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Research Article

Synthesis, labelling and biological characteristics of derivatives of Mercaptoacetyltriglycine with two amide functions

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Summary

Eight novel derivatives of MAG_3, containing in their structure two instead of three amide functions, have been synthesized and evaluated. The synthesized ligands were labelled with ^{99m}Tc by exchange labelling at 100°C and/or by a direct labelling method at room temperature. After labelling with ^{99m}Tc, the reaction mixtures were analyzed by radio-HPLC. Biological evaluation of different ^{99m}Tc-diamide complexes in mice and a baboon indicated that substitution in one amide function of MAG_3 structure greatly influences the renal handling of these compounds. It was found that the diamide derivatives containing at least one inverted amide exhibit biological behavior comparable to that of ^{99m}Tc-MAG_3. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: technetium-99m; MAG_3; diamides; renal agents; radiolabelling

Introduction

During the past three decades, a large number of radiolabelled compounds have been developed and evaluated as potential tracer

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agents for renal function studies.[1–8] The extensive search for a suitable renal radiopharmaceutical can be explained from the viewpoint that the kidney is a vital organ responsible for the maintenance of the composition of blood and extracellular fluid and thus deserves an optimal diagnostic agent for its precise evaluation. Since 1960, ^{131}I-Hippuran (OIH, Figure 1) has been used as the radiopharmaceutical of choice for the determination of effective renal plasma flow (ERPF) and functional evaluation of the kidneys by radioisotopic scintigraphy.[9,10] Despite the drawbacks of the isotope ^{131}I (i.e. high gamma-energy of 364 KeV, long physical half-life and the beta-component of its radiation), OIH is still considered the golden standard in view of its ideal biological characteristics.[10–12] However, an agent labelled with ^{99m}Tc and having renal extraction characteristics comparable to or even greater than OIH is highly desirable for practical and dosimetric reasons. Over the last 15 years, several compounds labelled with ^{99m}Tc have been proposed as alternatives to OIH.[13–26] Until now, the most appropriate and successful ^{99m}Tc-labelled tracer agent for renal function studies is ^{99m}Tc-mercaptoacetyltriglycine (^{99m}Tc-MAG_3, Figure 1). This chelate is based on a N_3S donor ligand system and can easily be prepared by using a commercially available kit.[27] The superior physical characteristics of the ^{99m}Tc label and its favorable biological properties make ^{99m}Tc-MAG_3, the agent of choice at present, especially for the evaluation of transplant kidney, tubular necrosis and kidney function in general.[28–30]

Nonetheless, ^{99m}Tc-MAG_3 is still not the ideal replacement for OIH, and improvements are still possible. The plasma-protein binding of ^{99m}Tc-MAG_3 is high,[30,31] and its plasma clearance in humans is not higher than about 60–65% of the OIH value. Therefore, accurate determination of the ERPF is rather difficult using ^{99m}Tc-MAG_3, although formulae have been proposed to calculate ERPF from the

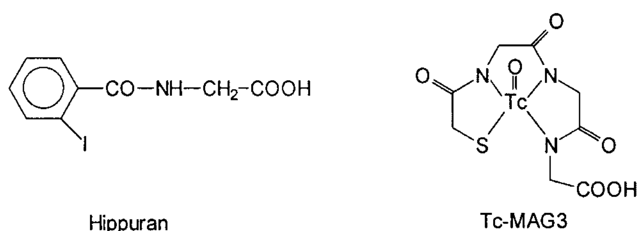

Figure 1. Structure of 2-iodohippuric acid, OIH) and ^{99m}Tc-mercaptoacetyl-triglycine ^{99m}Tc-MAG_3)

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acquired data.[31,32] Moreover, ^{99m}Tc-MAG$_3$ shows some hepatobiliary uptake.[33] For these reasons, the development of a ^{99m}Tc-labelled renal function agent that approaches OIH more closely (with respect to biological properties) than ^{99m}Tc-MAG$_3$ would still constitute an improvement.

The present study was undertaken in an attempt to improve the renal handling of ^{99m}Tc-MAG$_3$-like agents, to evaluate the effect of derivatization of MAG$_3$ on the biological behavior, and to elucidate the structural requirements for an efficient interaction of ^{99m}Tc-MAG$_3$-like agents with the tubular transport system. For this purpose, we have synthesized a number of S-protected derivatives of MAG$_3$ bearing in their structures only two instead of three amide functions. In these derivatives, one of the amides of MAG$_3$ sequence has been replaced by an amine function, and the remaining amides were also inverted (except for the terminal amide). In this way, eight different diamide derivatives of MAG$_3$ are possible to synthesize (Figure 2).

Compounds **B**, **C** and **D** are called the "direct derivatives" of MAG$_3$, in which one amide has been replaced by an amine. Derivatives **F**, **G, H** and **I** also contain one and derivative **E** two inverted amides, as compared to MAG$_3$. The new ligands were labelled with ^{99m}Tc by the exchange reaction and/or direct labelling method and the labelling mixtures were analyzed by reversed-phase HPLC. Their biodistribution studies were performed in mice and a baboon. This paper presents the chemistry, labelling and biological characteristics of diamide derivatives of MAG$_3$.

Results and discussion

Labelling with ^{99m}Tc

Exchange labelling of the studied ligands with ^{99m}Tc in the presence of stannous chloride and sodium tartrate produced for five of the chelates (**B**–**G**) one main radiochemical product with a retention time on HPLC close to that of ^{99m}Tc-MAG$_3$. However, the diamides **B**, **H** and **I** showed poor labelling characteristics with ^{99m}Tc. The precursors of these three *S*-protected ligands (27a, 41a and 32a) contain in their structure an *N*-tosyl group for reasons of feasibility of the chemical synthesis. Prior to labelling, the ligands were treated with sodium in liquid ammonia in order to remove *S*-protecting (benzyl or trityl) and

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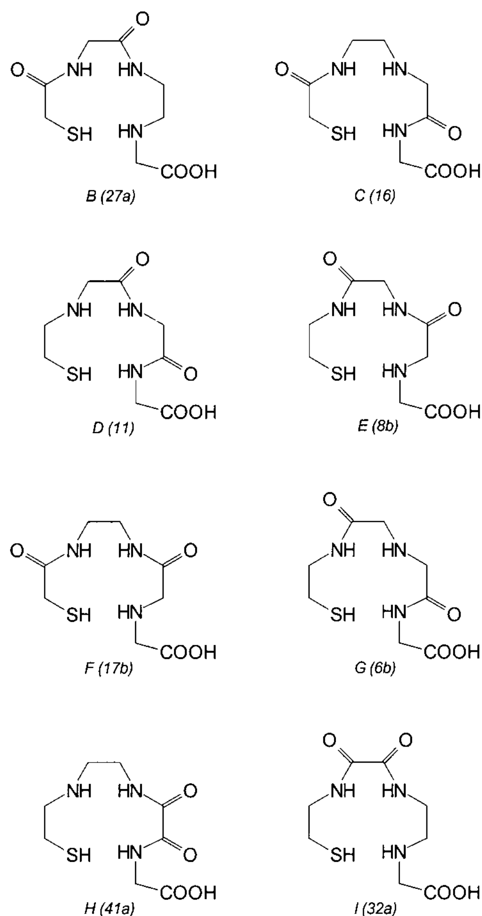

Figure 2. Structure of MAG_3 derivatives with two amides and one amine

N-protecting (tosyl) groups simultaneously. After this complete deprotection, labelling was performed under alkaline conditions (pH 12) at room temperature (direct labelling) and/or in the presence of tartrate at 100°C (exchange labelling). Both the direct and exchange labelling unexpectedly resulted in poor labelling efficiency for these derivatives that can be characterized by a mixture of peaks as revealed by HPLC analysis. The reason for this poor labelling is not yet clear. No doubt exists about the identity of the precursors, as both NMR and mass spectroscopy have confirmed this. One of the possibilities could be that the treatment with sodium in liquid ammonia for removal of the *N*-tosyl and *S*-benzyl or *S*-trityl group causes decomposition of the tetraligand diamide. This deprotection method is, however, a common

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procedure in peptide chemistry and has been performed with high yields by different authors.[34,35] Another possibility is, of course, that a combination of a thiol, two amides and one amine in the backbone of these three ligands has no efficient Tc complexing properties and hence, will not result in one single and/or stable radiochemical species.

Biodistribution in mice

The biodistribution studies of the ^{99m}Tc-labelled diamide derivatives were performed in mice. For each ^{99m}Tc-compound, the main component of the labelling reaction mixture was isolated by HPLC analysis and after appropriate dilution with saline, it was co-injected with [^{131}I]-OIH in mice (n = 5) at 10 and 30 min post-injection. 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. The results of the *in vivo* biodistribution of different diamide derivatives (except for derivative **I**) are summarized in Table 1.

To be a suitable tracer agent for renal function studies, a compound should have biological characteristics that approach OIH as closely as possible. This means high plasma clearance, a rapid uptake in the kidneys with immediate transport to the urine and a negligible retention in the liver, intestines and other body organs after the perfusion phase. Compounds **B**, **C** and **D** (Figure 2) are the direct derivatives of MAG_3, in which one of the amides has been replaced by an amine, whereas the other amides are not affected. They show poorer extraction from the

Table 1. Biodistribution of the different diamide derivatives of ^{99m}Tc-MAG_3 in mice (n = 5) at 10 and 30 min p.i. The results for ^{99m}Tc-I are not shown (see text)

	per cent 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
OIH	81	91	3	1	2	1	1	1	3	1
A (Tc-MAG_3)	77	89	8	2	3	2	2	5	2	1
B	36	34	4	3	4	6	4	10	21	48
C	47	63	6	5	3	4	3	4	8	4
D	64	78	5	1	6	5	2	3	5	2
E	80	85	3	1	5	2	2	8	2	1
F	74	84	3	1	2	5	2	9	3	1
G	75	82	4	1	2	1	2	4	3	2
H	44	66	3	2	4	3	3	3	13	14

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blood than the parent compound at both studied time points. At 10 min post-injection, the percentage of injected dose remaining in the blood was about 21, 8 and 5% for the ^{99m}Tc-complexes of **B**, **C** and **D**, respectively, as compared to 2% for ^{99m}Tc-MAG$_3$. At 10 min post-injection, the activity in the urine was about 95, 44, 58 and 78%, respectively, of the OIH value for **A**, **B**, **C** and **D**. As could be expected from the slow blood clearance of ^{99m}Tc-complex **B**, this derivative also shows slowest urinary excretion and significant elimination through the hepatobiliary system. No major retention was observed for these compounds in the kidneys, liver or intestines at 10 min post-injection. For these three derivatives, it was found that the replacement of an amide by an amine greatly impairs the renal excretion characteristics of these derivatives in general and derivative **B** in particular, in which the terminal amide is altered. It is well known that the presence of a terminal carbonylglycine sequence (-CO–N–CH$_2$–COOH) is necessary in OIH for an efficient interaction of the compound with the renal tubular transport proteins.[36] The terminal carbonylglycine sequence is also present in ^{99m}Tc-MAG$_3$, while it is lacking in derivative **B**. Furthermore, it is notable that the renal excretion characteristics improve from **B** to **D**, the derivatives in which both the second and third amide bond are unaffected. Although these results do not yet allow a clear understanding of the structural requirements for an efficient interaction of MAG$_3$-like compounds with the tubular receptor proteins, they nevertheless, seem to suggest that the presence of one or more amide bonds close to the terminal glycine part is an important requirement for the efficient tubular handling of MAG$_3$-like agents.

The ^{99m}Tc-complexes **E**, **F** and **G** are different from the parent ^{99m}Tc-MAG$_3$ complex with respect to the presence of only two amide bonds. In addition, one or more remaining amides are inverted, but they do not contain an oxamide moiety in their structure. The renal excretion characteristics of the complexes **E**, **F** and **G** are more comparable with ^{99m}Tc-MAG$_3$, as appears from their more rapid clearance from the blood and higher urinary excretion at both time points. At 10 min post-injection, the percentage of injected dose in the urine was about 93, 92 and 91%, respectively, of the OIH value versus 98% for ^{99m}Tc-MAG$_3$. Compared to ^{99m}Tc-MAG$_3$, their renal retention was slower and the radioactivity remaining in the blood was almost the same (Table 1).

From the biodistribution data, it appears that the ^{99m}Tc-complex of derivative **E** displayed superior biological characteristics than ^{99m}Tc-MAG$_3$ as a renal agent, except for the fact that its excretion to the

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intestines at 30 min post-injection was somewhat higher. Also the complexes **F** and **G** approach the parent compound closely and it may be concluded that the differences in biological behavior between ^{99m}Tc-MAG_3 and its derivatives **E**, **F** and **G** are insignificant (Table 1).

It is striking that the derivative with the most favorable renal excretion characteristics in mice is the ^{99m}Tc-complex of **E**, in which the two-amide functions are reversed as compared to the structure of ^{99m}Tc-MAG_3. Both diamides **E** and **F** are lacking the terminal amide bond of the parent compound, as is the case for compound **B**. Nevertheless, the renal handling of **E** and **F** is very efficient, unlike that of **B**. This indicates that the terminal amide is not indispensable for efficient interaction at the tubular cells. Comparison of the structures of compound **B** (poor clearance of the ^{99m}Tc-complex from the blood) with **E**, **F** and **G** (efficient extraction and renal excretion) shows in **B** the absence of a carbonyl group on the ethylene bridge connecting the second and third nitrogen atom, whereas the other three derivatives do contain such a carbonyl group in their structure. The only other diamide derivative without such a carbonyl group in this part of the molecule is compound **I**. As mentioned above, derivative **I** showed poor labelling characteristics with ^{99m}Tc, combined with unfavorable biological characteristics such as slow blood clearance, poor urinary excretion and significant uptake in the liver and intestines. These results support the hypothesis that the presence of at least one carbonyl group between the second and third nitrogen atom is essential in MAG_3-like compounds for an efficient extraction and secretion by the tubular system. It appears, however, that the presence of an amide carbonyl at other sites of the molecule is also important. Derivative **H** has an oxamide moiety just before the terminal glycine but no other carbonyl groups in the remaining part of its structure. It also showed poor extraction from the plasma and slow urinary excretion, but no significant uptake or retention in the liver, intestines or kidneys. It thus seems that for an efficient handling by the tubular system, ^{99m}Tc-MAG_3-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.

Biodistribution in a baboon

On the basis of the results obtained in mice, five of the ^{99m}Tc-labelled diamide derivatives (**C**, **D**, **E**, **F** and **G**) have also been evaluated in a

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

Derivative	1-h plasma clearance[a]
A (MAG$_3$)	51
C	6
D	13
E	38
F	53
G	30

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

baboon. The 1-h plasma clearance values of all tested compounds are presented in Table 2. These results show that ^{99m}Tc-labelled **F**, which also showed a favorable renal excretion in mice, has the highest plasma clearance in baboon, comparable to that of ^{99m}Tc-MAG$_3$. The same was true for ^{99m}Tc-labelled **E**. On the other hand, the plasma clearance was very low for the ^{99m}Tc-labelled derivatives **C** and **D** (Table 2), which also exhibited poor renal excretion characteristics and slow blood clearance in mice. It appears that the data obtained in the baboon confirm the findings in mice although there are some inter-species differences.

Materials and methods

General

Commercially available chemicals were of reagent grade and were used without purification except for *S*-benzylthioglycolic acid (recrystallized from ethyl acetate). Liquid ammonia was distilled from sodium into the reaction flask. TLC was carried out using precoated-TLC silica gel plates (Merck, 60F 254). 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 ^{1}H NMR spectroscopy obtained on a Joel FX 90Q spectrometer (Joel, Japan) using $CDCl_3$, DMSO-d_6 or D_2O as solvents. The final products were dried in a vacuum desiccator over phosphorus pentoxide. Animal studies were performed according to the Belgian code of practice for the care and use of experimental animals.

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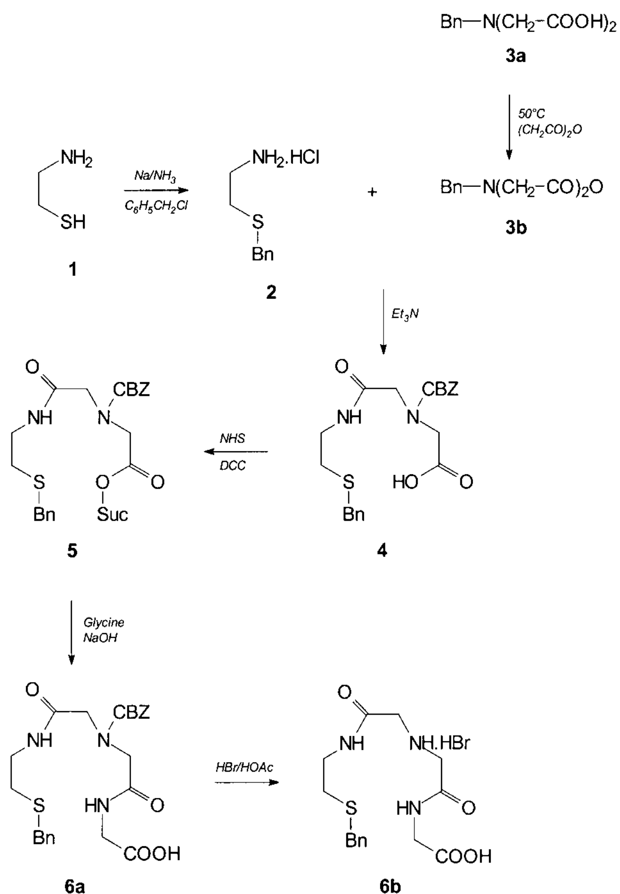
Synthesis/chemistry

Detailed synthetic pathways of the ligands is described elsewhere.[37] For reasons of simplicity only the chemistry part is mentioned here. The chemistry necessary for the synthesis of the diamides was mainly based on standard peptide chemistry techniques, e.g. use and choice of protecting groups, standard activation, coupling and deprotection procedures. The general chemistry for the preparation of different diamides is described below.

*N-Benzylmercaptoethyl-N′-acetic acid iminodiacetamide.HBr (***6b***)*

The synthesis of compound **6b** (derivative **G**) is given in Scheme 1. Several methods have been described for the preparation of *S*-benzyl-



Scheme 1. Synthesis of N-benzylmercaptoethyl-*N*-acetic acid iminodiacetamide. HBr (6b)

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cysteamine.[38–42] For example, reaction of sodium *S*-benzylmercaptan with bromoethylamine in boiling ethanol (yield 75%).[39–41] Coupling of benzyl chloride with mercaptoethylamine (**1**,cysteamine)[40] in DMF in the presence of potassium carbonate (yield 86%), or with the sodium mercaptide of (**1**) in boiling ethanol[38] (yield 75%) or in liquid ammonia[42] (yield 94%). An initial attempt to synthesize this compound according to the method of Goldberg and Kelly[38] was unsuccessful. Finally, the procedure of Balasubramaniam *et al.*[42] was adapted that afforded a pure product with a high yield (94%). Condensation of carbobenzyloxyimino acetic anhydride (**3b**) (prepared by warming the corresponding acid (**3a**) with acetic anhydride) with *S*-benzylcysteamine (**2**) resulted in (**4**), which was activated by reaction with *N*-hydroxysuccinimide (NHS) and 1–3-dicyclohexylcarbodiimide (DCC) to give the *N*-hydroxysuccinimidyl ester (**5**). Coupling of this active ester with glycine yielded (**6a**), which was deprotected with 33% hydrogen bromide in acetic acid (HBr/HOAc) to give (**6b**) in an overall yield of 43%.

*Imino-N-acetic acid-N-acetylglycyl-S-benzylcysteamine.HBr (***8b***)*

The preparation of **8b** (derivative **E**) is outlined in Scheme 2. Reaction of carbobenzyloxyglycine with *S*-benzylcysteamine (**2**) using DCC and 1-hydroxybenzotriazole (HBT) resulted in (**7a**), which was deprotected to give glycyl-*S*-benzylcysteamine. HBr (**7b**). Condensation of *N*-carbobenzyloxyimino acetic anhydride (**3b**) with the free amine of (**7b**) gave (**8a**), which was deprotected to afford the hydrobromide (**8b**) in an overall yield of 16%.

*N1-Benzylmercaptoethyltriglycine (***11***)*

The method used to prepare **11** (derivative **D**) is described in Scheme 3. The first intermediate (**9a**), i.e., *N*-2-benzylmercaptoethylglycine has previously been prepared from 2-benzylmercaptoethyl chloride and glycine.[43] Condensation of *S*-benzylcysteamine (**2**) with bromomalonic acid at pH 10 in 50% ethanol,[44] followed by thermal decarboxylation of one carboxyl group resulted in (**9a**). This intermediate was *N*-protected by reaction with carbobenzyloxy chloride in alkaline medium to give *N*-2-benzylmercaptoethyl-*N*-carbobenzyloxyglycine (**9b**). Activation of (**9b**) with DCC/HBT and subsequent coupling with glycylglycine ethyl ester provided the fully protected benzylmercaptoethyl tripeptide (**10a**). Alkaline hydrolysis produces the corresponding acid (**10b**), which was

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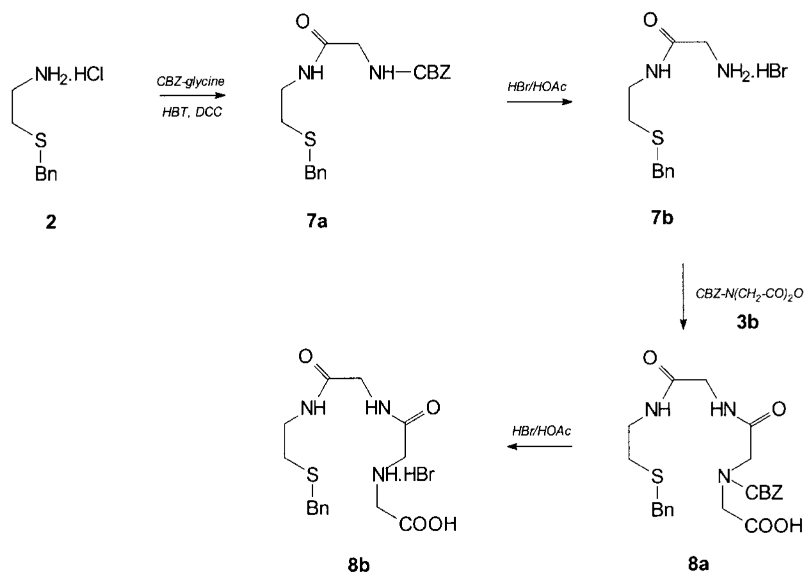
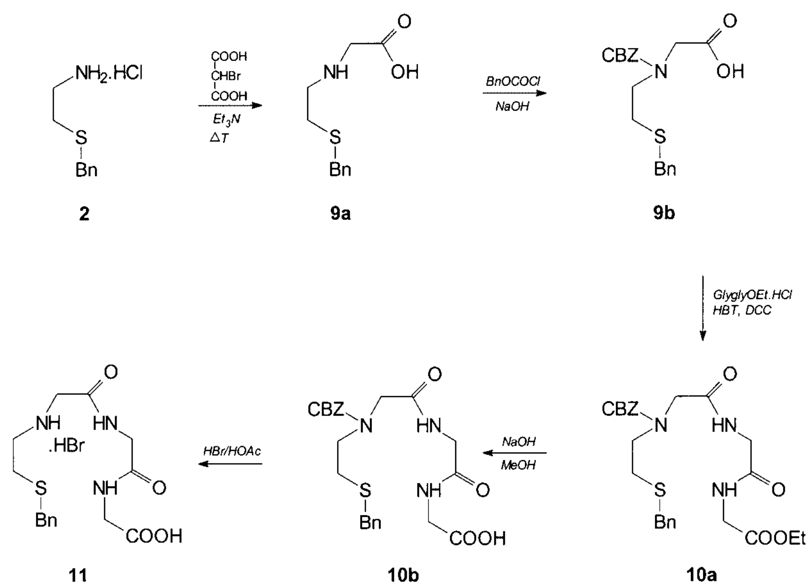


Scheme 2. Synthesis of Imino-N-acetic acid-N′-acetylglycyl-S-benzylcysteamine. HBr (8b)



Scheme 3. Synthesis of *N*1-Benzylmercaptoethyltriglycine (11)

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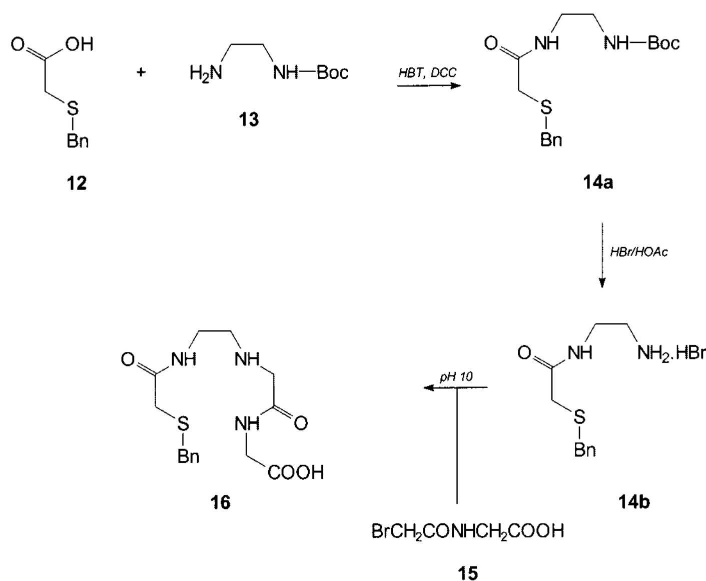
converted to *N*-2-benzylmercaptoethyltriglycine (**11**) by acidolysis in overall yield of 13%.

*S-Benzylmercaptoacetamidoethylenediglycine (***16***)*

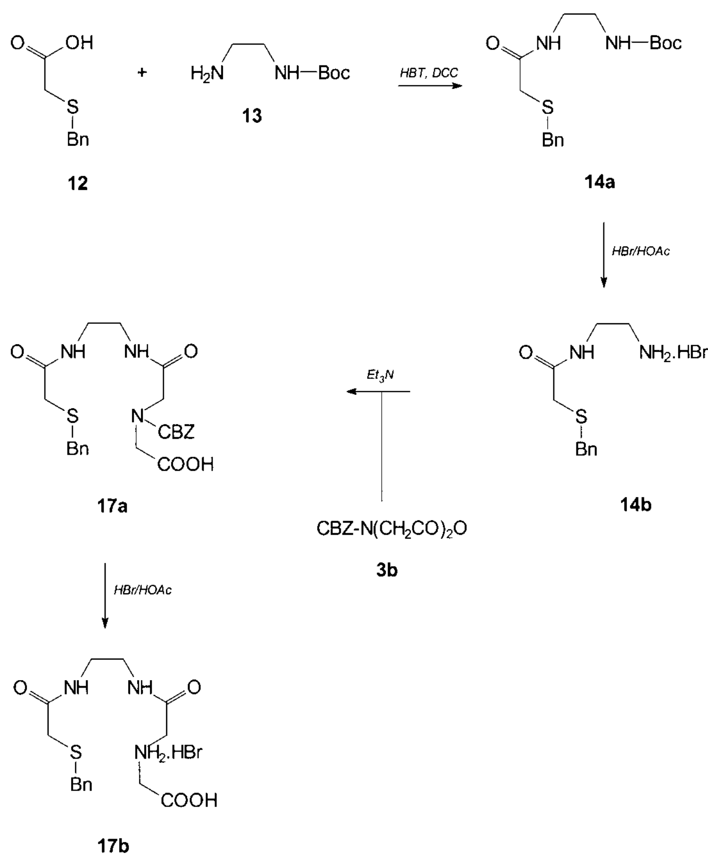
For the preparation of **16** (derivative **C**, Scheme 4), commercially available *S*-benzylthioglycolic acid (**12**) was reacted in the presence of HBT/DCC with *N*-tert-butoxycarbonylethylenediamine (**13**). This chemical was obtained from the reaction of ethylenediamine and di-tert-butyldicarbonate in 1 : 9 ratios in the presence of triethylamine.[45] *N-S*-benzylmercaptoacetyl-*N*′-tert-butoxycarbonylethylenediamine (**14a**) was the first intermediate. Removal of the BOC group with acid resulted in (**14b**), which was alkylated with bromoacetylglycine (**15**) (prepared from glycine and bromoacetyl chloride in aqueous alkaline medium) to give *S*-benzylmercaptoacetamidoethylenediglycine (**16**) in an overall yield of 29%.

N-2-Benzylmercaptoacetyl-N′*-imino (N*″*-acetic acid-N*″*-acetyl) ethylene diamine (***17b***)*

Compound **17b** (derivative **F**) was prepared according to Scheme 5. Coupling of (**14b**) (intermediate in the synthesis of **16**) with carboben-



Scheme 4. Synthesis of *S*-Benzylmercaptoacetamidoethylenediglycine (16)



Scheme 5. Synthesis of *N*-2-benzylmercaptoacetyl-*N*-imino (*N*″-acetic acid-*N*″ acetyl) ethylene diamine (17b)

zyloxyimino acetic anhydride (**3b**) gave (**17a**) The carbobenzyloxy protecting group was removed by treatment with HBr/HOAc to afford (**17b**) in an overall yield of 17%.

*S-Benzylmercaptoacetylglycyl-2-aminoethylene-N-tosylglycine (***27***)*

The method for the preparation of **27** is depicted in Schemes 6a and 6b. *S*-Benzyl-mercaptoacetylglycine (**19**) was prepared from the *N*-hydroxysuccinimidyl ester (**18a**) of *S*-benzylmercaptoacetic acid (**12**) and glycine and was then converted to its *N*-hydroxysuccinimidyl ester (**19a**, Scheme 6a). Ethanolamine was converted to the *N*-BOC protected compound (**20**), which was further transformed to the *O*-tosyl ester (**21**)

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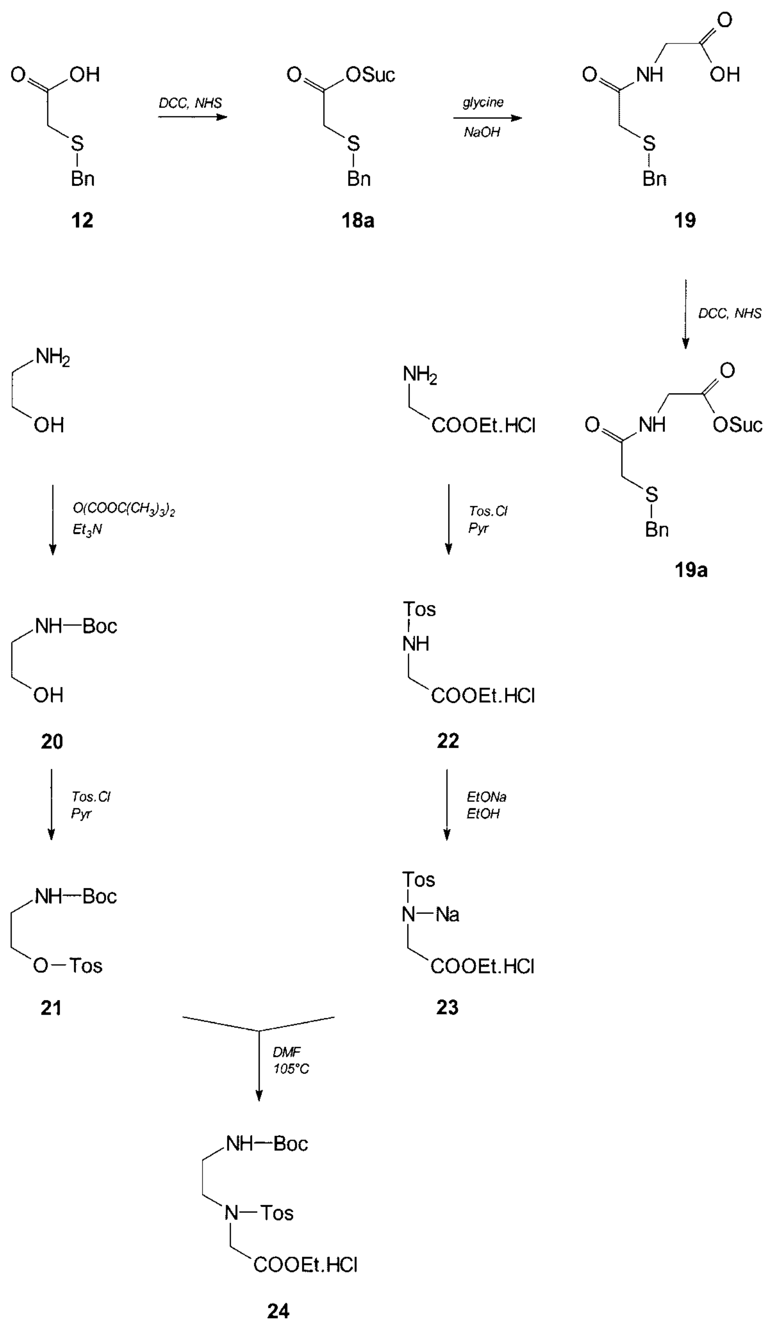

Scheme 6. a Synthesis of succinimidyl benzylmercaptoacetylglycinate (19a), and *N*(2-tert-buytoxylcarbonylaminoethyl)-*N*-tosylglycine ethyl ester (24) Scheme 6b. Synthesis of *S*-Benzylmercaptoacetylglycyl-2-aminoethylene-*N*-tosylglycine (27a)

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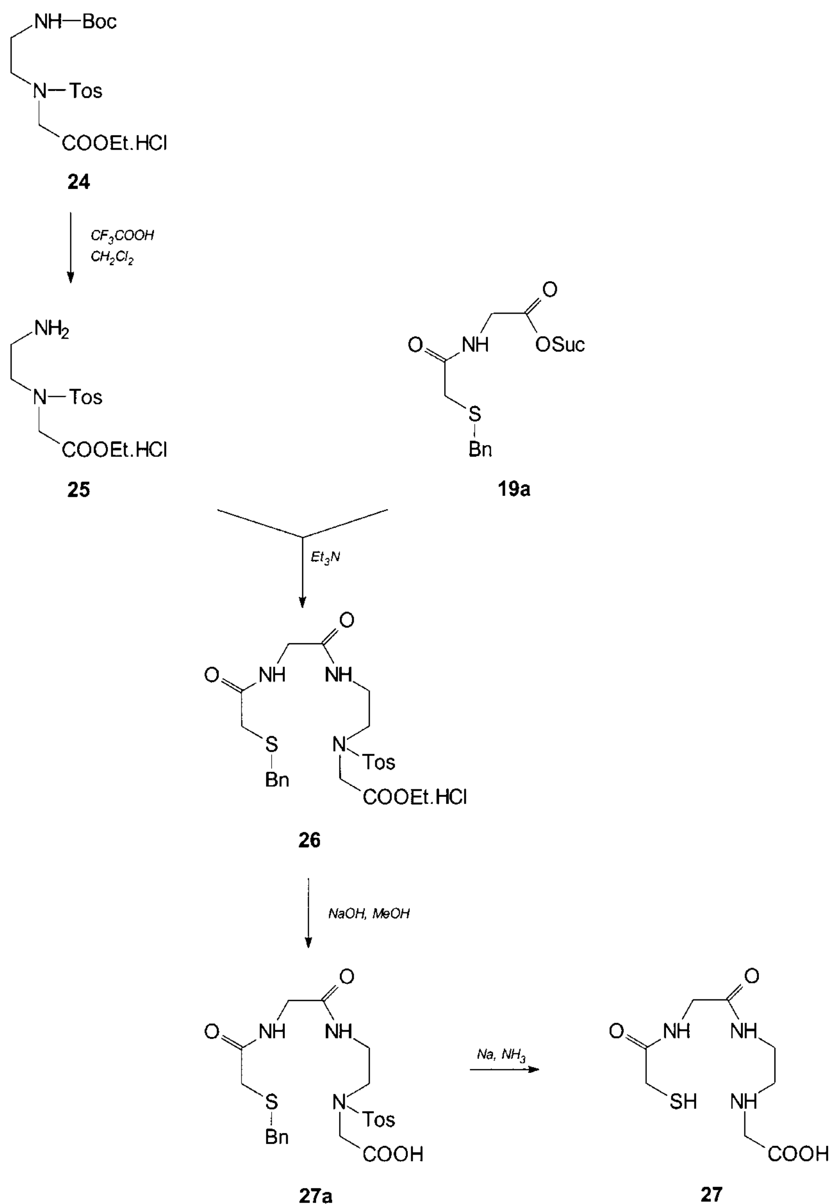

Scheme 6. Continued.

using tosyl chloride in pyridine. Glycine ethyl ester was protected as the *N*-tosyl derivative (**22**) from which the sodium salt (**23**) was prepared with sodium ethoxide in ethanol. Reaction of (**21**) and (**23**) in DMF at 105°C following the method of Atkins *et al.*[46] for the synthesis of hexacyclen, resulted in (*N*-2-tert-butoxycarbonylaminoethyl)-*N*-tosyl-

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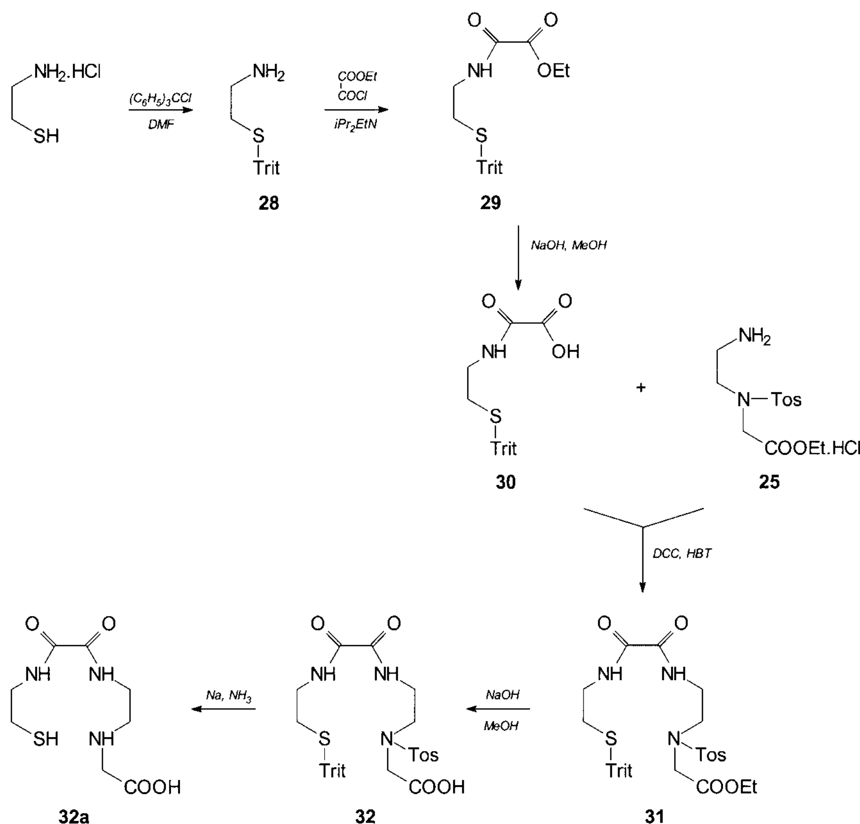
glycine ethyl ester (**24**) (Scheme 6a). This product was deprotected by TFA to give compound (**25**), which was isolated as the tosyl salt. Coupling of (**25**) with the *N*-hydroxysuccinimidyl ester (**19a**) provided (**26**) from which the ethyl ester was removed by alkaline hydrolysis to give (**27**) in overall yield of 23%. Compound (**27**) was further deprotected by sodium in liquid ammonia immediately before labelling (**27a**, Scheme 6b, derivative **B**).

*N-2-Tritylmercaptoethyl-N′-ethylene-N-tosylglycine oxamide (***32***)*

Compound **32** was prepared according to Scheme 7. Different methods for the preparation of *S*-tritylcysteamine (**28**) have been described, starting either from bromoethylamine and sodium trityl mercaptan[38]



Scheme 7. Synthesis of *N*-2-Tritylmercaptoethyl-*N′*-ethylene-*N*-tosylglycine oxamide (32)

(yield 93%) or from mercaptoethylamine and trityl chloride[47] (yield 63%) or trityl alcohol[48] (yield 70%). We performed however, the reaction of trityl chloride with cysteamine and obtained in this way a high yield of 76% of (**28**). Reaction of (**28**) with ethyl oxalyl chloride resulted in (**29**) from which the acid (**30**) was obtained by alkaline hydrolysis. Condensation of this acid with the 2-aminoethylglycine derivative (**25**, intermediate of Scheme 6b) produced the oxamide ester (**31**), which was hydrolyzed to the corresponding acid (**32**) in an overall yield of 7%. Compound (**32**) was fully deprotected by the treatment of sodium in liquid ammonia prior to radiolabelling to afford (**32a**) (derivative **I**).

*N-(2-Benzylmercaptoethyl-N-tosyl)-N′-(oxalylglycine) ethylenediamine (**41**)*

Compound **41** was obtained as represented in Scheme 8. Condensation of the sodium salt (**34**) of *S*-benzyl-*N*-tosyl cysteamine (**33**) with *N*-tert-butoxycarbonyl aminoethanol tosylate (**21**) (intermediate of Scheme 6a) resulted in a fully protected derivative of ethylene diamine (**35**). The BOC-group was removed with acid to give compound (**36**), which was converted to the tosylate salt (**37**). Coupling of this salt with *N*-oxalylglycine ethyl ester (**39**) (obtained from the corresponding mono-tert-butyl ester by reaction with HBr/HOAc) afforded the oxamide ethyl ester (**40**), which was hydrolyzed to the corresponding acid (**41**) in an overall yield of 4%. This compound was fully deprotected by sodium in liquid ammonia immediately before labelling (**41a**)(derivative **H**).

Overall yields were rather good for six end-products, especially considering the lengthy and complex procedures required for the syntheses. The other two diamides (with *N*-tosyl protective group) were however, hampered with a low yield coupling step. On the other hand, alkylation of the *N*-tosyl sodium salt with an *O*-tosyl leaving group seems to be a useful approach especially when compared with direct alkylation of the bromo compounds that resulted in impure mixtures.

It was our intention to use an *S*-benzyl protecting group for the preparation of all the compounds in this study. However, one of the intermediates, i.e. oxalyl-*S*-benzylcysteamine was found to be insoluble in organic solvents and hence became completely useless in DCC-mediated coupling reactions with amines. However, the solubility problem was resolved by the utilization of a more lipophilic *S*-trityl protecting group.

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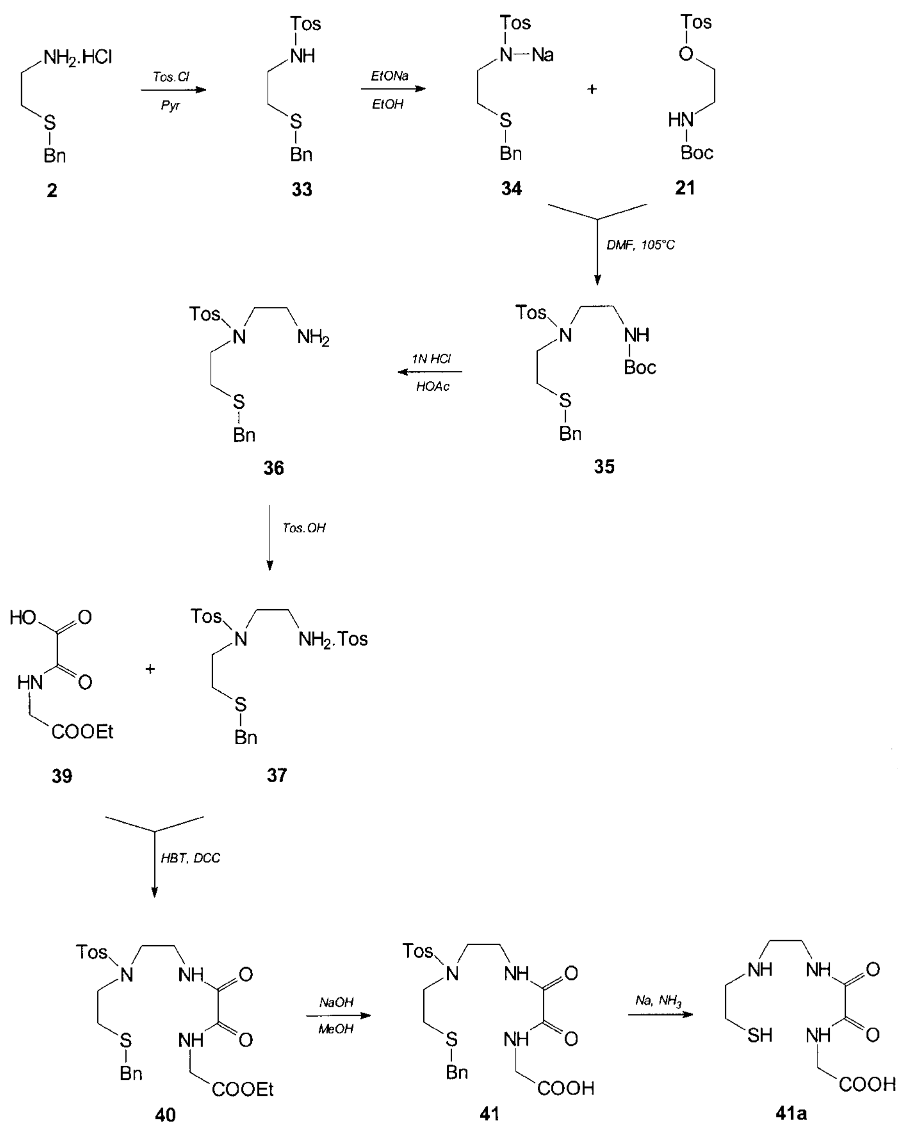

Scheme 8. Synthesis of *N*-(2-benzylmercaptoethyl-*N*-tosyl)-*N*-(oxalylglycine) ethylenediamine (41)

Initially, we intended to synthesize the *N*- and *S*-protected diamides (**B**, **H** and **I**) in the form of precursors bearing an *S*-protective group (but with no tosyl protection on the amine) as it has been done for the five others studied derivatives. All our efforts were unsuccessful mainly due to the formation of impure products, hence an alternative strategy

to use tosyl and trityl protection on amine and thiol functions, respectively, was employed that resulted in essentially pure products.

Labelling with ^{99m}Tc

Labelling of the synthesized diamide ligands was carried out by ligand exchange and/or direct method. For exchange labelling, the ligand (1–2 mg) and sodium tartrate (5 mg) were dissolved in 0.5 M phosphate buffer pH 10–11 (0.5 ml) followed by the addition of 200 μg of $SnCl_2 \cdot 2H_2O$ in 50 μl of 0.05N HCl. After the addition of 1–3 ml of eluate (UltraTechnekow generator, Mallinckrodt Medical, The Netherlands) containing 370 to 740 MBq (10 to 20 mCi) of $^{99m}TcO_4^-$, 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). For the direct labelling, 1–2 mg of the ligand was dissolved in 0.5 ml of 0.5 M phosphate buffer pH 12, followed by 200 μg of $SnCl_2 \cdot 2H_2O$ and 1 ml of $^{99m}TcO_4^-$ (370 MBq). The mixture was kept at room temperature for 1 h prior to radio-HPLC analysis.

Analysis of ^{99m}Tc-labelled compounds

Analysis of labelled reaction mixtures was performed by 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 × 4.6 mm reversed phase column. Radioactivity in the column effluent was monitored using a 2-inch NaI(Tl) scintillation detector coupled *via* a single channel analyzer to a Rachel-5-LS integrator (Raytest).

Biological evaluation of ^{99m}Tc-labelled compounds

Evaluation in mice. Biodistribution of diamides was studied in 5 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 144 kBq/ml with sterile saline and ^{131}I-OIH (Mallinckrodt Medical, The Netherlands) was added as an internal biological standard to a concentration of 14.4 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 ware sacrificed by decapitation at fixed time

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intervals. The organs were dissected and the radioactivity in each organ was measured. Corrections were made for background radiation, physical decay during counting and crossover of the ^{131}I-radiation into the ^{99m}Tc-channel. Radioactivity in each organ was expressed as a percentage of the total radioactivity, which was equal to the sum of the net counts in all body parts. To calculate the radioactivity in blood, blood mass was assumed to be 7% of body mass.

Evaluation in a baboon. For baboon studies, a male baboon (body mass about 12 kg) was anesthetized by intramuscular injection of 75 mg ketamine (Imagène®, Rhône Mérieux). Anesthesia was sustained by intravenous injection of 15 mg sodium pentobarbital (Nembutal®, Sanofi) at time intervals of 15 min. Approximately 18.5 MBq (0.5 mCi) of the ^{99m}Tc-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 (Monoject® H10, Sherwood Medical). After centrifugation, 500 μl plasma samples were pipetted out in tubes, weighed and radioactivity was measured using a γ-counter. One-hour plasma clearance was calculated using a double exponential fitting method.[36]

Conclusions

From the biodistribution studies in mice, it may be concluded that the renal excretion properties of the direct derivatives of ^{99m}Tc-MAG$_3$ (^{99m}Tc-complexes of **B**, **C** and **D**) in which one amide is replaced by an amine, are inferior to that of the parent compound. The results suggested that these derivatives do not fulfill the requirements for a suitable tracer agent for dynamic kidney function studies. On the other hand, the diamide derivatives of ^{99m}Tc-MAG$_3$, with at least one inverted amide and without an oxamide structure (^{99m}Tc-complexes of **E**, **F** and **G**), exhibited the most efficient renal handling in mice, characterized by their rapid renal excretion along with their negligible retention in other body organs. From these results, it seems that the presence of three amide groups is not strictly required for an efficient extraction of ^{99m}Tc-MAG$_3$-like agents by the kidneys and its excretion to the urine. Finally, it is evident that the position of the remaining amides, and especially of the carbonyl functionality, greatly determines the interaction of the tracer agent with the tubular receptor proteins.

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References

1. Hansen L, Marzilli LG, Eshima D, Malveaux EJ, Folks R, Taylor Jr A. J *Nucl Med* 1994; **35**: 1198–1205.
2. Eshima D, Taylor Jr A, Fritzberg AR, Kasina S, Hansen L, Sorenson JF. *J Nucl Med* 1987; **28**: 1180–1186.
3. Wang AY, Liu RS. *Nucl Med Biol* 1995; **22**: 937–942.
4. Chattopadhyay M, Jalan KN, Pal AK, Banerjee SN. *Nucl Med Biol* 1987; **14**: 599–604.
5. Arnold RW, Subramanian G, McAfee JG, Blair RJ, Thomas FD. *J Nucl Med* 1975; **16**: 357–367.
6. Subramanian G, Singh MV, Chander J, Singh B. *Eur J Nucl Med* 1976; **1**: 243–245.
7. Fukuoka M, Kiyohara T, Kobayashi T, Kojima S, Tanaka A, Kubodera A. *Nucl Med Biol* 1995; **22**: 181–191.
8. Fritzberg AR, Whitney WP, Kuni CC, Klingensmith III W. *Int J Nucl Med Biol* 1982; **9**: 79–82.
9. Tubis M, Posnick E, Nordyke RA. *Proc Soc Exp Biol Med* 1960; **103**: 497–498.
10. Burbank MK, Tauxe WN, Moher F, Hunt JC. *Proc Staff Meet Mayo Clin* 1961; **36**: 372–386.
11. Schwartz FD, Madeloff MS. *Clin Res* 1961; **9**: 208.
12. Fritzberg AR, Klingensmith III WC, Whitney WP, Kuni CC. *J Nucl Med* 1981; **22**: 258–263.
13. Jones AG, Davison A, LaTegola MR, Brodack JW. *J Nucl Med* 1982; **23**: 801–809.
14. Schneider RF, Subramanian G, Feld TA, McAfee JG, Zapf-Longo C, Palladino E, Thomas FD. *J Nucl Med* 1984; **25**: 223–229.
15. McAfee JG, Subramanian G, Schneider RF, Roskopf M, Lyons B, Ritter C, Zapf-Longo C, Palladino E, Thomas FD. *J Nucl Med* 1985; **26**: 375–386.
16. Kasina S, Fritzberg AR, Johnson DL, Eshima D. *J Med Chem* 1986; **29**: 1933–1940.
17. Fritzberg AR, Kuni CC, Klingensmith III WC, Stevens J, Whitney WP. *J Nucl Med* 1982; **23**: 592–598.
18. Klingensmith III WC, Fritzberg AR, Spitzer VM, Johnson DL, Kuni CC, Williamson MR, Washer G, Weil III R. *J Nucl Med* 1984; **25**: 42–48.

Copyright © 2002 John Wiley & Sons, Ltd.

19. Kuni CC, Klingensmith WC, Fritzberg AR, Spitzer VM, Latteier JL. *Clin Nucl Med* 1985; **10**: 810–813.
20. Bormans G, Cleynhens B, José D, Hoogmartens M, De Roo M, Verbruggen A. *Nucl Med Biol* 1990; **17**: 499–506.
21. Chervu LR, Sundoro BM, Blaufox MD. *J Nucl Med* 1984; **25**: 1111–1115.
22. Summerville DA, Packard AB, Bartynski B, Lim KS, Chervu LR, Treves ST. *J Nucl Med* 1987; **28**: 907–909.
23. Moran JK. *Semin Nucl Med* 1999; **39**: 91–101.
24. Fritzberg AR, Kasina S, Eshima D, Johnson DL. *J Nucl Med* 1986; **27**: 111–116.
25. Verbruggen AM, Nosco DL, Van Nerom CG, Bormans GM, Adriaens PJ, De Roo MJ. *J Nucl Med* 1992; **33**: 551–557.
26. Taylor Jr A, Eshima D, Fritzberg AR, Christian PE, Kasina S. *J Nucl Med* 1986; **27**: 795–803.
27. Nosco DL, Wolfangel RG, Bushman MJ, Grummon GD, Marmion ME, Pipes DW. *J Nucl Med Technol* 1993: **21**: 69–74.
28. Bubeck B, Brandau W, Steinbächer M, Reinbold F, Dreikorn K, Eisenhut M, Georgi P. *Nucl Med Biol* 1988; **15**: 109–118.
29. Jafri RA, Britton KE, Nimmon CC, *et al. J Nucl Med* 1988; **29**: 147–158.
30. Taylor Jr A, Eshima D, Christian PE, Milton W. *Radiology* 1987; **162**: 365–370.
31. Bubeck B, Brandau W, Weber E, Kälble T, Parekh N, Georgi P. *J Nucl Med* 1990; **31**: 1285–1293.
32. Britton KE, Jafri RA, Nimmon CC (Letters to Editor). *J Nucl Med* 1988; **29**: 1878–1879.
33. Shattuck LA, Eshima D, Taylor Jr A, Anderson TL, Graham DL, Latino FA, Payne SE. *J Nucl Med* 1994; **35**: 349–355.
34. Yamazaki N, Kitayashi C. *J Am Chem Soc* 1989; **3**: 1396–1408.
35. Schultz AG, McCloskey PJ, Court JJ. *J Am Chem Soc* 1987; **101**: 6493–6502.
36. Despopoulos A. *J Theor Biol* 1965; **8**: 163–192.
37. Okarvi SM. *Ph.D. Thesis* 1992; Faculty of Pharmaceutical Sciences. KUL, Belgium.
38. Goldberg AA, Kelly W. *J Chem Soc* 1948; 1919–1926.
39. Baddiley J, Thain EM. *J Chem Soc* 1952; 800–803.
40. Johnston TP, Gallagher A. *J Org Chem* 1963; **28**: 1305–1308.
41. Carroll FI, Dickson HM, Wall ME. *J Org Chem* 1965; **30**: 33–38.
42. Balasubramaniam A, Burt RJ, Christou G, Ridge B, Rydon HN. *J Chem Soc Perkin* 1981; **1**: 310–317.
43. Du Vigneaud V, Stevens CM, McDuffie Jr HF, Wood JL, McKennis Jr H. *J Am Chem Soc* 1948; **70**: 1620–1624.
44. Lutz O. *Ber* 1902; **35**: 2549–2554.

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45. Moroder L, Hallett A, Wünsch E, Keller O, Wersin G. *Hoppe-Seyler's Z Physiol Chem* 1976; **357**: 1651–1653.
46. Atkins TJ, Richman JE, Oettle WF. *Org Syn Coll* 1988: **6**: 652–662.
47. Conrad RA, Cullinan GJ, Gerzon K, Poore GA. *J Med Chem* 1979; **22**: 391–400.
48. Brenner D, Davison A, Lister-James J, Jones AG. *Inorg Chem* 1984; **23**: 3793–3797.

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