Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **42**, 553-565 (1999)

Preparation and biological evaluation of technetium-99m-L,L-propylenedicysteine

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SUMMARY

In this study we have investigated the influence of replacement of the ethylene moiety in ^{99m}Tc-L,L-ethylenedicysteine (^{99m}Tc-L,L-EC) by a propylene moiety on the physical and biological properties.
S,S'-dibenzyl-N,N'-1,3-propylenediylbis-L-cysteine was synthesised by reaction of S-benzyl-L-cysteine with 1,3-dichloropropane. The thiol groups were deprotected with sodium in liquid ammonia. The resulting L,L-propylenedicysteine (L,L-PC) was directly labelled with ^{99m}Tc at pH 12. Whereas labelling of L,L-EC results in a single radiochemical species, labelling of L,L-PC yields two ^{99m}Tc-complexes in a 3/1 ratio, probably two isomers with the central propylene carbon atom syn or anti to the oxotechnetium core. These isomers are stable and do not interconvert at neutral pH.
In mice, both isomers of ^{99m}Tc-L,L-PC showed a slower urinary excretion and a higher hepatobiliary uptake than ^{99m}Tc-L,L-EC. Furthermore, ^{99m}Tc-L,L-PC showed some renal retention. In a baboon, both conformers of ^{99m}Tc-L,L-PC were excreted rapidly in the urine without visualisation of liver or intestines, but their plasma clearance was only 33.6 % of hippuran clearance for isomer A and 25.6 % for isomer B, as compared to 75 % in the case of ^{99m}Tc-L,L-EC.
The results indicate that, besides the oxotechnetium-glycine sequence, also the length and orientation of the alkylene bridge between the two amines are important for the interaction of this type of compounds with the renal tubular transport proteins.

Key-words: ^{99m}Tc-L,L-ethylenedicysteine, ^{99m}Tc-L,L-propylenedicysteine, renal imaging agents, tubular transport, renal clearance

CCC 0362–4803/99/060553–13$17.50

Received 9 October 1998
Revised 20 October 1998
Accepted 21 December 1998

INTRODUCTION

Ortho-iodohippurate (OIH, hippuran) labelled with radioiodine was developed by Tubis and co-workers in 1960 as a radiolabelled derivative of *p*-aminohippuric acid (PAH) for the radioisotopic evaluation of renal function (1) and has for a long time been the standard radiopharmaceutical for measurement of effective renal plasma flow (ERPF). Since the seventies, an intensive search was started to substitute this radioiodinated renal tracer agent by a technetium-99m labelled compound that approaches the biological behaviour of OIH. In most Nuclear Medicine departments in Europe and the US, technetium-99m mercaptoacetyltriglycine (^{99m}Tc-MAG$_3$), developed by Fritzberg and co-workers (2), has now become the radiopharmaceutical of choice for the evaluation of transplant kidney, diagnosis of tubular necrosis and radioisotopic study of tubular function. However, ^{99m}Tc-MAG$_3$ is not yet the ideal replacement for OIH, and improvements are still possible. The plasma protein binding of ^{99m}Tc-MAG$_3$ is high, and its plasma clearance in humans is not higher than about 60 - 65 % of the OIH value (3,4). Therefore, determination of ERPF is not always accurate using ^{99m}Tc-MAG$_3$, although formulae have been proposed to calculate ERPF from the acquired data (4,5).

A second ^{99m}Tc-labelled compound with biological characteristics even more closely related to OIH (plasma clearance of about 75 % of the OIH value) has been developed, namely the ^{99m}Tc-complex of L,L-ethylenedicysteine (^{99m}Tc-L,L-EC) (6,7). Both ^{99m}Tc-MAG$_3$ and ^{99m}Tc-L,L-EC contain in their structure an oxotechnetium-glycine sequence (Figure 1). It is assumed that this structural moiety mimics the carbonylglycine sequence in the side-chain of hippuran, which is generally believed to be essential for an efficient fit with the tubular transport proteins (8).

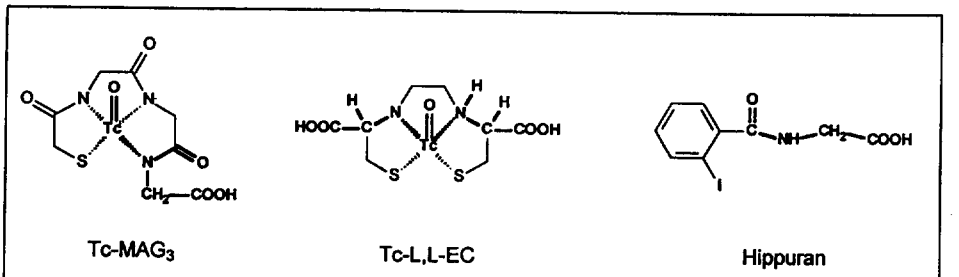
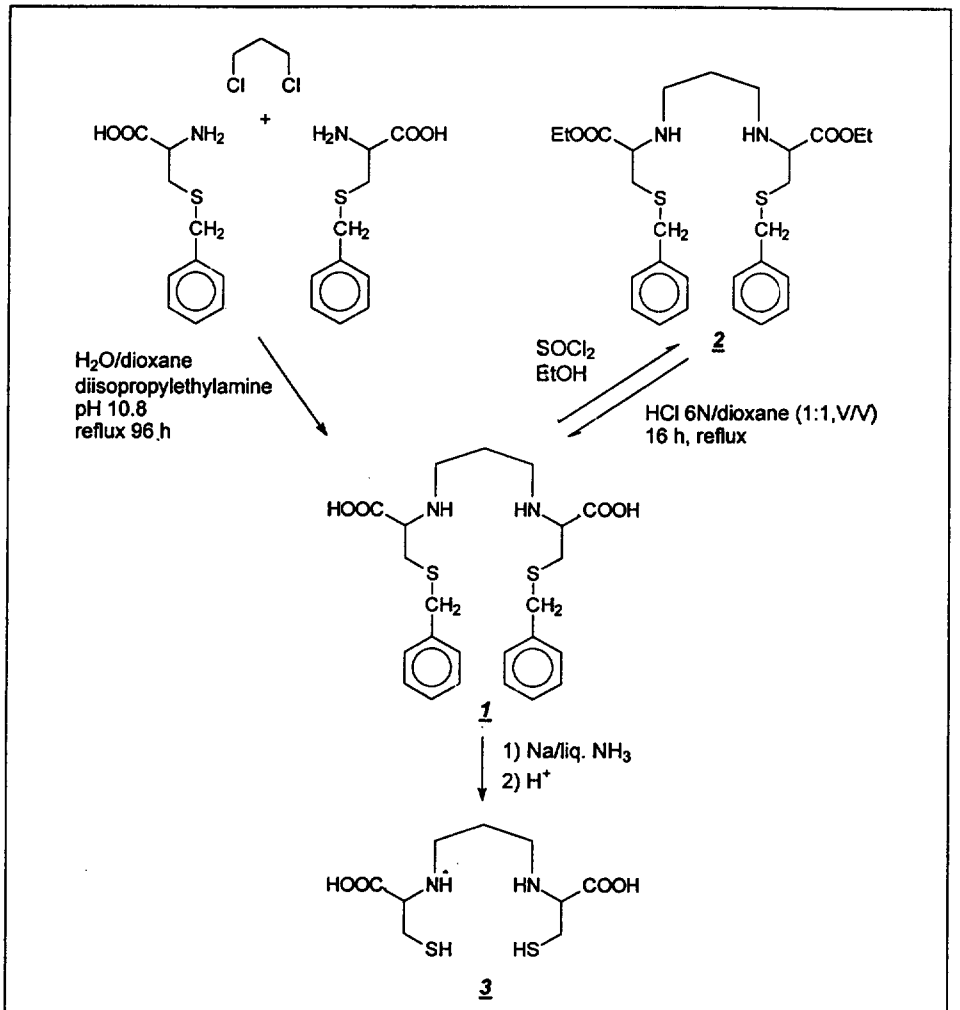


Figure 1. Structure of Tc-MAG$_3$, Tc-L,L-EC and hippuran

We have now studied a derivative of ^{99m}Tc-L,L-EC in which the ethylene moiety has been replaced by a propylene bridge to obtain a better understanding of the structural requirements for optimal renal handling of this type of compounds.

RESULTS AND DISCUSSION



Scheme 1. Synthesis of L,L-propylenedicysteine (3).

Synthesis

For the synthesis of L,L-propylenedicysteine (L,L-PC) (3) we used a slight modification of a method used by Blondeau et al. (9) for the synthesis of L,L-ethylenedicysteine, as shown in Scheme 1. The N-alkylation of S-benzyl-L-cysteine with 1,3-dichloropropane was performed under homogeneous conditions in dioxane/water. The pH was maintained at 10.8. In order to facilitate purification, both carboxylic groups were esterified. Because alkaline hydrolysis of the ester groups led mainly to intramolecular amide formation, both esters were removed under acidic conditions by heating in a 1:1 mixture

of 6N hydrochloric acid and dioxane. S,S'-dibenzyl-N,N'-1,3-propylenediylbis-L-cysteine was isolated in a 13.4 % overall yield. The S-benzyl groups were removed with sodium in liquid ammonia. The reaction product after debenzylation, containing L,L-PC (3) in the presence of sodium chloride and ammonium chloride as impurities, was used without further purification for the labelling experiments.

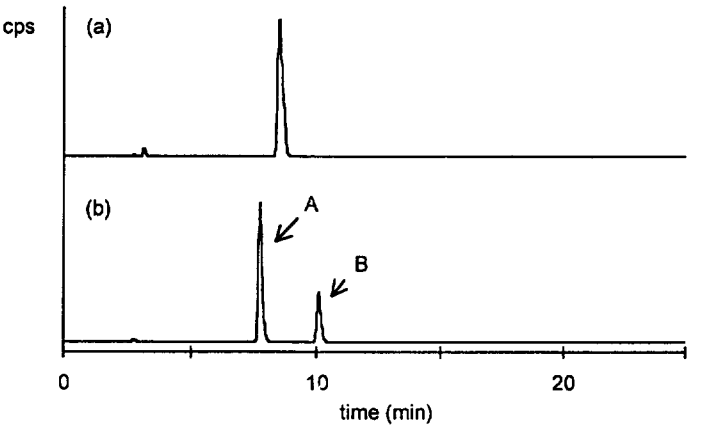


Figure 2. RP-HPLC chromatograms of ^{99m}Tc-L,L-EC (a) and ^{99m}Tc-L,L-PC (b)

Labelling with ^{99m}Tc and analysis

L,L-propylenedicysteine can be labelled efficiently with ^{99m}Tc via direct labelling at pH 12 or exchange labelling in the presence of a weak chelating agent at different pH values (pH 4 – 12). TLC analysis revealed the presence of only minor amounts of pertechnetate and colloidal ^{99m}Tc. The radiochemical purity was found to be higher than 95 % up to 6 hours after labelling.

Reversed phase HPLC. RP-HPLC analysis showed that labelling of L,L-PC (3) with ^{99m}Tc yielded two ^{99m}Tc-complexes (Figure 2). It can be assumed that these two peaks are two diastereomers, in which the oxotechnetium core is oriented in the same (syn) or opposite (anti) direction with respect to the central carbon atom of the propylene bridge of L,L-PC (Figure 3). Similar isomerism has been proposed for Tc(V)O complexes with propylenediaminedioxime tetraligands with certain substituents (-F, -CN, -OMe, -COOMe) on the central propylene carbon atom (10). To exclude the possibility that the isomers were a result of racemisation during the synthesis of L,L-PC, the optical rotation

of L,L-PC (*3*) was determined. The fact that a rotation significantly different from zero was found is an indication that the ligand was not completely racemised, although a partial racemisation cannot be excluded in this way. However, racemisation at one or both chiral carbon atoms of L,L-PC would result in the presence of three isomers in the ligand, namely with an L,L-; D,D- and D,L-configuration. After labelling, this would result in more than two ^{99m}Tc-complexes (theoretically, 8 isomers of which 6 diastereomers are possible). The presence of just two peaks in the RP-HPLC chromatograms is therefore another indication that no racemisation of the ligand occurred during synthesis. The relative amount of isomer A formed during exchange labelling was almost independent on the pH of the reaction mixture and varied between 80 and 85 %. On the other hand, direct labelling at pH 12 yielded 72% of isomer A. Re-analysis of the RP-HPLC isolated peaks of the isomers of ^{99m}Tc-L,L-PC, stored at neutral pH, did not show any interconversion up to 4 hours after isolation.

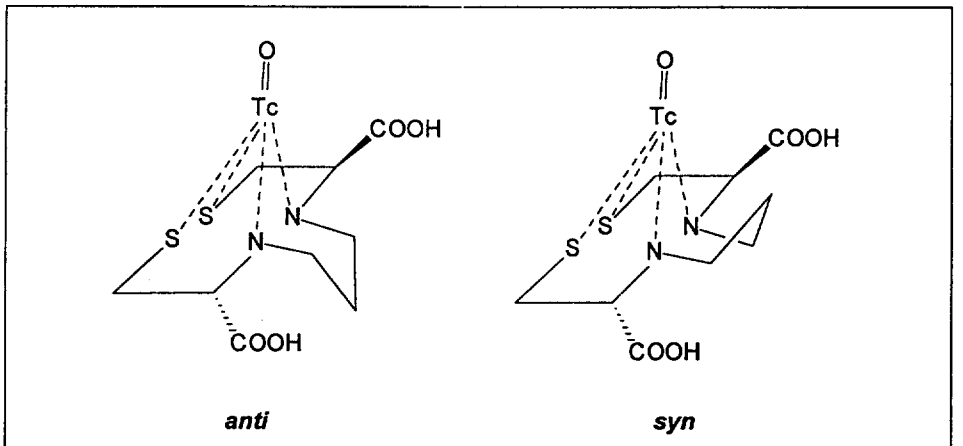


Figure 3. Proposed structures of ^{99m}Tc-L,L-propylenedicysteine (^{99m}Tc-3)

Biodistribution studies

Evaluation in mice. The results of the biodistribution study of the two isomers of ^{99m}Tc-L,L-PC in mice at 10 and 30 min p.i. are compared with the values obtained for ^{131}I labelled hippuran and ^{99m}Tc-L,L-EC (6) in Table 1. Both isomers of ^{99m}Tc-L,L-PC are cleared from the blood mainly via the renal and to a lower extent by the hepatobiliary system at a significantly lower rate than ^{99m}Tc-L,L-EC and ^{131}I-hippuran. At 10 and 30 min p.i., both isomers demonstrated a significantly lower urinary excretion than ^{99m}Tc-L,L-EC and ^{131}I-hippuran and a significantly higher retention in the kidneys. Only for

isomer B of ^{99m}Tc-L,L-PC, the kidney retention at 10 min p.i. was not significantly higher than that of ^{99m}Tc-L,L-EC, but it was still increased relative to that of ^{131}I-hippuran. Also the difference in urinary excretion and kidney retention between the two isomers of ^{99m}Tc-L,L-PC was significant, characterised by a higher retention in the kidneys and a lower urinary excretion for isomer A. The hepatobiliary uptake of both isomers of ^{99m}Tc-L,L-PC was significantly higher than that of ^{99m}Tc-L,L-EC and ^{131}I-hippuran while there was no significant difference between the two isomers of ^{99m}Tc-L,L-PC.

Table 1. Biodistribution in mice (n = 5) of ^{131}I-Hippuran (OIH), ^{99m}Tc-L,L-EC and the two isomers of ^{99m}Tc-L,L-PC at 10 and 30 min p.i. (values are % of I.D. ± S.D.)

	Time (p.i.) min	% Injected Dose ± S.D.				
		Blood	Liver	Intestines	Kidneys	Urine
^{131}I-Hippuran	10	3.3 ± 0.6	2.0 ± 0.5	1.7 ± 0.3	3.9 ± 1.6	75.8 ± 2.5
	30	1.2 ± 0.5	0.7 ± 0.3	0.9 ± 0.3	0.7 ± 0.3	91.9 ± 2.7
^{99m}Tc-EC (6)	10	2.0 ± 0.2	1.9 ± 0.2	1.4 ± 0.2	4.9 ± 2.5	78.7 ± 2.5
	30	0.3 ± 0.1	1.0 ± 0.4	1.1 ± 0.3	0.7 ± 0.2	94.0 ± 1.4
^{99m}Tc-L,L-PC,(A)	10	7.7 ± 0.9	15.3 ± 1.8	4.1 ± 0.3	18.0 ± 1.5	23.6 ± 4.9
	30	4.6 ± 0.4	16.3 ± 5.3	4.2 ± 1.0	9.4 ± 1.1	46.4 ± 6.9
^{99m}Tc-L,L-PC,(B)	10	7.5 ± 1.3	11.5 ± 3.4	4.0 ± 0.5	7.2 ± 0.4	39.2 ± 7.9
	30	4.4 ± 1.3	7.4 ± 1.1	4.1 ± 0.6	3.4 ± 0.5	62.8 ± 5.4

Evaluation in a baboon. In the baboon study, both isomers of ^{99m}Tc-L,L-PC showed a rapid excretion through the kidneys into the urine. There was no visible sign of uptake in the liver or the intestines on the baboon images. However, the renal handling of both isomers of ^{99m}Tc-L,L-PC was not as efficient as it is the case for ^{99m}Tc-L,L-EC and ^{131}I-hippuran. Figure 4 shows the left kidney renogram obtained for both isomers of ^{99m}Tc-L,L-PC and for ^{99m}Tc-L,L-EC in the same baboon. The time to reach the renal maximum (T_{max}) is the same for the three ^{99m}Tc-complexes, but compared to ^{99m}Tc-L,L-EC, the two isomers of ^{99m}Tc-L,L-PC have a lower renal maximum and a slower wash-out from the kidneys. The 1-h plasma clearance of both isomers of ^{99m}Tc-L,L-PC was lower than that of ^{99m}Tc-L,L-EC (Table 2) and represents not more than 33.6 % of the hippuran clearance for isomer A and 25.6 % for isomer B, as compared to 75.4 % in the case of ^{99m}Tc-L,L-EC. The protein binding of the two isomers of ^{99m}Tc-L,L-PC was lower than that of ^{99m}Tc-L,L-EC (Table 2). As a result, a higher fraction can be excreted by glomerular

filtration. As the fraction that can be excreted by glomerular filtration is higher for both isomers of ^{99m}Tc-L,L-PC than for ^{99m}Tc-L,L-EC while their 1-h plasma clearance is lower, it can be concluded that the fraction of ^{99m}Tc-L,L-PC, which is tubularly excreted, is lower.

Table 2. Plasma clearance and protein binding of both isomers of ^{99m}Tc-L,L-PC and ^{99m}Tc-L,L-EC in a baboon

	^{99m}Tc-L,L-PC,(A)	^{99m}Tc-L,L-PC,(B)	^{99m}Tc-L,L-EC (6)
Clearance (ml/min/1.73 m^2)	163	122	541
Clearance (as % of coinj. ^{131}I-OIH)	33.6	25.6	75.4
Protein binding (% of total amount)	10.5	18.3	28.0
Protein binding (as % of coinj. ^{131}I-OIH)	17.5	32.0	46.0

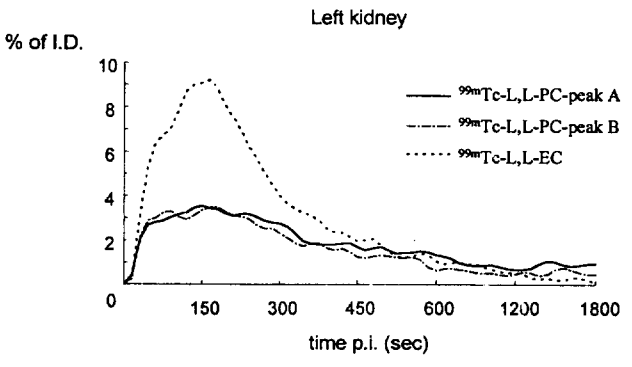


Figure 4. Computer-generated renograms (left kidney) of both isomers of ^{99m}Tc-L,L-PC and ^{99m}Tc-L,L-EC in a baboon from 0-30 min p.i.

EXPERIMENTAL

During synthesis, thin-layer chromatography (TLC) was carried out using precoated silica TLC plates (Alugram SIL G/UV254, Macherey-Nagel, Düren, Germany). Column chromatography was performed on silica gel with a particle size varying between 0.060 mm and 0.200 mm (Chromatographiegel C-gel C-560, CU Chemie Uetikon, Uetikon, Switzerland). The structure of the synthesised ligands was confirmed with ^{1}H-and ^{13}C-NMR on a Varian 200 MHz spectrometer (Varian, Palo Alto, CA). Chemical shifts are reported in ppm relative to tetramethylsilane (δ=0). The splitting patterns are designed as

follows: s (singlet); d (doublet); t (triplet); q (quadruplet), p (pentet), m (multiplet). Electron impact mass spectra (EI-MS) were recorded using an Engine mass spectrometer (Hewlett-Packard, Palo Alto, CA) and liquid secondary ion mass spectra (LSIMS) were recorded on a Kratos Concept IH mass spectrometer (Kratos Analytical, Manchester, UK) equipped with a Masspec II data system (MSS, Manchester, UK). Positive ion spectra were obtained by dissolving the sample in thioglycerol (thgly) as matrix doped with sodium acetate (NaOAc) and setting the accelerating voltage to 7 kV. The final products were dried in a vacuum desiccator over phosphorus pentoxide. Optical rotations were measured at room temperature on a Thorn Automation-NPL Automatic Polarimeter type 243 (Thorn Automation, Nottingham, UK).

Solutions were filtered through a 0.22-μm pore membrane filter (Acrodisc, Gelman Sciences, Ann Arbor, MI) before high performance liquid chromatographic (HPLC) analysis. HPLC-systems consisted of a Merck-Hitachi ternary gradient pump (model L-6200 intelligent pump, Merck, Overijse, Belgium), a Valco N6 injector (Alltech, Laarne, Belgium) and a column filled with Hypersil BDS (Alltech, Laarne, Belgium). The column eluate was monitored for radioactivity with a 2-in. NaI(Tl) scintillation detector coupled to a single channel analyser and radiometric detected signals were fed into a Rachel integration system (version 1.40, LabLogic, Sheffield, UK). Generator eluate containing 99mTc in the form of sodium pertechnetate was obtained from an Ultratechnekow generator (Mallinckrodt Medical, Petten, Holland).

Synthesis

S,S'-dibenzyl-N,N'-1,3-propylenediylbis-L-cysteine diethyl ester (2)

To a solution of S-benzyl-L-cysteine (8.45 g, 40 mmol) in 100 ml water at pH 10.8, a solution of N,N-diisopropylethylamine (6.95 ml, 40 mmol) and 1,3-dichloropropane (2.26 g, 20 mmol) in dioxane (50 ml) was added and the mixture was refluxed for 96 hours. The solution was then concentrated under reduced pressure and the resulting residue was redissolved in water (200 ml). The solution was extracted with ether (200 ml), acidified to pH 2 with hydrochloric acid (12N) and stored at 4°C for five days. The resulting precipitate was filtered off, washed successively with water (100 ml), ethanol (100 ml) and ether (100 ml) and dried in a vacuum desiccator to obtain 3.5 g of the crude S,S'-dibenzyl-N,N'-1,3-propylenediylbis-L-cysteine (1) as a white powder.

The crude precipitate of (*1*) (3.5 g) was dissolved at room temperature in a previously prepared mixture of thionylchloride (30 ml) and ethanol (150 ml) at 0°C and the solution was refluxed overnight. The solution was cooled to room temperature and evaporated under reduced pressure. To the residue was added saturated sodium hydrogen carbonate solution (50 ml) and water (50 ml) and the solution was extracted with ether (3 x 100 ml). The combined organic layers were dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue (3.25 g) was purified by column chromatography with tetrahydrofuran/hexane (4:6, V/V) as eluent to yield 2.10 g (20.3 %) of S,S'-dibenzyl-N,N'-1,3-propylenediylbis-L-cysteine diethyl ester as a dark yellow oil. ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.2 (6H, t, 2 x $COOCH_2C\underline{H}_3$); 1.6 (2H, p, $CH_2C\underline{H}_2CH_2$); 1.8 (2H, s, 2 x N<u>H</u>); 2.4-2.7 (4H, m, $C\underline{H}_2CH_2C\underline{H}_2$); 2.7 (4H, d, 2 x $CHC\underline{H}_2S$); 3.35 (2H, t, 2 x C<u>H</u>COOEt); 3.7 (4H, s, 2 x $SC\underline{H}_2C_6H_5$); 4.2 (4H, q, 2 x $COOC\underline{H}_2CH_3$); 7.2-7.4 (10H, m, 2 x $C_6\underline{H}_5$). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 14.25; 30.19; 34.23; 36.68; 46.36; 60.89; 61.20; 127.03; 128.46; 128.87; 138.01; 173.60. EI-MS (direct inlet 235 °C, 70 eV): MH^+ m/z = 519.

S,S'-dibenzyl-N,N'-1,3-propylenediylbis-L-cysteine (1)

A solution of 2.10 g (4.05 mmol) S,S'-dibenzyl-N,N'-1,3-propylenediylbis-L-cysteine diethyl ester (*2*) in 35 ml of a 1:1 mixture of 6N hydrochloric acid and dioxane was refluxed overnight. The solution was concentrated to 10 ml under reduced pressure and then diluted with 60 ml water. The precipitate was filtered off, washed successively with water, ethanol and ether and dried under reduced pressure over phosphorus pentoxide to yield 1.43 g (66.0 % as the bis-HCl salt) of a slightly yellow product. ^{1}H-NMR (DMSO + 15 % CD_3OD, 200 MHz): δ 1.8 (2H, p, $CH_2C\underline{H}_2CH_2$); 2.5-2.9 (8H, m, $C\underline{H}_2CH_2C\underline{H}_2$ and 2 x $CHC\underline{H}_2S$); 3.6-3.8 (6H, m, 2 x $SC\underline{H}_2C_6H_5$ and C<u>H</u>COOH); 7.1-7.4 (10H, m, 2 x $C_6\underline{H}_5$).
+ LSIMS (thgly doped with NaOAc):
$[M+H]^+ = 463$, $[M+Na]^+ = 485$, $[M+2Na-H]^+ = 507$.

N,N'-1,3-propylenediylbis-L-cysteine (L,L-propylenedicysteine; L,L-PC) (3)

In a two-necked flask provided with a Dewar cooler, 1.43 g of *1* was dissolved in 30 ml liquid ammonia. The mixture was stirred vigorously and sodium (0.29 g) was added in small portions until a blue colour persisted for 15 min. NH_4Cl was added until the blue colour disappeared. After removal of the ammonia by evaporation at room temperature,

the residue was dissolved in 10 ml water and the solution was extracted with ether (2 x 10 ml). The solution was acidified to pH 2 with 2N hydrochloric acid and concentrated under reduced pressure to yield 1.66 g of a white product. To determine the mass percentage of L,L-propylenedicysteine in this product, a solution in 0.05N NaOH was analysed for the number of sulfhydryl groups using the Ellman procedure (11,12). Analysis with flame emission spectrometry for the amount of sodium (IL 943, Instrumentation Laboratory, Lexington, MA) and chloride ions (Chloride analyser 925 Corning, Cibo Corning, Essex, UK) demonstrated that sodium chloride was a main impurity in the final product and the amount of L,L-PC was found to be 11.2 %.
$[\alpha]_D^{20} = -206.6°$ (0.05N NaOH).

Labelling with ^{99m}Tc

Exchange labelling procedure

In a 10-ml labelling vial containing a solution of 2 mg of reaction product containing L,L-PC (3) in 1 ml 0.5M phosphate buffer of the desired pH (pH 4 - 12) were added successively 10 mg sodium potassium tartrate tetrahydrate dissolved in 0.25 ml water, 100 µg $SnCl_2.2H_2O$ dissolved in 25 µl 0.05N HCl and 1 ml generator eluate containing 370-740 MBq $^{99m}TcO_4^-$.The mixture was heated for 10 min in a boiling water bath.

Direct labelling procedure

In a 10-ml labelling vial, 2 mg of reaction product containing L,L-PC was dissolved in 1 ml 0.5M phosphate buffer pH 12. A solution of 100 µg $SnCl_2.2H_2O$ in 25 µl 0.05N HCl was added immediately followed by the addition of 1 ml generator eluate containing 370-740 MBq $^{99m}TcO_4^-$. The mixture was incubated for 15 min at room temperature.

Analysis

Thin layer chromatography (TLC). TLC of the reaction product after labelling was performed on two strips (13 cm x 1 cm) of Whatman 4 Chr paper (Whatman International, Maidstone, England) eluted respectively with acetone (system A) or normal saline (system B) over a distance of 10 cm. The R_f-values of ^{99m}Tc-L,L-PC, $^{99m}TcO_2$, and $^{99m}TcO_4^-$ are respectively 0, 0, and 1 in system A and 1, 0, and 1 in system B. A quantitative analysis of the chromatograms was performed by cutting the strips at 5 cm from the application

point for system A and 2 cm for system B and counting the radioactivity on each part with a 2-in. NaI(Tl) scintillation detector connected to a single channel analyser and scaler.

Reversed phase high performance liquid chromatography (RP-HPLC). The RP-HPLC-system for analysis and preparative separation of the labelled compounds consisted of a column filled with Hypersil BDS C18 (250 mm x 4.6 mm, 5 μm). 20 to 100 μl of the preparation was applied on the column which was eluted at a flow rate of 1 ml/min with gradient mixtures of 0.0125M phosphate buffer pH 2.5 and ethanol. The percentage ethanol in the mobile phase was linearly increased from 0 % to 9 % during the first 6 min and maintained at that level for 19 min.

Stability of the ^{99m}Tc-complexes. RP-HPLC-isolated peaks were reinjected on a similar RP-HPLC-system at different time intervals after isolation and the relative amount of the ^{99m}Tc-complexes was calculated by integration of the resulting chromatograms.

Biodistribution studies

Evaluation in mice. The ^{99m}Tc-complexes isolated by HPLC were diluted with normal saline to a concentration of 144 kBq/ml. Iodine-131 labelled hippuran (Mallinckrodt Medical) was added as an internal biological standard to a concentration of 14.4 kBq/ml. Male NMRI mice (body mass 25-30 g) were sedated by i.m. injection of 0.25 mg fluanisone and 0.005 mg fentanyl (0.1 ml of a 1 to 4 diluted solution of Hypnorm®, Duphar, The Netherlands) and their body mass was determined. Then, 0.1 ml of the diluted tracer solution was injected via a tail vein. The mice were sacrificed by decapitation at 10 min (n = 5) or 30 min (n = 5) post injection (p.i.) and the organs and other body parts were dissected and weighed in a tarred tube. Blood was collected in a tarred tube and weighed. The activity in all organs and other body parts was counted in a 3-in. NaI(Tl) well crystal coupled to a dual-channel analyser and scaler. Corrections were made for background radiation, physical decay during counting and the crossover of the ^{131}I γ-rays into the ^{99m}Tc channel. Results were expressed as percentage of injected dose, equal to the sum of the net counts in all organs. For calculation of total blood activity, blood mass was assumed to be 7 % of the body mass (13). Statistical analysis was performed using a Student t-test. Results were considered significantly different for $p<0.05$.

Evaluation in a baboon. Kidney time-activity curves and plasma clearance of the RP-HPLC-purified ^{99m}Tc-complexes were studied in a male baboon according to a published

procedure (6). The 1-h plasma clearance was calculated using a double exponential fitting method (14). Plasma protein binding was determined using ultrafiltration on plasma samples collected 10 min after injection (Ultrafree-PF filter units, type UFP1 LGC24, Millipore, Bedford, MA) (15). Aspecific adsorption to the membrane was determined by ultrafiltration of the RP-HPLC isolated ^{99m}Tc-complex.

CONCLUSIONS

L,L-propylenedicysteine (L,L-PC) was efficiently labelled with ^{99m}Tc either by direct or exchange labelling. Two ^{99m}Tc-labelled isomers were formed in relative amounts not dependent on the pH. In mice, both complexes were cleared from the blood at a lower rate than ^{99m}Tc-L,L-EC. The clearly higher hepatobiliary uptake of both isomers of ^{99m}Tc-L,L-PC as compared to that of ^{99m}Tc-L,L-EC was striking in mice, but was not visible on the images of the baboon study. As in mice, both isomers of ^{99m}Tc-L,L-PC showed in the baboon study a lower plasma clearance, a lower renal handling and a higher renal retention than ^{99m}Tc-L,L-EC. These results indicate that, besides the oxotechnetium-glycine sequence, also the length and orientation of the alkylene bridge between the two amines are important for the interaction and handling of this type of compound with the renal tubular transport proteins.

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