

Research Article

Synthesis, radiolabelling and biological evaluation of terminal oxamide derivatives of mercaptoacetyltriglycine

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Summary

^{99m}Tc -MAG₃ is widely used in clinical nuclear medicine as a potential replacement of ^{131}I -OIH for renal function studies. The terminal carbonylglycine in the MAG₃ backbone is assumed to be essential for maintaining its efficient renal handling characteristics. A number of MAG₃-derivatives have been prepared and evaluated in which the terminal carbonylglycine sequence is substituted by an oxamide moiety in order to study the effect of the modified carbonylglycine sequence on the renal handling characteristics. These 'oxamide' derivatives have been synthesized starting from mercaptoacetic acid or cysteamine using the common synthetic procedures of peptide chemistry. These thiol-protected MAG₃-precursors were labelled with ^{99m}Tc by an exchange method using tartrate as a complexing agent. Biodistribution studies in mice showed that some of these agents were cleared rapidly from the blood and efficiently excreted into the urine and displayed comparable renal excretion characteristics to those of ^{99m}Tc -MAG₃. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: technetium-99m; oxamide derivatives; MAG₃; radiolabelling; renal agents

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1. Introduction

$^{99\text{m}}\text{Tc-MAG}_3$ (mercaptoacetyltriglycine) has been developed as a potential substitute for ^{123}I and ^{131}I -Hippuran (OIH) and it is now considered as the radiopharmaceutical of choice for the evaluation of transplant kidney, tubular necrosis, routine renal function and for imaging studies in clinical nuclear medicine.¹⁻³

It has been hypothesized that the terminal carbonylglycine sequence in the MAG_3 backbone is largely responsible for its efficient renal characteristics, since it is likely to be involved in the interaction with the renal tubular transport proteins.^{4,5} The gold standard OIH and *p*-amino hippuric acid (PAH) also contain the same carbonylglycine sequence. This structural entity was generally believed to be essential in these compounds for an efficacious fit with the receptor proteins of the tubular transport system.⁶ However, it is now apparent that a free carboxylate in the MAG_3 backbone is one of the key features required for receptor recognition by the transporter proteins.⁷ Thus, besides some specific distribution of the amide bonds, the presence of a free carboxylate group seems to be necessary in MAG_3 -like compounds for preserving the efficient renal excretion characteristics. In the light of the above observations, it seemed of interest to investigate the renal characteristics of MAG_3 derivatives in which the terminal carbonylglycine sequence ($-\text{CO}-\text{N}-\text{CH}_2-\text{COOH}$) has been modified by an oxamide moiety ($-\text{CH}_2-\text{N}-\text{CO}-\text{COOH}$).

The present study was undertaken in order to evaluate the effect that modification of the terminal carbonylglycine of MAG_3 would have on the biological behavior and to elucidate the structural requirements for an efficient interaction of $^{99\text{m}}\text{Tc-MAG}_3$ -like agents with the tubular transport system. For this purpose, we have synthesized a number of S-protected derivatives of MAG_3 . These are called 'oxamide' derivatives of MAG_3 (OMAG_3), and differ from the parent MAG_3 compound with respect to the positioning of one or both of the amido groups and, besides the terminal oxamide moiety, contain only two amido groups in their structure. In this way, it is possible to synthesize four different OMAG_3 -derivatives (Figure 1). These new ligands were labelled with $^{99\text{m}}\text{Tc}$ by an exchange method and the labelling mixtures were analyzed by reversed-phase HPLC. The biodistribution studies were performed on mice. This paper describes the chemistry, labelling and biological characteristics of the OMAG_3 -derivatives.

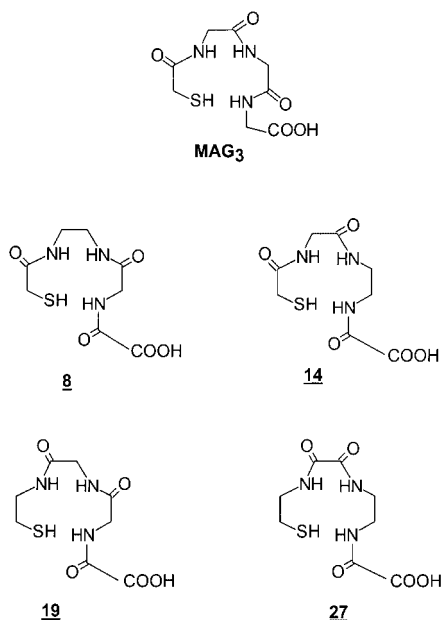


Figure 1. Structure of MAG₃ and its oxamide derivatives

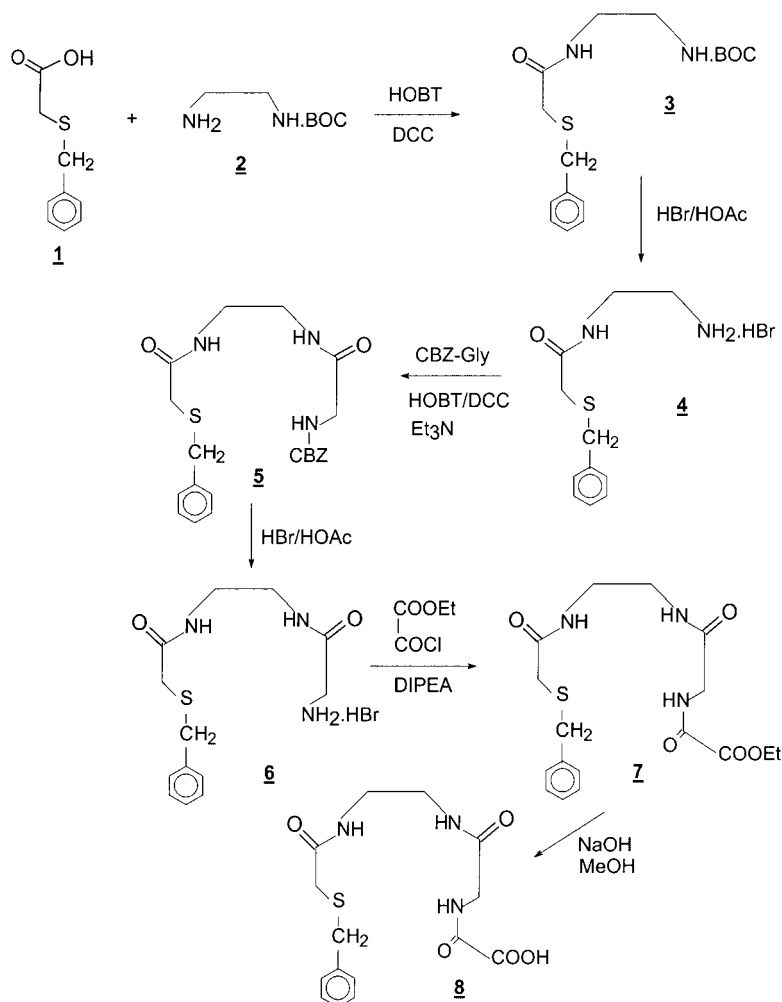
Results and discussion

Synthesis/chemistry

Due to the poor chemical stability of the thiol group, mercaptoacetyl-triglycine and analogs are usually synthesized as thiol-protected precursors. An ideal S-protecting group for the study of MAG₃-derivatives, which may require relatively complex and harsh synthesis procedures, should be stable during the chemical synthesis and withstand the strong reagents used for the removal of amine or carboxylate protective groups (CBZ and BOC, etc.). Moreover, it should be sufficiently removable, either prior to, or during, the radiolabelling process.⁸

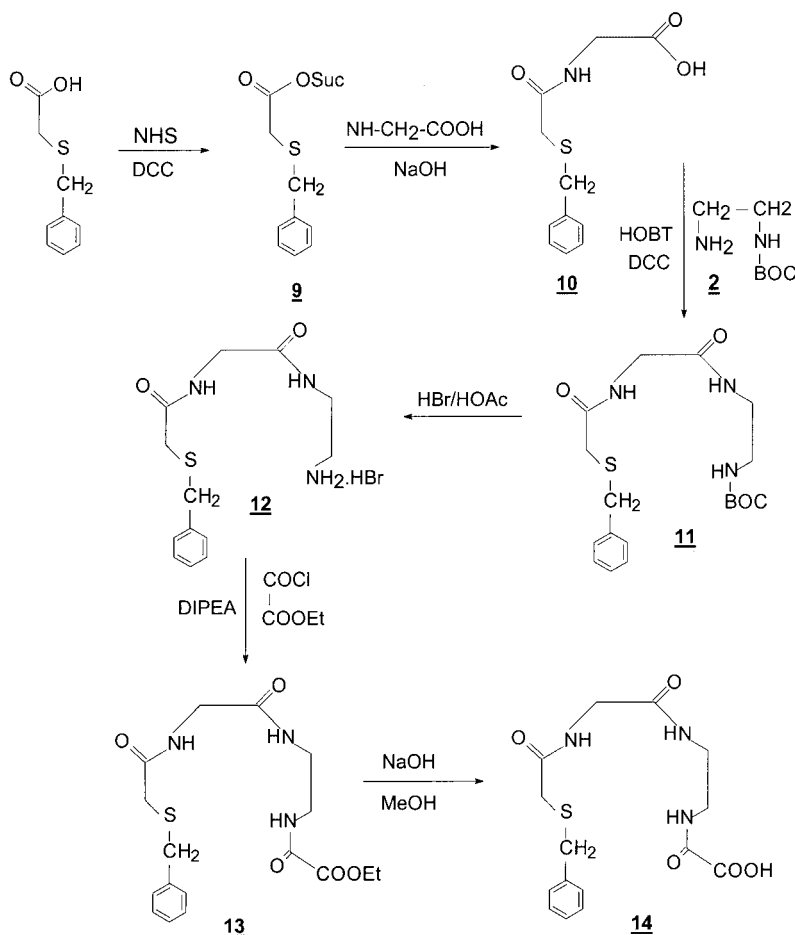
As outlined in Schemes 1 and 2, the synthesis of the oxamide derivatives **8** and **14** started from mercaptoacetic acid, and the thiol-function of the oxamide derivatives was protected with an S-benzyl group in order to ensure the feasibility of the chemical synthesis.

The synthesis of the other two derivatives **19** and **27** (Schemes 3 and 4, respectively), started from cysteamine, and the thiol-groups of the oxamide derivatives were protected with the more stable and lipophilic



Scheme 1. Synthesis of *N*-(*S*-benzylmercaptoacetyl)*N'*-oxalylglycylethylenediamine (**8**)

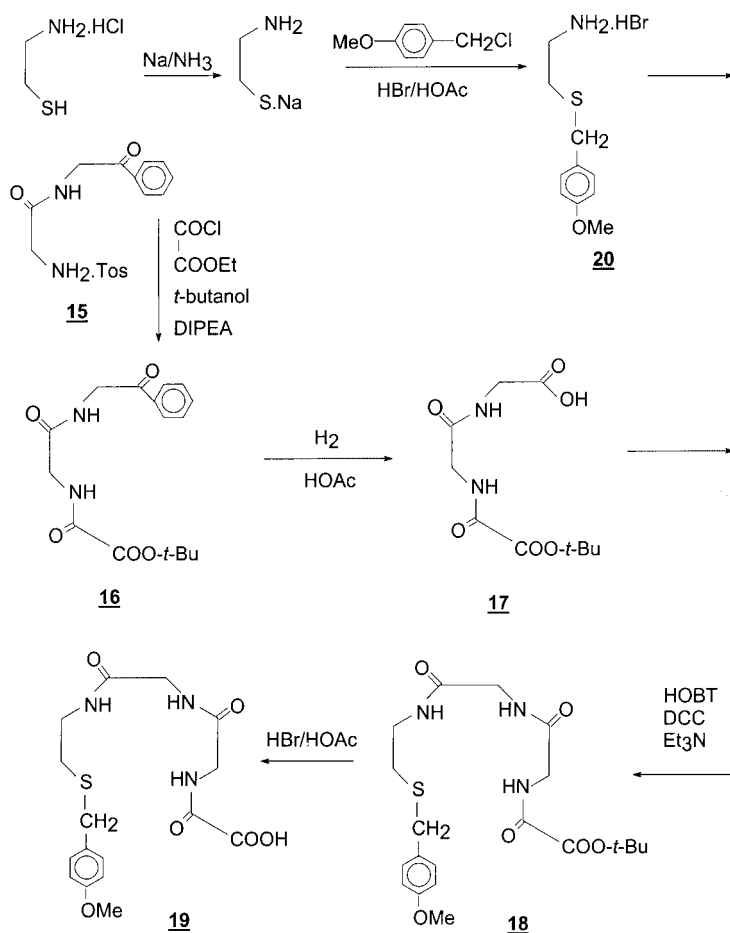
p-methoxybenzyl and diphenylmethyl groups so as to enhance the solubility of the intermediates in organic solvents. The synthesis of compound **19** was complicated, mainly due to the poor solubility of certain intermediate reagents in the organic solvents used. Finally, a different strategy was employed (i.e., preparation of intermediates **17** and **20**, Scheme 3) for the efficient synthesis of this compound. Unlike the synthesis of other oxamide derivatives, *tert*-butyloxalyl chloride was used to introduce a terminal oxamide group in **19**; this was done for reasons of chemical stability.



Scheme 2. Synthesis of *N*-Benzylmercaptoacetylglcyl-*N'*-oxalylethylenediamine (**14**)

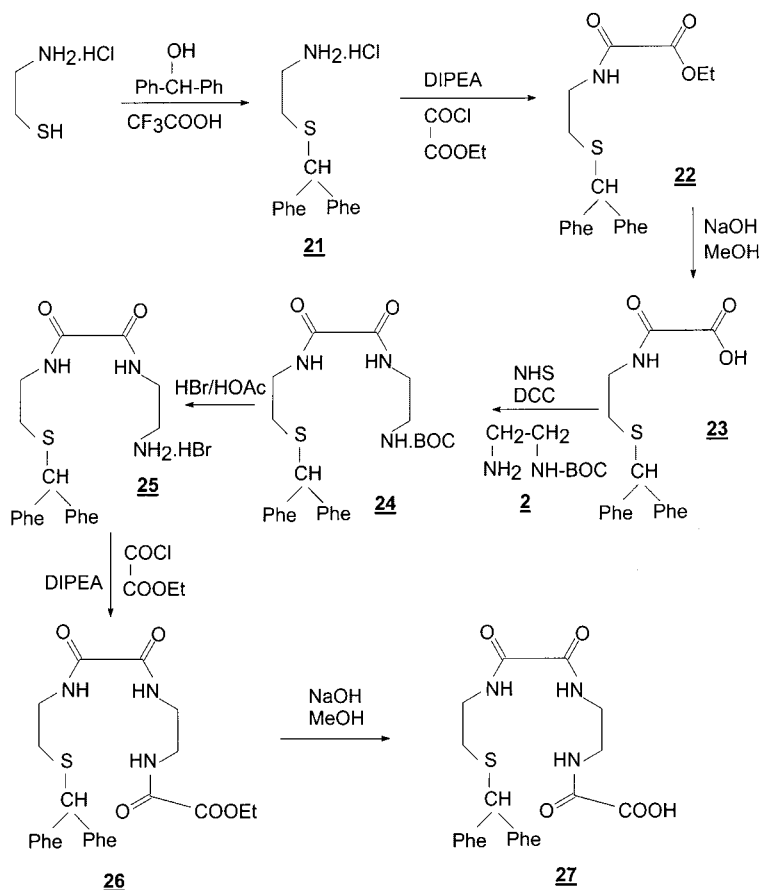
In the preparation of compound **27** with an *S*-benzyl protective group, one of the intermediates, i.e., *S*-benzyl-*N*-oxalylcysteamine ($\text{C}_6\text{H}_5\text{-CH}_2\text{-S-CH}_2\text{-CH}_2\text{-N-CO-COOH}$), was found to be insoluble in organic solvents and was ineffective in DCC-mediated coupling reactions with amines. However, the solubility problem was resolved by the use of a more lipophilic *S*-diphenylmethyl protecting group.

The BOC-group was found to be a useful protecting group for the amino function. In addition, the ease with which it can be removed—by the simple treatment with HBr/HOAc without affecting the other protecting groups (*S*-benzyl and ethyl ester, etc.)—makes it a protecting



Scheme 3. Synthesis of *N*-oxalylglycylglycyl-*S*-*p*-methoxybenzylcysteamine (**19**)

group of choice for the synthesis of MAG_3 -derivatives. Activation of the carboxylic function was realized by the conventional HOBT/DCC or NHS/DCC-mediated reactions. For introduction of a terminal oxamide moiety, it was necessary that only one of the carboxylic functions of oxalic acid construct an amide function. For this purpose, ethyloxalyl chloride was used (one step prior to the final synthesis step, Schemes 1, 2 and 4), enabling the formation of an amide bond on the terminal carbonylglycine moiety in developing the oxamide derivatives (**8**, **14** and **27**). The ethyl ester protection was selectively removed in the last step of the synthesis (Schemes 1, 2 and 4) by mild alkaline hydrolysis, without affecting the integrity of the molecules.



Scheme 4. Synthesis of *N*-oxalyl-*N'*-oxalyl-*S*-diphenylmethylcysteamine-ethylenediamine (**27**)

Labelling with $^{99\text{m}}\text{Tc}$

Initially it was our intention to synthesize all the OMAG_3 -derivatives in this study with an *S*-benzyl protective group—known for its high stability during synthesis, as well as the ease of removal (to provide free thiol) during radiolabelling process at elevated temperature.⁸ However, two of the OMAG_3 -derivatives contain in their structure an *S*-diphenylmethyl or *p*-methoxybenzyl protecting groups to ensure the feasibility of the chemical synthesis. Labelling of different OMAG_3 -derivatives with $^{99\text{m}}\text{Tc}$ was achieved by an exchange method in the presence of stannous chloride and sodium tartrate at different pH

values. Preferentially, a rather high pH (10 or above) of the labelling mixtures was chosen for the oxamide derivatives (except for **27**) in order to promote deprotonation of the amide nitrogens; this enhances the labelling yield as well as minimizes the formation of side-products. For derivatives **8** and **19** (with an *S*-benzyl and *p*-methoxybenzyl group, respectively, and containing in their structure one carbonyl group on the ethylene-bridge preceding the terminal glycine moiety), chelation with $^{99\text{m}}\text{Tc}$ resulted in the formation of one main radiochemical species with a relatively short retention time on RP-HPLC (about 6 min for complexes **8** and **19** versus 13 min for $^{99\text{m}}\text{Tc-MAG}_3$). The other two complexes **14** and **27** on the other hand, with a terminal *N*-oxalylethylenediamine moiety ($-\text{N}-\text{CH}_2-\text{CH}_2-\text{N}-\text{CO}-\text{COOH}$), showed relatively long retention times (13 and 16 min) and the optimal labelling efficiency for these derivatives was obtained at pH 11 and 4, respectively. The radio-HPLC analysis revealed that besides the formation of a single peak, a few minor peaks were also formed. It appears that the presence of an ethylene-bridge sequence (without a carbonyl group) prior to the terminal glycine moiety has a pronounced effect on the labelling characteristics of these ligands with $^{99\text{m}}\text{Tc}$, as it was also the case with the diamide derivatives of $^{99\text{m}}\text{Tc-MAG}_3$.⁹

In another series of experiments, mixtures of equimolar amounts of MAG_3 and OMAG_3 after exchange labelling (under the same conditions as described above) with $^{99\text{m}}\text{Tc}$ were analyzed by HPLC in order to determine the relative labelling strength of $^{99\text{m}}\text{Tc-OMAG}_3$ derivatives versus $^{99\text{m}}\text{Tc-MAG}_3$. The results suggest that the $^{99\text{m}}\text{Tc}$ -complex of **19** (where all the amides bonds are reversed compared to $^{99\text{m}}\text{Tc-MAG}_3$) showed a higher labelling strength than that of $^{99\text{m}}\text{Tc-MAG}_3$, whereas the other $^{99\text{m}}\text{Tc-OMAG}_3$ derivatives, particularly **14** and **27**, clearly exhibit a lower labelling strength than that of $^{99\text{m}}\text{Tc-MAG}_3$, as the percentage of these $^{99\text{m}}\text{Tc-OMAG}_3$ derivatives formed were rather low in $\text{MAG}_3\text{-OMAG}_3$ competition experiments. Although these results indicate the weaker binding strength of most $^{99\text{m}}\text{Tc-OMAG}_3$ ligands compared to the parent $^{99\text{m}}\text{Tc-MAG}_3$ complex, under the optimized labelling conditions (high pH and no competition with other strong chelating agents) sufficiently stable complexes of OMAG_3 -derivatives with $^{99\text{m}}\text{Tc}$ can be formed. In addition, HPLC-isolated peaks of $^{99\text{m}}\text{Tc-OMAG}_3$ complexes were re-analyzed at different time intervals by HPLC and it was found that these complexes remained stable for at least 5 h after isolation.

In vivo biodistribution

The biodistribution studies of the ^{99m}Tc -labelled oxamide derivatives were performed in mice at 10 and 30 min post-injection. For each ^{99m}Tc -compound, the main component of the labelling reaction mixture was isolated by radio-HPLC analysis and after appropriate dilution with saline, it was co-injected with [^{131}I]-OIH. The OIH was added as an internal biological standard with a well-known biodistribution profile to allow rejection of results in animals with impaired renal function. An ideal tracer agent for renal function studies should possess biological characteristics such as a high plasma clearance, rapid uptake in the kidneys and immediate transport to the urine and negligible retention in the liver, intestines and other body organs after the perfusion phase.

The results of the biodistribution of different ^{99m}Tc -OMAG₃ derivatives are summarized in Table 1. Both the ^{99m}Tc -complexes of **8** and **19** (containing a carbonyl on the ethylene-bridge prior to the terminal glycine moiety) exhibited renal excretion characteristics that are comparable with ^{99m}Tc -MAG₃, as shown by their rapid clearance from the blood and high urinary excretion at both time points. The percentage of injected dose for compounds **8** and **19** in the urine was 90 and 87% at 30 min post-injection, respectively, versus 87% for ^{99m}Tc -MAG₃. The radioactivity in other body organs at both time points was almost the same (Table 1). In a baboon, no distinct liver uptake was observed for these derivatives but clearance from the blood was slower than in mice, which underlines the inter-species variability. In contrast, the ^{99m}Tc -complexes of **14** and **27** (both are lacking a carbonyl group on the ethylene-bridge which is connecting the second and third nitrogen atoms), displayed poor affinity for the renal system, as shown by their poor renal excretion (15%) at both studied time points (Table 1). It was

Table 1. Biodistribution ($n = 5$) of different oxamide derivatives of ^{99m}Tc -MAG₃ at 10 and 30 min post-injection in mice

	% of injected dose in the organs									
	Urinary system									
	10	30	Kidneys		Liver		Intestines		Blood	
	10	30	10	30	10	30	10	30	10	30
^{99m}Tc -MAG ₃	75	87	7	2	3	2	2	4	2	1
^{99m}Tc - 8	78	90	4	1	1	1	1	1	3	1
^{99m}Tc - 19	79	87	4	1	2	1	1	1	2	1
^{99m}Tc - 14	14	15	1	1	40	10	32	51	6	1
^{99m}Tc - 27	12	15	1	1	3	3	50	76	1	1

found that these complexes excreted mainly via the hepatobiliary system (liver and intestines). The results demonstrate that the absence of an amide bond on the ethylene-bridge prior to the terminal glycine moiety greatly impaired the renal excretion characteristics of these derivatives.

From the *in vivo* biodistribution, it may be concluded that the ^{99m}Tc -complexes of **8** and **19** show an identical biological behavior in mice, both at 10 and 30 min post-injection. Moreover, these results resembled closely those of ^{99m}Tc -MAG₃ as a renal agent (i.e., rapid blood clearance, high urinary excretion and negligible retention in other body organs). These findings are also in agreement with our previous study of the diamide derivatives of ^{99m}Tc -MAG₃⁹ and further support the hypothesis that the presence of at least one carbonyl group between the second and third nitrogen atoms is essential in MAG₃-like compounds for a favorable biological profile. Furthermore, these results seem to suggest that the presence of a terminal carbonylglycine sequence is not strictly required for efficient extraction of ^{99m}Tc -MAG₃-like agents by the kidneys and excretion to the urine.

As described above, the complexes of ^{99m}Tc -**8** and ^{99m}Tc -**19** were eluted earlier on HPLC (~6 min versus 13 min for ^{99m}Tc -MAG₃) consistent with the higher polarity of these compounds compared to ^{99m}Tc -MAG₃. However, these complexes displayed a comparable migration pattern in the electrophoresis experiments (Table 2). Furthermore, they showed a biological behavior similar to that of ^{99m}Tc -MAG₃. On the other hand, the complexes (^{99m}Tc -**14** and ^{99m}Tc -**27**) displayed comparable HPLC retention times to ^{99m}Tc -MAG₃ but exhibited a significantly different biological behavior and migration pattern during electrophoresis at pH 9 (Table 2). It is worth mentioning that the HPLC analysis of the unlabelled ligands **8** and **14** (with an S-benzyl-group) revealed a retention time similar to S-benzyl-MAG₃. These results indicate a significant variation in forming the complexes with ^{99m}Tc by these two sorts of ligands. However, the structural

Table 2. Migration of ^{99m}Tc -OMAG₃-derivatives by electrophoresis at different pH of electrolyte solution (migration towards the anode in cm)

	pH3	pH6	pH9
^{99m}Tc -MAG ₃	1.7	4.3	3.0
^{99m}Tc - 8	2.3	3.6	3.0
^{99m}Tc - 19	1.9	3.4	3.7
^{99m}Tc - 14	1.5	3.5	1.5
^{99m}Tc - 27	1.7	3.5	1.5

differences between these two sets of OMAG₃-derivatives (**8** and **19** versus **14** and **27**) appear to be minimal, namely one amide function is reversed in **14** compared to **8** and one amide function is reversed in **27** compared to **19** (Figure 1). One major difference, however, is the presence of a terminal oxalylglycinamide moiety ($-\text{N}-\text{CO}-\text{CH}_2-\text{N}-\text{CO}-\text{COOH}$) in the compounds **8** and **19** versus an *N*-oxalylethylenediamine-*N'*-amide sequence ($-\text{CO}-\text{N}-\text{CH}_2-\text{CH}_2-\text{N}-\text{CO}-\text{COOH}$) in **14** and **27**, which may be responsible for the differences both in radiochemical and biological characteristics of these complexes.

Materials and methods

General

Commercially available chemicals were of reagent grade and were used without purification unless otherwise stated. Thin-layer chromatography (TLC) with different solvent mixtures was carried out using precoated-TLC silica gel plates (Merck, 60F 254) to verify the purity of the products. Compounds were detected with UV light at 254 nm, or by exposing the TLC-chromatograms to iodine vapors. Column chromatography was run on a silica gel 60A (Merck 230–400 meshes). The structures of the synthesized ligands were confirmed with ¹H NMR spectroscopy obtained on a Jeol FX 90Q spectrometer (Jeol, Japan) using CDCl₃, DMSO-d₆ or D₂O as solvents. The 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.

Synthesis/chemistry

Detailed synthetic pathways of the oxamide derivatives are described elsewhere.¹⁰ For reasons of simplicity only the chemistry part is mentioned here. The chemistry necessary for the synthesis of the oxamides was mainly based on standard peptide chemistry techniques, e.g., use and choice of protecting groups, standard activation, coupling and deprotection procedures.^{11,12} The general chemistry for the preparation of different oxamide derivatives of MAG₃ is described below.

N-(*S*-benzylmercaptoacetyl)*N'*-oxalylglycylethylenediamine (**8**). The method for the preparation of **8** is depicted in Scheme 1. Condensation of *S*-benzylmercaptoacetic acid with *N*-*tert*-butoxycarbonyl-ethylenediamine (BOC-EDA) **2** (prepared from ethylenediamine and di-*tert*-butyldicarbonate in 1:9 molar ratios in triethylamine) in the presence of 1-hydroxybenzotriazole (HOBT) and 1,3-dicyclohexylcarbodiimide (DCC) produced **3**, which was deprotected by HBr/HOAc to give *N*-benzylmercaptoacetyl-ethylenediamine hydrobromide **4**. This product was reacted with carbobenzyloxyglycine (CBZ-glycine) in the presence of HOBT/DCC to **5**, followed by the selective removal of the CBZ-group with HBr/HOAc **6**. Reaction of ethyloxalyl chloride with **6** under Schotten–Baumann conditions¹³ resulted in the ethyl ester derivative **7**, which was hydrolyzed with base to the corresponding acid **8**.

N-(*S*-benzylmercaptoacetyl)glycyl *N'*-oxalylethylenediamine (**14**). The synthesis of compound **14** is outlined in Scheme 2. Commercially available *S*-benzylmercaptoacetic acid was recrystallized from ethyl acetate and converted to *N*-hydroxysuccinimidyl ester **9** with NHS/DCC. Coupling of this active ester with glycine under alkaline conditions produced *S*-benzylmercaptoacetyl-glycine (MAG₁) **10**. Activation of the carboxylic group with HOBT/DCC and subsequent coupling of **10** with BOC-EDA **2** provided **11**, which was deprotected by HBr/HOAc to **12**. Reaction of ethyloxalyl chloride in the presence of diisopropylethylamine (DIPEA) with **12** under Schotten–Baumann conditions followed by alkaline hydrolysis of the ester group **13** gave the oxamide derivative **14**.

N-oxalylglycylglycyl-*S*-*p*-methoxybenzylcysteamine (**19**). The preparation of compound **19** is shown in Scheme 3. Cysteamine hydrochloride was converted into *S*-*p*-methoxybenzylcysteamine hydrobromide **20** by the treatment of Na/NH₃ in the presence of *p*-methoxybenzyl chloride. The intermediate, glycylglycinebenzyl ester *p*-toluenesulfonate **15**, was prepared by the condensation of *p*-toluenesulfonic acid with glycylglycine and benzyl alcohol in benzene. Reaction of this compound with ethyloxalyl chloride and *tert*-butanol provided diester **16**. Hydrogenation of the benzyl ester resulted in monoester/monoacid derivative **17**. Coupling of **17** with **20** in the presence of HOBT/DCC and Et₃N gave *tert*-butyl ester derivative **18**. Lastly, the *tert*-butyl ester was hydrolyzed by reaction with HBr/HOAc to give the corresponding acid **19**.

N-oxalyl-N'-oxalyl-S-diphenylmethylcysteamine ethylenediamine (27). Compound **27** was prepared according to Scheme 4. Condensation of *S*-diphenylmethyl-cysteamine hydrochloride **21** (prepared from aminoethanethiol hydrochloride and benzhydrol in TFA) with ethyloxalyl chloride in DIPEA gave the ethyl ester derivative **22**, which after alkaline hydrolysis was converted into the acid **23**. This acid was activated on its carboxylic function by NHS/DCC and was then coupled to BOC-EDA **2** to provide **24**. Removal of the BOC-group by HBr/HOAc produced the hydrobromide **25**, which was reacted with ethyloxalyl chloride to form the ethyl ester derivative **26**. Alkaline hydrolysis of the ester resulted in the formation of the diphenyl-protected oxamide derivative **27**.

Labelling with ^{99m}Tc

Labelling of the synthesized oxamide ligands was carried out by the ligand exchange method. The ligand (1–2 mg) and sodium tartrate (5 mg) were dissolved in 0.5 ml of phosphate buffer 0.5 M of the desired pH (4–11) followed by addition of 100 μg of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 25 μl of 0.05 M HCl. After the addition of 1–3 ml of generator eluate (UltraTechnekow generator, Mallinckrodt Medical, The Netherlands) containing 370–740 MBq (10–20 mCi) of $^{99m}\text{TcO}_4^-$, the reaction mixture was heated for 10 min at 100°C and cooled to room temperature and filtered through a 0.2- μm pore membrane filter (Acrodisc, Gelman Sciences) prior to radio-HPLC analysis.

Analysis of ^{99m}Tc -labelled compounds

Analysis of labelled reaction mixtures was performed using RP-HPLC on a Hypersil 5 μm ODS column (Shandon Scientific, UK) 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. The HPLC system consisted of a Merck-Hitachi L6200 intelligent pump and a 250 mm \times 4.6 mm reversed phase column. Radioactivity in the column effluent was monitored using a 2-in NaI(Tl) scintillation detector coupled to a single-channel analyzer and a Rachel analysis program.

Electrophoresis of ^{99m}Tc -MAG₃ and ^{99m}Tc -OMAG₃ complexes was performed by applying a potential of 300 V for 30 min using a 0.025 M phosphate buffer (pH 3, 6 and 9) as the electrolyte solution. The

distribution of radioactivity was measured by cutting the strips into 1 cm segments and counting each segment in a γ -counter.

Biological evaluation in mice

Biodistribution of the oxamides was studied in male NMRI mice (body mass 25–35 g) at 10 and 30 min post-injection. The HPLC purified product was diluted to a concentration of 148 kBq/ml (4 μ Ci/ml) with sterile saline and ^{131}I -OIH (Mallinckrodt Medical, The Netherlands) was added as an internal biological standard to a concentration of 14.8 kBq/ml. The diluted tracer solution (0.1 ml) was injected via a tail vein after sedation of the animals with Hypnorm[®] (Duphar, The Netherlands). The animals were sacrificed by decapitation at fixed time intervals. The organs were dissected and the radioactivity in each organ was measured. Radioactivity in each organ was expressed as a percentage of injected radioactivity, which was equal to the sum of the radioactivity in all organs. For calculation of radioactivity in total blood, blood mass was assumed to be 7% of total body mass. Results were corrected for background radiation, physical decay during counting, and ^{131}I crossover into the $^{99\text{m}}\text{Tc}$ -channel.

Conclusions

The results suggest that the renal excretion properties of the $^{99\text{m}}\text{Tc}$ -complexes of **14** and **27** (without a carbonyl on the ethylene-bridge prior to the terminal glycine) are inferior to those of $^{99\text{m}}\text{Tc}$ -MAG₃. Moreover, these compounds exhibited relatively poor labelling characteristics with $^{99\text{m}}\text{Tc}$ and hence do not satisfy the requirements for a suitable renal agent. In contrast, the $^{99\text{m}}\text{Tc}$ -complexes of **8** and **19** (with one inverted amide on the ethylene-bridge) labelled efficiently with $^{99\text{m}}\text{Tc}$ and displayed the most efficient renal handling of the studied compounds in mice, as characterized by their rapid renal excretion and negligible retention in other body organs. It thus seems that for efficient handling by the tubular system, $^{99\text{m}}\text{Tc}$ -MAG₃-like compounds need in their structure some specific distribution of the amide bonds and at least one carbonyl group on the ethylene-bridge preceding the terminal glycine moiety. Furthermore, it appears that the presence of a terminal carbonylglycine sequence in MAG₃-like compounds is not necessary for the observation of efficient renal excretion characteristics.

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