Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **44**, 99-111 (2001)

Structural Requirements for Stable Binding of Technetium-99m to Derivatives of Triglycine

Hubert P. Vanbilloen[1], Roger Busson[2], Alfons M. Verbruggen[1]

[1] Laboratory of Radiopharmaceutical Chemistry, Faculty of Pharmaceutical Sciences,
[2] Laboratory of Medicinal Chemistry, Faculty of Pharmaceutical Sciences,
University of Leuven, Belgium

SUMMARY

To study the importance of the thiol function of mercaptoacetyltriglycine (MAG3) for complexing technetium, we investigated a number of derivatives wherein the thiol function of MAG3, is replaced by other metal binding groups such as 3-nitropropionyl, thiophene-2-carbonyl, pyrrole-2-carbonyl, acetoacetyl, hydroxyacetyl, picolyl or aminoacetyl. These groups have the potential to form a bond with Tc via a free electron pair or the loss of a proton.
3-Nitropropionyltriglycine, thiophene-2-carbonyltriglycine and pyrrole-2-carbonyl-triglycine were not able to form a single complex with ^{99m}Tc. Under the experimental conditions, acetoacetyltriglycine forms a rather unstable ^{99m}Tc-complex. On the other hand, the acetoacetyl group possesses sufficient complexing capacity to serve as the fourth coordination site of a tetraligand with a strongly technetium binding thiol and two amides. Hydroxyacetyltriglycine and derivatives of MAG3 in which the thiol group is substituted by an aromatic amine, as in picolyltriglycine, or an aliphatic amine, as in tetraglycine and other tetrapeptides, form complexes with technetium-99m which are stable for several hours.

Key-words: ^{99m}Tc-MAG3, ^{99m}Tc-complexes, synthesis, HPLC, triglycine derivatives

INTRODUCTION

^{99m}Tc-mercaptoacetyltriglycine (^{99m}Tc-MAG3), has become the standard radio-pharmaceutical for radioisotopic kidney studies such as renal imaging, relative renal function studies and determination of renal transit time (1-4). In ^{99m}Tc-MAG3 an oxotechnetium(V) core $[(TcO)^{3+}]$ is bound via three deprotonated amide nitrogen atoms and a deprotonated thiol sulphur atom of the SN_3 tetraligand (5). A thiol group has a high affinity for transition metals such as technetium and it provides a strong binding site in the ligand for the radionuclide.

Correspondence to: A.M. Verbruggen, U.Z. Gasthuisberg, Radiopharmacy, Herestraat 49, B-3000 Leuven, Belgium. Tel: (32) 16 343714. Fax: (32) 16 343759.
E-mail: alfons.verbruggen@uz.kuleuven.ac.be

Received 31 July 2000
Revised 16 August 2000
Accepted 1 October 2000

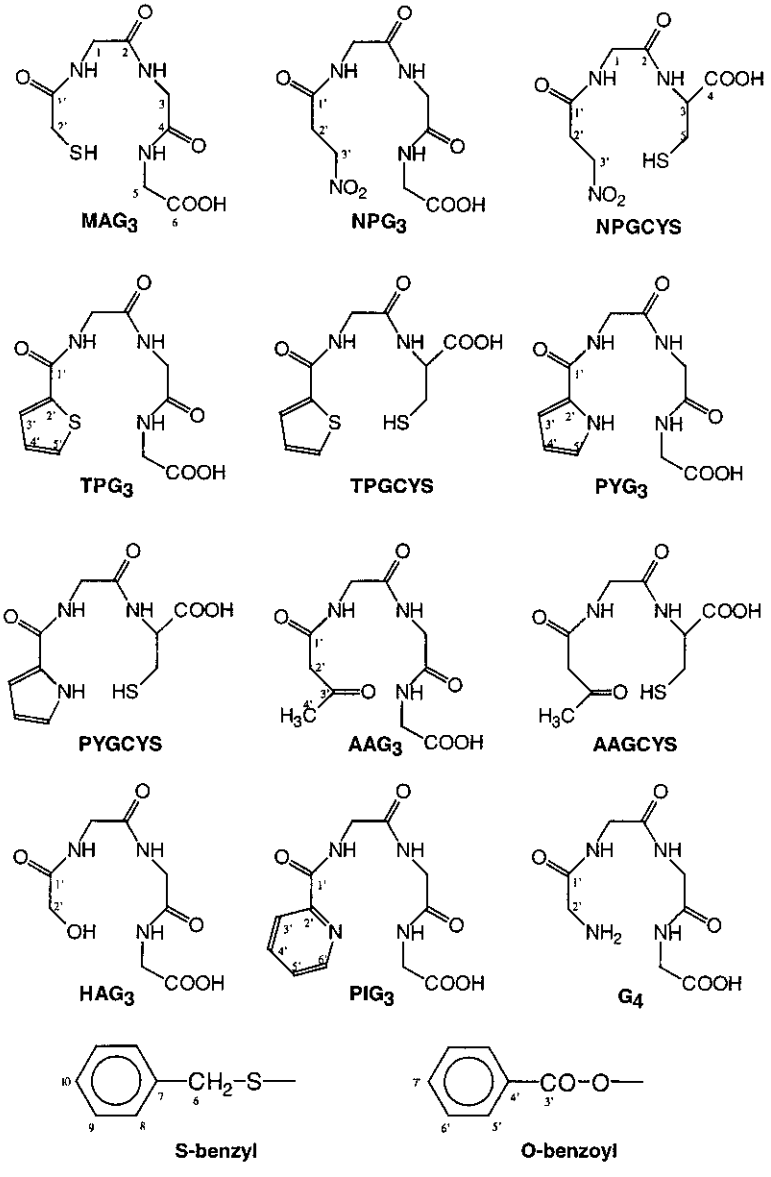

Figure 1. Structure of MAG3 and the newly synthesized ligands: 3-nitropropionyltriglycine (NPG_3), 3-nitropropionylglycylcysteine (NPGCYS), thiophene-2-carbonyltriglycine (TPG_3), thiophene-2-carbonylglycylcysteine (TPGCYS), pyrrole-2-carbonyl-triglycine (PYG_3), pyrrole-2-carbonylglycylcysteine (PYGCYS), acetoacetyltriglycine (AAG_3), acetoacetyl-glycylcysteine (AAGCYS), hydroxyacetyltriglycine (HAG_3), picolyltriglycine (PIG_3) and tetraglycine (G_4)

On the other hand, the thiol group is also the reason for some stability problems of the ligand, due to its sensitivity to oxidation. In this study we have replaced the thiol function of MAG3 by other metal binding groups, in order to find other groups with a higher stability which have sufficient metal binding capacity to form a stable complex with ^{99m}Tc in combination with three amides. This could possibly also lead to new interesting applications in diagnostic nuclear medicine.

Some other functional groups with the potential to form a bond with Tc either via a free electron pair of a donor atom, or by the loss of a proton, are 3-nitropropionyl, thiophene-2-carbonyl, pyrrole-2-carbonyl, acetoacetyl, hydroxyacetyl, picolyl and aminoacetyl (Fig. 1). 3-Nitropropionyl and acetoacetyl groups can be assumed to have this potential because of the relatively strong acidic character of the proton on the α-carbon atom and the other groups contain nitrogen, sulphur or oxygen atoms, known for their ability to bind Tc. Indeed, numerous ligands have been reported in which complex formation with technetium is based on amine nitrogen atoms (tetra-amines, e.g. cyclam (6)), a combination of amines and oximes (exametazime or HM-PAO (7)), a combination of amines and thiols (ethylene dicysteine diethyl ester (ECD) (8-9), ethylene dicysteine (EC) (10-11)) or other combinations of nitrogen and oxygen atoms (e.g. MRP20 (12-13)).

This paper describes the technetium-99m complexing characteristics of some new derivatives of MAG3 in which one of the above mentioned groups is substituted for the sulphydryl group. Also included are derivatives of MAG3 where, in addition to replacement of the sulphydryl group, the terminal diglycine moiety is replaced by a cysteine amino acid. This was done to investigate whether the new functional groups which do not bind technetium in combination with three amides can contribute to the complex formation when a stongly Tc binding thiol function, provided by the cysteinyl moiety, is present.

RESULTS AND DISCUSSION

Mercaptoacetyltriglycine is able to strongly bind technetium, a property which is largely attributed to the high affinity of the thiol for this transition metal and the presence of three amide nitrogen atoms. The affinity of thiol sulphur atoms for metals in labelling peptides and proteins with technetium-99m is taken advantage of by introducing mercapto groups through reduction of the cystine disulphide bridges or via coupling with thiol-containing bifunctional chelating agents (14-16). Amide nitrogen atoms possess less Tc chelating

capacities. In our experience, acetyltetraglycine and other tetra-amide ligands do not form a complex with ^{99m}Tc under the experimental reaction conditions used in this study (results not shown). However, in combination with a thiol, amides serve as useful additional coordination sites of the tetraligand.

An inportant disadvantage of thiols is their marked sensitivity to oxidation and this hampers their synthesis, purification and storage. For this reason, we have synthesized and studied a number of derivatives of MAG3 in which the mercaptoacetyl moiety is replaced by an other group which has, in principle, the capacity to form a bond to Tc.

Synthesis

With the exception of acetoacetyltriglycine and acetoacetylglycyl-L-cysteine, the other not commercially available ligands were synthesized by reaction of the activated acid (in the form of the N-hydroxysuccinimide ester or the acid chloride) of the mercaptoacetyl replacing group with triglycine or glycyl-S-benzylcysteine. Acetoacetyltriglycine and acetoacetylglycyl-L-cysteine were synthesized by activation of commercially available acetoacetylglycine to its N-hydroxysuccinimide ester followed by reaction with diglycine or S-benzylcysteine. Hydroxyacetyltriglycine was synthesized from O-benzoylglycolic acid (17). The O-benzoyl protective group was removed *in situ* just before labelling by incubation of the ligand for 30 min with 0.1M NaOH at room temperature. The ligands were obtained in overall yields that varied from 18% to 90%.

Labelling with ^{99m}Tc and analysis

The reaction mixtures, after labelling of the ligands with ^{99m}Tc, were analysed using a rapid two-strip TLC method to determine the amount of $^{99m}TcO_4^-$ and ^{99m}Tc in colloidal form. A more precise analysis using reversed phase HPLC allowed the differentiation of other radiochemical species.

Complex labelling mixtures were found after labelling nitropropionyltriglycine (NPG_3) with technetium-99m, as well as after direct labelling and after exchange labelling at different pH values. The amounts of colloidal ^{99m}Tc were rather limited (2-5%), but RP-HPLC chromatograms showed the presence of many compounds besides pertechnetate.

Although NPG_3 possesses an acidic proton on the carbon atom adjacent to the nitro group, this ligand was not able to form a single ^{99m}Tc-chelate, even in alkaline medium when deprotonation should proceed very easily. Even in combination with a mercapto group,

provided by the replacement of the terminal glycylglycine by a cysteinyl moiety in nitropropionylglycylcysteine (NPGCYS) the ligand was not able to form a complex with ^{99m}Tc.

Also thiophene-2-carbonyltriglycine (TPG_3) and pyrrole-2-carbonyltriglycine (PYG_3) failed to form a single complex with ^{99m}Tc. This is most probably due to the fact that the free electron pairs of respectively the sulphur and nitrogen atom of the heterocyclic rings are involved in the formation of the low energetic electron orbital of the ring, resulting in the aromatic character of the compound. The additional presence of a thiol in the tetraligand as in thiophene-2-carbonylglycylcysteine (TPGCYS) and pyrrole-2-carbonylglycylcysteine (PYGCYS) improved the chelating capacity, but besides the predominantly formed expected two isomers, important amounts of pertechnetate, ^{99m}Tc-tartrate and other impurities could be detected after exchange labelling of these ligands, indicating that the complexes formed are rather weak.

Acetoacetyltriglycine (AAG_3) could be labelled efficiently with technetium-99m using a direct labelling method. TLC-analysis showed that the amount of remaining pertechnetate and colloidal ^{99m}Tc formed during the direct labelling reaction was negligible (Table 1).

Table 1. TLC-analysis of the labelling reaction mixtures after direct labelling of AAG_3, HAG_3, PIG_3 and G_4 with ^{99m}Tc at pH 12

ligand	% colloidal ^{99m}Tc	% ^{99m}Tc-TcO_4^-
AAG_3	4.2	0.1
HAG_3	0.6	0.1
PIG_3	1.2	0.2
G_4	0.8	0.1

Reversed phase HPLC-analysis of ^{99m}Tc-labelled AAG_3, however, allowed us to differentiate other radiochemical species besides the main peak which was formed for about 55% (Fig. 2). The nature of this complex is not yet unequivocally confirmed but a reasonable explanation for the Tc-binding capacity of the acetoacetyl group is its keto-enol tautomery, resulting in several tautomeric forms. The tautomeric form with the enol on the carbon atom which also bears the methyl group can be considered as the most likely candidate for complex formation. Therefore, the structure of the complex formed upon labelling in alkaline medium and eluting from the HPLC column as the main peak probably consists of an oxotechnetium core bound to the three deprotonated amide

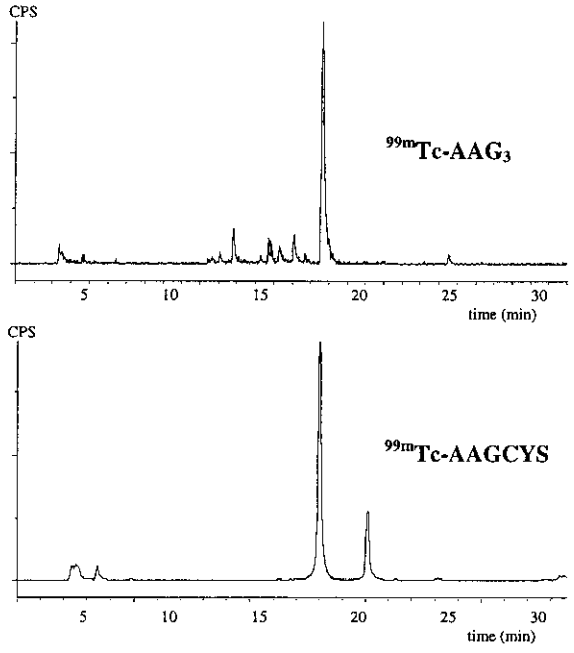

Figure 2. HPLC-chromatograms of the labelling reaction mixtures after direct labelling of AAG_3 at pH 12 and exchange labelling of AAGCYS at pH 10 with ^{99m}Tc

nitrogen atoms and the deprotonated enol. Nevertheless, this complex, which has two five-membered and one six-membered rings, as compared to three five-membered rings in the case of ^{99m}Tc-MAG3, seems to be rather weak as suggested by the various side-products formed. This indicates that other complexes with different configurations are possible. One of the possibilities would be a complex in which Tc is bound to three deprotonated amide nitrogen atoms and the terminal carboxylate.

Replacement of the terminal diglycyl moiety by a cysteine residue to introduce a mercapto group in the ligand (acetoacetylglycylcysteine, AAGCYS) resulted in clearly more efficient labelling and mainly two radiochemical species were formed in a yield of more than 90% (Fig. 2). Here, an oxotechnetium core is bound to the deprotonated thiol, the two deprotonated amides and the deprotonated enol. The two species formed are probably two diastereomers with the oxotechnetium core oriented respectively *syn* and *anti* with respect to the carboxyl group of the cysteinyl moiety. This indicates that when the tetraligand contains a group with strong Tc-binding properties, such as a thiol group, the deprotonated acetoacetyl moiety is capable of being directed in one tautomeric form so that the complex

with ^{99m}Tc is formed in one way only. When, however, the strong complexing group is absent as in AAG_3, Tc is bound in different ways by the combination of five potential Tc binding atoms, i.e. an enol, three amide nitrogen atoms and a carboxylate.

Hydroxyacetyltriglycine (HAG_3), obtained from O-benzoylhydroxyacetyltriglycine by hydrolysis of the benzoyl protective group was efficiently labelled using a direct labelling method at pH 12, resulting in the formation of mainly one radiochemical species with a radiochemical purity over 95 %. No important amounts of colloidal ^{99m}Tc were formed and no remaining pertechnetate was detected (Table 1). Lowering the pH to 8 after the labelling did not affect the nature or purity of the formed ^{99m}Tc-complex, as a radiochemical purity of more than 95 % could be maintained for at least four hours. On the basis of the fact that the retention time of ^{99m}Tc-HAG_3 on RP-HPLC is nearly identical to that of ^{99m}Tc-MAG3 when analysed under the same conditions, it is assumed that the structure of both complexes are similar, with the evident understanding that the deprotonated thiol function of ^{99m}Tc-MAG3 is replaced by a deprotonated hydroxyl group in ^{99m}Tc-HAG_3.

Replacement of the thiol group of MAG3 by an aromatic amine, as in picolyltriglycine (PIG_3), or by an aliphatic amine, as in tetraglycine (G_4), resulted in ligands which were able to bind ^{99m}Tc efficiently by direct labelling at a pH ≥11.5 without residual pertechnetate or formation of substantial amounts of colloidal ^{99m}Tc (Table 1). Reversed phase HPLC chromatograms showed one single radiochemical species with a radiochemical purity over 95 % (Fig. 3) for both ^{99m}Tc-PIG_3 and ^{99m}Tc-G_4. In these complexes, it is very unlikely that a proton is withdrawn from the basic amine. In PIG_3 there is even no hydrogen atom bound to the aromatic amine. Also Su and co-workers described complexes with dimethylamine containing ligands (18). If these amine-containing tetraligands do form Tc(V)oxo-complexes in a similar way as MAG3, it can be supposed that this complex formation between the oxotechnetium core and the ligand occurs through the three deprotonated amides and the free electron pair of the amine function. This assumption is supported by the fact that when no electron pair is available on the amine, as is the case for PYG_3, the ligand is not able to complex technetium-99m.

EXPERIMENTAL

Tetraglycine (Fluka, Bornem, Belgium) was commercially available.

Glycyl-S-benzyl-L-cysteine

yield 20.90 g (78%) of a white powder; mp. 172-174 °C (dec); ^{1}H-NMR, (D_2O) δ 2.6 (2H, d, S-CH_2-CH); 3.6-3.8 (4H, m, S-CH_2-Ar, NHCH_2CO); 4.5 (1H, m, NH-CH(COOH)-CH_2); 7.3 ppm (5H, s, Ar).

3-Nitropropionylglycyl-S-benzyl-L-cysteine

yield 0.66 g (18%) of a white powder; mp. 115-116 °C; ^{1}H-NMR, (DMSO-d_6) δ 2.7 (2H, d, S-CH_2-CH); 3.5 (2H, t, CH_2-$\underline{CH_2}$-CO); 3.7 (2H, s, S-CH_2-Ar); 3.8 (2H, d, NH-$\underline{CH_2}$-CO); 4.3 (2H, t, NO_2-CH_2);.4.5 (1H, m, NH-CH(COOH)-CH_2); 7.3 (5H, s, Ar); 8.2-8.3 ppm (2H, m, 2xNH).

^{13}C-NMR, (DMSO-d_6) δ 31.4 ($C_{2'}$), 32.5 (C_5), 35.5 (C_6), 41.8 (C_1), 51.9 (C_3), 70.7 ($C_{3'}$), 127.0 (C_{10}), 128.5 (C_8), 129.1 (C_9), 138.3 (C_7), 168.9 (C_2), 169.1 ($C_{1'}$), ppm 172.1 (C_4).

Thiophene-2-carbonyltriglycine (TPG_3)

yield 17.63g (88.1%) of a white powder; mp. 236-237 °C (dec); ^{1}H-NMR, (DMSO-d_6) δ 3.7 (4H, d, 2x NH-$\underline{CH_2}$-CO); 3.9 (2H, d, NH-$\underline{CH_2}$-COOH); 7.2 7.7-7.8 (3H, m, CH-CH=CH-S); 8.1-8.2 (2H, 2x t, 2x CO-$\underline{NH}$-CH_2); 8.8 ppm (1H, s, S-C-CO-NH).

^{13}C-NMR, (DMSO-d_6) δ 40.8 (C_5), 42.0 (C_1), 42.6 (C_3), 128.1 ($C_{3'}$), 128.7 ($C_{4'}$), 131.1($C_{5'}$), 139.6 ($C_{2'}$), 161.7 ($C_{1'}$), 169.4 (C_2 and C_4), 171.2 ppm (C_6).

Thiophene-2-carbonylglycyl-S-benzyl-L-cysteine (TPGCYS)

yield 3.17g (42.0%) of a white powder; mp. 142-143 °C (dec); ^{1}H-NMR, (DMSO-d_6) δ 2.7 (2H, d, S-$\underline{CH_2}$-CH); 3.7 (2H, s, S-CH_2-Ar); 3.8 (2H, s, NH-$\underline{CH_2}$-CO); 4.5 (1H, m, NH-$\underline{CH}$(COOH)-CH_2); 7.3 (5H, s, Ar); 7.2-7.8 (3H, t m, CH-CH=CH-S); 8.2 (1H, d, CO-$\underline{NH}$-CH); 8.8 ppm (1H, s, S-C-CO-NH).

^{13}C-NMR, (DMSO-d_6) δ 32.5 (C_5), 35.6 (C_6), 42.1 (C_1), 52.0 (C_3), 127.0 (C_{10}), 128.0 ($C_{3'}$), 128.5 (C_8 and $C_{4'}$), 129.0 (C_9), 131.0 ($C_{5'}$), 138.3 (C_7), 139.7 ($C_{2'}$), 161.6 ($C_{1'}$), 169.1 (C_2), 172.1 ppm (C_4).

Pyrrole-2-carbonyltriglycine (PYG_3)

yield 1.44g (39.5%) of a white powder; mp. 229-230.5 °C; ^{1}H-NMR, (DMSO-d_6) δ 3.6-3.9 (6H, s, 3x NH-$\underline{CH_2}$-CO); 6.7-6.9 (4H, 2x s, NH-CH=CH-CH); 8.1-8.4 ppm (3H, s, 3x CO-$\underline{NH}$-CH_2).

^{13}C-NMR, (DMSO-d_6) δ 40.8 (C_5), 42.0 (C_1), 42.4 (C_3), 108.9 ($C_{4'}$), 110.7 ($C_{3'}$), 121.8($C_{5'}$), 126.0 ($C_{2'}$), 161.3 ($C_{1'}$), 169.5 (C_2 and C_4), 171.3 ppm (C_6).

Melting points were determined using open capillaries immersed in a IA9100 digital melting point apparatus (Electrothermal, Southend-on-Sea, U.K.) and were not corrected.

The structure of the synthesized ligands was confirmed by ^{1}H nuclear magnetic resonance (NMR) spectroscopy on a Jeol FX 90Q (Jeol, Tokyo, Japan) spectrometer and ^{13}C-NMR (numbering see Fig. 1) on a Gemini 200 spectrometer (Varian, Palo Alto, California). Chemical shifts are reported in δ units (ppm) relative to TMS. The splitting patterns are denoted as follows: s (singlet); d (doublet); t (triplet); q (quadruplet), m (multiplet). Carbon resonances are assigned mainly by mutual comparison between the different compounds. The final products were dried in a vacuum desiccator over phosphorus pentoxide.

HPLC analyses were performed using a 250 mm x 4.6 mm column filled with Hypersil ODS 5μm and eluted at a flow rate of 1 mL/min with gradient mixtures of phosphate buffer 0.025M pH 5.85 and ethanol as described by Bormans and co-workers (19)

Synthesis of the tetraligands

3-Nitropropionyltriglycine (NPG_3)

yield 1.17 g (20.1%) of a white powder; mp. 192-194 °C (dec); ^{1}H-NMR, ($NaOD/D_2O$) δ 3.5 (2H, t, CH_2-$\underline{CH_2}$-CO); 3.7-4.1 (6H, s, 3xNH-CH_2-CO); 4.3 ppm (2H, t, NO_2-CH_2).

^{13}C-NMR, (DMSO-d_6) δ 31.5 ($C_{2'}$), 40.7 (C_5), 41.9 (C_1), 42.3 (C_3), 70.7 ($C_{3'}$), 169.3 ($C_{1'}$, C_2 and C_4), 171.3 ppm (C_6).

3-Nitropropionylglycyl-S-benzyl-L-cysteine (NPGCYS)

N-Carbobenzyloxyglycine-N'-hydroxysuccinimide ester

yield 44.55 g (97%) of a white powder; mp. 79-81 °C; ^{1}H-NMR, ($CDCl_3$) δ 2.8 (4H, s, CH_2-CH_2); 4.3 (2H, d, CH_2CO); 5.1 (2H, s, CH_2OCO); 5.5 (1H, t, NH); 7.3 ppm (5H, s, Ar).

N-Carbobenzyloxyglycyl-S-benzyl-L-cysteine

yield 42.21 g (75%) of a white powder; mp. 150-152 °C; ^{1}H-NMR, (DMSO-d_6) δ 2.7 (2H, d, S-CH_2-CH); 3.7 (2H, s, S-CH_2-Ar); 3.8 (2H, d, $NHCH_2CO$); 4.5 (1H, m, NH-CH(COOH)-CH_2); 5.1 (2H, s, CH_2OCO); 7.3 (10H, s, 2xAr); 7.5 (1H, t, NHCOO); 8.3 ppm (1H, d, NH).

Pyrrole-2-carbonylglycyl-S-benzyl-L-cysteine (PYGCYS)

yield 3.05 g (64%) of a white powder; mp. 123-126 °C (dec); ^{1}H-NMR, (DMSO-d_6) δ 2.7 (2H, d, S-$\underline{CH_2}$-CH); 3.7 (2H, s, S-CH_2-Ar); 3.8 (2H, s, NH-$\underline{CH_2}$-CO); 4.5 (1H, m, NH-CH(COOH)-CH_2); 6.7-6.9 (4H, 2x s, NH-CH=CH-CH); 7.3 (5H, s, Ar); 8.1-8.3 ppm (2H, m, 2x CO-NH-CH_2).

^{13}C-NMR, (DMSO-d_6) δ 32.9 (C_5), 35.6 (C_6), 41.9 (C_1), 52.3 (C_3), 108.8 ($C_{4'}$), 110.6 ($C_{3'}$), 121.7 ($C_{5'}$), 126.0 ($C_{2'}$), 126.9 (C_{10}), 128.5 (C_8), 129.0 (C_9), 138.4 (C_7), 161.0 ($C_{1'}$), 169.4 (C_2), 172.4 ppm (C_4).

Acetoacetyltriglycine (AAG_3)

Acetoacetylglycine N-hydroxysuccinimide ester

yield 21.00 g (82%) of an off-white powder; mp. 80-82 °C; ^{1}H-NMR, (DMSO-d_6) δ 2.2 (3H, s, CH_3); 2.8 (4H, s, CH_2-CH_2); 3.4 (2H, s, CO-CH_2-CO); 4.3 (2H, d, NH-$\underline{CH_2}$-CO); 8.7 ppm (1H, t, NH).

Acetoacetyltriglycine

yield 0.87g (21.3%) of the title compound in the form of white needles; mp. 195-197 °C (dec); ^{1}H-NMR, (DMSO-d_6) δ 2.2 (3H, s, CH_3); 3.4 (2H, s, CO-CH_2-CO); 3.6-3.8 (6H, d, 3xNH-$\underline{CH_2}$-CO); 8.1-8.3 ppm (3H, m, 3xNH).

^{13}C-NMR, (DMSO-d_6) δ 30.1 ($C_{4'}$), 40.7 (C_5), 41.9 (C_1), 42.3 (C_3), 51.2 ($C_{2'}$), 166.8, 169.2, and 169.3 ($C_{1'}$, C_2 and C_4); 171.3 (C_6); 204.3 ppm ($C_{3'}$).

Acetoacetylglycyl-S-benzyl-L-cysteine (AAGCYS)

yield 11.58 g (82%); mp. 121-122 °C; ^{1}H-NMR, (DMSO-d_6) δ 2.2 (3H, s, CH_3); 2.7 (2H, d, S-CH_2-CH); 3.4 (2H, s, CO-CH_2-CO); 3.7 (2H, s, S-CH_2-Ar); 3.8 (2H, s, NH-CH_2-CO); 4.5 (1H, m, NH-$\underline{CH}$(COOH)-CH_2); 7.3 (5H, s, Ar); 8.2-8.3 ppm (2H, m, 2xNH).

^{13}C-NMR, (DMSO-d_6) δ 30.1 ($C_{4'}$), 32.5 (C_5), 35.5 (C_6), 41.9 (C_1), 51.2 ($C_{2'}$), 52.0 (C_3), 127.0 (C_{10}), 128.5 (C_8), 129.0 (C_9), 138.3 (C_7), 166.6 and 168.9 ($C_{1'}$ and C_2), 172.1 (C_4); 204.7 ppm ($C_{3'}$).

O-benzoylglycoloyltriglycine (HAG_3)

yield 4.79 g (91%) of a white powder; mp. 192-195 °C (dec.); ^{1}H-NMR, (DMSO-d_6) δ 3.6-3.9 (6H, m, 3xNH-CH_2-CO); 4.8 (2H, s, CH_2-O); 7.5-8.1 (5H, m, Ar); 8.1-8.4 ppm (3H, 3x t, 3x NH-CO).

^{13}C-NMR, (DMSO-d_6) 40.4 (C_5), 41.9 (C_1), 42.4 (C_3), 63.0 ($C_{2'}$), 128.8 ($C_{6'}$), 129.3 ($C_{5'}$), 129.6 ($C_{4'}$), and 133.6 ($C_{7'}$), 167.0 ($C_{3'}$), 168.2 (C_2 and C_4), 171.1 (C_6), 172.5 ppm ($C_{1'}$).

Picolyltriglycine (PIG_3)

Yield 2.51g (57%); mp. 201-203 °C. ^{1}H-NMR, (NaOD/D_2O) δ 3.7-4.1 (6H, 3xNH-CH_2-CO); 7.5-8.7 ppm (4H, m, Ar).

^{13}C-NMR, (DMSO-d_6) δ 40.8 (C_5), 42.1 (C_1), 42.6 (C_3), 122.1 ($C_{5'}$), 126.9 ($C_{3'}$), 138.0 ($C_{4'}$), 148.7 ($C_{6'}$), 149.7 ($C_{2'}$), 164.3 ($C_{1'}$) 169.2 and 169.4 (C_2 and C_4), 171.4 ppm (C_6).

Labelling with ^{99m}Tc

Direct labelling procedure

In a 10-mL labelling vial 1 mg of the ligand was dissolved in 0.5 mL of 0.5 M phosphate buffer pH 12. A solution of 100 μg of $SnCl_2.2H_2O$ in 25 μL of HCl 0.05N was added, immediately followed by the addition of 1 mL of generator eluate (Ultratechnekow™ generator, Petten, Mallinckrodt Medical, Holland) containing 370-740 MBq ^{99m}Tc in the form of sodium pertechnetate.

Exchange labelling procedure

In a 10-mL labelling vial containing 1 mg of the ligand were added consecutively 0.5 mL of phosphate buffer 0.5M of the desired pH, 10 mg of sodium potassium tartrate tetrahydrate dissolved in 0.25 mL of water, 100 μg of $SnCl_2.2H_2O$ dissolved in 25 μL of HCl 0.05N and finally 1 mL of generator eluate containing 370-740 MBq ^{99m}Tc in the form of sodium 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 (Gelman Sciences, Ann Arbor, Michigan).

Analysis of ^{99m}Tc-labelled preparations

Paper chromatography

2 μL of the reaction mixtures after labelling were applied on each of two chromatography strips (13 cm x 1 cm) of Whatman 4 Chr paper (Whatman International Ltd., Maidstone, U.K.) at the application point 2 cm from the bottom. The strips were eluted over a distance of 10 cm with, respectively, acetone or normal saline. After drying, the strips were cut 2 cm above the application point and the radioactivity on each part was measured with a 2-in. NaI(Tl) scintillation detector, connected to a single channel analyser and scaler. Results were corrected for background radioactivity. The new ^{99m}Tc-complexes migrated with the solvent front when normal saline was used and stayed at the application point during elution with acetone.

The percentage $^{99m}TcO_4^-$ (R_f = 1 in both systems) and ^{99m}Tc in colloidal form (R_f = 0 in both systems) was determined in each case.

CONCLUSIONS

From this study it can be concluded that a hydroxyl group in hydroxytriamides (ON_3) and an aromatic or aliphatic amine in aminetriamides (N_4) in the correct configuration are interesting alternatives for the oxidation-sensitive thiol function in mercaptoacyltripeptide ligands (SN_3) in terms of their ability to form a bond with Tc. By analogy with MAG3, hydroxyacetyltriglycine probably binds to the oxotechnetium core via three deprotonated amide nitrogen atoms and the deprotonated hydroxyl group. To bind Tc, the aminetriamides or tetrapeptides use probably three deprotonated amides and in addition the free electron pair of the amine to form the fourth bond .

The acetoacetyl moiety is only partially satisfactory as a replacement for the mercaptoacetylgroup of mercaptotriamides, probably due to the distorted geometry of the complex as the oxygen atom of the aceto moiety is one atom further away. Consequently, the chelating capacity of this species is insufficient to direct the ligand to form a single complex. On the other hand, the acetoacetyl group possesses sufficient complexing capacity to serve as the fourth coordination site of a tetraligand with a strongly Tc binding thiol and two amides.

References

1 Abdel-Dayem H. M., Sadek S., al-Bahar R., Sabha M. and el-Sayed M. *Nucl. Med. Commun.* **10**: 99-107 (1989)

2 Fritzberg A. R., Kasina S., Eshima D. and Johnson D. L. *J. Nucl. Med.* **27**: 111-116 (1986)

3 Lee H.-B. and Blaufox M. D. *J. Nucl. Med.* **30**: 666-671 (1989)

4 Russell C. D., Thorstad B., Yester M. V., Stutzman M., Baker T. and Dubovsky E. V. *J. Nucl. Med.* **29**: 1189-1193 (1988)

5 Nosco D. L. and Manning R. G. *J. Nucl. Med.* **27**: 939 (1986)

6 Troutner D. E., Simon J., Ketring A. R., Volkert W. A., and Holmes R. A. *J. Nucl. Med.* 21: 443-448 (1980)

7 Neirinckx R. D., Canning L. R., Piper I. M., Nowotnik D. P., Pickett R. D., Holmes R. A., Volkert W. A., Forster A. M., Weisner P. S., Marriott J. A. and Chaplin S. B. *J. Nucl. Med.* 28: 191-202 (1987)

8 Cheesman E. H., Blanchette M. A., Calabrese J. C., Ganey M. V., Maheu L. J., Morgan R. A., Walovitch R. C., Watson A. D., Williams S. J. and Miller S. J. *J. Labelled Compd. Radiopharm.* **26**: 421-423 (1989)

9 Walovitch R. C., Hill T. C., Garrity S. T., Cheesman E. H., Burgess B. A., O'Leary D. H., Watson A. D., Ganey M. V., Morgan R. A. and Williams S. J. *J. Nucl. Med.* **30**: 1892-1901 (1989)

10 Van Nerom C. G., Bormans G. M., De Roo M. J. and Verbruggen A. M. *Eur. J. Nucl. Med.* **20**: 738-746 (1993)

11 Verbruggen A. M., Nosco D. L., Van Nerom C. G., Bormans G. M., Adriaens P. J. and De Roo M. J. *J. Nucl. Med.* **33**: 551-557 (1992)

12 Morgan G. F., Abram U., Evrard G., Durant F., Deblaton M., Clemens P., Van Den Broeck P. and Thornback J. R. *J. Chem. Soc. Chem. Commun.* 1772-1773 (1990)

13 Morgan G. F., Deblaton M., Clemens P., Van Den Broeck P., Bossuyt A. and Thornback J. R. *J. Nucl. Med.* **32**: 500-505 (1991)

14 Delmon-Moingeon L. I., Mahmood A., Davison A. and Jones A. G. *J. Nucl. Biol. Med.* **35**: 47-59 (1991)

15 Verbeke K. A., Vanbilloen H. P., De Roo M. J. and Verbruggen A. M. *Eur. J. Nucl. Med.* **20**: 473-482 (1993)

16 Verbruggen A. M. *Eur. J. Nucl. Med.* **17**: 346-364 (1990)

17 Ringshaw D. J. and Smith H. J. *J. Am. Chem. Soc.* **86**: 1559-1562 (1964)

18 Su Z.-F., Zhang X., Ballinger J. R., Rauth A. M., Pollak A. and Thornback J. R. *Bioconj. Chem.* **10**: 897-904 (1999)

19 Bormans G., Cleynhens B., Hoogmartens M., De Roo M. and Verbruggen A. *Nucl. Med. Biol.* **19**: 375-388 (1992)

