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Synthesis and Evaluation of β-Homocysteine Derivatives of ^{99m}Tc-*L,L*-EC and ^{99m}Tc-*L,L*-ECD

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SUMMARY

Bis-amine bis-thiol tetraligands such as ethylene dicysteine (EC) and its diethylester (ECD) bind ^{99m}Tc efficiently at room temperature and neutral to alkaline pH to form stable complexes. The use of bis-amine bis-thiol ligands as bifunctional chelating agents (BCAs) for labelling of bioactive compounds (peptides, diphosphonates, etc.) looks promising. To study the effect of extending the carboxylic side-group in ^{99m}Tc-*L,L*-EC and ^{99m}Tc-*L,L*-ECD, we have synthesised ethylene *bis*-*L*-β-homocysteine (*L,L*-EhC) and its diethylester derivative *L,L*-EhCD, incorporating a methylene group between each of the carboxyl groups and the N_2S_2 tetraligand core. The more distant carboxyl groups could offer reduced steric hindrance in the use of *L,L*-EhC and *L,L*-EhCD as BCAs.

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As for ^{99m}Tc-*L,L*-ECD, ^{99m}Tc-*L,L*-EhCD is neutral on electrophoresis at pH 6.0. In mice, brain uptake of ^{99m}Tc-*L,L*-EhCD is lower than that of ^{99m}Tc-*L,L*-ECD. Blood clearance of the two complexes is similar. The diacid ^{99m}Tc-*L,L*-EhC migrates to the same extent as the corresponding ^{99m}Tc-*L,L*-EC on electrophoresis at pH 3.2, 9.0 and 12 but it migrates 25 % further at pH 6. Urine levels for ^{99m}Tc-*L,L*-EhC in mice are lower than those for ^{99m}Tc-*L,L*-EC (65 % versus 74 % of I.D. at 10 min p.i. and 85 % versus 95 % at 30 min p.i., respectively).

The results show that the β-homocysteine derivatives retain the key characteristics of ^{99m}Tc-*L,L*-EC and ^{99m}Tc-*L,L*-ECD, i.e. easy formation of stable complexes with ^{99m}Tc, a high urinary excretion for ^{99m}Tc-*L,L*-EhC, and in the case of ^{99m}Tc-*L,L*-EhCD a neutral compound with appreciable brain uptake. These properties indicate that *L,L*-EhC and *L,L*-EhCD merit further evaluation as BCAs with attractive conjugation properties.

KEY WORDS

L,L-EC, *L,L*-ECD, Technetium, Homocysteine, Bifunctional chelating agents

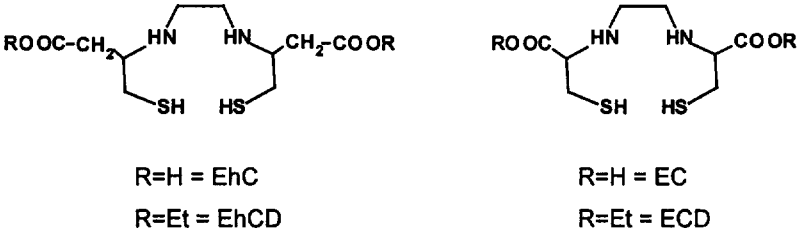
INTRODUCTION

The ^{99m}Tc-complexes of the bis-amine bis-thiol tetraligands ethylene-*bis*-*L*-cysteine (*L,L*-EC) and its diethyl ester derivative *L,L*-ECD are valuable tracer agents for determination of renal function and regional cerebral blood flow, respectively [1,2]. Bis-amine bis-thiol tetraligands such as EC and ECD readily form stable complexes with ^{99m}Tc by covalent bonding at the thiol groups and by covalent and/or coordinate

bonding at the amine groups. Attempts have also been made to use bis-amine bis-thiol derivatives as bifunctional chelating agents (BCAs) [3-8].

In this application the BCA is conjugated to a molecule of biological interest. In the case of EC and ECD it can be done at either or both of the amines or at a carboxyl side group, and the conjugate can then easily and efficiently be labelled with a metal radionuclide such as technetium-99m.

In this study we were interested in the synthesis of a derivative of both *L,L*-EC and *L,L*-ECD containing an extra methylene group between each of the carboxyl side groups and the N_2S_2 tetraligand core. These derivatives can be considered to contain β-homocysteine in place of cysteine groups and can therefore be named ethylene-*bis*-β-homocysteine (EhC) and ethylene-*bis*-β-homocysteine diethylester (EhCD) (Fig. 1). In the context of their potential use as BCAs, the β-homocysteinyl derivatives contain less sterically hindered carboxyl groups for coupling to bio-active molecules. The β-homocysteinyl derivatives could therefore be more convenient for use as BCAs in comparison to *L,L*-EC and *L,L*-ECD.



R=H = EhC
R=Et = EhCD

R=H = EC
R=Et = ECD

Fig. 1. Structures of the synthesised ligands EC, ECD, EhC and EhCD

The objectives of the study were to achieve chemical synthesis of the β-homocysteine derivatives of *L,L*-EC and *L,L*-ECD, to label these derivatives with ^{99m}Tc and to

evaluate the influence of the presence of β-homocysteine in place of cysteine on electrophoretic properties and on biological behavior in mice.

RESULTS AND DISCUSSION

Chemical synthesis

Chemical synthesis of *L,L*-EhC is achieved by extension of the side chains of *L,L*-EC (**1**) following a modified Arndt-Eistert procedure [9,10,11] (Fig. 2). *L,L*-EC was first protected at the amine groups with *N*-benzyloxycarbonyl (CBO) and at the thiols with *S*-benzyl protective groups [12].The carboxyl groups were then activated with ethyl choroformate and reacted with diazomethane to yield the *bis*-α-diazoketone **3**. In the next step, this intermediate then underwent rearrangement in the presence of silver benzoate and methanol to yield the dimethylester of *S,S'-bis*-benzyl-*N,N'-bis*-CBO *L,L*-EhC (**4**). The ester groups of **4** were hydrolysed at alkaline pH and the protective groups were removed by treatment with sodium in liquid ammonia to obtain *L,L*-EhC (**6**)

L,L-ECD was obtained by esterification of *L,L*-EC in HCl/ethanol.

Labelling with technetium-99m and analysis

The synthesised ligands were efficiently labelled with ^{99m}Tc at room temperature, following a direct labelling procedure. For the diacid compounds EC and EhC, labelling was performed at pH 11-12. At such high pH the amines of the bis-aminoacids are no longer positively charged due to zwitterion formation, which would interfere with an efficient binding of technetium. The diester *L,L*-ECD was

labelled at neutral pH. As zwitterion formation is not possible with the diester, the amines are sufficiently deprotonated at neutral pH, which also avoids hydrolysis of one or both of the ester groups.

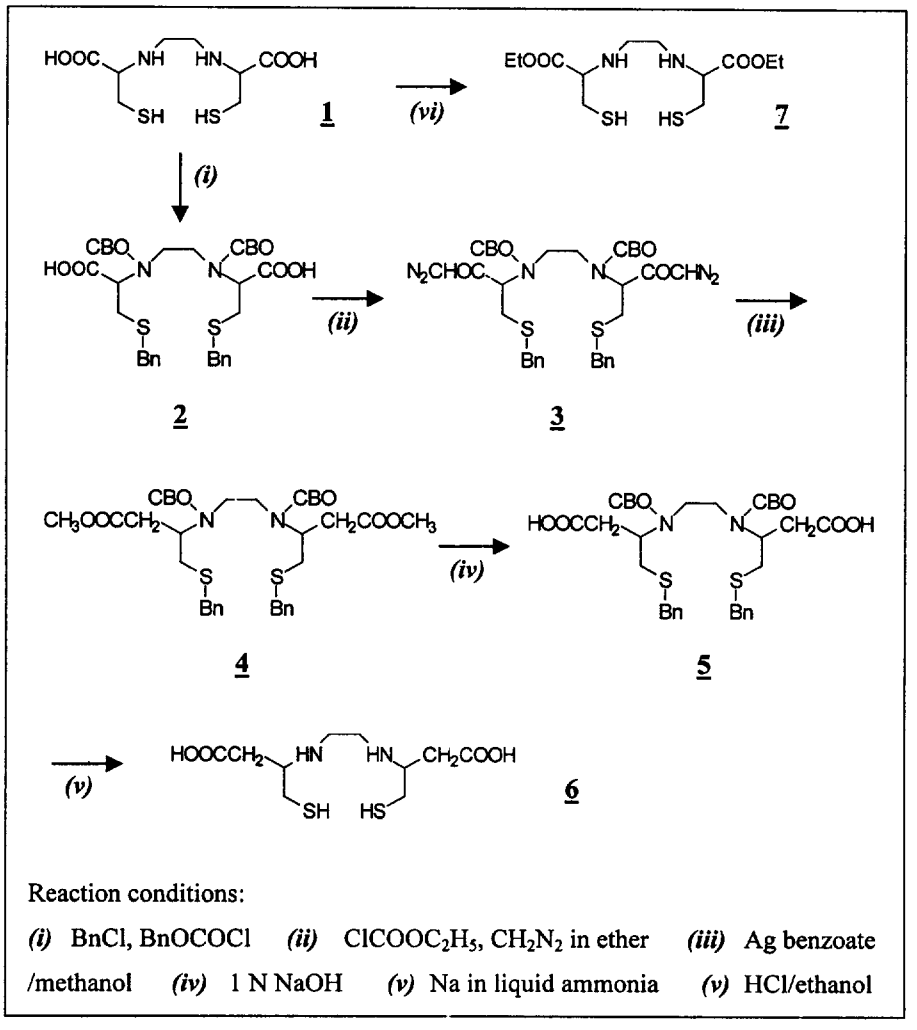


Fig. 2. Chemical synthesis of *L,L*-EhC and *L,L*-ECD, starting from *L,L*-EC

The β-homocysteine diester derivative ^{99m}Tc-*L,L*-EhCD was obtained by non-aqueous esterification of ^{99m}Tc-*L,L*-EhC using triethyloxonium tetrafluoroborate, following the method described by Yonemitsu and co-workers [13]. This option was prefered because of the low amounts of *L,L*-EhC obtained in the chemical synthesis. The product obtained eluted with a retention time close to that obtained for the corresponding cysteine derivative ^{99m}Tc-*L,L*-ECD. Additionally, alkaline hydrolysis of a HPLC-isolated diester (^{99m}Tc-*L,L*-EhCD) peak yielded on HPLC a peak eluting with the retention time obtained fo the starting diacid ^{99m}Tc-*L,L*-EhC.

During electrophoresis, HPLC-isolated ^{99m}Tc-*L,L*-ECD and ^{99m}Tc-*L,L*-EhCD did not migrate at pH 6.0, indicating that they are uncharged around neutral pH. The diacid derivatives ^{99m}Tc-*L,L*-EhC and ^{99m}Tc-*L,L*-EC are anionic. ^{99m}Tc-*L,L*-EhC migrated to the same extent as ^{99m}Tc-*L,L*-EC when electrophoresis was performed at pH 3.2, 9.0 and 12 (25 mm, 50 mm, 50 mm, respectively). However, at pH 6 ^{99m}Tc-*L,L*-EhC migrates 25 % further than ^{99m}Tc-*L,L*-EC (50 mm and 40 mm, respectively).

It has been reported that in solution at pH 6 to 8, ^{99m}Tc-*L,L*-EC exists in two forms [14,15] (Fig. 3). The two forms are (a) a monoanionic six-coordinate distorted-octahedral form with both amine groups protonated and the *anti*- carboxyl group co-ordinated to the ^{99m}TcO core, and (b) a dianionic five-coordinate square-pyramidal form deprotonated at the *anti*- amine group and with the carboxyl group de-ligated from the TcO core.

Taylor and co-workers reported that at pH 7.4, 20 % of ^{99m}Tc-*L,L*-EC is in the monoanionic form [16]. Interestingly, the two forms that co-exist at physiological pH might conceivably have differing biological behaviour. It is not clear if the greater

migration of ^{99m}Tc-*L,L*-EhC during electrophoresis at pH 6 is due to a higher proportion of the dianionic form than that given by ^{99m}Tc-*L,L*-EC.

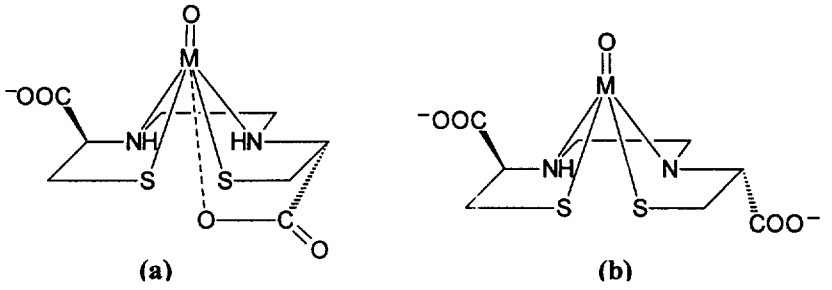


Fig. 3. The two forms of the complexes derived from *L,L*-EC that co-exist at approximately physiological pH (M = Tc or Re) [15]. In (a) both amine groups are protonated, the *anti*-carboxyl group is ligated; and in (b) the *anti*-amine group is deprotonated, the *anti*-carboxyl group is deligated.

Biological behaviour in mice

Results obtained for biodistribution of ^{99m}Tc-*L,L*-ECD and ^{99m}Tc-*L,L*-EhCD in important organs in mice are given in Table 1. Brain uptake in mice for ^{99m}Tc-*L,L*-EhCD is significantly lower than that for ^{99m}Tc-*L,L*-ECD (maximum level in brain = 0.2 % of I.D. versus 1.2 % for ^{99m}Tc-*L,L*-ECD). Excretion into urine is much higher for ^{99m}Tc-*L,L*-EhCD than for ^{99m}Tc-*L,L*-ECD, probably due to hydrolysis of ^{99m}Tc-*L,L*-EhCD at a faster rate to its mono- and diacid derivatives than the similar hydrolysis of ^{99m}Tc-*L,L*-ECD. Excretion into the intestines is correspondingly lower for ^{99m}Tc-*L,L*-EhCD. The disappearance profile from blood of the two diethylester complexes is similar. A faster hydrolysis of the ester groups of ^{99m}Tc-*L,L*-EhCD could be an

explanation for the lower brain uptake, as a considerable fraction of the diester is possibly already hydrolysed to the mono-ester mono-acid or to the diacid metabolites before arrival of the tracer agent at the brain.

Table 1. Biodistribution in mice (n=5) of ^{99m}Tc-*L,L*-EhCD and ^{99m}Tc-*L,L*-ECD. Results are expressed as percentage of injected dose per organ (mean ±sd).

	^{99m}Tc-*L,L*-EhCD			^{99m}Tc-*L,L*-ECD		
Time p.i	2 min	10 min	30 min	2 min	10 min	30 min
Urine	2.2 ±0.9	26.0 ±3.2	41.4 ±2.3	0.3 ±0.1	3.4 ±1.2	8.8 ±1.5
Kidneys	22.2 ±3.2	12.1 ±2.3	3.1 ±0.5	7.3 ±0.4	9.0 ±1.9	6.3 ±0.9
Liver	34.9 ±1.2	39.3 ±2.0	31.2 ±0.2	27.2 ±0.5	42.8 ±2.4	39.0 ±2.5
Intestines	6.3 ±0.2	9.4 ±2.9	18.0 ±0.4	10.5 ±0.2	13.9 ±1.2	28.1 ±5.0
Brain	0.21 ±0.0	0.11 ±0.0	0.03 ±0.0	1.2 ±0.3	0.9 ±0.3	0.3 ±0.0
Blood	9.2 ±0.5	3.1 ±0.3	1.9 ±0.2	6.3 ±0.4	3.7 ±0.8	2.1 ±0.1

Table 2. Biodistribution in mice (n=5) of ^{99m}Tc-*L,L*-EhC and ^{99m}Tc-*L,L*-EC. Results are expressed as percentage of injected dose per organ (mean ±sd).

	^{99m}Tc-*L,L*-EhC		^{99m}Tc-*L,L*-EC	
Time p.i.	10 min	30 min	10 min	30 min
Urine	65.0 ±3.1	85.7 ±3.6	73.6 ±2.4	95.3 ±1.4
Kidneys	6.8 ±2.7	2.5 ±1.2	7.0 ±3.5	0.8 ±0.2
Liver	7.3 ±1.4	4.9 ±0.9	1.9 ±0.2	1.0 ±0.4
Intestines	2.5 ±0.5	1.3 ±0.2	1.4 ±0.2	1.1 ±0.3
Blood	3.7 ±0.4	1.3 ±0.4	2.0 ±0.2	0.3 ±0.1

Results obtained for biodistribution in mice for the diacid derivatives ^{99m}Tc-*L,L*-EC and ^{99m}Tc-*L,L*-EhC are given in Table 2. The rate of excretion of ^{99m}Tc-*L,L*-EhC into urine is slower than that of ^{99m}Tc-*L,L*-EC at 10 min and 30 min p.i. However, the values for urine levels of ^{99m}Tc-*L,L*-EhC at 10 min and 30 min p.i. remain quite high. ^{99m}Tc-*L,L*-EhC shows a relatively slower blood disappearance and higher liver levels. However, the rate and extent of excretion into the intestines for the two diacid complexes is minimal (approximately 1 %) and this shows that both ^{99m}Tc-*L,L*-EhC and ^{99m}Tc-*L,L*-EC rely mainly on renal handling for their excretion.

EXPERIMENTAL

All reagents obtained from commercial sources were of analytical grade. Structures were confirmed by ^{1}H-NMR spectroscopy recorded on a Gemini 200 MHz spectrometer (Varian, Palo Alto, CA) and acquired relative to TMS (δ=0). Melting points were determined in open capillaries immersed in an oil bath (Electrothermal, Southend-on-Sea, U.K.) and are not corrected.

Chemical Synthesis

Ethylene-*bis*-*L*-cysteine (*L,L*-EC, **1**) was synthesised by dimerisation of *L*-(-) thiazolidine-4-carboxylic acid following the procedure described by Blondeau and co-workers [17]. *L,L*-ECD (**7**) was obtained by gently refluxing *L,L*-EC over 18 h in anhydrous ethanol previously saturated with hydrogen chloride. *L,L*-ECD precipitates as the dihydrochloride salt [18].

S,S'-Bis-benzyl-N,N'-bis-CBO ethylene bis-L-cysteine (**2**)

L,L-EC (**1**, 21.7 g, 81.0 mmol) is dissolved in liquid ammonia and sodium metal is added in small portions till a blue colour persists for at least 15 min. Excess sodium is quenched by addition of ammonium chloride. Benzyl chloride (19.0 ml, 163.5 mmol) is added, the mixture stirred for 30 min, the ammonia evaporated, water added and the mixture washed with ether. The pH of the aqueous layer is then adjusted to 2 with 37 % HCl and the mixture is left to stand at 4-8°C for 18 h. The precipitate formed is filtered off, washed well with water and dried thoroughly at 40 °C under reduced pressure. *S,S'-Bis*-benzyl *L,L*-EC is obtained in quantitative yield as a white powder, melting point 229 °C (dec.).

To a solution of *S,S'-bis*-benzyl *L,L*-EC(15.0 g, 33.5 mmol) in 200 ml water at pH 10 (adjusted using 1N NaOH), Na_2CO_3 (8.1 g, 75 mmol) is added followed by a solution of benzyl chloroformate (15.6 ml, 105.6 mmol) in 5 ml dioxane. The pH is maintained at 10 by addition of solid Na_2CO_3, and at constant pH the mixture is stirred for a further 2 h. The mixture is basified to pH 12 and washed with ether, the pH is lowered to 2 and the mixture extracted with ethyl acetate. The organic layer is concentrated under reduced pressure and the residue is purified on a silica gel column using as the mobile phase a gradient from dichloromethane:acetic acid (99:1) to dichloromethane:methanol:acetic acid (94:5:1). *S,S'-Bis*-benzyl-*N,N'-bis*-CBO ethylene *bis-L*-cysteine (**2**) is obtained as a pale yellow oil (yield 87%).

^{1}H-NMR ($CDCl_3$) δ 8.95 (2 H, s, broad), δ 7.2 (20 H, m, Phe Ar), δ 5.1 (4 H, m), δ 4.4 (2 H, m), δ 3.4-3.8 (8 H, m), δ 2.8-3.1 (4 H, m).

S,S'-Bis-benzyl-N,N'-bis-CBO ethylene bis-L-cysteinoyl diazomethane (**3**)

Isobutyl chloroformate (2.3 ml, 17.6 mmol) is added to a solution of **2** (5.0 g, 7 mmol) and *N*-methyl morpholine (2.05 ml, 18.6 mmol) in 300 ml THF at –5 °C under nitrogen. The mixture is stirred for 1h at –5°C. The *N*-methyl morpholine hydrochloride formed is filtered off and washed with cold THF. The filtrate is added over 30 min to a cold (0°C) solution of diazomethane in ether previously prepared using the equipment set-up developed by Hudlicky [19]. The mixture is stirred for 4 h at 0°C. Excess diazomethane is bubbled off with nitrogen and the solvents are evaporated. The residue is dissolved in ethyl acetate, washed successively with 0.25 N citric acid, 1 N $NaHCO_3$ and brine, and the mixture evaporated to give **3** as a yellowish oil (yield 84 %).

S,S'-Bis-benzyl-N,N'-bis-CBO ethylene bis-L-β-homocysteine dimethylester (**4**)

To a solution of **3** (1.55 g, 2 mmol) in 10 ml anhydrous methanol is added 1.5 ml of a 50 mg/ml solution of silver benzoate in triethylamine. An exothermic reaction occurs and the solution turns dark. The mixture is stirred for 20 min, 1 g celite, 1 g charcoal and 5 ml brine are added, and after 15 min the mixture is filtered through 1 g celite to obtain a faintly yellow solution. Silica gel column purification is performed with hexane:ethyl acetate (70:30) as mobile phase to obtain **4** as a yellow oil (yield 10%).

^{1}H-NMR ($CDCl_3$) δ 7.1-7.4 (20 H, m, Phe Ar), δ 5.2 (4 H, m), δ 4.6 (1 H, t), δ 4.4 (1 H, t), δ 3.1-3.8 (14 H, m), δ 2.5-2.8 (4 H, m), δ 2.2-2.4 (4 H, double duplet).

S,S'-Bis-benzyl-N,N'-bis-CBO ethylene bis-L-β-homocysteine (**5**)

450 mg of **4** (0.6 mmol) is dissolved in 5 ml methanol, 1 ml 2 N NaOH is slowly added and the mixture incubated at 50°C for 12 h. 20 ml water is added, the methanol is evaporated under reduced pressure, the mixture is acidified to pH 2.5 – 3 and extracted with ethyl acetate. The organic solvent is evaporated and the residue purified by silica gel column chromatography eluted with hexane:ethyl acetate:acetic acid (60:40:1), to obtain **5** in 61 % yield.

^{1}H-NMR ($CDCl_3$) δ 8.5 (2 H, s, broad), δ 7.0-7.4 (20 H, m, Phe Ar), δ 5.05 (4 H, m), δ 4.6 (1 H, s, broad), δ 4.4 (1 H, s, broad), δ 3.1-3.8 (8 H, m), δ 2.1-2.8 (8 H, m).

Ethylene bis-L-β-homocysteine (**6**)

To a solution of **5** (130 mg, 0.2 mmol) in 20 ml liquid ammonia is added sodium metal in small portions till a blue colour persists for 15 min. Excess sodium is quenched by addition of ammonium chloride. The ammonia is then allowed to evaporate and the residue is further dried under reduced pressure. The obtained product **6** (1.8 g) is used as such for labelling with ^{99m}Tc.

Labelling with ^{99m}Tc

L,L-EC and *L,L*-ECD were labelled following reported procedures [1,2]. *L,L*-EhC is labelled by adding pertechnetate solution (eluate of an UltraTechnekow™ generator (Mallinckrodt Medical, Petten, The Netherlands, diluted with saline) to a mixture containing the equivalent of 1 mg *L,L*-EhC in 1 ml water, 0.2 ml phosphate buffer pH

11-12 (0.5 M) and 50 μg $SnCl_2.2H_2O$ (12.5 μl of a 4 mg/ml solution in 0.05 M HCl). The mixture is incubated for 30 min for maximum yield.

^{99m}Tc-*L,L*-EhCD is obtained from ^{99m}Tc-*L,L*-EhC by non-aqueous esterification using triethyloxonium tetrafluoroborate [13]. For this purpose, a C18 Sep-Pak™ mini-column (Waters Corporation, Mass., U.S.A) is first pre-conditioned by rinsing successively, with 10 ml ethanol and 10 ml water. Then, the pH of a ^{99m}Tc-*L,L*-EhC preparation is adjusted to 3 using 1N HCl. ^{99m}Tc-*L,L*-EhC in a volume of the preparation equivalent to 555 MBq ^{99m}Tc (approximately 0.5 ml) is applied onto the Sep-Pak™ mini-column. The column is then rinsed with water and dried by blowing in dry nitrogen. The ^{99m}Tc-*L,L*-EhC is eluted with 1.5 ml absolute ethanol and esterified by incubation for 30 min of the ethanolic eluate with 50 mg triethyloxonium tetrafluoroborate and 36 mg diisopropyl ethylamine in 0.2 ml ethanol (yield 85 %, as determined using HPLC, see further).

Analysis and electrophoresis

The radiolabelled reaction mixtures were analysed by instant thin layer chromatography (ITLC) strips (Gelman Sciences, U.S.A). Elution was done with acetone for the diester complexes (^{99m}Tc-*L,L*-ECD and ^{99m}Tc-*L,L*-EhCD) and with physiological saline for the diacids (^{99m}Tc-*L,L*-EC and ^{99m}Tc-*L,L*-EhC) to rule out formation of colloidal technetium (R_f=0) in significant amounts.

HPLC analyses of the labelled reaction mixtures were performed on a 250 mm x 4.6 mm column filled with Hypersil BDS 5 μm (Alltech, Laarne, Belgium) and eluted with linear gradient mixtures of (a) phosphate buffer (pH 2.5; 0.025 M), (b) 30 % v/v

ethanol in phosphate buffer (pH 2.5; 0.025 M) and (c) ethanol, at a flow rate of 1 ml/min. The gradient mixtures at given time points are: at 0 min, 100 % solvent (a), at 20 min, 100 % solvent (b), and at 20.1 min to 35 min, a mixture of 57 % solvent (b) and 43 % solvent (c).

HPLC-isolated ^{99m}Tc-*L,L*-EC and ^{99m}Tc-*L,L*-EhC, and the ^{99m}Tc-*L,L*-ECD and ^{99m}Tc-*L,L*-EhCD complexes were analysed concurrently on electrophoresis. The samples were applied on 2.54 cm x 17 cm Whatman #1 chromatographic paper. Electrophoresis was done at 300 V over 30 min and using 0.025 M phosphate buffer pH 7.4 as electrolyte solution for the diacid complexes and using 50 % methanol in 0.025 M phosphate buffer pH 7.4 for the diesters.

Evaluation in mice

Male NMRI mice (body mass 25-35 g) were sedated by intramuscular 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). They were weighed and 0.1 ml of a HPLC-purified tracer solution, diluted with saline to a radioactive concentration of 148 kBq/ml was injected via a tail vein. For ^{99m}Tc-*L,L*-EC and ^{99m}Tc-*L,L*-EhC, five mice were sacrificed by decapitation at 10 min and another five at 30 min p.i.. For ^{99m}Tc-*L,L*-ECD and ^{99m}Tc-*L,L*-EhCD three times five mice were sacrificed at 2 min, 10 min and at 30 min p.i., respectively. Blood was collected in a tared tube and weighed. The mice were dissected and the activity in all organs and body parts was determined using a 3-in. NaI(Tl) scintillation detector coupled to a dual channel analyser and scaler.

Results were corrected for background radiation, physical decay during counting and for amount of activity localised in the tail. The activity in each organ was expressed as a percentage of the injected dose (ID), equal to the sum of net counts in all organs. For calculation of radioactivity in total blood, blood mass was assumed to be 7 % of total body mass.

CONCLUSION

A chemical synthesis procedure for chain extension at both the carboxyl side groups of *L,L*-EC is described, whereby β-homocysteine can be considered to replace cysteine in the *L,L*-EC structure. The chain extension succeeds with a low overall yield (4.5 %, calculated with respect to the conversion of *L,L*-EC to *S,S'-bis*-benzyl-*N,N'-bis*-CBO *L,L*-EhC), mainly due to the poor yield (10 %) in the conversion of *S,S'-bis*-benzyl-*N,N'-bis*-CBO ethylene *bis-L*-cysteinoyl diazomethane to *S,S'-bis*-benzyl-*N,N'-bis*-CBO ethylene *bis-L*-β-homocysteine dimethylester. Optimisation of this synthetic step is therefore necessary if the β-homocysteine derivatives are to be used as substrates in subsequent reactions, for instance as bifunctional chelating agents.

The two diester complexes ^{99m}Tc-*L,L*-ECD and ^{99m}Tc-*L,L*-EhCD behave in a similar way on electrophoresis at physiological pH. However, introduction of β-homocysteine in place of cysteine groups apparently causes reduction of brain uptake and retention for ^{99m}Tc-*L,L*-EhCD. Urinary excretion for ^{99m}Tc-*L,L*-EhC is slower than that of ^{99m}Tc-*L,L*-EC. However, this extended chain derivative ^{99m}Tc-*L,L*-EhC remains almost exclusively excreted through the urinary system.

The results show that the key characteristics of ^{99m}Tc-*L,L*-EC and ^{99m}Tc-*L,L*-ECD are retained in the β-homocysteine derivatives (i.e. a high urinary excretion for ^{99m}Tc-*L,L*-EhC, and in the case of ^{99m}Tc-*L,L*-EhCD a neutral molecule with appreciable brain uptake). These properties allow for *L,L*-EhC and *L,L*-EhCD to be used as BCAs in place of ^{99m}Tc-*L,L*-EC and ^{99m}Tc-*L,L*-ECD, respectively. The extended side chain in the case of *L,L*-EhC makes it potentially more attractive for use as a bifunctional chelating agent due to lower steric hindrance for conjugation to bioactive molecules.

REFERENCES

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

2 Léveillé J., Demonceau G., De Roo M., Rigo P., Taillefer R., Morgan R.A., Kupranick D. and Walovitch R.C. - J. Nucl. Med. 30: 1902 (1989)

3 Baidoo K.E., Lever S.Z. and Scheffel U. – Bioconjug. Chem. 5: 114 (1994)

4 Verbruggen A. – In: Current Directions in Radiopharmaceutical Development, Mather S.J. Ed., Kluwer Academic Publications, Dordrecht, 1996, p 31-46.

5 Del Rosario R.B., Yung J-W., Baidoo K.E., Lever S.Z., and Wieland D.M. – Nucl. Med. Biol. 21: 197 (1994)

6 Eisenhut M., Lehmann W.D., Becker W., Behr T., Elser H., Strittmatter-W; Steinstrasser A., Baum R.P., Valerius T., Repp R., and Deo Y. - J. Nucl. Med. 37: 362 (1996)

7 Samnick S., Brandau W., Sciuk J., Steinsträsser A., and Schober O. Nucl. Med. Biol. 22: 573 (1995)

8 DiZio J.P., Anderson C.J., Davison A., Ehrhardt G.J., Carlson K. E., Welch M.J. and Katzenellenbogen J.A. - J. Nucl. Med. 33: 558 (1992)

9 Balenović K. and Fleš D. - J. Org. Chem. 17: 347 (1952)

10 Gordon E.M., Godfrey J.D., Delaney N.G., Asaad M.M., Von Langen D. and Cushman D.W. - J. Med. Chem. 31: 2199 (1988)

11 Jefford C.W., Tang Q. and Zaslona A. - J. Am. Chem. Soc. 113: 3513 (1991)

12 Greenstein J.P. and Winitz M. - Chemistry of the Amino Acids, volume 2, Robert E. Krieger Publishing Company, Malabar, 1984.

13 Yonemitsu O., Hamada T. and Kanaoka Y. - Tetrahedron Lett. 23: 1819 (1969)

14 Edwards D.S., Cheesman E.H., Watson M.W., Watson M.W., Maheu L.J., Nguyen S.A., Dimitre L., Nason T., Watson A.D. and Walovitch R. – In: Technetium and Rhenium in Chemistry and Nuclear Medicine 3; Nicolini M., Bandoli G., Mazzi U, Eds., Cortina International, Verona, 1990, p 433-444.

15 Marzilli L.G., Banaszczyk M.G., Hansen L., Kuklenyik Z., Cini R. and Taylor A. - Inorg. Chem. 33: 4850 (1994)

16 Taylor A., Hansen L., Eshima D., Malveaux E., Folks R., Shattuck L., Lipowska M. and Marzilli L.G. - J. Nucl. Med. 38: 821 (1997)

17 Blondeau P., Berse C. and Gracel D. - Can. J. Chem. 45: 49 (1967)

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

19 Hudlicky M. - J. Org. Chem. 45: 5377 (1980)

