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Development of ^{99m}Tc-thioflavin-T derivatives for detection of systemic amyloidosis

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In a search for a ^{99m}Tc-labelled tracer agent for imaging of amyloid plaques in patients with systemic amyloidosis (SA), we have conjugated three bifunctional chelating ligands, namely *S*-benzyl-mercaptoacetyl-l-aspartyl(tBu)-glycine, HYdrazino-NICotinic acid and nitrilotriacetic acid, to the 2-phenylbenzothiazole core of thioflavin-T (ThT), which has known affinity for amyloid. The compounds were successfully synthesized and labelled with technetium-99m. Their structure was confirmed by radio-LC-MS analysis. After i.v. injection in normal mice, all three ^{99m}Tc-labelled ThT derivatives were excreted almost exclusively via liver and intestines. This indicates that the new tracer agents have not the required biokinetics for application as a probe for detection of SA in the abdominal region.

Keywords: amyloid; thioflavin-T; technetium-99m; SPECT; 2-phenylbenzothiazoles

Introduction

Amyloidosis is an acquired or hereditary disorder of protein folding, in which whole or fragments of normally soluble proteins are deposited extracellularly as abnormal, insoluble fibrils that accumulate, disrupt the structure and function of organs and tissues and cause disease.[1] In systemic amyloidosis (SA) the deposits may be present in the parenchyma of the viscera and of all tissues except the brain as well as in the walls of blood vessels throughout the body. The depositions consist of proteins with a typical β-sheet form called amyloid β (Aβ).[2,3] The most accepted theory concerning the aetiology of the disease is the amyloid cascade theory. SA is usually fatal, although the prognosis has been improved by haemodialysis, kidney, liver and heart transplantation and the increasing capacity to effectively treat the acquired primary diseases that are complicated by amyloid deposition. It is, however, essential that the diagnosis is made as early as possible, if these benefits are to be recognized.[4]

Clinical diagnosis of SA is now done by immunohistochemical staining of tissue sections obtained by invasive biopsy, surgical resection or autopsy. Congo red still remains the gold standard in staining Aβ.[5] Other histological techniques using antibodies, immunoelectron microscopy and Western blotting give good results.[6] However, histology cannot provide information about the overall whole body load or distribution of amyloid deposits, nor does it permit *in vivo* monitoring of the natural history of amyloidosis or its response to treatment. Therefore, ^{123}I-labelled serum amyloid P component has been used to rapidly and specifically localize amyloid deposits *in vivo* allowing diagnosis and quantification of deposits by scintigraphy.[7] This technique has 90–100% diagnostic sensitivity and is the only *in vivo* method available nowadays. However, this tracer agent is not available as a licenced radiopharmaceutical, is only in use in a very few centres and has the disadvantage of its proteinacous origin with all associated risks.

In view of the nearly optimal physical and imaging characteristics of technetium-99m, the aim of this study was the development of a ^{99m}Tc-labelled tracer agent for visualization of SA, based on the structure of thioflavin-T (ThT, Figure 1(A)). ThT has a high affinity for the β-pleated sheet structure of Aβ[8] and is a rather polar compound due to the presence of a quaternary amine. Therefore, radiolabelled derivatives of ThT could be useful for non-invasive *in vivo* detection of amyloid plaques in the human body. Such a radiolabelled compound should have a relatively low molecular mass and should be hydrophilic, as the excretion from the body should be fast and mainly through the kidneys to avoid pronounced uptake in the abdominal region.

In this study, three bifunctional chelating ligands (BCLs) were coupled to the 2-phenylbenzothiazole core of ThT.[9] In a first approach, *S*-benzyl-mercaptoacetyl-l-aspartyl(tBu)-glycine (Figure 2(A)) was coupled to 2-(4′-aminophenyl)-1,3-benzothiazole (Figure 1(B)) in order to obtain a negatively charged ^{99m}Tc complex.[10] It was hypothesized that such a negatively charged ^{99m}Tc-labelled ThT derivative could be useful for detection of SA in view of its polar characteristics, which would favour renal excretion and decrease uptake in the hepatobiliary system. In a second approach, the phenylbenzothiazole core was conjugated to HYdrazinoNICotinic acid (HYNIC) (Figure 2(B)). The HYNIC chelating group was chosen in view of the favourable behaviour of ^{99m}Tc-HYNIC-octreotide, i.e. high and rapid excretion through the kidneys and low hepatic uptake.[11] In a third approach the technetium-tricarbonyl labelling method was used to also

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obtain a negatively charged ^{99m}Tc-labelled ThT derivative.[12] In this case a conjugate of nitrilotriacetic acid (Figure 2(C)) and 2-(4′-aminophenyl)-1,3-benzothiazole was made and used as the Tc-binding ligand.

This paper describes the synthesis of three conjugates of the 2-phenylbenzothiazole core of ThT, deprotection of the technetium-binding groups, labelling and purification, structure confirmation with radio-LC-MS and a preliminary biological evaluation of the new ^{99m}Tc-labelled ThT derivatives.

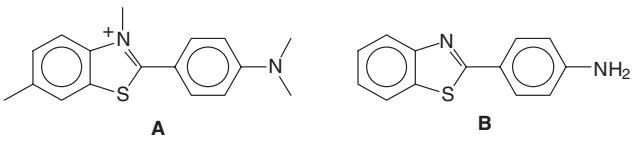


Figure 1. Structure of (A) thioflavin-T (ThT) and (B) 2-(4′-aminophenyl)-1,3-benzothiazole.

Results and discussion

Synthesis, labelling and structure confirmation

As ThT does not contain sufficient metal-binding donor atoms in its structure to bind ^{99m}Tc, we have attached three BCLs to the 2-phenylbenzothiazole core of ThT.

For preparing ^{99m}Tc-**2**, 2-(4′-aminophenyl)-1,3-benzothiazole was coupled to *S*-benzyl-mercaptoacetyl-l-aspartyl(tBu)-glycine to obtain a precursor of a negatively charged ^{99m}Tc(V)O complex (Scheme 1). The BCL was synthesized according to published data[10] and contains an *S*-benzyl-protected thiol and a *tert*-butyl-protected carboxylate in the aspartyl moiety.

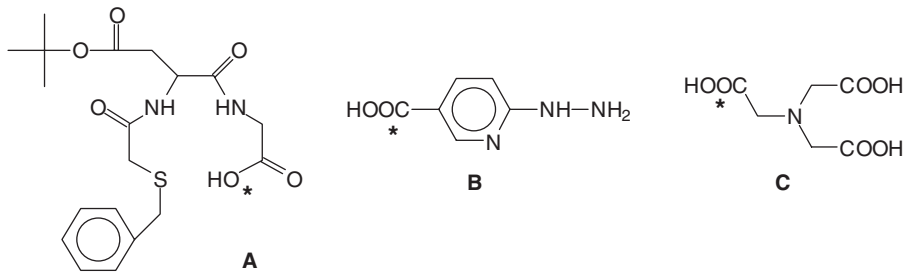
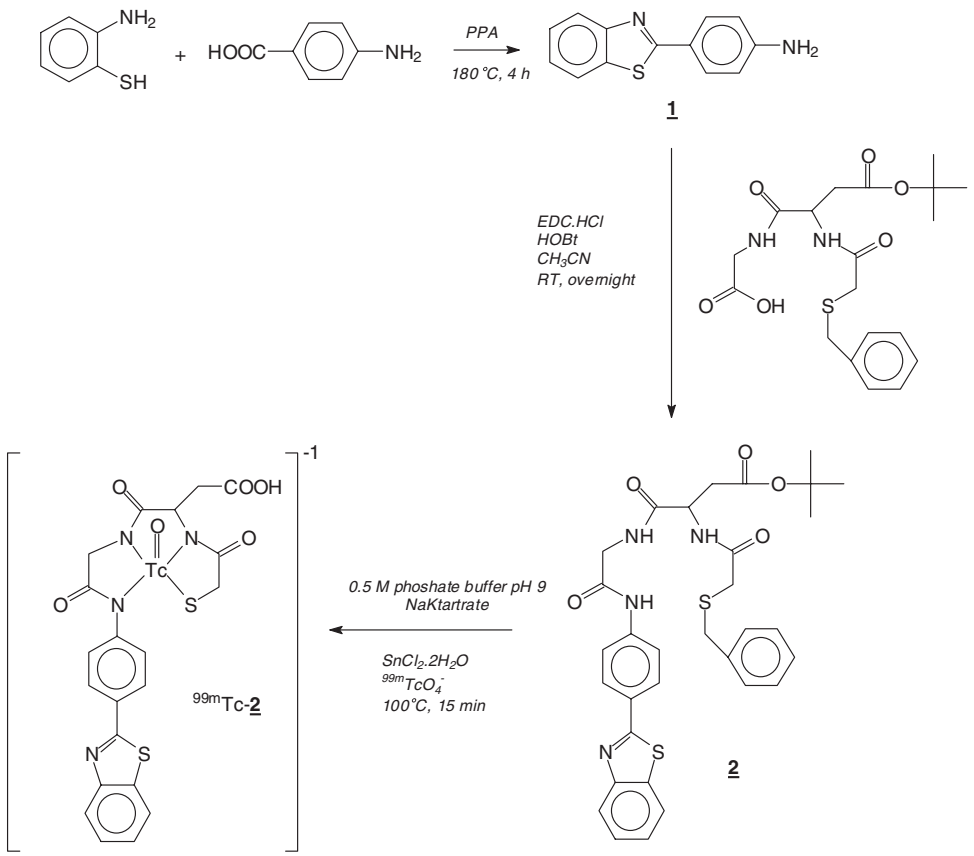


Figure 2. Bifunctional chelating agents: (A) *S*-benzyl-mercaptoacetyl-l-aspartyl(tBu)-glycine; (B) HYDrazinoNICotinic acid (HYNIC); and (C) nitrilotriacetic acid. ∗ indicates the site of coupling with the phenylbenzothiazole core of ThT.



Scheme 1. Synthesis and labelling with ^{99m}Tc of 2-[4′-(S-benzyl-mercaptoacetyl-l-aspartyl(tBu)-glycinamido)phenyl]-1,3-benzothiazole (**2**).

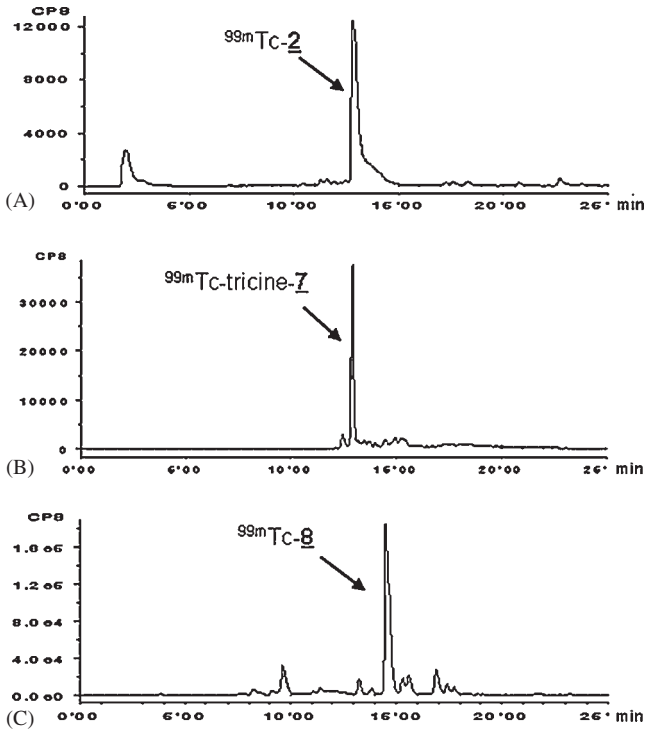


Figure 3. RP-HPLC chromatograms of the labelling reaction mixtures of: (A) 2-[4′-(*S*-benzyl-mercaptoacetyl-l-aspartyl(tBu)-glycinamido)phenyl]-1,3-benzothiazole (**2**) with technetium-99m at pH 9; (B) 2-[2′-(N-BOC-6-hydrazinonicotinamido-3-propoxy)-4′-amino]phenyl-1,3-benzothiazole (**7**) with technetium-99m in the presence of tricine as co-ligand; and (C) 2-[4′-(*N*,*N*-diacetic acid)-*N*-acetamidophenyl]-1,3-benzothiazole (**8**) with $[^{99m}Tc(CO)_3(OH_2)_3]^+$ solution at pH 7. HPLC conditions: XTerra™ RP C18 column (5 μm, 250 mm × 4.6 mm) eluted with gradient mixtures of CH_3CN and 0.05 M ammonium acetate (for **2** and **8**: 0 min: 0:100 v/v, 20 min: 90:10 v/v, 25 min: 90:10 v/v, 30 min: 0:100 v/v; for **7**: 0 min: 0:100 v/v, 15 min: 90:10 v/v, 20 min: 90:10 v/v, 25 min: 0:100 v/v) at a flow rate of 1 ml/min.

Removal of the *tert*-butyl ester of **2** in a mixture of CH_2Cl_2/trifluoroacetic acid (TFA) (5:1 v/v) at room temperature (RT), as described in literature,[10] provided only low yields of the derivative of **2** with a deprotected carboxylate group. However, we found that it was not necessary to remove the *tert*-butyl protective group prior to the radiolabelling reaction, as the combination of pH 9 and a heating step of 15 min during the labelling reaction appeared sufficient for efficient formation of the ^{99m}Tc-**2** complex with a deprotected carboxylate. The labelling yield was 80% (Figure 3(A)).

The use of liquid chromatography with UV and radiometric detection in combination with mass spectrometry allows one to determine which molecular ion mass corresponds to a peak observed in the radiometric channel. Small amounts of carrier ^{99}Tc (about 1.5 μg in the form of $NH_4^{99}TcO_4$) were added to the preparation to be able to determine the mass of the selected peaks.[13–16]

Figure 4(A) shows the single ion mass chromatogram of the reaction product after labelling of **2** over the mass range 583.406–583.474 Da and Figure 4(B) the radiometric signal of the radio-LC-MS analysis. The peak at 11.38 min is supposed to be the intended Tc(V)-oxo complex with deprotected **2** (^{99m}Tc-**2**, Scheme 1). The relatively large peak at 0.87 min, corresponding to the void volume of the column, is supposed to be $^{99m}TcO_4^-$ as a radiochemical impurity because the yield of the carrier-added labelling reaction was lower than that of the no-carrier-added procedure. In Figure 4(D), the background subtracted mass spectrum over the peak with retention time (Rt) 11.38 min shows a molecular mass of 583 Da, which is in perfect agreement with the theoretical molecular ion mass for the supposed structure of ^{99m}Tc-**2** (Figure 4(C)). This mass is in accordance with a negatively charged complex in which a $[Tc(V)O]^{3+}$ core is bound to the deprotonated thiol sulphur atom and three deprotonated amide nitrogen atoms of the

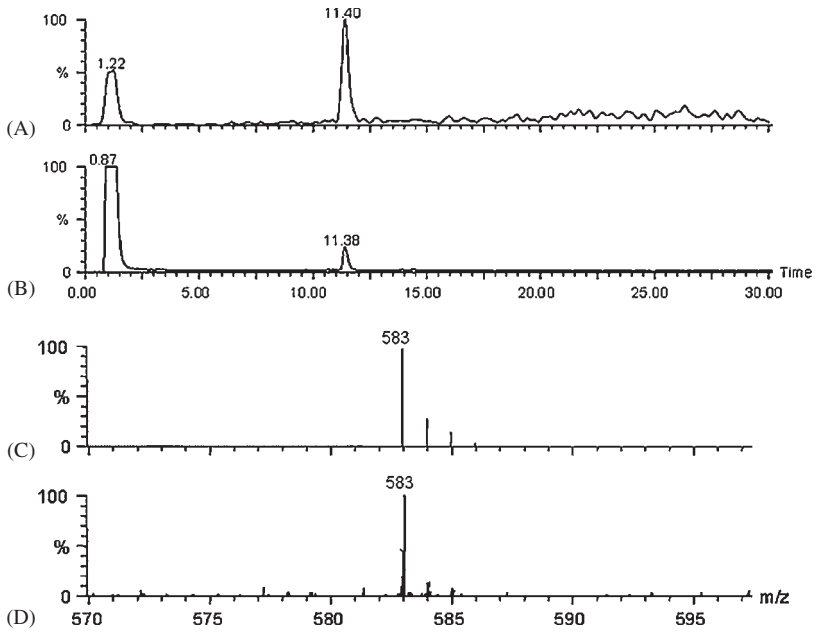


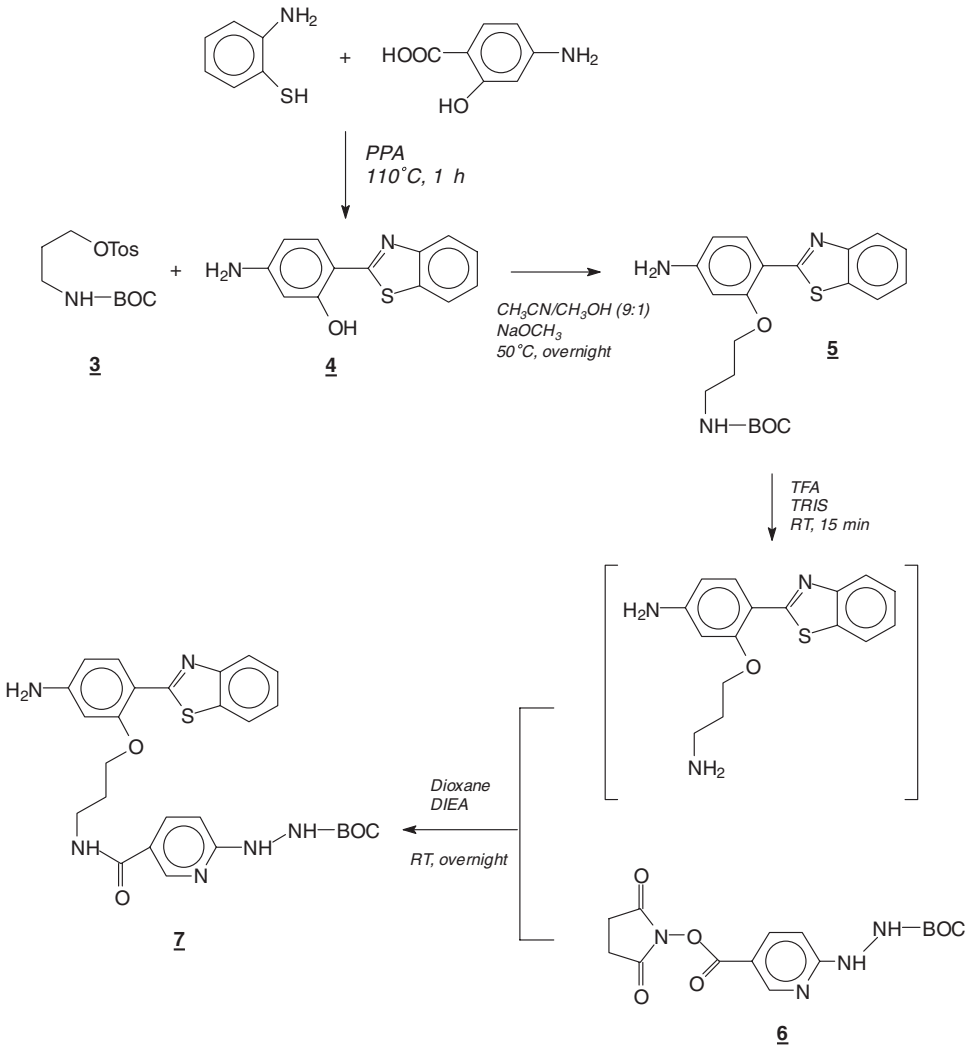
Figure 4. (A) Single ion mass chromatogram (ES^-, 583.406–583.474 Da); (B) radiometric chromatogram obtained by LC-MS analysis of ^{99m}Tc-2-[4′-(*S*-benzyl-mercaptoacetyl-l-aspartyl(tBu)-glycinamido)phenyl]-1,3-benzothiazole (^{99m}Tc-**2**); (C) theoretical molecular ion mass of ^{99m}Tc-**2**; and (D) background subtracted mass spectrum of the peak at 11.38 min. HPLC conditions: XTerra™ MS C18 column (3.5 μm, 50 mm × 2.1 mm) eluted with gradient mixtures of CH_3CN and 0.05 M ammonium acetate (0 min: 0:100 v/v, 20 min: 90:10 v/v, 25 min: 90:10 v/v, 30 min: 0:100 v/v) at a flow rate of 0.3 ml/min.

mercaptotriamide tetraligand. The presence of a non-coordinated carboxylic group of the aspartyl moiety, which is almost fully ionized at pH 7, renders an overall charge of −2 to ^{99m}Tc-**2** at physiological pH.

For preparing ^{99m}Tc-**7**, 2-(2′-hydroxy-4′-aminophenyl)-1,3-benzothiazole was coupled to BOC-protected HYNIC (Scheme 2) via a 3-aminopropyl spacer.

^{99m}Tc-labelled HYNIC conjugates are known to possess polar properties, especially in the presence of a polar co-ligand[17] such as tricine. The spacer precursor N-BOC-3-amino-1-propyl-*p*-tosylate (**3**) and the HYNIC precursor (succinimidyl-6-N-BOC-hydrazinopyridine-3-carboxylic acid, **6**) were synthesized according to published data.[17,18] To allow conjugation of the HYNIC precursor **6** with **5**, the N-BOC protective group on the amine of the spacer of **5** was removed. Initially, this was attempted with a mixture of TFA, triisopropylsilane and a few drops of water, but analysis of the reaction product by reversed phase high-performance liquid chromatography (RP-HPLC) revealed that compound **5** was not stable in these conditions. Removal of the N-BOC group in the absence of water was more successful and resulted in a complete deprotection of the amine group of the spacer. Deprotected **5** was coupled to the succinimidyl activated HYNIC precursor in dioxane with diethylamine (DIEA) as base and two equivalents of the HYNIC ligand and yielded the desired conjugate **7**. Although **5** also bears an aromatic amine, coupling of the HYNIC precursor with this aromatic amine was not observed. This can be explained in view of the much lower nucleophilic character of the aromatic amine as compared with the aliphatic amine.

Deprotection of the N-BOC-protected HYNIC moiety in **7** was performed in an acidic dioxane solution following a described procedure,[19] which yielded the HCl salt of deprotected **7**. Labelling of HYNIC derivatized compounds with ^{99m}Tc requires the presence of a co-ligand with metal-binding donor atoms, as the HYNIC moiety as such does not provide a sufficient number of donor atoms for the formation of a stable complex with ^{99m}Tc.[20] In this study, tricine, ethylene diamine-*N*,*N*′-diacetic acid (EDDA) and a mixture of tricine and nicotinic acid have been used as different co-ligands. With tricine as the co-ligand, the labelling reaction yielded 70% of ^{99m}Tc-tricine-**7** (Figure 3(B)). Yield of the labelling reaction in the presence of a mixture of tricine and nicotinic acid was 77% and in the presence of EDDA it was 52%. The RP-HPLC chromatograms show that ^{99m}Tc-EDDA-**7** (Rt of approximately 15 min) is more lipophilic than ^{99m}Tc-tricine/nicotinic acid-**7** and ^{99m}Tc-tricine-**7** (both have an Rt of approximately 13 min). As the goal was to obtain a more



Scheme 2. Synthesis of 2-[2′-(N-BOC-6-hydrazinonicotinamido-3-propoxy)-4′-aminophenyl]-1,3-benzothiazole (**7**).

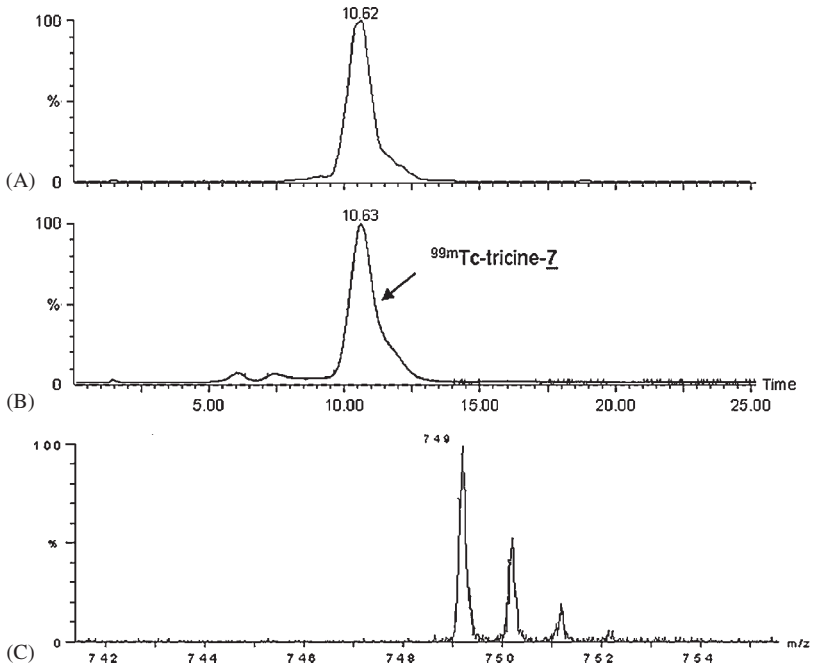


Figure 5. (A) Single ion mass chromatogram (ES^+, 749.084–749.312 Da); (B) radiometric chromatogram obtained by LC-MS analysis of ^{99m}Tc-tricine-2-[2′-(N-BOC-6-hydrazinonicotinamido-3-propoxy)-4′-amino]phenyl-1,3-benzothiazole (^{99m}Tc-tricine-**7**); and (C) background subtracted mass spectrum of the peak at 10.63 min. HPLC conditions: XTerra™ MS C18 column (3.5 μm, 50 mm × 2.1 mm) isocratic eluted with a mixture of CH_3CN and 0.05 M ammonium acetate (25:75 v/v) at a flow rate of 0.3 ml/min.

hydrophilic complex, we did not focus further on the technetium-99m complex using EDDA as co-ligand. Although the labelling yield using tricine/nicotinic acid as co-ligand was somewhat higher than with tricine alone (77 versus 70%), the labelling procedure is more complex and requires a boiling step. Therefore, we only used ^{99m}Tc-tricine-**7** for further evaluation.

Figure 5(B) shows the radiometric signal of the radio-LC-MS analysis of the reaction mixture after labelling of deprotected **7** with ^{99m}Tc with tricine as the co-ligand. The peak at 10.63 min is supposed to be the intended radiolabelled compound ^{99m}Tc-tricine-**7**. In Figure 5(C), the background subtracted mass spectrum of the peak eluting at 10.63 min is depicted and the mass obtained in this spectrum is 749 Da. Figure 5(A) depicts the single ion mass over the mass range 749.084–749.312 Da. There has been a lot of speculation and different hypotheses have been proposed about the structure of Tc-HYNIC-co-ligand complexes without a clear conclusion.[21] Recent literature reports suggest that with tricine as co-ligand, Tc-HYNIC/tricine complexes can contain one or two tricine molecules, depending on the presence or not of additional donor atoms at a suitable position in the conjugate.[22] The radio-LC-MS result of ^{99m}Tc-tricine-**7** (molecular mass of 749 Da) is not in agreement with a structure containing two tricine molecules, which would result in a mass of 884 Da. The mass found for ^{99m}Tc-tricine-**7** may correspond with the disodium salt of a complex in which a Tc-ion is bound to one molecule of **7** and one molecule of tricine with the loss of seven protons.

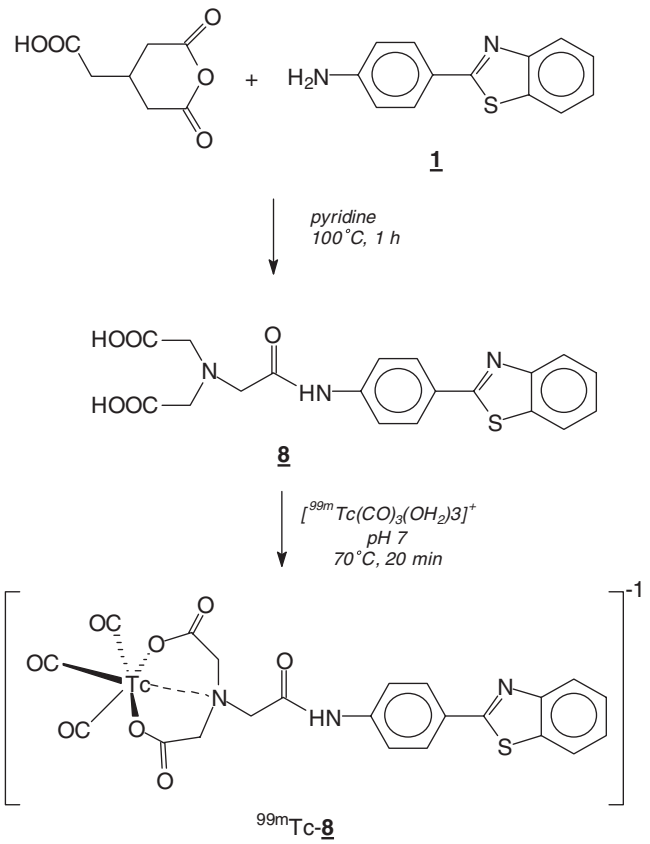
The synthesis of 2-[4′-(*N*,*N*-diacetic acid)-*N*-acetamidophenyl]-1,3-benzothiazole (**8**) was carried out starting from nitrilotriacetic acid anhydride, which was formed *in situ* by mixing acetic acid anhydride and nitrilotriacetic acid in pyridine[23] (Scheme 3).

For ^{99m}Tc-labelling of **8** we used the tricarbonyl method in which $[^{99m}Tc(CO)_3(OH_2)_3]^+$ is reacted with a ligand that has in its structure preferentially three suitable metal-binding donor atoms (such as an amine nitrogen or a carboxylate oxygen) each separated by two or three atoms. Such donor atoms can easily replace the three loosely bound water molecules of the $[^{99m}Tc(CO)_3(OH_2)_3]^+$ precursor, resulting in the formation of two 5- (or 6-) rings. The iminodiacetic acid (IDA) chelating group in compound **8** contains two carboxylate oxygen atoms and one amine nitrogen atom, which function as donor atoms. Their binding results in a negatively charged ^{99m}Tc-tricarbonyl-IDA complex. Labelling of **8** with a ^{99m}Tc-tricarbonyl core starting from a Isolink™ (Mallinckrodt Medical B.V., Pettten, The Netherlands) kit or using the CO-bubbling method[24] yielded in both cases a Tc complex with identical Rt on RP-HPLC. Because of the alkaline pH of the $[^{99m}Tc(CO)_3(OH_2)_3]^+$ solution, it was necessary to adjust its pH to 7 with 1 M HCl before precursor **8** was added to the radioactive solution because of degradation of **8** at higher pH values. The yield of the labelling reaction at pH 7 was 64% (Figure 3(C)).

Figure 6(B) shows the radiometric signal of the radio-LC-MS analysis of the reaction mixture after labelling of **8** with $^{99m}Tc(CO)_3$. The peak at 12.01 min is supposed to be the intended radiolabelled compound ^{99m}Tc-**8**. In Figure 6(C), the background subtracted mass spectrum shows a mass of 579 Da for this compound, which corresponds to the theoretical molecular ion mass for the supposed structure of ^{99m}Tc-**8** (Figure 6(D)). Figure 6(A) depicts the single ion mass chromatogram over the mass range 579.523–580.051 Da. These data strongly support the assumption that the peak eluting at 12.01 min in the radiometric channel corresponds to a structure as shown in Scheme 3. In such a $Tc(CO)_3$ complex, Tc has a valence of +1, whereas two protons of the IDA moiety are lost, resulting in an overall anionic complex (charge −1).

Partition coefficients

Depending on the functional groups of the BCL and the oxidation state of technetium in the Tc complex, the new ^{99m}Tc-



Scheme 3. Synthesis and labelling with a ^{99m}Tc-tricarbonyl core of 2-[4′-(N,N-diaceticacid)-N-acetamidophenyl]-1,3-benzothiazole (**8**).

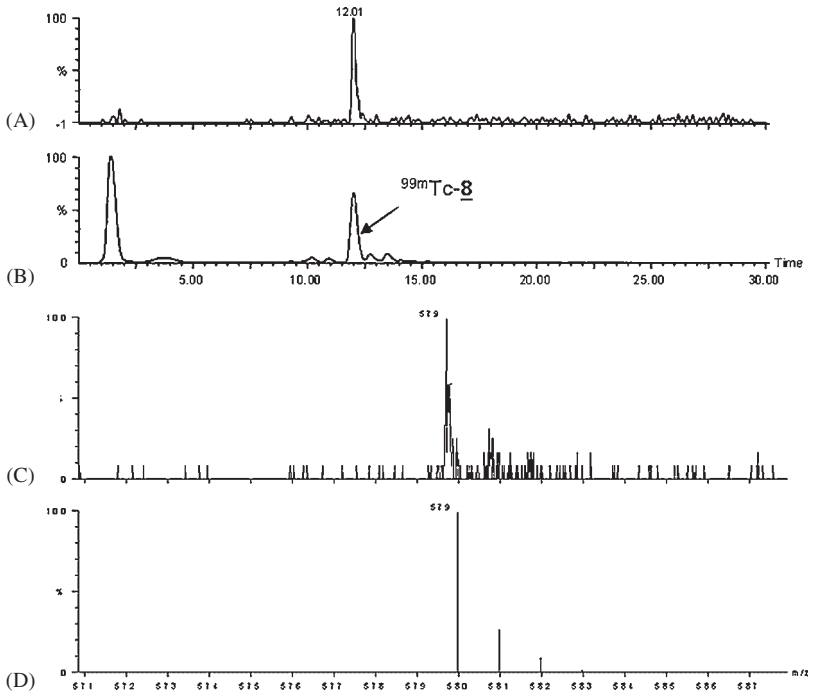


Figure 6. (A) Single ion mass chromatogram (ES$^-$, 579.523–580.051 Da); (B) radiometric chromatogram obtained by LC-MS analysis of ^{99m}Tc-2-[4′-(*N*,*N*-diacetic acid)-*N*-acetamidophenyl]-1,3-benzothiazole (^{99m}Tc-**8**); (C) background subtracted mass spectrum of the peak at 12.01 min; and (D) theoretical molecular ion mass of ^{99m}Tc-**8**. HPLC conditions: XTerra^{TM} MS C18 column (3.5 μm, 50 mm × 2.1 mm) eluted with gradient mixtures of CH_3CN and 0.05 M ammonium acetate (0 min: 0:100 v/v, 20 min: 90:10 v/v, 25 min: 90:10 v/v, 30 min: 0:100 v/v) at a flow rate of 0.3 ml/min.

labelled compound is negatively charged or neutral. The new tracer agents developed for this study should preferentially be hydrophilic as they have been designed for detection of SA, for which renal clearance is an important requirement. The log *P* values were found to be -0.49 ± 0.02 for ^{99m}Tc-**2**, which is rather hydrophilic, 0.81 ± 0.01 for ^{99m}Tc-tricine-**7** and 1.1 ± 0.02 for ^{99m}Tc-**8**. Only ^{99m}Tc-**2** has a value in accordance with a relatively hydrophilic compound, whereas the log *P* values of ^{99m}Tc-tricine-**7** and ^{99m}Tc-**8** indicate that these two radiolabelled compounds are rather hydrophobic.

Biodistribution studies

The results of the biodistribution studies of the technetium-99m-labelled phenylbenzothiazoles **2**, **7** and **8** in normal mice at 2 and 60 min p.i. are summarized in Tables 1–3. Although the nature of the technetium-binding ligand and of the final ^{99m}Tc complexes in these ^{99m}Tc-labelled phenylbenzothiazoles is quite different, their biological behaviour is rather similar.

Surprisingly, none of the compounds is excreted efficiently through the renal system. The kidney value at 2 min p.i. varies from 4 to 9% ID. These low values are solely the result of a low extraction efficiency and not of a rapid transit into the urine as the urinary activity at 60 min p.i. is almost negligible. The assumption that the presence of negative charges on the complex at physiological pH and thus of more polar characteristics would promote extraction and excretion by the kidneys was found invalid.

Moreover, the three ^{99m}Tc-labelled phenylbenzothiazoles are efficiently taken up by the liver and excreted into the intestines. Liver uptake (in % ID) at 2 min p.i. varies from 48% (^{99m}Tc-tricine-**7**) to 77% (^{99m}Tc-**8**) and decreases at 60 min p.i. to values between 20% (^{99m}Tc-**2**) and 43% (^{99m}Tc-**8**). The activity in the intestines at 60 min p.i. varies between 39% (^{99m}Tc-tricine-**7**) and 73% (^{99m}Tc-**2**). The most polar complex, i.e. ^{99m}Tc-**2** ($\log P = -0.49$), is concentrated most efficiently and rapidly in the hepatobiliary system (activity in liver+intestines ranging from 75% at 2 min p.i. to 93% at 60 min p.i.). The clearance from blood and liver is efficient and rapid for ^{99m}Tc-**2** and ^{99m}Tc-**8**, but more than three times faster for ^{99m}Tc-**2** than for ^{99m}Tc-**8**. The HYNIC derivative **7**, labelled with ^{99m}Tc in the presence of tricine, shows a slower clearance. The general assumption that polar compounds are extracted from the blood mainly by the kidneys and lipophilic compounds by the liver is clearly not valid for the ^{99m}Tc-labelled phenylbenzothiazoles of this study. The non-expected biodistribution results might be due to a rapid metabolism of the compounds.

Experimental section

Materials and methods

All reagents and solvents used in synthesis were obtained from Acros Organics (Geel, Belgium), Aldrich, Fluka or Sigma (Sigma-Aldrich, Bornem, Belgium) and were used without further purification. Purification of reaction mixtures was done by column chromatography using silica gel with a particle size varying between 0.04 and 0.063 mm (230–400 Mesh) (MN Kieselgel 60 M, Macherey-Nagel, Düren, Germany) as the stationary phase. The structure of the synthesized products was confirmed with ^{1}H-nuclear magnetic resonance (NMR) spectroscopy on a Gemini 200 MHz spectrometer (Varian, Palo Alto, CA, USA). Chemical shifts are reported as δ-values (parts per million) relative to tetramethylsilane ($\delta = 0$). Coupling constants are reported in Hertz. Splitting patterns are defined by s (singlet), d (doublet), dd (double doublet), t (triplet) and m (multiplet). Melting points (Mps) were determined with an IA9100 digital Mp apparatus (Electrothermal, Essex, UK) in open capillaries and are reported uncorrected.

Generator eluate containing ^{99m}Tc in the form of pertechnetate was obtained from an Ultratechnekow generator (Tyco Healthcare, Petten, The Netherlands). $NH_4^{99}TcO_4$ and Isolink™ labelling kits were a gift from Tyco Healthcare.

Quantitative determination of radioactivity in samples was done using an automatic γ-counter coupled to a multi-channel analyser (Wallac 1480 Wizard® 3″, Wallac, Turku, Finland). The values were corrected for physical decay and background radiation.

RP-HPLC runs were performed with a system consisting of a TSP SpectraSeries P4000 quaternary pump (Thermo Separation Products, San Jose, CA, USA) connected with a TSP SpectraSeries UV 100 detector (Thermo Separation Products) and a 3-in NaI(Tl) crystal connected with a Medi-Lab Select SC7II single channel analyzer (Medi-Lab Select, Mechelen, Belgium). The output signal was recorded and analyzed using a RaChel data acquisition system (Lablogic, Sheffield, UK).

Radio-HPLC combined with mass spectrometry (radio-LC-MS) was performed on a system consisting of a Waters Alliance 2690

Table 1. Tissue distribution of ^{99m}Tc-2-[4′-(*S*-benzyl-mercaptoacetyl-l-aspartyl(tBu)-glycinamido)phenyl]-1,3-benzothiazole (^{99m}Tc-**2**) after i.v. injection in normal mice at 2 and 60 min p.i. ($n = 4$ at each time point)

	% ID ± s.d.		% ID/g tissue ± s.d.	
	2 min p.i.	60 min p.i.	2 min p.i.	60 min p.i.
Urine	0.1 ± 0.0	0.8 ± 0.3		
Kidneys	7.6 ± 0.1	2.3 ± 0.5	11.0 ± 0.5	3.3 ± 0.6
Liver	71.7 ± 1.1	20.4 ± 11.5	31.4 ± 3.2	8.1 ± 4.4
Intestines	3.3 ± 1.2	72.7 ± 12.6		
Spleen+pancreas	0.4 ± 0.1	0.1 ± 0.0	1.1 ± 0.2	0.2 ± 0.1
Lungs	0.6 ± 0.3	0.1 ± 0.1	2.1 ± 0.6	0.4 ± 0.3
Heart	0.3 ± 0.1	0.1 ± 0.0	1.8 ± 0.6	0.4 ± 0.1
Stomach	0.4 ± 0.1	0.5 ± 0.4	0.4 ± 0.1	0.6 ± 0.5
Cerebrum	0.09 ± 0.03	0.06 ± 0.02	0.28 ± 0.08	0.20 ± 0.10
Cerebellum	0.07 ± 0.02	0.05 ± 0.02	0.63 ± 0.19	0.50 ± 0.14
Blood	8.6 ± 1.8	0.9 ± 0.4	3.1 ± 0.7	0.3 ± 0.2

Table 2. Tissue distribution of ^{99m}Tc-tricine-2-[2′-(N-BOC-6-hydrazinonicotinamido-3-propoxy)-4′-amino]phenyl-1,3-benzothiazole (^{99m}Tc-tricine-**7**) after i.v. injection in normal mice ($n = 4$) at 2 and 60 min p.i. ($n = 4$ at each time point)

	% ID ± s.d.		% ID/g tissue ± s.d.	
	2 min p.i.	60 min p.i.	2 min p.i.	60 min p.i.
Urine	0.4 ± 0.3	2.0 ± 0.1		
Kidneys	3.5 ± 0.3	2.6 ± 0.3	5.4 ± 0.4	3.9 ± 0.2
Liver	48.0 ± 1.8	32.6 ± 3.6	19.3 ± 2.7	13.3 ± 2.0
Intestines	5.0 ± 0.4	39.4 ± 3.1		
Spleen + pancreas	0.7 ± 0.1	0.3 ± 0.1	1.6 ± 0.2	0.9 ± 0.2
Lungs	1.3 ± 0.3	0.9 ± 0.2	5.1 ± 0.6	3.2 ± 0.5
Heart	0.4 ± 0.1	0.2 ± 0.0	2.5 ± 0.2	1.4 ± 0.2
Stomach	0.6 ± 0.2	1.0 ± 0.5	0.9 ± 0.2	1.6 ± 1.1
Cerebrum	0.12 ± 0.03	0.05 ± 0.01	0.38 ± 0.07	0.17 ± 0.05
Cerebellum	0.07 ± 0.02	0.03 ± 0.01	0.59 ± 0.19	0.23 ± 0.06
Blood	26.1 ± 1.4	14.9 ± 0.9	9.3 ± 0.5	5.6 ± 0.6

Table 3. Tissue distribution of ^{99m}Tc-2-[4′-(*N*,*N*-diacetic acid)-*N*-acetamidophenyl]-1,3-benzothiazole (^{99m}Tc-**8**) after i.v. injection in normal mice ($n = 4$) at 2 and 60 min p.i. ($n = 4$ at each time point)

	% ID ± s.d.		% ID/g tissue ± s.d.	
	2 min p.i.	60 min p.i.	2 min p.i.	60 min p.i.
Urine	0.1 ± 0.1	0.3 ± 0.1		
Kidneys	8.8 ± 1.0	3.3 ± 0.2	13.0 ± 0.2	5.1 ± 0.4
Liver	77.0 ± 1.0	43.3 ± 3.0	36.5 ± 1.6	23.4 ± 1.6
Intestines	2.6 ± 1.3	46.3 ± 4.9		
Spleen + pancreas	0.2 ± 0.1	0.1 ± 0.1	0.7 ± 0.3	0.3 ± 0.1
Lungs	0.4 ± 0.1	0.2 ± 0.0	1.7 ± 0.4	0.6 ± 0.1
Heart	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.2	0.4 ± 0.2
Stomach	0.2 ± 0.1	3.0 ± 3.8	0.4 ± 0.3	5.0 ± 5.4
Cerebrum	0.02 ± 0.00	0.01 ± 0.00	0.07 ± 0.02	0.03 ± 0.01
Cerebellum	0.02 ± 0.01	0.01 ± 0.00	0.12 ± 0.05	0.08 ± 0.06
Blood	4.9 ± 0.9	1.6 ± 0.1	1.7 ± 0.4	0.6 ± 0.0

separation module (Waters, Milford, MA, USA) coupled to a reverse phase XTerra™ MS C18 3.5 μm column (2.1 mm × 50 mm) (Waters). The column eluate was first analyzed by a UV spectrometer (Waters 2487 dual wavelength absorbance detector), subsequently by radiometric detection consisting of a 3-in NaI(Tl) crystal coupled to a single channel analyzer (The Nucleus, Oak Ridge, TE, USA) and finally by a time-of-flight mass spectrometer (LCT, Micromass, Manchester, UK) equipped with an electrospray ionization (ESI) source. Acquisition and processing of data were performed with Masslynx software (version 3.5).

Animal studies were performed according to the Belgian code of practice for the care and use of animals, after approval from the university ethics committee for animals.

Synthesis of 2-[4′-(*S*-benzyl-mercaptoacetyl-l-aspartyl(tBu)-glycinamido)phenyl]-1,3-benzothiazole (2)

*2-(4′-Aminophenyl)-1,3-benzothiazole (**1**). Method A*

2-Aminothiophenol (6.26 g, 50 mmol) and *p*-aminobenzoic acid (6.86 g, 50 mmol) were added to 80 g of polyphosphoric acid and the mixture was stirred at 180°C for 4 h. The reaction mixture was allowed to cool down to RT and poured into a 10% (m/V) solution of Na_2CO_3. The white precipitate was filtered off and dried in a vacuum oven. Crystallization from methanol yielded 7.24 g (32 mmol, 64%) of **1** as light yellow crystals.

^{1}H-NMR (DMSO, 200 MHz): δ 5.8 (2H, s, NH_2); δ 6.68 (2H, d, 3′-H 5′-H); δ 7.32 (1H, t, 6-H); δ 7.45 (1H, t, 5-H); δ 7.76 (2H, d, 2′-H 6′-H); δ 7.89 (1H, d, 4-H); δ 7.98 (1H, d, 7-H). Mass: $[M-H]^-$ 225 (calculated: 225). Mp: 147.5–150°C.

*2-[4′-(S-benzyl-mercaptoacetyl-l-aspartyl(tBu)-glycinamido)phenyl]-1,3-benzothiazole (**2**)*

To a solution of *S*-benzyl mercaptoacetyl-l-aspartyl(tBu)-glycine (410 mg, 1 mmol) in 10 ml of CH_3CN, **1** (226 mg, 1 mmol) was added together with 1-hydroxybenzotriazole hydrate (HoBt) (141 mg, 1 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC · HCl) (192 mg, 1 mmol). The reaction mixture was stirred at RT overnight and the precipitate was filtered off and washed with CH_3CN. The residual yellow oil was dissolved in CH_2Cl_2 and the solution was washed with 10% $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and evaporated to dryness. The residue was purified with silica column chromatography using gradient mixtures of CH_2Cl_2 and CH_3OH (up to 5%) yielding 80 mg of **2** (0.13 mmol, 13%).

^{1}H-NMR ($CDCl_3$): δ 1.46 (9H, s, tBu); δ 2.69 and 2.88 (2H, 2 × dd, CH–COOtBu); δ 3.20 (2H, s, CO–CH_2–SBz); δ 3.76 (2H, s, S–CH_2–Bz); δ 4.01 and 4.18 (2H, 2 × dd, CO–CH_2–NH); δ 4.65 (1H,

m, CO–CH); δ 7.04 (1H, t, CH_2–NH–CO); δ 7.28 (5H, s, C_6H_5–CH_2); δ 7.36 (1H, t, 5-H); δ 7.48 (1H, t, 6-H); δ 7.57 (1H, d, CH–NH–CO); δ 7.80 (2H, d, 2′-H 6′-H); δ 7.87 (1H, d, 7-H); δ 8.01 (2H, d, 3′-H 5′-H); δ 8.04 (1H, d, 4-H); δ 8.72 (1H, s, Φ-NH–CO). Mass: $[M+H]^+$ 619 (calculated: 619). Mp: 141.1–143.6°C.

Synthesis of 2-[2′-(N-BOC-6-hydrazinonicotinamido-3-propoxy)-4′-amino]phenyl-1,3-benzothiazole (7)

*2-(2′-Hydroxy-4′-aminophenyl)-1,3-benzothiazole (**4**)*

Compound **4** was synthesized following method A starting from 4-aminosalicylic acid (7.70 g, 50 mmol) and 2-aminothiophenol (6.26 g, 50 mmol) and yielding 4 g (17 mmol, 33%) of **4** as a slightly yellow solid.

^{1}H-NMR (DMSO): δ 5.97 (2H, s, NH_2); δ 6.18 (1H, d, 3′-H); δ 6.26 (1H, dd, 5′-H); δ 7.34 (1H, t, 5-H); δ 7.45 (1H, t, 6-H); δ 7.63 (1H, d, 6′-H); δ 7.89 (1H, dd, 7-H); δ 8.03 (1H, dd, 4-H); δ 11.76 (1H, s, OH). Mass: $[M-H]^-$ 241 (calculated: 241). Mp: 186.9–187°C.

*2-[2′-(N-BOC-3-aminopropoxy)-4′-aminophenyl]-1,3-benzothiazole (**5**)*

Compound **4** (4 g, 17 mmol) was dissolved in 50 ml of CH_3CN/CH_3OH (9:1 v/v) and 50 mmol of freshly prepared $NaOCH_3$ was added. To this solution, N-BOC-3-amino-1-propyl-*p*-tosylate (**3**)[13] (11.86 g, 36 mmol) dissolved in 50 ml CH_3CN/CH_3OH (9:1 v/v) was added dropwise and the mixture was stirred overnight at 50°C. After cooling down to RT, water was added and the mixture was extracted three times with CH_2Cl_2. The residue was purified with silica column chromatography using gradient mixtures of CH_2Cl_2 and CH_3OH (up to 2%) to yield 3.32 g of **5** (8.3 mmol, 49% yield).

^{1}H-NMR (CD_3OD): δ 1.38 (9H, s, tBu); δ 2.17 (2H, m, CH_2–CH_2–CH_2); δ 3.40 (2H, m, CH_2–NH); δ 4.20 (2H, m, O–CH2); δ 5.92 (2H, s, NH_2); δ 6.33 (1H, d, 5′-H); δ 6.35 (1H, d, 3′-H); δ 7.00 (1H, s, NH–CO); δ 7.30 (1H, t, 5-H); δ 7.44 (1H, t, 6-H); δ 7.88 (1H, d, 7-H); δ 7.98 (1H, d, 4-H); δ 8.13 (1H, d, 6′-H). Mass: $[M+H]^+$ 400 (calculated: 400). Mp: 170.2–171.6°C.

*2-[2′-(N-BOC-6-hydrazinonicotinamido-3-propoxy)-4′-amino]phenyl-1,3-benzothiazole (**7**)*

A solution of **5** (100 mg, 0.25 mmol) in a mixture of 19.5 ml TFA and 0.5 ml of triisopropylsilane (TRIS) was stirred at RT for 15 min. TFA was removed under reduced pressure and the residue was co-evaporated four times with hexane. The residue was taken up in CH_2Cl_2 (50 ml). The solution was extracted twice with 50 ml 10% (m/V) $NaHCO_3$, washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue (100 mg, 0.334 mmol) was dissolved in 20 ml of dioxane and DIEA (60 µl, 100 mmol) and succinimidyl-6-BOC-hydrazinopyridine-3-carboxylic acid (**6**)[13] (235 mg, 0.67 mmol) were added. The mixture was stirred overnight at RT. The organic solvent was evaporated and the residue was purified by silica column chromatography using 5% CH_3OH in CH_2Cl_2 as the eluent to yield 80 mg of green–yellow solid **7** (0.15 mmol, 45%).

^{1}H-NMR (CD_3OD): δ 1.47 (9H, s, tBu); δ 2.28 (2H, m, CH_2–CH_2–CH_2); δ 3.71 (2H, m, CH_2–NH); δ 4.22 (2H, m, O–CH_2); δ 6.37 (1H, d, 5-HPh); δ 6.40 (1H, d, 3-HPh); δ 6.65 (1H, d, 5-HYNIC); δ 6.92 (1H, s, NH–CO); δ 7.28 (1H, t, 5-HBe); δ 7.42 (1H, t, 6-HBe); δ 7.87 (2H, d, 4-HBe 7-HBe); δ 7.95 (1H, dd, 4-HYNIC); δ 8.09 (1H, dd, 6-HPh); δ 8.55 (1H, d, 2-HYNIC). Mass: $[M+H]^+$ 535 (calculated: 535). Mp: 147.4–149.5°C.

Synthesis of 2-[4′-(*N*,*N*-diacetic acid)-*N*-acetamidophenyl]-1,3-benzothiazole (8)

A solution of nitrilotriacetic acid (129 mg, 0.67 mmol) in 1.75 ml pyridine was heated at 50°C for 10 min. Acetic acid anhydride (0.12 ml, 1.2 mmol) was added and the mixture was heated further at 100°C for 30 min. The mixture was allowed to cool to 50°C and **1** (226 mg, 1 mmol) was added. The mixture was heated again at 100°C for 1 h and, after cooling, pyridine was evaporated under reduced pressure. The pH was adjusted to 9.5 by the addition of 2 M NH_4OH. The mixture was extracted three times with diethylether and the organic layer was discarded. After acidification of the water layer to pH 2 with 6 M HCl, the precipitate was filtered off and crystallized from ethanol/water (50:50 v/v) to yield 50 mg of **8** (0.13 mmol, 19.4%).

^{1}H-NMR (DMSO): δ 3.51 and 3.55 (6H, 3 × s, 3 × CH_2); δ 7.42 (1H, t, 5-H); δ 7.53 (1H, t, 6-H); δ 7.69 (1H, s, NHCO); δ 7.86 (2H, d, 3′-H 5′-H); δ 8.02 (2H, d, 2′-H 6′-H); δ 8.10 (2H, d, 4-H 7-H); δ 10.92 (1H, s, COOH). Mass: $[M-H]^-$ 398 (calculated: 398). Mp: 183.9–185°C.

Deprotection and labelling of 2

In a labelling vial were successively mixed 100 µl of a 1 mg/ml solution of **2** in CH_3CN, 500 µl of 0.5 M phosphate buffer pH 9, 250 µl of a 40 mg/ml solution of NaKtartrate in water, 25 µl of a 4 mg/ml solution of $SnCl_2 \cdot 2H_2O$ in 0.05 M HCl and about 600 MBq $^{99m}TcO_4^-$ in 1 ml saline. The mixture was heated in a boiling water bath for 15 min and RP-HPLC was performed after cooling to RT. The labelling yield was 80%.

Two-step procedure for deprotection and labelling of 7

For deprotection purpose, a solution of 6 mg of **7** in a mixture of 2 ml of dioxane and 2 ml of HCl/dioxane (prepared by bubbling HCl gas at a moderate rate through dioxane for about 10 min) was stirred at RT for 4 h after which the precipitate was filtered off and washed several times with ether. The precipitate (the HCl salt of deprotected **7**) was used for labelling without further purification.

For labelling with ^{99m}Tc three different co-ligands were used. The first attempt (A) used tricine as co-ligand. To a labelling vial were successively added 100 µl of a 1 mg/ml solution of deprotected **7** (HCl salt) in ethanol/water (50:50 v/v), 200 µl of 0.5 M phosphate buffer pH 7, 15 µl of a 100 mg/ml solution of tricine in water, 12.5 µl of a 4 mg/ml solution of $SnCl_2 \cdot 2H_2O$ in 0.05 M HCl and about 600 MBq $^{99m}TcO_4^-$ in 1 ml of saline. The mixture was incubated at RT for 15 min. The second attempt (B) used EDDA as co-ligand and was performed in the same way as A, but 5 mg EDDA was used instead of 1.5 mg tricine. The third attempt (C) used a mixture of tricine and nicotinic acid as co-ligand and was also performed in the same manner as A, but 15 mg tricine and 2 mg nicotinic acid were used and the mixture was heated in a boiling water bath for 15 min. RP-HPLC was performed after cooling to RT. The labelling yield varied from 52% (method B) to 77% (method C).

Labelling of 8

When using an Isolink™ kit, about 600 MBq of $^{99m}TcO_4^-$ in a maximum of 1 ml saline was added to a labelling kit, which was heated in a boiling water bath for 20 min. The pH of the reaction mixture was adjusted to 7 with 1 M HCl and 250 µl of this neutralized solution was added to 100 µl of a 1 mg/ml solution of **8** in $CH_3CN/CH_3OH/H_2O$ (3:3:4 v/v). The mixture was heated at 70°C for 20 min and RP-HPLC analysis was performed after cooling to RT.

As an alternative, $[^{99m}Tc(I)(CO)_3(OH_2)_3]^+$ was prepared by the CO-bubbling method.[20] Briefly, CO gas was bubbled through a mixture containing 4.5 mg Na_2CO_3, 20–40 mg $NaBH_4$ and 20 mg NaKtartrate. Then, about 600 MBq $^{99m}TcO_4^-$ in a maximum of 1 ml saline was added and the mixture was heated at 75°C for 20 min. The pH of the resulting $[^{99m}Tc(I)(CO)_3(OH_2)_3]^+$ solution was adjusted to pH 7 by the addition of approximately 250 µl of a 1 M HCl solution and 250 µl of this mixture was added to 100 µl of a 1 mg/ml solution of **8** in $CH_3CN/CH_3OH/H_2O$ (3:3:4 v/v). The mixture was heated at 70°C for 20 min and RP-HPLC analysis was performed after cooling to RT. The mean labelling yield for both procedures was 64%.

Analysis of ^{99m}Tc-labelled compounds with radio-LC-MS

Radio-LC-MS was carried out using an XTerra™ MS C18 column (3.5 µm, 50 mm × 2.1 mm, Waters) with gradient mixtures of CH_3CN and 0.05 M ammonium acetate as eluent at a flow rate of 0.3 ml/min.

Labelling of 2 and 7 for LC-MS analysis

Deprotection and labelling of **2** and **7** were performed as described earlier, but to the solution of $^{99m}TcO_4^-$ 0.1 ml of a solution of 150 µg $NH_4^{99}TcO_4$ in 10 ml of water (1.5 µg Tc) was added. In addition, 25 and 50 µl of the 4 mg/ml solution of $SnCl_2 \cdot 2H_2O$ in 0.05 M HCl were used for **2** and **7**, respectively. For ^{99m}Tc-**7**, mass determination was only carried out after labelling with tricine as co-ligand.

Labelling of 8 for LC-MS analysis

To perform a carrier-added labelling, the amount of reducing agent in the Isolink™ kit is too low. To obtain mass data of a ^{99m}Tc-tricarbonyl labelled compound, it is therefore necessary to perform the preparation of the $[^{99m}Tc(CO)_3(OH_2)_3]^+$ precursor via the CO-bubbling method. The amount of $NaBH_4$ was increased because of the high amount of technetium-(99m + 99) in the reaction mixture. To the solution of 600 MBq $^{99m}TcO_4^-$ in 0.9 ml saline, 0.1 ml of a solution of 150 µg $NH_4^{99}TcO_4$ in 10 ml of water was added (1.5 µg Tc). The rest of the procedure was followed as described earlier.

Partition coefficients

The lipophilicity of the RP-HPLC isolated ^{99m}Tc complexes was determined using a modification of the method described by Yamauchi and co-workers.[25] To a test tube containing 2 ml of 1-octanol and 2 ml of 0.025 M phosphate buffer pH 7.4, 25 µl of the RP-HPLC isolated ^{99m}Tc complex was added. The test tube was vortexed at RT for 3 min followed by centrifugation at 2700 g for 10 min. Aliquots of 60 and 500 µl were drawn from the 1-octanol and buffer phases, respectively, and weighed. The radioactivity in each aliquot was counted using a γ-counter and the partition coefficient *P* was calculated using the following equation:

$$P = \frac{\text{cpm/ml octanol}}{\text{cpm/ml buffer}}$$

with cpm being counts per minute. Experiments were performed in triplicate.

Biodistribution studies

The peak containing the desired labelled compound was isolated on RP-HPLC and diluted with saline to a concentration of 150 kBq/ml. Male NMRI mice were sedated with an intraperitoneal injection of 0.1 ml Hypnorm® (fentanyl citrate 63 µg/ml + fluanisone 2 mg/ml) and injected with 100 µl of the compound solution via a tail vein in a series of eight mice. The mice were sacrificed by decapitation at 2 or 60 min p.i. ($n = 4$ at each time point). Blood was collected in a tared tube and weighed. All organs and other body parts were dissected and weighed and their activity was counted in a γ-counter. Results were corrected for background activity and are expressed as percentage of the injected dose per organ (% ID) or as percentage of the injected dose per gram tissue (% ID/g). To calculate the activity in the blood, blood mass was assumed to be 7% of the total body mass.

Conclusion

Three derivatives of the amyloid-binding agent ThT with a technetium-binding moiety have been successfully synthesized in sufficient yields and their identity and structure were confirmed. Deprotection and labelling of the studied compounds with technetium-99m were done using either direct labelling, exchange labelling or $Tc(CO)_3$ labelling, providing good labelling yields for all three compounds as confirmed by RP-HPLC. Results of radio-LC-MS analysis support the hypothesized structures of two of the complexes, whereas the structure of the Tc-HYNIC-tricine complex remains speculative. Despite the fact that adding a negative charge to the neutral phenylbenzothiazole core will have an unknown effect on the binding of the derivative to Aβ, all compounds were evaluated in normal mice. The results of the biodistribution studies of these ^{99m}Tc-labelled phenylbenzothiazoles indicate that none of these tracer agents has ideal biological characteristics. The target-to-background activity ratio in the abdominal organs would be unfavourable due to the pronounced uptake in liver and intestines. Further evaluation should investigate the potential usefulness for detection of SA in body parts other than the abdomen.

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References

[1] M. B. Pepys, Amyloidosis in *Oxford Textbook of Medicine*, Vol. 2 (Eds.: D. J. Weatherall, J. G. G. Ledingham, D. A. Warrell), Oxford University Press, Oxford, **1995**, pp. 1512–1524.
[2] A. S. Cohen, *Bull. Rheum. Dis.* **1991**, *40*, 1–12.

[3] G. Verdile, S. Fuller, C. S. Atwood, S. M. Laws, S. E. Gandy, R. N. Martins, *Pharmacol. Res.* **2004**, *50*, 397–409.
[4] M. B. Pepys, *Philos. Trans. R. Soc. B* **2001**, *356*, 203–211.
[5] J. D. Gillmore, *Br. J. Haematol.* **1997**, *99*, 245–256.
[6] M. M . Picken, *Curr. Opin. Nephrol. Hypertension* **2007**, *16*, 196–203.
[7] P. N. Hawkins, M. J. Myers, A. A. Epenetos, D. Caspi, M. B. Pepys, *J. Exp. Med.* **1988**, *167*, 903–913.
[8] M. R. H. Krebs, E. H. C. Bromley, A. M. Donald, *J. Struct. Biol.* **2005**, *149*, 30–37.
[9] C. A. Mathis, B. J . Bacskai, S. T. Kajdasz, M. E. McLellan, M. P. Frosch, B. T. Hyman, D. P . Holt, Y. Wang, G.-F. Huang, M. L. Debnath, W. E. Klunk, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 295–298.
[10] K. Verbeke, K. Snauwaert, B. J. Cleynhens, W. Scheers, A. M. Verbruggen, *Nucl. Med. Biol.* **2000**, *27*, 769–779.
[11] C. Decristoforo, S. J. Mather, W. Cholewinski, E. Donnemiller, G. Riccabona, R. Mancayo, *Eur. J. Nucl. Med.* **2000**, *27*, 1318–1325.
[12] R. Alberto, *Eur. J. Nucl. Med. Mol. I* **2003**, *30*, 1299–1302.
[13] T. Verduyckt, D. Kieffer, D. Huyghe, B. Cleynhens, K. Verbeke, A. Verbruggen, *J. Pharm. Biomed.* **2003**, *32*, 669–678.
[14] D. Vanderghinste, M. Van Eeckhoudt, C. Terwinghe, L. Mortelmans, G. M. Bormans, A. M. Verbruggen, *J. Pharm. Biomed.* **2003**, *32*, 679–685.
[15] D. Kieffer, B. Cleynhens, K. Verbeke, H. Vanbilloen, T. de Groot, C. Terwinghe, *J. Labelled Compd. Radiopharm.* **2004**, *47*, 199–208.
[16] G. Bormans, B. Cleynhens, T. J. de Groot, L. Mortelmans, A. Verbruggen, *J. Labelled Compd. Radiopharm.* **2003**, *46*, 575–585.
[17] C. Decristoforo, S. Mather, *Bioconjugate Chem.* **1999**, *10*, 431–438.
[18] D. Heerding, M. Rouse, M. A. Seefeld, Patent WO 2006/113837 77, **2006**.
[19] M. Abrams, M. Juweid, C. I. tenKate, D. A. Schwartz, M. M. Hauser, F. E. Gaul, *J. Nucl. Med.* **1990**, *31*, 2022–2028.
[20] C. Decristoforo, S. J. Mather, *Eur. J. Nucl. Med.* **1999**, *26*, 869–876.
[21] D. S. Edwards, S. Liu, J. A. Barrett, A. R. Harris, R. J. Looby, M. C. Ziegler, *Bioconjugate Chem.* **1997**, *8*, 146–154.
[22] R. C. King, M. B. U. Surfraz, S. C. C. Biagini, P. J. Blower, S. J. Mather, *Dalton Trans.* **2007**, *43*, 4998–5007.
[23] R. L. Folman, S. Marburg, W. J. Leanza, U.S. 5274122 A 19931228, **1993**.
[24] D. Rattat, B. Cleynhens, G. Bormans, C. Terwinghe, A. Verbruggen, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4192–4195.
[25] H. Yamauchi, J. Takahashi, S. Seri, H. Kawashima, H. Koike, M. Kato-Azuma in *Technetium and Rhenium in Chemistry and Nuclear Medicine*, Vol. 3 (Eds.: M. Nicolini, G. Bandoli, U. Mazzi), Cortina International, Verona, Italy, **1989**, pp. 475–502.