Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIII, No. 11

SYNTHESIS AND LABELLING CHARACTERISTICS OF ^{99m}Tc–MERCAPTOACETYLTRIPEPTIDES

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Keywords : technetium-99m mercaptoacetyltripeptides, renal tracer agents, labelling with technetium-99m, synthesis of S-benzyl-mercaptoacetyltripeptides

Abstract

A number of derivatives of mercaptoacetyltriglycine in which one or more of the three glycyl moieties are replaced by other amino acids such as D- or L-alanine, D-serine, D-2-aminobutyric acid, D-valine or D-phenylglycine have been synthesized and labelled with technetium-99m. Labelling with technetium yielded in each case a mixture of mainly two radioactive species, which probably are diastereomeric oxotechnetium (V) complexes originating from the presence of a chiral carbon atom in the ligands. The diastereomers were separated by reversed phase HPLC. Except for the serine derivative, all radiolabelled derivatives have a longer retention time than the parent compound ^{99m}Tc-MAG_3. The relative amount of the two diastereomers formed upon labelling is dependent on the nature of the ligand but can be influenced to a limited extent by the pH of the exchange labelling mixture.

INTRODUCTION

The intensive search for a technetium-99m labelled alternative to [^{131}I]-Hippuran lead to the successive development of ^{99m}Tc-DADS (1,2) , ^{99m}Tc-CO_2DADS (3), ^{99m}Tc-MAG_3 (4) and recently ^{99m}Tc-L,L-EC (5,6) (Fig. 1).

^{99m}Tc-DADS shows a significant excretion through the hepatobiliary system which has precluded its clinical use. ^{99m}Tc-CO_2DADS, which has a carboxyl group on the DADS ligand backbone, can exist in the form of four isomers (7) of which only the "syn" isomers exhibit efficient renal excretion in the baboon (8).

0362–4803/93/111065–14$12.00

Received 3 June, 1993
Revised 7 June, 1993

^{99m}Tc-mercaptoacetyltriglycine (^{99m}Tc-MAG$_3$), which in fact occurs as a mixture of two enantiomers (9), has passed a period of extensive clinical investigation and is now generally accepted as the agent of choice for routine renal imaging, relative renal function and transit time studies, and for the estimation of renal plasma flow. ^{99m}Tc-MAG$_3$ labelling kits are commercially available both in Europe and the United States from Mallinckrodt Medical.

In an attempt to improve the renal excretion characteristics of ^{99m}Tc-MAG$_3$, Eshima and coworkers synthesized and evaluated the technetium-99m complexes of several MAG$_3$ derivatives with modification of the terminal amino acid (10). However, none of these agents showed a more efficient renal clearance and handling than that of the parent compound ^{99m}Tc-MAG$_3$.

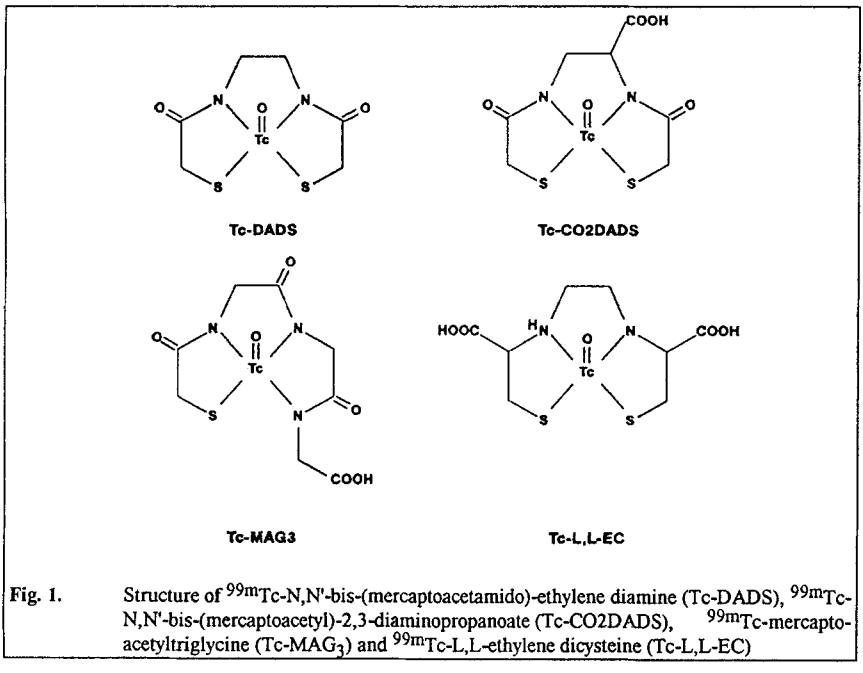
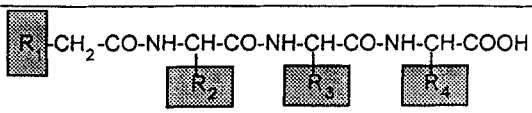


Fig. 1. Structure of ^{99m}Tc-N,N'-bis-(mercaptoacetamido)-ethylene diamine (Tc-DADS), ^{99m}Tc-N,N'-bis-(mercaptoacetyl)-2,3-diaminopropanoate (Tc-CO2DADS), ^{99m}Tc-mercaptoacetyltriglycine (Tc-MAG$_3$) and ^{99m}Tc-L,L-ethylene dicysteine (Tc-L,L-EC)

In order to study the general influence of substitution of the MAG$_3$ ligand core on the biological characteristics of the corresponding ^{99m}Tc-complexes, we have synthesized 15 derivatives of MAG$_3$ in which one, two or three glycyl groups are substituted by other amino acids such as D- or L-alanine, D-serine, D-2-aminobutyric acid, D-valine and D-phenylglycine (Table 1).

Due to the presence of a chiral carbon atom in the resulting mercaptoacetyltripeptides, labelling with technetium-99m produced for each of the ligands a pair of diastereomers that could be separated on RP-HPLC.

In this paper we describe the synthesis of the thiol protected ligands, the labelling characteristics and the influence of labelling conditions on the relative amount of the two diastereomers that are formed during labelling. The results of the biodistribution study of the ^{99m}Tc-complexes have already been reported (11).



	R1	R2	R3	R4
S-benzoyl-MAG$_3$ S-benzoyl-mercaptoacetylglycylglycylglycine	C_6H_5CO	H	H	H
S-benzoyl-MAAGG S-benzoyl-mercaptoacetylalanylglycylglycine	C_6H_5CO	CH_3	H	H
S-benzoyl-MAGAG S-benzoyl-mercaptoacetylglycylalanylglycine	C_6H_5CO	H	CH_3	H
S-benzyl-MAGGA S-benzyl-mercaptoacetylglycylglycylalanine	C_6H_5	H	H	CH_3
S-benzyl-MAAAG S-benzyl-mercaptoacetylalanylalanylglycine	C_6H_5	CH_3	CH_3	H
S-benzyl-MAGAA S-benzyl-mercaptoacetylglycylalanylalanine	C_6H_5	H	CH_3	CH_3
S-benzyl-MAAAA S-benzyl-mercaptoacetylalanylalanylalanine	C_6H_5	CH_3	CH_3	CH_3
S-benzyl-MAGSERG S-benzyl-mercaptoacetylglycylserylglycine	C_6H_5	H	CH_2OH	H
S-benzyl-MAGABUG S-benzyl-mercaptoacetylglycyl-2-aminobutyrylglycine	C_6H_5	H	C_2H_5	H
S-benzyl-MAGVALG S-benzyl-mercaptoacetylglycylvalylglycine	C_6H_5	H	$(CH_3)_2CH$	H
S-benzyl-MAGPHEG S-benzyl-mercaptoacetylglycylphenylglycyl-glycine	C_6H_5	H	C_6H_5	H

Fig. 2. Structure of the different S-protected mercaptoacetyltripeptides

EXPERIMENTAL PART

SYNTHESIS OF S-PROTECTED MERCAPTOACETYLTRIPEPTIDES

GENERAL PROCEDURES

Melting points were determined using open capillaries immersed in an oil bath (Büchi-Tottoli) and are not corrected. Thin layer chromatography was carried out using precoated TLC silicagel plates (Merck, 60F254) eluted with the mixture chloroform-methanol-acetic acid (60:40:1). The compounds were detected with U.V.-light of 254 nm, or by exposing the chromatograms to iodine vapours. Optical rotation was measured at room temperature on a Thorn 243 polarimeter. ^{1}H nuclear magnetic resonance (NMR) spectra were obtained on a Jeol FX 90Q spectrometer. All chemicals were of reagent grade and were used without purification unless otherwise indicated. Di- and tripeptides that were commercially available were purchased from Sigma or Serva.

Method A for the preparation of S-benzoyl and S-benzyl protected mercaptoacetyltripeptides.

Example : preparation of *S-benzoylmercaptoacetyl-D-alanylglycylglycine* (1).

200 mg (1 mmol) of D-alanylglycylglycine, dissolved in 5 ml of sodium hydroxide 0.2N, was added to a solution of 290 mg (1 mmol) of succinimidyl-S-benzoylthioglycolate (12) in 5 ml of

acetonitrile. The reaction mixture was stirred for 4 h and the pH was adjusted to pH 2 by the addition of hydrochloric acid 12N. The resulting suspension was stirred for an additional 2 h and filtered. The filter cake was washed with two portions of 5 ml ice-cold water and finally recrystallized from acetone-water (60:40) to yield 200 mg (51%) of a white powder; mp 180-182°C ; $[\alpha]^{25} = 22°$ (c=1, methanol) ; R_f 0.61 ; ^{1}H-NMR (DMSO) TMS δ 1.3 (d, CH_3, 3H), δ 3.7-3.8 (m, NH-$\underline{CH_2}$-CO, 4H), δ 3.9 (s, S-CH_2-CO, 2H), δ 4.1-4.5 (m, CH, 1H), δ 7.4-8.1 (m, ϕ-CO, 5H), δ 8.2-8.4 (m, CO-$\underline{NH}$-CH_2, 2H), δ 8.5 (d, CO-$\underline{NH}$-CH, 1H).

Other compounds synthesized following method A :

S-Benzoylmercaptoacetyl-L-alanylglycylglycine (2). L-alanylglycylglycine was used instead of D-alanylglycylglycine; yield 75% ; mp 184-185°C ; $[\alpha]^{25}$ - 24° (c=1, methanol) ; R_f 0.61 ; ^{1}H-NMR (DMSO) TMS δ 1.3 (d, CH_3, 3H), δ 3.7-3.8 (m, NH-$\underline{CH_2}$-CO, 4H), δ 3.9 (s, S-CH_2-CO, 2H), δ 4.1-4.5 (m, CH, 1H), δ 7.4-8.1 (m, ϕ-CO, 5H), δ 8.2-8.4 (m, CO-$\underline{NH}$-CH_2, 2H), δ 8.5 (d, CO-$\underline{NH}$-CH, 1H).

S-Benzoylmercaptoacetyl-D-alanyl-D-alanyl-D-alanine (3). D-alanyl-D-alanyl-D-alanine was used instead of D-alanylglycylglycine; yield 66% ; mp 187-189°C ; $[\alpha]^{25}$ + 57° (c=1, methanol) ; R_f 0.73 ; ^{1}H-NMR (DMSO) TMS δ 1.3 (d, 3x CH_3-, 9H), δ 3.9 (s, S-CH_2-CO, 2H), δ 4.1-4.5 (m, 3x CH, 3H), δ 7.4-8.2 (m, ϕ-CO, 5H), δ 8.4-8.6 (d, 3x CO-$\underline{NH}$-CH, 3H).

S-Benzoylmercaptoacetylglycine (4). Glycine was used instead of D-alanylglycylglycine; yield 88% ; mp 122-124°C ;(Lit : 138-139.5 °C (12)) R_f 0.73.

Method B for the preparation of S-benzoyl and S-benzyl protected mercaptoacetyltripeptides

Example : Preparation of *S-benzylmercaptoacetylglycyl-D-alanylglycine* (12).

B1 : *S-Benzylmercaptoacetylglycine* (5). 7.51 g (0.1 mol) of glycine was dissolved in 50 ml of potassium hydroxide 2N. The solution was cooled to 0°C and vigorously stirred during the dropwise addition of 22.06 g (0.11 mol) of benzylmercaptoacetyl chloride (39) in 30 ml of acetone in the course of 30 min. Potassium hydroxide 6N in water was continuously added at a rate sufficient to maintain pH 10. The mixture was stirred for 3 h at room temperature and acetone was evaporated. The pH was then adjusted to pH 2 with hydrochloric acid 12N. The precipitate formed was filtered off and recrystallized from ethanol to yield 20.35 g (85%) of a white powder; mp 120-122°C ; R_f 0.77.

Other compounds synthesized following method B1 :

S-Benzylmercaptoacetyl-D-alanine (6). D-alanine was used instead of glycine; yield 89% ; mp 128-131°C.

S-Benzylmercaptoacetyl-L-alanine (7). L-alanine was used instead of glycine; yield 80% ; mp 126-129°C.

B2 : *Succinimidyl-S-benzylmercaptoacetylglycine* (8). 10.2 g (43 mmol) of benzylmercaptoacetylglycine (5) and 4.95 g (43 mmol) of N-hydroxysuccinimide were dissolved in 50 ml of tetrahydrofuran. 10.7 g (52 mmol) of dicyclohexylcarbodiimide was added and the reaction mixture was stirred overnight at room temperature. Dicyclohexylureum was filtered off and washed twice with 15 ml of boiling tetrahydrofuran. The filtrate was evaporated and the residue was recrystallized from ethyl acetate to yield 6.64 g (46%) of the product; mp 101-103°C.

Other compounds synthesized following method B2 :

Succinimidyl-S-benzoylmercaptoacetylglycine (9). Benzoylmercaptoacetylglycine (4) was used instead of benzylmercaptoacetylglycine; yield 40% ; mp 158-161°C (Lit. : 161-163°C (12)).
Succinimidyl-S-benzylmercaptoacetyl-D-alanine (10). Benzylmercaptoacetyl-D-alanine (6) was used instead of benzylmercaptoacetylglycine; yield 89% ; mp 138-140°C.
Succinimidyl-S-benzylmercaptoacetyl-L-alanine (11). Benzylmercaptoacetyl-L-alanine (7) was used instead of benzylmercaptoacetylglycine; yield 46% ; mp 140-144°C.

B3 : *S-Benzylmercaptoacetylglycyl-D-alanylglycine* (12). 146 mg (1 mmol) of D-alanylglycine, dissolved in 5 ml of sodium hydroxide 0.2N was added to 334 mg (1 mmol) of succinimidyl-S-benzylmercaptoacetylglycine (8) dissolved in 5 ml of acetonitrile. The mixture was stirred for 2 hours at room temperature. The pH was adjusted to pH 2 with hydrochloric acid 12N and the solvents were evaporated. The residue was dispersed in water and filtered. The filter cake was washed twice with 10 ml of water. The product was purified by recrystallization from acetone-water (70:30) to yield 140 mg (38%) of a white product; mp 154-156°C ; $[\alpha]^{25}$ + 29° (*c*=1, methanol) ; R_f 0.62 ; ^{1}H-NMR (DMSO) TMS δ 1.2 (d, CH_3, 3H), δ 3.1 (s, S-CH_2-CO, 2H), δ 3.7 (m, NH-CH_2-CO, 4H), δ 3.8 (s, CH_2-ϕ, 2H), δ 4.2-4.5 (m, CH-CH_3, 1H), δ 7.3 (s, ϕ-, 5H), δ 8-8.5 (m, CO-NH, 3H).

Other compounds synthesized following method B3 :

S-Benzylmercaptoacetylglycyl-L-alanylglycine (13). L-alanylglycine was used instead of D-alanylglycine; yield 65% ; mp 156-158°C ; $[\alpha]^{25}$ - 30° (*c*=1, methanol) ; R_f 0.62 ; ^{1}H-NMR (DMSO) TMS δ 1.2 (d, CH_3, 3H), δ 3.1 (s, S-CH_2-CO, 2H), δ 3.7 (m, NH-CH_2-CO, 4H), δ 3.8 (s, CH_2-ϕ, 2H), δ 4.2-4.5 (m, CH-CH_3, 1H), δ 7.3 (s, ϕ-, 5H), δ 8-8.5 (m, CO-NH, 3H).
S-Benzoylmercaptoacetylglycyl-D-alanylglycine (14). Succinimidyl-S-benzoylmercaptoacetylglycine (9) was used instead of succinimidyl-S-benzylmercaptoacetylglycine ; yield 63% ; mp 154-156°C ; $[\alpha]^{25}$ + 22° (*c*=1, methanol) ; R_f 0.55 ; ^{1}H-NMR (DMSO) TMS δ 1.2 (d, CH_3, 3H), δ 3.5-3.8 (m, NH-CH_2-CO, 4H), δ 3.9 (s, S-CH_2-CO, 2H), δ 4.2-4.5 (m, CH-CH_3, 1H), δ 7.3-7.9 (m, ϕ-CO, 5H), δ 8-8.2 (m, CO-NH-CH_2, 2H), δ 8.4 (t, CO-NH-CH_2, 1H).
S-Benzoylmercaptoacetylglycyl-L-alanylglycine (15). Succinimidyl-S-benzoylmercaptoacetylglycine (9) was used instead of succinimidyl-S-benzylmercaptoacetylglycine and L-alanylglycine was used instead of D-alanylglycine; yield 66% ; mp 156-159°C ; $[\alpha]^{25}$ - 19° (*c*=1, methanol) ; R_f 0.56 ; ^{1}H-NMR (DMSO) TMS δ 1.2 (d, CH_3, 3H), δ 3.5-3.8 (m, NH-CH_2-CO, 4H), δ 3.9 (s, S-CH_2-CO, 2H), δ 4.2-4.5 (m, CH-CH_3, 1H), δ 7.3-7.9 (m, ϕ-CO, 5H), δ 8-8.2 (m, CO-NH-CH_2, 2H), δ 8.4 (t, CO-NH-CH_2, 1H).
S-Benzylmercaptoacetylglycylglycyl-D-alanine (16). Glycyl-D-alanine was used instead of D-alanylglycine; yield 68% ; mp 172-175°C ; $[\alpha]^{25}$ + 13° (*c*=1, DMSO) ; R_f 0.70 ; ^{1}H-NMR (DMSO) TMS δ 1.3 (d, CH_3, 3H), δ 3.1 (s, S-CH_2-CO, 2H), δ 3.5-3.9 (m, CO-CH_2-NH, ϕ-CH_2, 6H), δ 4.1-4.4 (m, NH-CH-CO, 1H), δ 7.3 (s, ϕ-, 5H), δ 8-8.4 (m, CO-NH, 3H).
S-Benzylmercaptoacetylglycylglycyl-L-alanine (17). Glycyl-L-alanine was used instead of D-alanylglycine; yield 65% ; mp 171-173°C ; $[\alpha]^{25}$ - 11° (*c*=1, DMSO) ; R_f 0.70 ; ^{1}H-NMR (DMSO) TMS δ 1.3 (d, CH_3, 3H), δ 3.1 (s, S-CH_2-CO, 2H), δ 3.5-3.9 (m, CO-CH_2-NH, ϕ-CH_2, 6H), δ 4.1-4.4 (m, NH-CH-CO, 1H), δ 7.3 (s, ϕ-, 5H), δ 8-8.4 (m, CO-NH, 3H).

S-Benzylmercaptoacetyl-D-alanyl-D-alanylglycine (18). Succinimidyl-S-benzylmercaptoacetyl-D-alanine (10) was used instead of succinimidyl-S-benzylmercaptoacetylglycine; yield 66% ; mp 138-140°C ; $[\alpha]^{25}$ + 32° (*c*=1, methanol) ; R_f 0.80 ; ^{1}H-NMR (DMSO) TMS δ 1.2 (d, -CH_3, 6H), δ 3.1 (s, S-CH_2-CO, 2H), δ 3.6 (s, $\underline{CH_2}$-COOH, 2H), δ 3.7 (s, $\underline{CH_2}$-ϕ, 2H), δ 4.1-4.5 (m, $\underline{CH}$-CH_3, 2H), δ 7.3 (s, ϕ-, 5H), δ 8-8.4 (m, CO-NH, 3H).

S-Benzylmercaptoacetyl-L-alanyl-L-alanylglycine (19). Succinimidyl-S-benzylmercaptoacetyl-L-alanine (11) was used instead of succinimidyl-S-benzylmercaptoacetylglycine and L-alanylglycine instead of D-alanylglycine; yield 77% ; mp 140-142°C ; $[\alpha]^{25}$ - 35° (*c*=1, methanol) ; R_f 0.80 ; ^{1}H-NMR (DMSO) TMS δ 1.2 (d, -CH_3, 6H), δ 3.1 (s, S-CH_2-CO, 2H), δ 3.6 (s, $\underline{CH_2}$-COOH, 2H), δ 3.7 (s, $\underline{CH_2}$-ϕ, 2H), δ 4.1-4.5 (m, $\underline{CH}$-CH_3, 2H), δ 7.3 (s,ϕ-CH_2, 5H), δ 8-8.4 (m, CO-NH, 3H).

S-Benzylmercaptoacetylglycyl-D-alanyl-D-alanine (20). D-alanyl-D-alanine was used instead of D-alanylglycine; yield 64% ; mp 135-138°C ; $[\alpha]^{25}$ + 30° (*c*=1, methanol) ; R_f 0.72 ; ^{1}H-NMR (DMSO) TMS δ 1.2-1.3 (2xd, $\underline{CH_3}$-CH, 6H), δ 3.1 (s, S-CH_2-CO, 2H), δ 7.4 (s, ϕ-, 5H), δ 7.9-8.3 (m, CO-NH, 2H), δ 8.6 (t, $\underline{NH}$-CO-CH_2, 1H).

S-Benzylmercaptoacetylglycyl-L-alanyl-L-alanine (21). L-alanyl-L-alanine was used instead of D-alanylglycine; yield 70% ; mp 137-140°C ; $[\alpha]^{25}$ - 32° (*c*=1, methanol) ; R_f 0.72 ; ^{1}H-NMR (DMSO) TMS δ 1.2-1.3 (2xd, $\underline{CH_3}$-CH, 6H), δ 3.1 (s, S-CH_2-CO, 2H), δ 7.4 (s, ϕ-, 5H), δ 7.9-8.3 (m, CO-NH, 2H), δ 8.6 (t, $\underline{NH}$-CO-CH_2, 1H).

S-Benzylmercaptoacetylglycyl-D-valylglycine (22). D-valylglycine (35) was used instead of D-alanylglycine; yield 64% ; mp 158-160°C ; $[\alpha]^{25}$ + 28° (*c*=1, methanol) ; R_f 0.80 ; ^{1}H-NMR (DMSO) TMS δ 0.9 (d, 2x CH_3-, 6H), δ 1.9-2.2 (m, CH-$\underline{CH_3}$, 1H), δ 3.1 (s, S-CH_2-CO, 2H), δ 3.6-3.9 (m, $\underline{CH_2}$-ϕ, 6H; d, NH-$\underline{CH_2}$-CO, 4H), δ 4.1-4.4 (m, CH-CO, 1H), δ 7.3 (s, ϕ-, 5H), δ 7.9 (d, CO-$\underline{NH}$-CH, 1H), δ 8.2-8.5 (m, CO-$\underline{NH}$-CH_2, 2H).

S-Benzylmercaptoacetylglycyl-D-phenylglycylglycine (23). D-phenylglycylglycine (36) was used instead of D-alanylglycine; yield 69% ; mp 142-144°C ; $[\alpha]^{25}$ - 68° (*c*=1, methanol) ; R_f 0.85 ; ^{1}H-NMR (DMSO) TMS δ 3.1 (s, S-CH_2-CO, 2H), δ 3.6-4.1 (m, NH-$\underline{CH_2}$-CO, CH_2-ϕ, 6H), δ 5.6 (d, -CH-, 1H), δ 7.1-7.6 (m, 2x ϕ-, 10H), δ 8.3 (d, CO-NH, 1H), δ 8.5-8.9 (m, CO-$\underline{NH}$-CH_2, 2H).

S-Benzylmercaptoacetylglycyl-D-serylglycine (24). D-serylglycine (37) was used instead of D-alanylglycine; yield 74% ; mp 152-155°C ; $[\alpha]^{25}$ + 6° (*c*=1, methanol) ; R_f 0.35 ; ^{1}H-NMR (DMSO) TMS δ 3.1 (s, S-CH_2-CO, 2H), δ 3.6-4.1 (m, NH-$\underline{CH_2}$-CO, $\underline{CH_2}$-ϕ, NH-$\underline{CH}$-CO, -$\underline{CH_2}$-OH, 9H), δ 4.4 (m, -OH, 1H), δ 7.3 (s, ϕ-, 5H), δ 8-8.4 (m, CO-$\underline{NH}$-, 3H).

Method C for the preparation of S-benzoyl and S-benzyl protected mercaptoacetyltripeptides

C1 : *N-carbobenzyloxy-D-2-aminobutyric acid* (25). 4.125 g (40 mmol) of D-2-aminobutyric acid was dispersed in 5 ml water and the pH was adjusted to pH 12 by the addition of sodium hydroxide 4N. The solution was cooled to 5°C and 7.48 g (44 mmol) of benzyl chloroformate was added in the course of 1 h. NaOH 4N was continuously added to maintain pH 10. The reaction mixture was stirred for an additional 30 min at 5°C, and was then extracted twice with 50 ml of diethyl ether. The aqueous phase was acidified to pH 2.5 with hydrochloric acid 12N

and was then extracted three times with 75 ml of ethyl acetate. The combined ethyl acetate layers were dried on anhydrous sodium sulphate and then evaporated to yield 6.9 g (67%) of a white powder; mp 72-74°C (Lit. : 78-79°C (13)).

Other compounds synthesized following method C1 :

N-carbobenzyloxy-D-valine (26). D-valine was used instead of D-2-aminobutyric acid; yield 67% ; mp 63-64°C (Lit. : 66-67°C (13)).

N-carbobenzyloxy-D-phenylglycine (27). D-phenylglycine was used instead of D-2-aminobutyric acid; yield 49% ; mp 57-59°C.

N-carbobenzyloxy-D-serine (28). D-serine was used instead of D-2-aminobutyric acid; yield 56% ; mp 105-109°C (Lit. : 119°C (13)).

C2 : *Glycine benzyl ester p-toluenesulphonate* (29). 37.6 g (0.5 mol) of glycine and 97 g (0.51 mol) of p-toluenesulphonic acid monohydrate were dispersed in a mixture of 200 ml benzyl alcohol and 100 ml benzene. The mixture was refluxed and the formed water was removed azeotropically with a Dean and Stark trap. Refluxing was continued until water no longer separated. After cooling to room temperature, 1 l of diethyl ether was added and the reaction mixture was cooled in the refrigerator for 2 h. The crystalline glycine benzyl ester p-toluenesulphonate was filtered off, washed twice with 300 ml of ether and recrystallized from methanol-ether to yield 150 g (89%) of white crystals; mp 131-133°C (Lit. : 132-134°C (13)).

C3 : *N_1-carbobenzyloxy-D-2-aminobutyrylglycine benzyl ester* (30). 6.5 g (27 mmol) of N-carbobenzyloxy-D-2-aminobutyric acid (25) and 9.23 g (27 mmol) of glycine benzyl ester p-toluenesulphonate (29) were dissolved in 50 ml of dichloromethane in the presence of 3.8 ml (27 mmol) of triethylamine. 4 g (30 mmol) N-hydroxybenzotriazole hydrate and 5.65 g (27 mmol) of dicyclohexylcarbodiimide were added and the reaction mixture was stirred overnight at room temperature. N,N'-dicyclohexylureum was filtered off and washed twice with 20 ml of boiling dichloromethane. The filtrate was evaporated and the residue was recrystallized from ethanol to yield 6.6 g (62.7%) of a white powder; mp 114-115°C.

Other compounds synthesized following method C3 :

N_1-carbobenzyloxy-D-valylglycine benzyl ester (31). N-carbobenzyloxy-D-valine (26) was used instead of N-carbobenzyloxy-D-2-aminobutyric acid; yield 47% ; mp 137-140°C (Lit. : 142-143°C (14)).

N_1-carbobenzyloxy-D-phenylglycylglycine benzyl ester (32). N-Carbobenzyloxy-D-phenylglycine (27) was used instead of N-carbobenzyloxy-D-2-aminobutyric acid; yield 56% ; mp 144-151°C.

N-Carbobenzyloxy-D-serylglycine benzyl ester (33). N-Carbobenzyloxy-D-serine (28) was used instead of N-carbobenzyloxy-D-2-aminobutyric acid; yield 62% ; mp 154-158°C.

C4 : *D-2-aminobutyrylglycine* (34). A suspension of 0.5 g of 10% palladium on activated carbon and 6.6 g (17 mmol) of N_1-carbobenzyloxy-D-2-aminobutyrylglycine benzyl ester (30) in 150 ml of methanol containing 0.5 ml of acetic acid was placed in a reaction flask. The reaction flask was flushed and pressurized (179 kPa) with hydrogen. The mixture was shaken for 10 h and again flushed and pressurized with hydrogen to 179 kPa. The reaction mixture was shaken during an additional period of 14 h and was then evaporated. The residue was

suspended in 50 ml of water and passed over a blue ribbon filter (Schleicher & Schuell 589) in order to remove the palladium/carbon catalyst. The filtrate was evaporated and the residue was dispersed in 30 ml of ethanol. The suspension was filtered, the filter cake was washed twice with 15 ml of an acetone-diethyl ether mixture (50:50) and then dried to yield 2.39 g (87%) of D-2-aminobutyrylglycine; mp 258-260°C.

Other compounds synthesized following method C4 :

D-valylglycine (35). N_1-carbobenzyloxy-D-valylglycine benzyl ester (31) was used instead of N_1-carbobenzyloxy-D-2-aminobutyrylglycine benzyl ester; yield 42% ; mp 264-266°C (Lit. : 271-272°C (15)).

D-phenylglycylglycine (36). N_1-carbobenzyloxy-D-phenylglycylglycine benzyl ester (32) was used instead of N_1-carbobenzyloxy-D-2-aminobutyrylglycine benzyl ester; yield 48% ; mp 205-210°C (Lit. : 226-228°C (16)).

D-serylglycine (37). N_1-carbobenzyloxy-D-serylglycine benzyl ester (33) was used instead of N_1-carbobenzyloxy-D-2-aminobutyrylglycine benzyl ester (45); yield 65% ; mp 208-211°C (Lit. : 215-216°C (17)).

C5 : ***S-Benzylmercaptoacetylglycyl-D-2-aminobutyrylglycine*** **(38). Procedure B3 was followed, using D-2-aminobutyrylglycine (34) instead of D-alanylglycine; yield 75% ; mp 160-162°C ;** $[\alpha]^{25}$ **+ 28° (*c*=1, methanol) ;** R_f **0.80 ;** ^{1}**H-NMR (DMSO) TMS δ 0.9 (t,** CH_3**-, 3H), δ 1.5-1.9 (m,** CH_3**-**CH_2**-, 2H), δ 3.2 (s, S-**CH_2**-CO, 2H), δ 3.6-3.9 (m,** CH_2**-ϕ,** CH_2**-COOH, NH-**CH_2**-CO, 6H), δ 4.1-4.4 (m, -CH-, 1H), δ 7.4 (s, ϕ-, 5H), δ 8.1 (d, CO-NH-CH, 1H), δ 8.3-8.5 (m, CO-NH-**CH_2**, 2H).**

S-Benzylmercaptoacetylchloride (39). 149 g (1.25 mol) of thionyl chloride was added to 54.6 g (0.3 mol) of benzylthioglycolic acid that had been recrystallized from ethyl acetate. The mixture was stirred overnight at room temperature and was then refluxed during 4 h. The excess of thionyl chloride was removed by evaporation. The residue was dissolved in 80 ml of benzene and the solvent was again evaporated. This treatment was repeated twice. The resulting S-benzylmercaptoacetylchloride was purified by distillation at reduced pressure (100°C, 0.25 mm Hg) to yield 55.6 g (93 %) of a yellow oil.

EXCHANGE LABELLING WITH ^{99m}Tc

The ligands were labelled with technetium-99m by mixing in a vial 1 mg of the S-protected mercaptoacetyltripeptide, 15 mg of tartaric acid, 0.1 ml of phosphate buffer 0.5M (pH 3.0 to pH 10.0), 100 µg of $SnCl_2.2H_2O$ in 25 µl of HCl 0.05N and 1 to 3 ml of generator eluate (Ultratechnekow FM^{TM}, Mallinckrodt Diagnostica, Holland) containing 0.37 to 3.7 GBq ^{99m}Tc in the form of pertechnetate. The mixture was heated for 10 min in a boiling water-bath, cooled to room temperature and filtered through a 0.22-µm pore membrane filter (Millipore).

DIRECT LABELLING WITH ^{99m}Tc

Direct labelling in alkaline conditions was carried out by incubating 1 mg of the S-benzoyl protected precursor in 1 ml of phosphate buffer 0.1M pH 10 to pH 13 during 10 min at room

temperature followed by the addition of 100 μg of $SnCl_2.2H_2O$ in 25 μl of HCl 0.05N and 2 ml of generator eluate (Ultratechnekow FM™, Mallinckrodt Diagnostica, Holland) containing 0.37 to 3.7 GBq ^{99m}Tc in the form of pertechnetate.

ANALYSIS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

All separations were performed on a HPLC-system, consisting of a Merck-Hitachi L 6200 intelligent pump, a Valco N6 injector and a 250 mm x 4.6 mm reversed phase column filled with Hypersil™ ODS 5 μm (Shandon) eluted with a ternary gradient mixture of ethanol (A)-0.025M phosphate buffer pH 5.85 (B) -water (C) at a flow rate of 1 ml/min.(Gradient : t=0, B=100%; t=15 min, A=20%, B=80%; t=20 min, A=50%, C=50%; t=25 min, A=90%, C=10%; t=30 min, A=90%, C=10%).

Radioactivity in the column effluent was monitored by a 2-inch NaI(Tl) crystal coupled via a single channel analyzer to a Ramona-5-LS integrator.

RESULTS AND DISCUSSION

SYNTHESIS OF S-PROTECTED MERCAPTOACETYLTRIPEPTIDES

In a first approach we studied the influence of the position and the configuration of an alanyl group which replaced one of the glycyl moieties of MAG_3. Therefore, the various glycine groups of MAG_3 were substituted by a D- or L-alanyl group in one of the three positions. Derivatives with D-serine, D-2-aminobutyric acid, D-valine and D-phenylglycine in position 2 of the tripeptide chain were also synthesized in order to investigate the influence of the type of amino acid on the renal handling of ^{99m}Tc-labelled mercaptoacetyltripeptides.

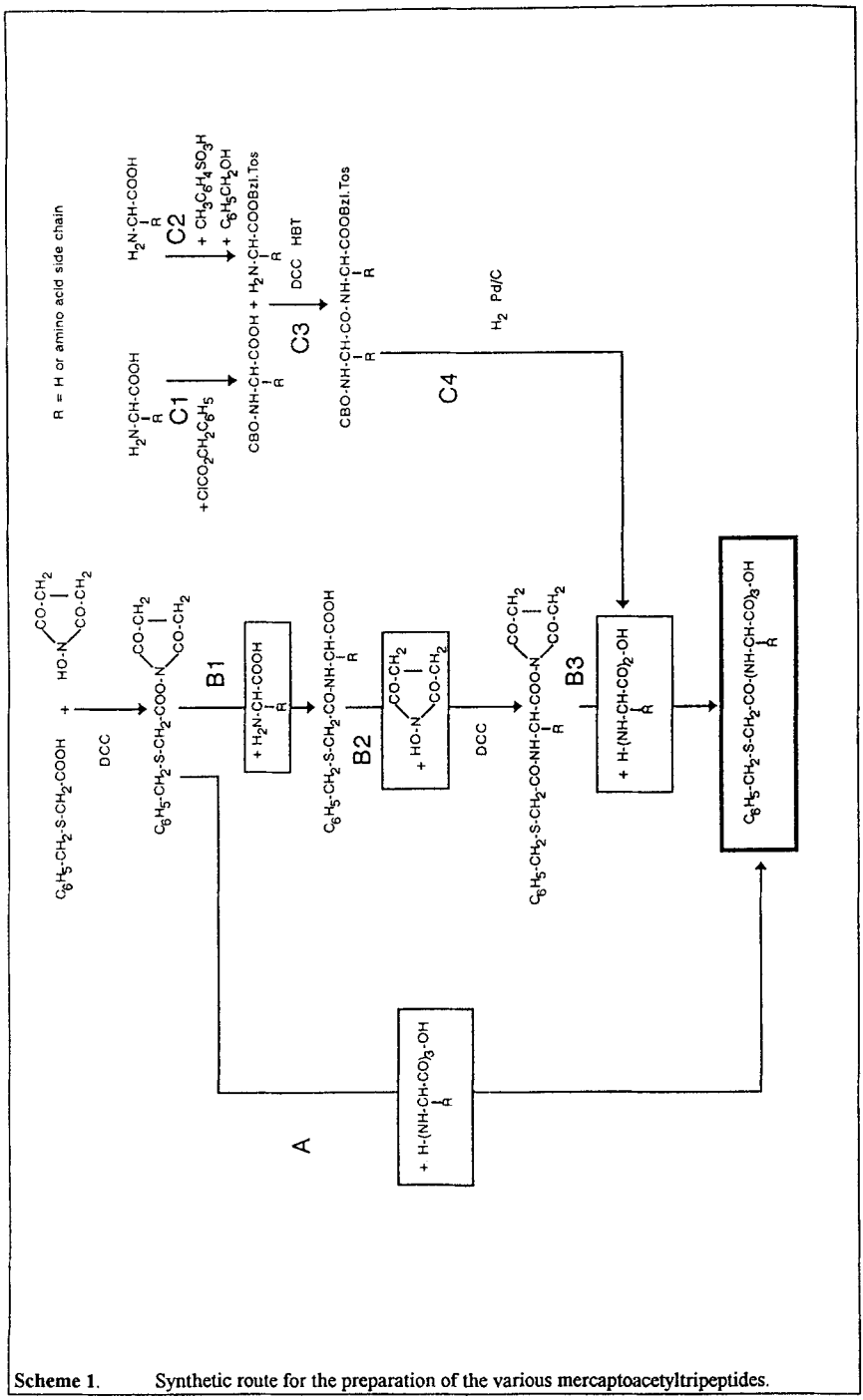
Thiol protected mercaptoacetyltripeptides of which the tripeptide is commercially available were synthesized by similar methods as described for MAG_3, i.e. by reacting either succinimidyl-S-benzylthioglycolate or S-benzylmercaptoacetyl chloride with the corresponding tripeptide (Scheme 1, A).

For the compounds of which not the tripeptide but the terminal dipeptide is commercially available, succinimidyl-S-benzylthioglycolate was coupled to the first amino acid. After activation of the terminal carboxyl group with N-hydroxysuccinimide the intermediate activated ester was further reacted with the appropriate dipeptide (Scheme 1, B).

Dipeptides which are not commercially available were synthesized by reaction of a carbobenzyloxy protected amino acid with an amino acid benzyl ester (17). The two protected amino acids were coupled in the presence of DCC and 1-hydroxybenzotriazole, the latter being added in order to improve the yield and to decrease racemization (18). The protective groups of the dipeptide were removed by reductive hydrogenation in the presence of a palladium catalyst (Scheme 1, C).

LABELLING WITH ^{99m}Tc

Labelling of mercaptoacetyltripeptides with technetium yields a complex in which an oxo-technetium core is coordinated by one sulfur and three amide nitrogen atoms. The atoms bound



Scheme 1. Synthetic route for the preparation of the various mercaptoacetyltripeptides.

to technetium form a square pyramid with the oxo group occupying the apical position. As an example a pluto drawing of the isomer B of ^{99}Tc-MAAGG is presented in Fig. 3. The synthesis and the structure determination of this complex are the subject of a separate report.

Labelling of each of the studied MAG_3 derivatives with ^{99m}Tc can yield two diastereomers with the amino acid substituent either in syn- or anti-position relative to the oxotechnetium core. Labelling of the ligand with D-configuration will yield two diastereomeric Tc-complexes that are the respective enantiomers of the two Tc-species formed by labelling of the corresponding L-configured ligand.

As it is the case for the parent compound MAG_3, the synthesized ligands can be labelled with technetium-99m by the exchange labelling technique in the presence of stannous tartrate. As could be expected from previous considerations and experience, RP-HPLC of the labelling

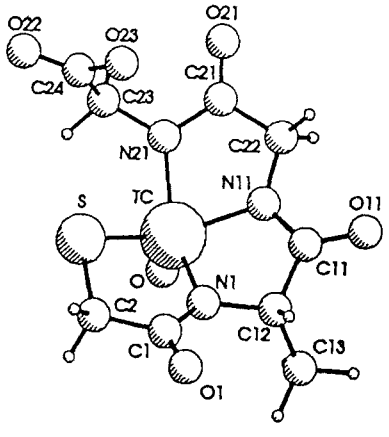


Fig. 3. Pluto drawing of isomer B of ^{99}Tc-MAAGG

Table 1. Retention times (RT, min) on gradient RP-HPLC for the diastereomeric ^{99m}Tc-complexes of the different mercaptoacetyl-tripeptides

	Isomer	RT
^{99m}Tc-MAG_3	-	13.0
^{99m}Tc-MAAGG	A	14.6
	B	16.0
^{99m}Tc-MAGAG	A	14.6
	B	16.4
^{99m}Tc-MAGGA	A	17.4
	B	18.9
^{99m}Tc-MAAAG	A	16.2
	B	18.2
^{99m}Tc-MAGAA	A	18.6
	B	19.3
^{99m}Tc-MAAAA	A	18.7
	B	22.0
^{99m}Tc-MAGSERG	A	11.4
	B	12.6
^{99m}Tc-MAGABUG	A	14.9
	B	18.1
^{99m}Tc-MAGVALG	A	18.0
	B	21.2
^{99m}Tc-MAGPHEG	A	16.8
	B	21.6

reaction mixture of each ligand indeed shows the presence of mainly two radiochemical species. The latter two compounds can be assumed to be the two diastereomeric ^{99m}Tc-complexes. Table 1 lists the different radiolabelled mercaptoacetyltripeptides with the retention time of their assumed diastereomers (designated A and B referring to the order of elution on gradient HPLC). The retention time is not only influenced by the nature, the size and the number of the substituents, but also to a high degree by the configuration of the Tc-complex (syn or anti) and the position of the substituent. Although MAAGG (mercaptoacetylalanylglycylglycine), MAGAG (mercaptoacetylglycylalanylglycine) and MAGGA (mercaptoacetylglycyl-glycylalanine) only differ by the position of the methyl substituent, their respective ^{99m}Tc-complexes can elute from the described system with a time difference of 4 minutes. Especially the complex with MAGGA is retained much longer on the column as compared to the parent compound MAG_3. This lets suppose that a substituent in α-position to the terminal carboxylate group masks the partially polar properties of the latter, which results in an apparent less polar character of the complex.

The configurational impact on the retention time is very pronounced for ^{99m}Tc-MAGPHEG for which the difference in retention time of the two diastereomers is larger than the difference in

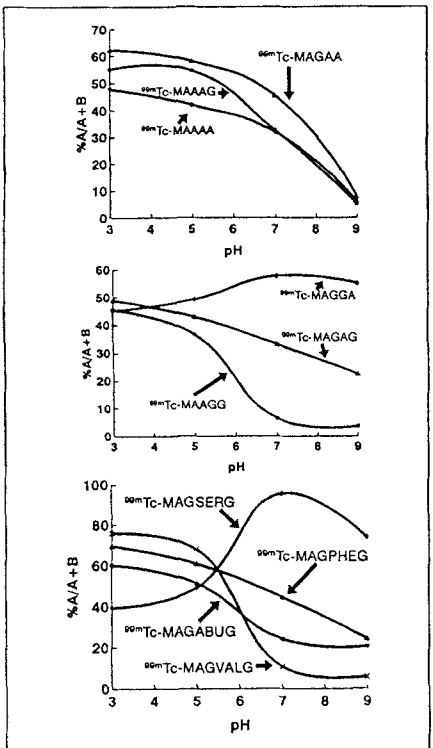


Fig. 4. Influence of pH on the relative amount of diastereomers formed during the labelling of mercapto-acetyl-tripeptides with ^{99m}Tc

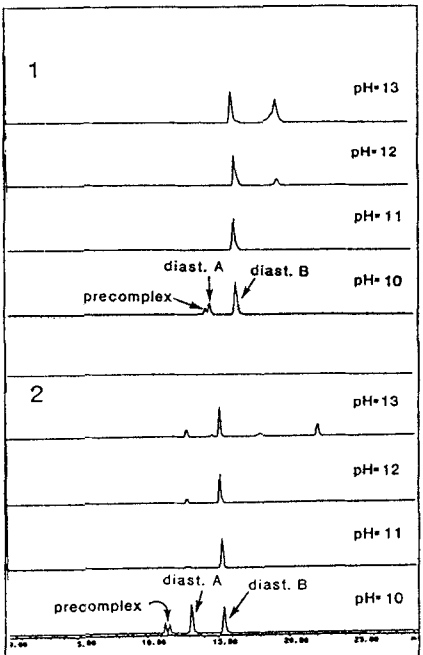


Fig. 5. RP-HPLC chromatograms of the direct labelling reaction mixtures at different pH values of ^{99m}Tc-MAAGG (1) and ^{99m}Tc-MAGAG (2)

retention time of its isomer A and that of ^{99m}Tc-MAG_3. The retention time of all derivatives was longer than that of the parent compound ^{99m}Tc-MAG_3, except for both diastereomers of the hydroxymethyl derivative ^{99m}Tc-MAGSERG, that seem to be more polar.

Variation of the exchange labelling conditions revealed that the pH during labelling influences significantly the relative amount of the diastereomers in the exchange reaction mixture (Fig. 4). The percentage of isomer A decreases with increasing pH for most of the compounds, except for the MAGSERG and MAGGA derivatives. In the case of ^{99m}Tc-MAGGA the chiral carbon atom is located outside of the complex ring and the relative amount of the diastereomers does not seem to be influenced to a great extent by variation of the pH of the exchange labelling mixture. MAGSERG is the only ligand for which diastereomer A can be formed in nearly quantative yield, namely by labelling at pH 7. Exchange labelling of the S-benzyl protected precursors of MAAGG, MAAAG, MAGAA, MAAAA, and MAGVALG at pH>9 yields on the other hand more than 90% of the B-isomer.

The technetium complexes of the mercaptoacetyltripeptides can also be obtained by direct labelling of the S-deprotected ligands. As an example we have labelled MAAGG and MAGAG by the successive addition of stannous chloride and pertechnetate to the S-benzoyl protected precursor from which the benzoyl group was removed by incubation in alkaline phosphate buffer (pH $\geq$ 10).

The direct labelling yields the corresponding ^{99m}Tc-complexes beside varying amounts of impurities. The amount and the type of impurities were found to be dependent on the pH of the labelling mixture (Fig. 5). The polar impurities designated on Fig. 5 as precomplex are only formed when labelling is performed at pH 10. The same polar impurities occur after exchange labelling at pH 7-9 in the presence of an excess of ligand (>10 mg). These compounds readily convert to the corresponding main peaks when they are isolated by HPLC and incubated in alkaline conditions (pH 12), but they remain stable in neutral environment. One of the assumptions is that the compound is a complex in which technetium is coordinated to the thiol groups of four different mercaptoacetyltripeptide molecules. A similar complex where technetium is coordinated to the thiol groups of different ligand molecules was found in the case of the dimercaptodiamide ligand DADS and was designated as the lantern dimer complex (18). This complex is also formed in excess of ligand, remains stable in neutral environment, but readily converts to the monomeric technetium complex in aqueous base.

Direct labelling at pH 13 of both MAAGG and MAGAG yields a lipophilic impurity with an identical retention time on HPLC as the technetium complex of the corresponding mercaptoacetyldipeptide. This impurity is probably generated by cleavage of the terminal amino acid of the ligand in aqueous base.

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