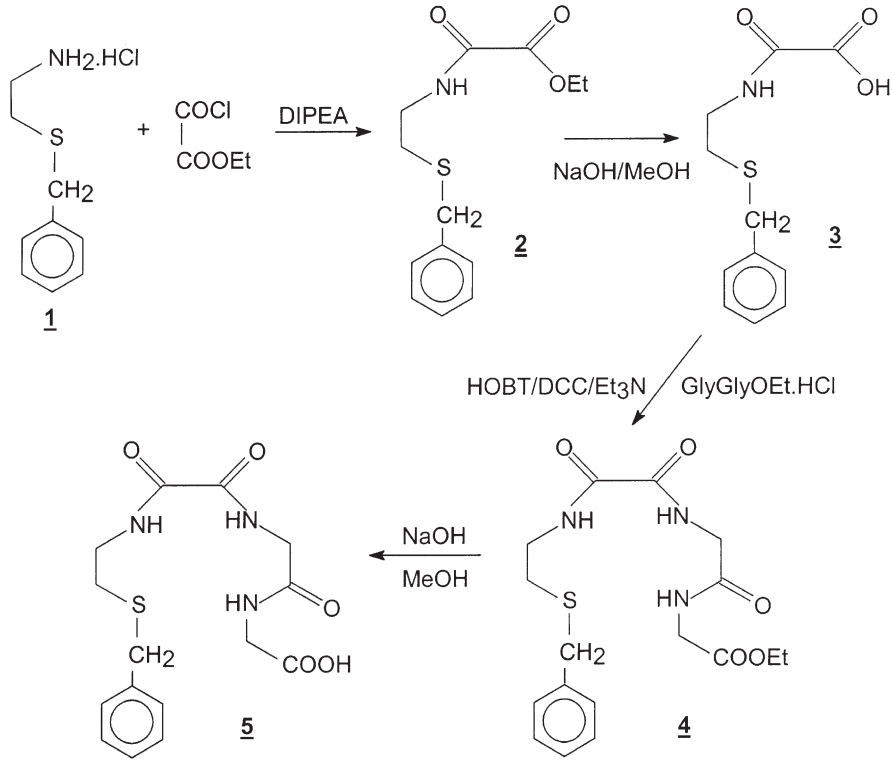
Figure 1. Structure of MAG_3 and its studied triamide derivatives (shown without an *S*-benzyl protective group)

(**5**, **13** and **18**, Figure 1) are possible in which the first or second or both amide functions are inverted compared to MAG_3. These novel ligands were labelled with ^{99m}Tc by an exchange method and analysed by reversed-phase HPLC. The biological behaviour of these complexes was studied in mice and a baboon. This paper describes the chemistry, labelling and biological characteristics of the MAG_3-derivatives.

Results and discussion

Chemistry/synthesis

The chemistry necessary for the synthesis of the MAG_3-derivatives was mainly based on standard peptide chemistry techniques.[18, 19] Due to the poor chemical stability of the thiol-group, MAG_3 and its derivatives were synthesized as the thiol-protected precursors, the *S*-benzyl protecting group being split off during chelation with ^{99m}Tc at elevated temperature.[20] Since all the derivatives (**5**, **13** and **18**) have an oxamide moiety with different substituents on the nitrogen atoms, ethyloxalyl chloride (Schemes 1 and 2) and oxalylglycine ethyl ester (Schemes 2 and 3) were used for the introduction of the oxamide moieties. The ethyl ester protection was selectively removed in the last step of the synthesis by alkaline hydrolysis without affecting the integrity of the molecules.

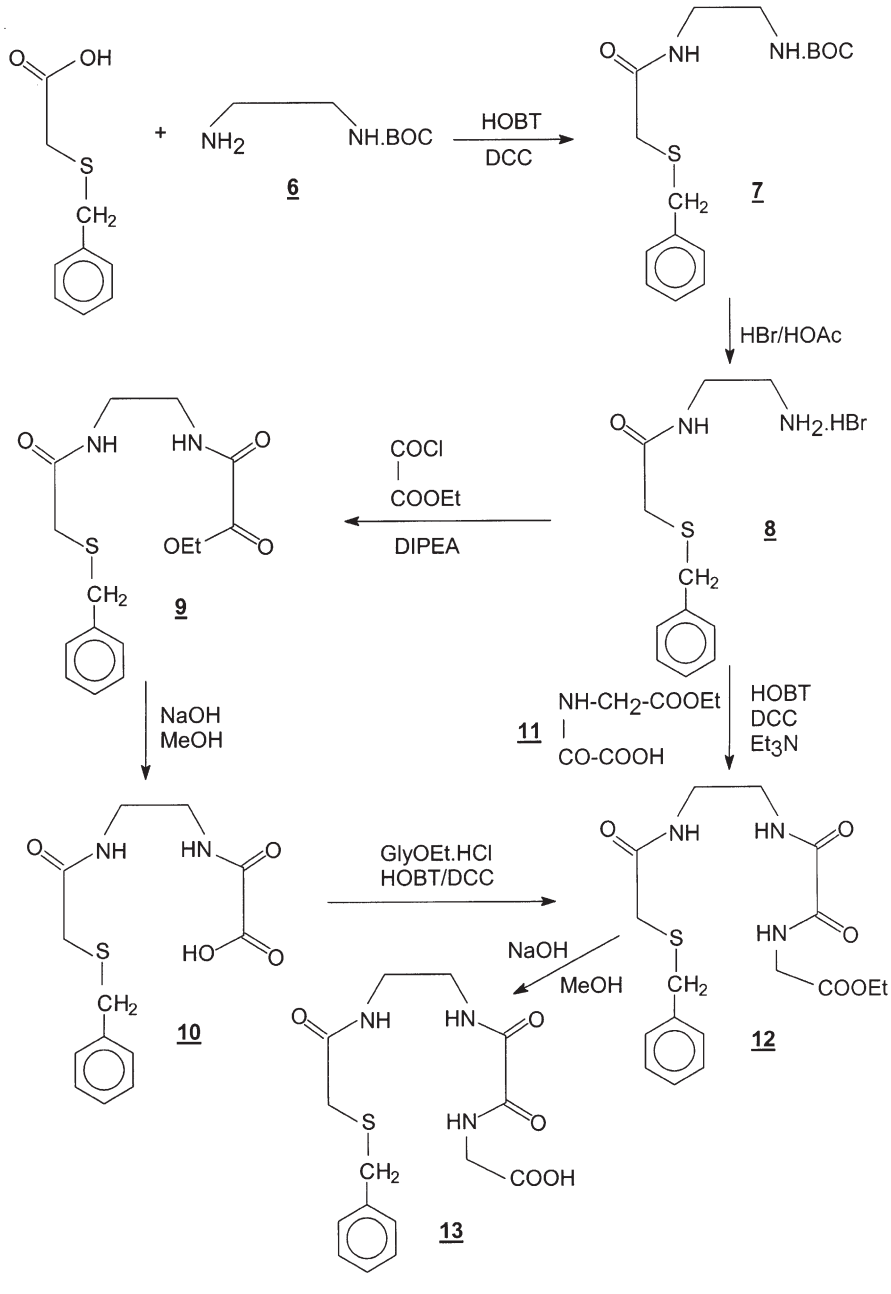


Scheme 1. Synthesis of MAG_3 derivative 5

The overall yield of the preparation of different derivatives of MAG_3 was rather low (see chemistry section) because many steps were required for the synthesis of these compounds, which often required extensive purification by recrystallization and/or by column chromatography. However, a sufficient amount of each ligand was obtained, permitting identification and confirmation of the structure, radiolabelling and animal experiments.

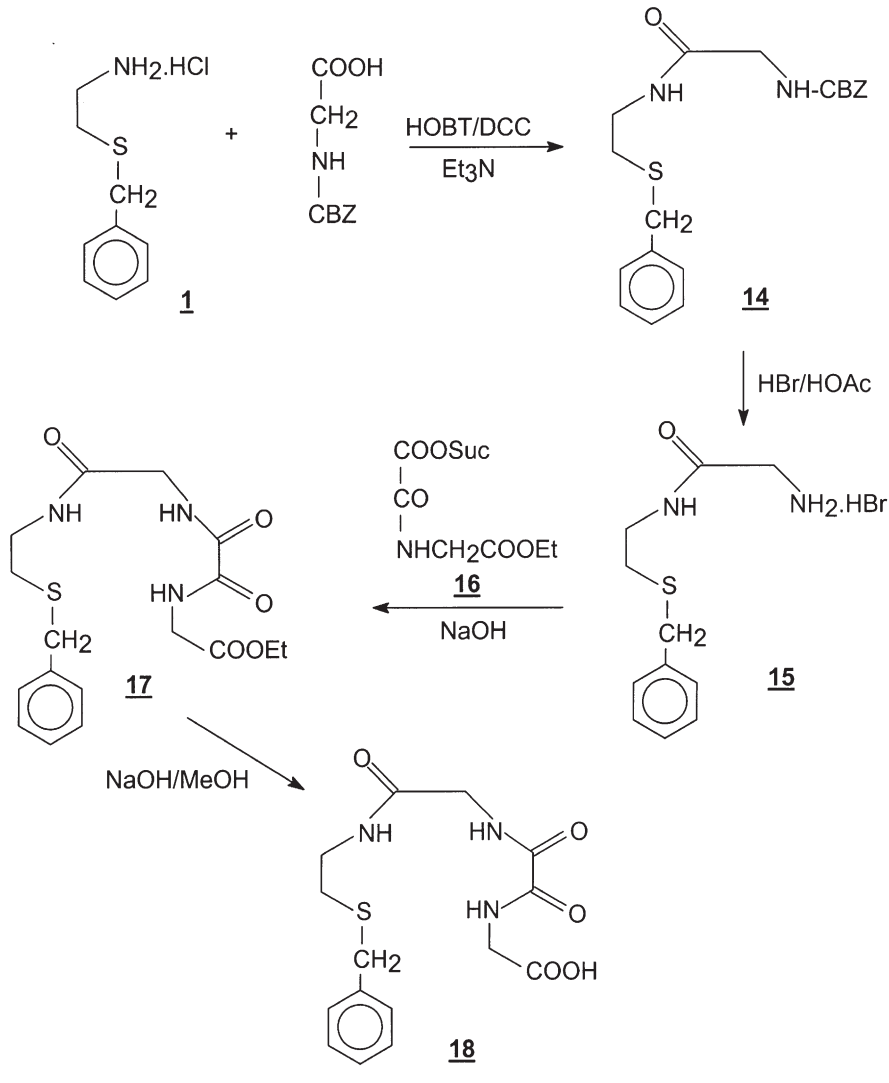
Labelling with ^{99m}Tc

The ^{99m}Tc complexes of the MAG_3-derivatives were obtained by the exchange labelling method in the presence of stannous tartrate at different pH values (pH 10–12). Since the deprotonation of the amide nitrogens and removal of the *S*-protecting group in MAG_3-like agents is facilitated under alkaline conditions, a relatively high pH (10 or above) for the labelling reaction mixtures was preferred in order to maximize



Scheme 2. Synthesis of MAG_3 derivative 13

the labelling yield as well as to minimize the formation of side products.[20] Radio-HPLC analysis of ^{99m}Tc-MAG_3-derivatives revealed that labelling with ^{99m}Tc resulted in the formation of one radiochemical



Scheme 3. Synthesis of MAG_3 derivative 18

species (> 95%) with similar HPLC-profiles and comparable retention times to that of the parent ^{99m}Tc-MAG_3, except for ^{99m}Tc-**13**, which showed a slightly shorter retention time (about 13 min versus 15 min). The comparable retention times of the ^{99m}Tc-MAG_3-derivatives indicate that the lipophilicity has not been drastically changed by the inversion of one- or two-amide functions in the MAG_3 structure.

Biodistribution in mice

The results of the biodistribution study of ^{99m}Tc-MAG$_3$ and its derivatives are summarized in Table 1. The differences in biological behaviour between ^{99m}Tc-MAG$_3$ and its derivatives (^{99m}Tc-**5**, ^{99m}Tc-**13**, and ^{99m}Tc-**18**) are rather limited. All three complexes showed an efficient clearance from the blood, a rapid excretion to the urine, a low retention in the liver and kidneys and negligible radioactivity in the intestines, except for ^{99m}Tc-**5**, which showed a slightly higher uptake in intestines (7% versus 4% for ^{99m}Tc-MAG$_3$ at 30 min post-injection). On the basis of these results, it seems that ^{99m}Tc-**13** has the most favourable properties as a renal function agent. At both studied times radioactivity in the urine was the highest (up to 92%) and radioactivity in the liver and intestines was clearly lower than that of the parent compound (Table 1). As mentioned above, the ^{99m}Tc-**13** showed a slightly shorter retention time on RP-HPLC and thus relatively higher polarity. Nonetheless, it has been shown that the polar characteristics of ^{99m}Tc-compounds are one of the factors, but certainly not the most important one, for determining the renal handling of the compounds.[8, 11] The results seem to suggest that an arrangement of the amides as in ^{99m}Tc-**13** is more suitable in ^{99m}Tc-MAG$_3$-like compounds for an efficient handling by the tubular cells. In this context, it is surprising to see that the inversion of the first amide in ^{99m}Tc-**13** (resulting in ^{99m}Tc-**18**) clearly retarded the clearance from the blood and subsequent excretion to the urine. It is not clear at this point whether this was due to the slightly higher lipophilicity of this compound or to a less efficient fit with the tubular receptor system, or a combination of both factors. From these results it appears that the

Table 1. Biodistribution in mice ($n = 5$) of ^{99m}Tc-MAG$_3$ and its derivatives at 10 and 30 min post-injection

	% of injected dose in the organs									
	Urinary system		Kidneys		Liver		Intestines		Blood	
	10 min	30 min	10 min	30 min	10 min	30 min	10 min	30 min	10 min	30 min
^{99m}Tc-MAG$_3$	76	89	7	2	4	2	2	4	2	1
^{99m}Tc-**5**	78	84	5	1	5	2	2	7	2	1
^{99m}Tc-**13**	82	92	4	1	1	1	1	1	2	1
^{99m}Tc-**18**	71	86	5	1	2	1	1	1	5	3

specific position of the amide-carbonyls in ^{99m}Tc-MAG$_3$ is important for an efficient fit of the tracer agent with tubular transport proteins and cannot be modified without impairing the interaction at this site.

Biological evaluation in a baboon

The 1-h plasma clearance of the ^{99m}Tc-MAG$_3$-derivatives is presented in Table 2. The results are expressed as a percentage of the relative clearance of ^{131}I-OIH, which was co-injected as an internal biological standard in each study to permit an inter-study comparison. From these results, it is clear that only ^{99m}Tc-**5** is extracted from the plasma as efficiently as ^{99m}Tc-MAG$_3$, whereas the disappearance rates were found to be much slower for the derivatives (^{99m}Tc-**13** and ^{99m}Tc-**18**) (Table 2).

Materials and methods

Commercially available chemicals were of reagent grade and were used without purification except for *S*-benzylmercaptoacetic acid, which was recrystallized from ethyl acetate. TLC analysis with different solvent mixtures was carried out using precoated-TLC silica gel plates (Merck, 60F 254) to verify the purity of the products. Column chromatography was run on a silica gel 60 A (Merck 230–400 mesh). All melting points were determined on a Büchi–Tottoli apparatus. The structures of the synthesized ligands were confirmed by ^{1}H NMR spectroscopy on a Jeol FX 90Q spectrometer (Joel, Japan) using $CDCl_3$, DMSO-d_6 or D_2O as solvents. The intermediates and final products were dried in a vacuum desiccator over phosphorus pentoxide. Animal experiments were performed according to the Belgian code of practice for the care and use of experimental animals.

Table 2. One-hour plasma clearance values in a baboon for ^{99m}Tc-MAG$_3$ and its triamide derivatives

Derivative	1 h plasma clearance[a]
^{99m}Tc-MAG$_3$	51
^{99m}Tc-**5**	54
^{99m}Tc-**13**	23
^{99m}Tc-**18**	9

[a] Expressed as percentage of the clearance of co-injected ^{131}I-OIH.

Chemistry

Detailed syntheses of the triamide derivatives are reported elsewhere,[21] only the synthetic schemes are described here. Compound names do not necessarily follow IUPAC system.

S-Benzylcysteamido-oxalyl-glycylglycine **5**

The synthesis of compound **5** is outlined in Scheme 1. *S*-benzylcysteamine hydrochloride **1** (prepared from cysteamine hydrochloride dissolved in liquid ammonia and benzyl chloride) was reacted with ethyloxalyl chloride in the presence of diisopropylethylamine (DIPEA) to afford **2**, which was saponified to oxalyl-*S*-benzylcysteamine **3**. Condensation of this compound with glycylglycine ethyl ester hydrochloride in the presence of 1-hydroxybenzotriazole (HOBT)/1, 3-dicyclohexylcarbodiimide (DCC) and triethylamine (Et_3N) resulted in the formation of ester **4**, which was hydrolyzed to the corresponding acid **5** in an overall yield of 5%.

S-Benzylmercaptoacetyl-ethylenediamido-N-oxalylglycine **13**

Preparation of the compound **13** is shown in Scheme 2. Condensation of benzyl-mercaptoacetic acid and *tert*-butyloxycarbonyl-ethylenediamine (BOC-EDA) **6** (obtained by the reaction of ethylenediamine and di-*tert*-butyldicarbonate in 1:9 molar ratios in Et_3N) in the presence of HOBT/DCC provided compound **7**, which was deprotected by HBr/HOAc to give the hydrobromide **8**. Reaction of this compound with ethyloxalyl chloride resulted in the formation of ethyl ester derivative **9**, which after alkaline hydrolysis gave the corresponding acid **10**. It was then coupled with glycine ethyl ester hydrochloride to afford **12**. The same compound **12** was obtained by the treatment of the intermediate **8** with oxalylglycine ethyl ester **11** (obtained by the reaction of equimolar amounts of *tert*-butyl alcohol, oxalyl chloride and glycine ethyl ester hydrochloride in the presence of DIPEA followed by the removal of *tert*-butyl group with TFA) in the presence of HOBT/DCC and Et_3N. Alkaline hydrolysis of **12** resulted in the formation of the desired derivative **13**. The overall yield for this preparation was about 9%.

S-Benzyl-N-(glycinamidooxalylglycyl)cysteamine **18**

Compound **18** was prepared according to Scheme 3. The intermediate *N*-carbobenzyloxyglycyl-*S*-benzylcysteamine **14** was produced by the

reaction of *N*-carbobenzyloxyglycine (CBZ-Gly) with *S*-benzylcysteamine hydrochloride **1**. Deprotection of the CBZ-group with HBr/HOAc provided **15**, which was condensed with *N*-hydroxysuccinimidyl oxalylglycine ethyl ester **16** (prepared from oxalylglycine ethyl ester **11** in the presence of NHS/DCC) to provide the ethyl ester **17** of the desired derivative. This ester was then hydrolyzed to the corresponding acid **18** in an overall yield of 31%.

Labelling with ^{99m}Tc

To 1–2 mg of the ligand, dissolved in 0.5 M phosphate buffer (500 µl) of the desired pH (10 or above) was added in succession: 10 mg sodium potassium tartrate dissolved in 0.25 ml of water, 100 µg of $SnCl_2 \cdot 2H_2O$ in 25 µl of 0.05 N HCl, and 1–3 ml of generator eluate (UltraTechnekow generator, Mallinckrodt Medical, The Netherlands) containing 370–740 MBq (10–20 mCi) of $^{99m}TcO_4^-$. The reaction mixture was heated in a boiling water-bath for 10 min, cooled to room temperature, and filtered through a 0.2−µm pore membrane filter (Acrodisc, Gelman Sciences, USA) prior to radio-HPLC analysis.

Radio-HPLC analysis

The HPLC analysis of labelled reaction mixtures was performed on a 250 × 4.6 mm reversed-phase column filled with Hypersil ODS 5 µm (Shandon Scientific, UK) and eluted with a ternary gradient mixture of ethanol, 0.025 M phosphate buffer pH 5.85 and water at a flow rate of 1 ml/min (Merck-Hitachi L6200 intelligent pump). Radioactivity in the column effluent was monitored using a 2-in NaI(Tl) scintillation detector coupled to a single-channel analyser and a Rachel analysis program.

Biological evaluation in mice

The biodistribution of the ^{99m}Tc-MAG_3-derivatives was studied in male NMRI mice (body mass 25–35 g) at 10 and 30 min post-injection, according to a published procedure.[8] Briefly, the HPLC-purified product was diluted to a concentration of 144 kBq/ml with saline and to this ^{131}I-OIH (Mallinckrodt Medical, The Netherlands) was added as an internal biological standard to a concentration of 15 kBq/ml. The mice were weighed and 0.1 ml of the diluted tracer solution was injected

via a tail vein after sedation of the animals with Hypnorm® (Duphar, The Netherlands). Five mice were sacrificed by decapitation at 10 and 30 min post-injection. Blood was collected and mice were dissected. Organs were isolated and radioactivity in all organs and body parts were measured using a γ-counter. Radioactivity in each organ is expressed as a percentage of the injected dose. For total blood radioactivity calculation, blood mass is assumed to be 7% of total body mass.

Evaluation in a baboon

The plasma clearance of HPLC-isolated peaks of the ^{99m}Tc-MAG$_3$-derivatives was studied in a male baboon according to a published procedure.[8] Briefly, a male baboon (body mass about 12 kg) was anesthetized by intramuscular injection of 75 mg ketamine (Imalgène®, Rhône Mérieux). Anesthesia was sustained by intravenous injection of 15 mg sodium pentobarbital (Nembutal®, Sanofi) at 10-min intervals. Approximately 18.5 MBq (0.5 mCi) of the ^{99m}Tc-labelled complex was co-injected with 1.85 MBq (50 μCi) of ^{131}I-OIH *via* a limb vein. Blood samples (2 ml) were collected at 2, 4, 6, 8, 10, 15, 20, 30, 45 and 60 min post-injection in heparinized tubes. After centrifugation, 500 μl plasma samples were pipetted, weighed and radioactivity was measured using a γ-counter. The 1-h plasma clearance was calculated using a double exponential fitting method based on a two-compartment model.[22]

Conclusions

The data seem to suggest that the inversion of the first and/or second amide bond in ^{99m}Tc-MAG$_3$ has no significant influence on the biological behaviour of ^{99m}Tc-MAG$_3$-like compounds, as these modified derivatives displayed biological behaviour comparable to ^{99m}Tc-MAG$_3$ in mice. The results from the baboon study, nevertheless, demonstrate that the data obtained in mice are poorly predictive for the worth of a compound as a tracer agent. The very high and rapid urinary excretion of ^{99m}Tc-**13** in mice did suggest that this compound is equal or even superior to ^{99m}Tc-MAG$_3$ for evaluation of the renal function; however, it showed clearly a less efficient renal extraction in a baboon. The inter-species variations observed between the mice and the baboon suggest that the real potential of these compounds as useful renal tracer agents would ultimately be determined by evaluating them in humans.

References

1. Fritzberg AR, Kasina S, Eshima D, Johnson DL. *J Nucl Med* 1986; **27**: 111–116.
2. Chatterjee M, Majurnder A, Iyer P, *et al. Nucl Med Biol* 1996; **23**: 867–872.
3. Hansen L, Marzilli LG, Eshima D, Malveaux J, Folks R, Taylor Jr A. *J Nucl Med* 1994; **35**: 1198–1205.
4. Taylor Jr A, Eshima D, Christian PE, Milton W. *Radiology* 1987; **162**: 365–370.
5. Taylor Jr A, Ziffer JA, Steves A, Eshima D, Delaney VB, Welchel JD. *Radiology* 1989; **170**: 721–725.
6. Bormans G, Cleynhens B, Adriaens P, De Roo M, Verbruggen A. *J Label Compd Radiopharm* 1993; **33**: 1065–1078.
7. Eshima D, Taylor Jr A, Fritzberg AR, Kasina S, Hansen L, Sorenson JF. *J Nucl Med* 1987; **28**: 1180–1186.
8. Okarvi SM, Verbeke K, Adriaens P, Verbruggen A. *J Label Compd Radiopharm* 2002; **45**: 115–137.
9. Okarvi SM, Torfs P, Adriaens P, Verbruggen A. *J Label Compd Radiopharm* 2002; **45**: 407–421.
10. Subhani M, Vanbilloen H, Cleynhens B, *et al. J Label Compd Radiopharm* 1991; **30**: 83–85.
11. Subhani M, Cleynhens B, Hoogmartens M, De Roo M, Verbruggen A. *Technetium and Rhenium in Chemistry and Nuclear Medicine 3*. Cortina International, Verona, Raven Press: New York, 1990; 453–461.
12. Okarvi SM, Adriaens P, Verbruggen A. *Radiochim Acta* 2002; **90**: 423–429.
13. Despopoulos A. *J Theoret Biol* 1965; **8**: 163–192.
14. Kasina S, Fritzberg AR, Johnson DL, Eshima D. *J Med Chem* 1986; **29**: 1933–1940.
15. Klingensmith III WC, Fritzberg AR, Spitzer VM, *et al. J Nucl Med* 1984; **25**: 42–48.
16. Moran JK. *Semin Nucl Med* 1999; **39**: 91–101.
17. Rao TN, Adhikesavalu D, Camerman A, Fritzberg AR. *J Am Chem Soc* 1990; **112**: 5798–5804.
18. Bodanszky M. *Principles of Peptide Synthesis.* Springer: New York, 1993.
19. Greenstein JP, Winitz M. *Chemistry of the Amino Acids*, vol. 3. Krieger: Florida, 1983.
20. Okarvi SM, Adriaens P, Verbruggen A. *J Label Compd Radiopharm* 1997; **39**: 853–874.
21. Okarvi SM. *Ph.D. Thesis*, Faculty of Pharmaceutical Sciences, KUL, Belgium, 1992.
22. Sapirstein LA, Vidt DG, Mandel MJ, Hanusek G. *Am J Physiol* 1955; **181**: 330–336.

