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Synthesis and metal complexation properties of Ph-DTPA and Ph-TTHA: novel radionuclide chelating agents for use in nuclear medicine

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We wish to report the synthesis and metal complexation properties of new radionuclide chelating agents for use in nuclear medicine. The strategy includes the facile preparation of rigid analogues of DTPA and TTHA possessing an aromatic ring. The aromatic structure used increased the stability of the complexes formed (pre-organization concept) and they are easily functionalised for attaching to any support. The poly(amino)poly(carboxylic) acids, Ph-DTPA (**5a**) and Ph-TTHA (**5b**) were obtained in five steps from phenylenediamine as the starting material with overall yields of 42 and 20%, respectively. The key step in this synthetic process is the preparation of tri- and tetra-amino compounds, **3a** and **3b**, respectively. In order to assess the ability of both ligands to complex with different metals (^{111}In, ^{153}Sm, ^{90}Y, ^{177}Lu, ^{213}Bi, ^{225}Ac), along with their suitability for use in nuclear medicine, we used a number of complementary tests. We were able to demonstrate the high complexation capacity of Ph-DTPA (**5a**) with a broad range of radionuclides in a slightly acidic medium. *In vitro* stability studies show the high stability of Ph-DTPA with ^{111}In in human serum, a necessary condition for all medical applications. The protonation constant (log K_i^H) of Ph-DTPA (**5a**) was determined by potentiometric methods.

Introduction

Radioimmunotherapy (RIT) is a developing and promising technique for the treatment of small tumors.[1] It consists of injecting patients with a radiopharmaceutical able to target and to selectively destroy tumor cells.[2] The radionuclides usually used are α or β^- emitters with a short half-life, coupled to the vector (an antibody or hapten) through a bifunctional chelating agent.[3–8] In order to deliver radionuclides to tumors whilst minimizing irradiation of healthy tissue,[9] the chelating agent must be able to form a stable complex in human serum. Classically used ligands are polyaminopolycarboxylic acid compounds with a sufficient number of O- and N-donor groups to satisfy the high coordination number of the radionuclides, which generally varies from 8 to 10.[10–15] Diethylenetriaminepentaacetic acid (DTPA) is particularly effective and this molecule is widely used for radiodiagnostic purposes in immunoscintigraphy (IS).[16–21] While not as frequently used,[22] the homologous triethylenetetraaminehexaacetic acid (TTHA) is nonetheless an interesting ligand and forms chelates with high complexation constants with a great number of lanthanides.[23,24]

It has been established that in order to improve the stability of the complexes formed, it is necessary to use ligands with a semi-rigid or rigid structure (pre-organization concept).[25–27] This is why nowadays many research projects actually focus on the synthesis of chelating agents with cyclohexane, cyclopentane, or macrocyclic structures.[28–35] Few teams however work on the development of ligands with a benzene skeleton.[36–39] Mederos *et al.* and Tanaka *et al.* have shown that aromatic analogues of ethylenediaminetetraacetic acid (EDTA) are effective chelating agents, notably in acidic conditions.[40,41] Moreover, aromatic rings have the property of being easily functionalized with a nitro group, the precursor of an isothiocyanate function, which enables chelating agents to be coupled to antibodies or haptens.[42]

These considerations brought us to use a benzene ring for the synthesis of novel rigid analogues of DTPA and TTHA, which were termed Ph-DTPA and Ph-TTHA, respectively.[43,44] The complexation capacity of these compounds was tested on a range of radionuclides used in nuclear medicine. Tests were also performed to determine the stability of these complexes in human serum, an absolute requirement for potential use in IS and RIT. Furthermore, potentiometric titrations have been used to determine the protonation constants of Ph-DTPA (**5a**).

Results and discussion

Chemical synthesis

Ph-DTPA and Ph-TTHA were synthesized from *ortho*-phenylenediamine according to similar protocols (Scheme 1). Amide **1a** was prepared by monoacylation of the starting compound with DCC activated Cbz-glycine, according to the method described by Bermejo *et al.*[45] The carbobenzyloxy group was then cleaved off by palladium-catalyzed hydrogenolysis which generated diamine **2a** with a 98% yield.[46] The carbonyl group was then effectively reduced by the BH_3·DMS complex in THF.[47] The triamine thus obtained was isolated and purified as its chlorohydrate **3a** form through bubbling HCl gas in ethanol.[48] In order to synthesize tetraamine **3b**, Cbz-glycine was activated into an acid chloride, then opposed to *ortho*-phenylenediamine to obtain the original product **1c** quantitatively, contrary to classical coupling by DCC. It was not possible to deprotect diamine **1c** by hydrogenolysis in neutral medium with palladium on charcoal as catalyst, probably due to the fact that **1c** is highly

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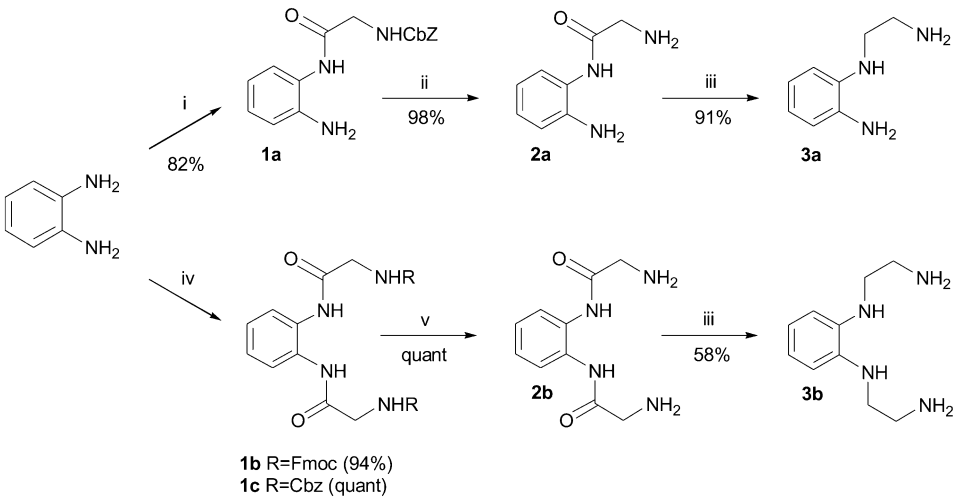
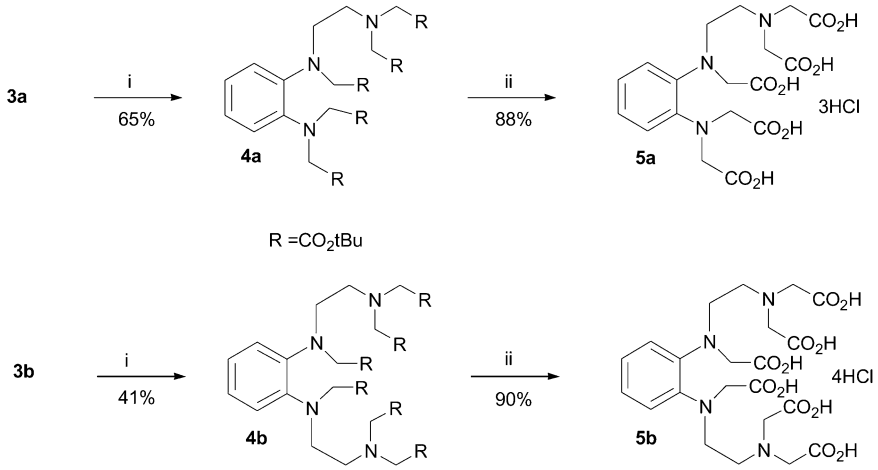


Scheme 1 Synthesis of polyamines **3a** and **3b**. Reagents and conditions: (i) Cbz-$NHCH_2CO_2H$, DCC, THF, 4 h, rt; (ii) Pd/C, H_2, EtOH, 24 h, rt; (iii) BH_3-DMS, THF, 28 h, reflux; (iv) Fmoc-$NHCH_2COCl$, $(i\text{-Pr})_2EtN$, THF, 1 h, rt for **1b**; Cbz-$NHCH_2COCl$, Et_3N, CH_2Cl_2, 1.5 h, rt for **1c**; (v) from **1b**, piperidine, DMF, 0.5 h, rt.



Scheme 2 Synthesis of Ph-DTPA (**5a**) and Ph-TTHA (**5b**). Reagents and conditions: (i) *tert*-buyl bromoacetate, KI, K_2CO_3, CH_3CN, 80 °C, 72 h for **4a** and 96 h for **4b**; (ii) NaOH 2 M, EtOH, 60 °C, 12 h then 6 M HCl.

insoluble in ethanol. This led us to change the nature of the protective group on glycine. Compound **1b** functionalized with a Fmoc group that can be cleaved in basic medium, was synthesized with a yield of 94%. Quantitative deprotection of both nitrogens was achieved using piperidine in DMF. Reduction of compound **2b** was then carried out as previously described. As opposed to triamine **3a**, tetraamine **3b** was not isolated as its chlorohydrate form which is too hygroscopic to provide convenient storage.

Alkylation of both compounds **3a** and **3b** was induced by *tert*-butyl bromoacetate according to the method described by Studer (KI, K_2CO_3). Products **4a** and **4b** were obtained with respectively 65 and 41% yields (Scheme 2).[49,50]

The uncompleted cleavage of ester groups of compounds **4a** and **4b** by using trifluoroacetic acid,[51,52] led us to proceed the deprotection step in basic medium in a mixture of ethanol and 2 M aqueous solution of sodium hydroxide. After acidification with hydrochloric acid, Ph-DTPA (**5a**) and Ph-TTHA (**5b**) were then isolated as their chlorohydrate forms with yields of 88 and 90%, respectively.

Complexation tests

In order to assess the ability of both ligands to complex different metals along with their suitability for use in nuclear medicine, three complementary tests were planned. The first test was designed to evaluate the chelating efficiency of the ligands and was conducted with UV-vis spectroscopy. The second test was intended to estimate the rate of complexation in an extremely diluted and slightly acidic medium. The last test would only be performed if the first two tests proved conclusive and was aimed at determining the stability of a metal complex in human serum, a requirement for its use in RIT or IS.

The first test was performed with non-radioactive metal cations and aimed at determining if ligands **5a** and **5b** were effective chelates. As both ligands have a benzenic chromophore, the corresponding absorption spectra exhibited an intense band around 230 nm, which corresponds to a $\Pi \rightarrow \Pi^*$ transition (ε_{max} = 8500 $dm^3\ mol^{-1}\ cm^{-1}$, λ_{max} = 228.4 nm for **5a** and ε_{max} = 12000 $dm^3\ mol^{-1}\ cm^{-1}$, λ_{max} = 229 nm for **5b**). Upon metal complexation, an electronic perturbation is generated, which usually translates into a modification of the absorption spectrum. The procedure consists of dissolving the metal trichloride in distilled water to a final concentration of 1 mM in neutral medium (pH *ca.* 7). After 1 h incubation with constant stirring at rt, a few ml of the solution are analyzed by UV-vis spectroscopy (200–800 nm). Fig. 1 summarizes results obtained with three metals (In, Y, and Sm) complexed with Ph-DTPA (**5a**) and Ph-TTHA (**5b**).

In these three examples, in the presence of an equimolar amount of In(III), Y(III) or Sm(III), an important modification of the UV absorption spectrum of Ph-DTPA (**5a**) and Ph-TTHA (**5b**) was observed (except for Ph-TTHA with samarium). The band around 230 nm disappears due to a hypochromic

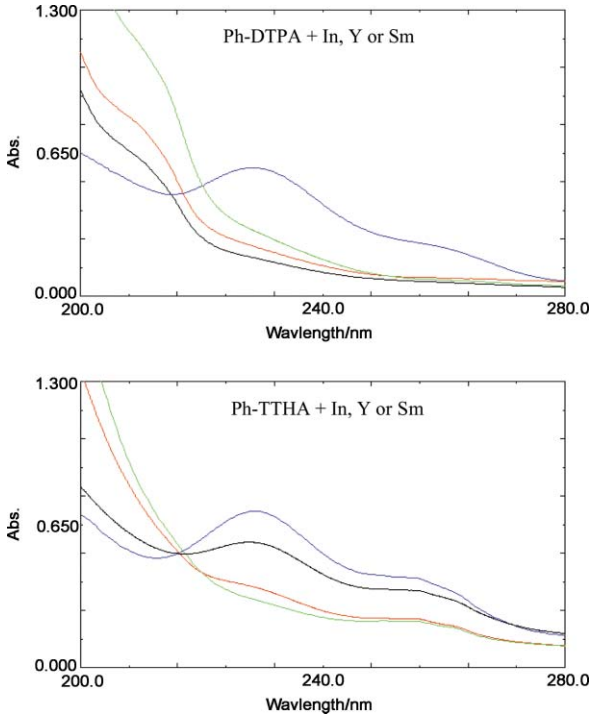


Fig. 1 UV-vis spectra of **5a** and **5b** and their complexes with indium, yttrium and samarium. Blue curve: **5a** or **5b**; red curve: Y complex; green curve: In complex; black curve: Sm complex.

effect, that is indicative of a perturbation produced by the complexation of the metal ion near the aromatic chromophore. As a consequence, Ph-DTPA should be an effective ligand to complex indium, yttrium, and samarium cations as well as the homologous compound Ph-TTHA for indium and yttrium. However, with the assumption that, for a given ligand, the conformation of the complexed species is comparable for In(III), Y(III), Sm(III) and thus should induce similar modification of the UV absorption spectrum (as is the case for Ph-DTPA), Ph-TTHA proved to be ineffective to chelate samarium cations. At last, similar experiments enable us to demonstrate effective chelation of both ligands with lutetium and bismuth. As non-radioactive actinium trichloride was not available, analysis by UV spectroscopy could not be performed.

After having verified the ability of ligands **5a** and **5b** to trap a given metal we assessed complexation effectiveness by determining the rate of complexation. In order to do so in labelling conditions close to those used in nuclear medicine, Ph-DTPA and Ph-TTHA were tested with photon emitting radionuclides used in immunoscintigraphy (^{111}In and ^{153}Sm), and β^- and α emitters used in RIT (^{90}Y, ^{177}Lu, ^{213}Bi and ^{225}Ac).

For each radionuclide studied, a series of samples at concentrations ranging from 10^{-6} to 10^{-10} M were prepared in a buffered aqueous solution (pH 5.5). For these tests, we used a slightly acidic medium to avoid precipitation of metal hydroxides. Increasing amounts of both ligands, Ph-DTPA (**5a**) and Ph-TTHA (**5b**) were then added and the resulting samples were incubated for 1 h at 37 °C. Thin-layer chromatography (TLC) associated with a judicious choice of eluent enabled the separation of the free form of the radionuclide from the complexed one, as the former does not migrate in the established conditions. Radioactivity on TLC plates was quantified using a phosphorimager. These experiments were also done with DTPA, Cy-DTPA, CHX-DTPA or DOTA (Fig. 2) as references for their similar number and nature of coordinating atoms as well as for their known high kinetics of complexation with the studied radionuclides.[53]

This study was done with two γ emitters used in IS, indium (^{111}In, $T_{1/2}$ = 2.83 d) and samarium (^{153}Sm, $T_{1/2}$ = 1.95 d), which also has a β^- component.[54] Both types of emission of this radionuclide could allow imaging and therapy to be combined. Because no perturbation of the UV absorption spectrum had been observed when samarium cation was incubated with Ph-TTHA ligand (Fig. 1), this ligand was not tested with the radioactive isotope (^{153}Sm).

A representative TLC plate with Ph-DTPA–indium and Ph-TTHA–indium complexes (ligand–indium ratio of 1 : 1) is shown in Fig. 3. As staining intensity is a function of the locally emitted radioactivity, the rate of complexation can be measured. Results are summarized in Table 1.

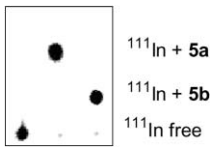


Fig. 3 Thin-layer chromatography analysis of **5a**-^{111}In and **5b**-^{111}In complexes.

Results showed that, under our experimental conditions, both chelating agents complexed indium cations quantitatively and proved to be as effective as the well known DTPA ligand. The strong chelating capacity of Ph-DTPA (**5a**) was also observed for samarium as the observed rates of complexation, 73% with a stoichiometric amount of ligand, and 95% with a two-fold molar excess, are comparable to those obtained with DTPA.

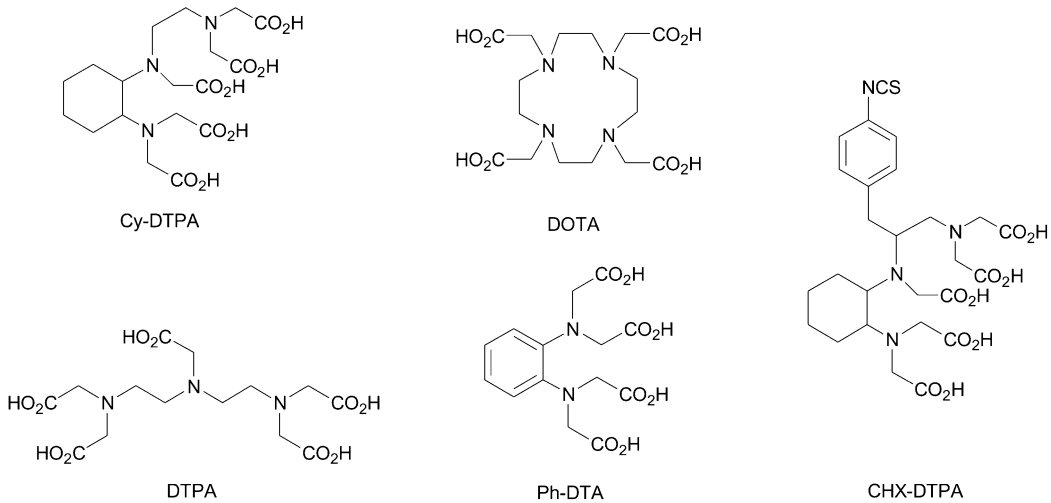


Fig. 2 Structure of polyaminocarboxylate ligands used as references.

Table 1 Complexation capacity of **5a** and **5b** with two photon emitters (^{111}In and ^{153}Sm)[a]

	^{111}In chelated/%			^{153}Sm chelated/%		
Molar equivalent of ligand	DTPA	Ph-DTPA (**5a**)	Ph-TTHA (**5b**)	DTPA	Ph-DTPA (**5a**)	Ph-TTHA (**5b**)
1	100	98	100	76	73	—
2	100	100	100	100	95	—
5	—	—	—	100	100	—

[a] [^{153}Sm] = 0.74 μmol dm^{-3}; [^{111}In] = 1.34 nmol dm^{-3}; incubation = 1 h; pH = 5.5–5.8 (0.1 M sodium acetate buffer); temperature = 37 °C; each point is the average of the percentage amount from three replicates.

Table 2 Complexation capacity of **5a** and **5b** with two β$^-$ particle emitters (^{90}Y and ^{177}Lu)[a]

	^{90}Y chelated/%			^{177}Lu chelated/%		
Molar equivalent of ligand	DTPA	Ph-DTPA (**5a**)	Ph-TTHA (**5b**)	DOTA	Ph-DTPA (**5a**)	Ph-TTHA (**5b**)
1	9	23	6	87	55	0
2	—	—	—	100	100	0
10	75	93	37	—	—	—
10^2	97	97	43	100	100	55
10^3	97	100	56	—	—	—

[a] [^{90}Y] = 44 nmol dm^{-3}; [^{177}Lu] = 200 μmol dm^{-3}; incubation = 1 h; pH = 5.5–5.8 (0.1 M sodium acetate buffer); temperature = 37 °C; each point is the average of the percentage amount from three replicates.

Table 3 Complexation capacity of **5a** and **5b** with two α particle emitters (^{213}Bi and ^{225}Ac)[a]

	^{213}Bi chelated/%			^{225}Ac chelated/%		
Molar equivalent of ligand	Cy-DTPA	Ph-DTPA (**5a**)	Ph-TTHA (**5b**)	CHX-DTPA	Ph-DTPA (**5a**)	Ph-TTHA (**5b**)
10^2	0	17	17	—	—	—
10^3	0	49	67	—	—	—
10^4	69	71	70	—	—	—
10^5	86	73	88	0	0	0
10^6	—	—	—	23	0	0

[a] [^{213}Bi] = 0.67 nmol dm^{-3}; [^{225}Ac] = 0.25 nmol dm^{-3}; incubation = 1 h; pH = 5.5–5.8 (0.1 M sodium acetate buffer); temperature = 37 °C; each point is the average of the percentage amount from three replicates.

Two β$^-$ emitters potentially used in RIT were then studied: yttrium (^{90}Y, $T_{1/2}$ = 2.67 d), which is currently used in clinical applications,[12,55] and lutetium (^{177}Lu, $T_{1/2}$ = 6.71 d). Results are presented in Table 2 and show important differences in the affinity of Ph-DTPA and Ph-TTHA for these radionuclides. When 93% of yttrium cation is complexed in a presence of ten-fold molar excess of ligand Ph-DTPA (**5a**), Ph-TTHA (**5b**) appeared to be less effective as only 37% of the radionuclide is chelated in the same conditions. Furthermore, even a 10^3-fold molar excess of ligand **5b** only chelates 56% of the yttrium cation. It should be noted that, in these experiments, the measured rate of complexation is not directly proportional to the number of equivalents of ligand as the solution of the metallic cation contains a small part of non-radioactive isotopes which are undetected by the radioactivity counter. However, it remains a method of choice for estimating the rate of complexation of radionuclides in high dilute medium.

Results obtained with lutetium (^{177}Lu), and DOTA as reference compound, showed even more marked differences between **5a** and **5b**.[56] With a two-fold molar excess of ligand, Ph-DTPA proved to be competitive to DOTA with a complete complexation of lutetium, as opposed to Ph-TTHA, which only complexed 55% of lutetium cation with a ligand–metal ratio of 100 : 1.

Experiments conducted with bismuth (^{213}Bi, $T_{1/2}$ = 45.6 min) and actinium (^{225}Ac, $T_{1/2}$ = 10 d)—both of them are being tested as α emitters in phase I and II of clinical trials—were carried out using solutions with even lower radionuclide concentrations. As radioactive sources and buffered solutions contain unknown and varying amounts of non-radioactive isotopes which compete with the radionuclide, a high excess of ligand has been used to ensure the total complexation of the cationic species. Results obtained with bismuth-213 showed the superior complexation ability of both Ph-DTPA (**5a**) and Ph-TTHA (**5b**) over Cy-DTPA (Table 3), which is currently considered the best chelating agent for bismuth.[13,57,58] Indeed, as opposed to **5a** and **5b**, a greater excess of Cy-DTPA must be used to observe complexation with bismuth. Conversely, Ph-DTPA (**5a**) and Ph-TTHA (**5b**) were ineffective at chelating actinium; no complexation was observed, even with high excess of ligand (10^6 mol equiv.). However, in such conditions, CHX-DTPA achieves complexation of actinium-225 with a rate of 23%. The poor results obtained with this radionuclide are in accordance with the reported results that showed improved complexation rates with macrocyclic ligands rather than with their linear analogues.[59]

In conclusion, these tests revealed the promising complexation properties of Ph-DTPA (**5a**) for the radionuclides studied. This ligand proved to be more effective than DTPA and Cy-DTPA, to complex yttrium-90 and bismuth-213, respectively. Results obtained with Ph-TTHA (**5b**) were not as clear cut. While being rather ineffective at complexing yttrium-90, lutetium-177, and actinium-225, this chelating agent was shown to chelate indium and bismuth effectively, as reflected by the high rates of complexation obtained. We thus envisaged the evaluation of the stability of these complexes in human serum, an essential condition for their use in IS and RIT.

A number of serum proteins and enzymes have the ability to *trans*-chelate radionuclides from metal complexes or to cleave off radiopharmaceuticals. In order to be used in IS and RIT, these decomplexation processes must be avoided as much as

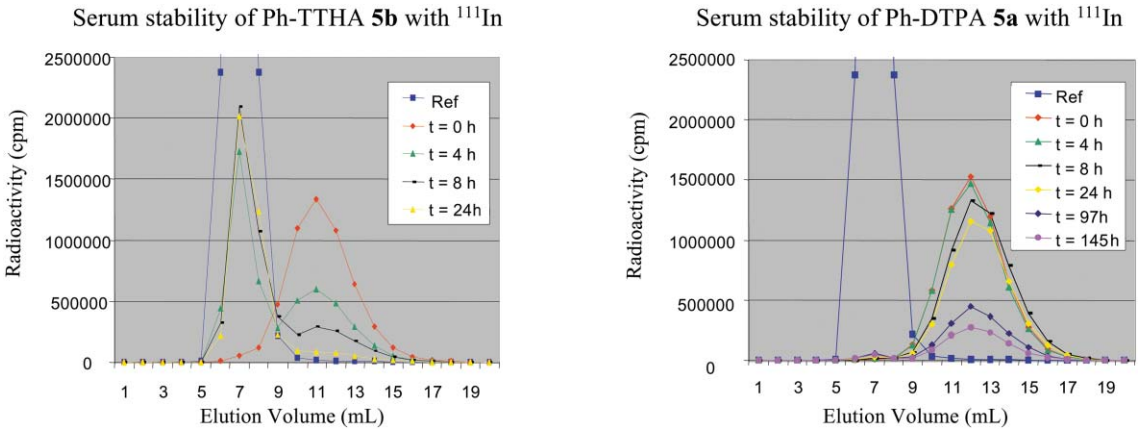


Fig. 4 Stability of ^{111}In–Ph-TTHA and ^{111}In–Ph-DTPA complexes in human serum.

possible. Very few novel chelating agents have been shown to form complexes with a good stability in blood. The kinetic inertness of a complex in serum appears to be a major property to evaluate this stability,[60] and we thus decided to study our complexes in such conditions.

After incubation of buffered solutions of radionuclides and ligand in human serum, the separation of the complexed forms of radionuclide (serum protein-bound *versus* ligand-bound) was realized by size exclusion chromatography and quantified by radioactivity measurements. Typical profiles obtained are presented in Fig. 4 where the blue curve corresponds to serum protein-bound metal, and the other curves reflect radioactivity emitted by metal complexes as a function of time of incubation. This study was realized only for metal chelates having a quantitative rate of complexation (Ph-DTPA–In, –Sm, –Y, –Lu, and Ph-TTHA–In).

The ^{111}In complex formed with Ph-TTHA (**5b**) exhibited poor stability in human serum as after 4 h of incubation (green curve), *trans*-complexation of more of 50% of ^{111}In from Ph-TTHA to human serum proteins had occurred. This phenomenon is amplified after 24 h incubation at 37 °C, as most of the radioactivity is detected on human serum proteins. Similar results were found for Ph-DTPA–Y, Ph-DTPA–Lu, and Ph-DTPA–Sm complexes as after 15 h of incubation, more than 50% of the radionuclide was bound to serum proteins.

However, with Ph-DTPA, the *trans*-complexation of ^{111}In did not occur and after 145 h of incubation (6 d) only 6% of the radionuclide (pink curve) had been displaced, which suggests that this complex is a good candidate for IS.

As a consequence, the evaluation of the stability constant of the complex formed with Ph-DTPA (**5a**) and ^{111}In should be fundamental for medical applications.

In order to do so, the determination of the acidity constants of the ligand is usually needed, so we decided to conduct potentiometric titrations of the fully protonated ligand (**5a**) as reported for other polyaminopolycarboxylate ligands.[61] In our conditions,[62] four stepwise protonation constants were found (Table 4).

By comparison with the aliphatic analogue DTPA, the first and the third protonation constants are very similar. The second one is lower than that of DTPA and is responsible of an overall decrease of basicity of the ligand. This result is in accordance with the decrease of basicity observed by rigidification of EDTA with a benzenic ring (Ph-DTA, Fig. 2).

Table 4 Comparison of the protonation constants of Ph-DTPA (**5a**) and Ph-DTA with the constants of linear analogues (DTPA and EDTA)

Protonation constants	Ph-DTPA **5a**[a]	DTPA[b]	Ph-DTA[c]	EDTA[d]
$-\log K_1^H$	10.29	10.06	6.64	9.79
$-\log K_2^H$	6.33	8.32	4.76	6.21
$-\log K_3^H$	4.05	4.13	3.60	2.45
$-\log K_4^H$	3.02	2.50	2.88	1.95
$-\log K_5^H$	—	2.29	0.60	1.50
$-\Sigma \log K_i^H$	23.69	27.30	17.88	21.90

[a] 0.1 M KCl, 25 °C. [b] Ref.63, 1 M KCl, 25 °C. [c] Ref.64, 0.1 M KCl, 25 °C. [d] Ref.65, 1 M KCl, 25 °C.

Conclusion

We have developed two original structures Ph-DTPA (**5a**) and Ph-TTHA (**5b**) that mimic the heteroatom linking of DTPA and TTHA. Both of them were synthesized in five steps with respective overall yields of 42 and 20%, the rate-limiting step being the peralkylation of compounds **3a** and **3b**. We demonstrated the high complexation capacity of **5a**, the rigid analogue of DTPA with a broad range of radionuclides (^{111}In, ^{153}Sm, ^{90}Y, ^{213}Bi, ^{177}Lu) in slightly acidic medium (pH = 5.5). Chelation rates obtained with this ligand proved to be of the same order of magnitude, and sometimes even greater, than those obtained with DTPA. We also noted an important difference in the chelation ability of both ligands with Sm, Y and Lu as Ph-DTPA (**5a**), with 8 coordination centers, usually has a higher complexation capacity than Ph-TTHA **5b**, with 10 coordination centers. This result is in accordance with observations made when comparing DTPA and Cy-DTPA complexes with the homologous TTHA and Cy-TTHA complexes, which are usually less stable. Indeed, when the metal coordination number is exceeded, an excessive increase in the number of chelating functions of a ligand would often would lead to the formation of more labile complexes.[66]

Moreover, Ph-DTPA (**5a**) was shown to form stable complexes with indium in human serum, a necessary condition for all medical applications. We report here the acidity constants of this ligand determined by potentiometric titration.

These preliminary results encouraged us to envision the synthesis of functionalized analogues of Ph-DTPA (**5a**) in order to develop a ligand that could be grafted to chemical or biological vectors. As Ph-DTPA (**5a**) is able to complex different metals, it may be used for applications other than nuclear medicine when functionalized. For instance, binding of this molecule to substrates such as silica gels may be considered in solid–liquid extraction systems used for the treatment of effluents.[67,68]

Experimental

Chemistry

All reagents were purchased from Acros Organics and Aldrich. All chemicals were reagent grade and used without further purification, and all solvents were freshly distilled before use. The CNRS Analysis Laboratory (Vernaison) performed the

elemental analyses. Column chromatography was conducted over silica gel 60 (40–63 μm), available from E. Merck. Melting points measured using a Reichert microscope are uncorrected. The ^{13}C and ^{1}H-NMR spectra were recorded at rt using a Bruker AC200 operating at 50 and 200 MHz, respectively. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane as internal standard. Mass spectra were determined with a Hewlett Packard 5989 spectrometer. The IR spectra were obtained using a Bruker Vector 22 spectrometer.

o-Phenylene-*N*,*N*-bis-[2-(fluoren-9-yl)methyloxycarbonylaminoethanamide] (1b)

N-(9-Fluorenylmethoxycarbonyl)glycine (1 g, 3.36 mmol) and oxalyl chloride (457 mg, 3.60 mmol) were added to anhydrous THF (15 cm^3) at 0 °C under inert atmosphere. A catalytic quantity of DMF (24 mg, 0.33 mmol) was added and the mixture was stirred for 45 min at rt. The solution was injected under inert atmosphere into a mixture of *o*-phenylenediamine (151 mg, 1.40 mmol), diisopropylethylamine (1.37 g, 10.6 mmol) in anhydrous THF (15 cm^3). The resulting solution was stirred for 1 h at rt. After evaporation under reduced pressure, the residue was taken up in dichloromethane (50 cm^3) and the organic phase is washed with water (40 cm^3). The organic phase precipitate was filtered on a frit and rinsed with dichloromethane (10 cm^3) to afford compound **1b** as a white solid. (898 mg, 94%): mp: 168–170 °C; IR (KBr): 3316, 1708, 1676, 1526, 1253 cm^{-1}; δ_H (DMSO) 3.88 (s, 4H, 2CH_2), 4.24 (s, 2H, 2C*H*), 4.32 (s, 4H, 2CH_2), 7.10–7.90 (m, 20H, 20$C_{ar}H$), 9.36 (s, 2H, 2C_{ar} N*H*CO); δ_C (DMSO) 44.2 (2*C*H), 46.7 (2*C*H_2), 65.9 (2*C*H_2), 120.1, 121.4, 124.7, 125.3, 127.1, 127.6, 128.9, 130.3, 140.7, 143.8 (10C_{ar} + 20C_{ar}H), 156.8 (2NH*C*O), 168.5 (2NH*C*O); L-SIMS$^+$ *m*/*z* 683 ([M + H]$^+$, 100); Anal. calcd. for $C_{40}H_{34}N_4O_6$: C, 72.12; H, 5.61; N, 8.21. Found: C, 72.27; H, 5.28; N, 8.10.

o-Phenylene-*N*,*N*-bis-(2-benzoxycarbonylaminoethanamide) (1c)

A round-bottom flask equipped with stirring bar and two addition funnels, containing *N*-carbobenzyloxyglycine (1 g, 4.78 mmol) in anhydrous dichloromethane (15 cm^3) was placed under nitrogen. The flask was submerged in an ice-water bath at 0 °C, and oxalyl chloride (669 mg, 4.9 mmol) and dimethylformamide (22 mg, 0.3 mmol) were added. The ice-water bath was removed but stirring continued for 90 min at rt. A mixture of *o*-phenylenediamine (258 mg, 2.39 mmol) and triethylamine (911 mg, 9 mmol) in anhydrous dichloromethane (20 cm^3) was added dropwise to the solution. The solution was stirred at rt for 1.5 h under nitrogen atmosphere, and then treated with 2 M hydrochloric acid (60 cm^3). The organic phase was separated and shaken with water (60 cm^3), dried over $MgSO_4$ and concentrated to afford **1c** as a white solid. (1.17 g, 100%): mp: 179–181 °C; IR (KBr): 3421, 3268, 1705, 1681, 1543 cm^{-1}; δ_H (DMSO) 3.84 (d, 4H, 2CH_2, $J = 5.6$ Hz), 5.06 (s, 4H, 2CH_2), 7.14–7.19 (m, 2H, 2C*H*), 7.35 (s, 10H, 10$C_{ar}H$), 7.52–7.63 (m, 4H, 2C*H* + 2CH_2N*H*), 9.35 (br s, 2H, 2C_{ar}N*H*); δ_C (DMSO) 44.2 (2*C*H_2), 65.7 (2*C*H_2), 124.7 (2*C*H_2), 125.1 (2*C*H_2), 127.8 (6*C*H), 128.4 (4*C*H), 130.3 (2C_{ar}N), 136.9 (2*C*), 156.7 (2N*H*COO), 168.4 (2N*H*CO); MS *m*/*z*: 152, 126 (100), 108; Anal. calcd. for $C_{26}H_{26}N_4O_6$: C, 63.66; H, 5.34; N, 11.42. Found: C, 63.60; H, 5.38; N, 11.42.

o-Phenylene-*N*,*N*-bis(2-aminoethanamide) (2b)

Compound **1b** (600 mg, 0.90 mmol) and piperidine (4 cm^3) were mixed in anhydrous DMF (20 cm^3). The solution was stirred at reflux 30 min under nitrogen. The solution was evaporated to dryness and the residue was dissolved in diethyl ether (10 cm^3). The solution was washed with water (60 cm^3), and the aqueous phase was evaporated to dryness. The residue was redissolved in water and the solution evaporated to dryness. This procedure was repeated three times to afford product **2b** as a colorless oil. (200.7 mg, 100%): IR (KBr): 3258, 1669, 1598, 1525, 1296 cm^{-1}; δ_H (CD_3OD) 3.48 (s, 4H, 2CH_2), 7.23 (s, 2H, 2C_a*H*), 7.60 (s, 2H, 2C_b*H*); δ_C (CD_3OD) 45.4 (2*C*H_2), 126.0 (2*C*H), 127.1 (2*C*H), 131.6 (2C_{ar}N), 173.9 (2*C*O); MS *m*/*z*: 223 (100, [M + H]$^+$), 180.

N-(2-Aminophenyl)ethylenediamine trichloride (3a)[69]

Compound **2a** (400 mg, 2.47 mmol) was dissolved in anhydrous THF (10 cm^3) and cooled to 0 °C before addition of BH_3·DMS (6.2 cm^3, 12.2 mmol) 2 M in THF. The solution was stirred at reflux for 28 h under nitrogen. The solution was evaporated to dryness and the residue, cooled in an ice bath. Methanol was added (25 cm^3) and the solution was saturated with HCl gas for 0.5 h at 0 °C. The solution was evaporated to dryness and the residue was redissolved in absolute ethanol (10 cm^3). The solution was saturated with HCl gas 0.5 h at 0 °C. The resulting pink precipitate was filtered on a frit under argon and vacuum dried. (579 mg, 91%): mp: 210–212 °C; IR (KBr): 3429, 2879, 1507, 1326 cm^{-1}; δ_H (CD_3OD) 3.27 (t, 2H, C$H_2$$NH_2$, $J = 5.8$ Hz), 3.58 (t, 2H, NHCH_2, $J = 5.8$ Hz), 7.00 (m, 2H, 2C_{ar}*H*), 7.40 (m, 2H, 2C_{ar}*H*); δ_C (CD_3OD) δ 39.7 (*C*$H_2$$NH_2$), 41.8 (NH*C*$H_2$), 115.7 (*C*$_{ar}$H), 119.4 (*C*$_{ar}$), 121.0 + 125.5 + 132.0 (3*C*$_{ar}$H), 142.9 (*C*$_{ar}$). L-SIMS$^-$ *m*/*z* 256.6 ([M − H]$^+$, 100), 403, 331.

o-Phenylene-*N*,*N*-bis(ethylenediamine) (3b)

Compound **2b** (680 mg, 3.05 mmol) and a 2 M solution of complex BH_3·DMS in THF (7.5 cm^3, 15.0 mmol) were added to anhydrous THF (25 cm^3) at 0 °C. The mixture was heated at reflux under nitrogen atmosphere for 23 h. After evaporation under reduced pressure, the residue was dissolved in methanol (25 cm^3). Bubbling of hydrochloric acid gas into the solution was carried out for 30 min at 0 °C. The solution was evaporated under reduced pressure and the residue was dissolved in absolute ethanol (18 cm^3). Bubbling of hydrochloric acid gas into the solution was repeated as previously described. The precipitate was filtered on a frit, dried under reduced pressure and added to a 5 M NaOH solution (80 cm^3). The product was extracted from the aqueous phase with dichloromethane (3 × 100 cm^3). The organic phase was dried over magnesium sulfate, filtered, and evaporated under reduced pressure to afford compound **3b** as a colorless oil. (343 mg, 58%): IR (KBr): 3345, 2936, 2861, 1598 cm^{-1}; δ_H ($CDCl_3$) 2.43 (br s, 4H, 2NH_2), 2.97 (t, 4H, 2CH_2, $J = 5.2$ Hz), 3.16 (t, 4H, 2CH_2, $J = 5.2$ Hz), 6.65 (m, 2H, 2C*H*), 6.78 (m, 2H, 2C*H*); δ_C ($CDCl_3$) 41.2 (2*C*H_2), 46.9 (2*C*H_2), 111.6 (2*C*H), 119.1 (2*C*H), 137.4 (2*C*N); L-SIMS$^+$ *m*/*z* 195.0 ([M + H]$^+$, 100).

N,*N*,*N*′-Tri-*tert*-butoxycarbonylmethyl-*N*′-*tert*-butoxycarbonylmethyl-*N*′-[2-di(*tert*-butoxycarbonylmethyl)aminophenyl]ethylenediamine (4a)

Potassium carbonate (3.21 g, 23 mmol) and potassium iodide (78 mg, 0.4 mmol) were added to a solution of **3a** (600 mg, 2.3 mmol) in freshly distilled acetonitrile (40 cm^3). The mixture was stirred at 80 °C, and *tert*-butylbromoacetate (4.48 g, 23 mmol) was added dropwise. The reaction mixture was kept at this temperature over a period of 72 h under argon, and then cooled to rt, filtered, and concentrated under reduced pressure. Dichloromethane was added (50 cm^3) and the solution washed with water (80 cm^3), dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography on silica using hexane–triethylamine (49 : 1) as eluent. This procedure was repeated with dichloromethane–ethyl acetate (19 : 1) as eluent to give **4a** as a colorless oil. (1.1 g, 65%): IR (KBr): 2978, 2931, 1740, 1367, 1149 cm^{-1}; δ_H ($CDCl_3$) 1.37 (s, 27H, 9CH_3), 1.44 (s, 18H, 6CH_3), 2.83 (t, 2H, CH_2, $J = 5.8$ Hz), 3.41 (m, 6H, 3CH_2), 4.10 (s, 4H, 2CH_2), 4.15 (s, 2H, CH_2), 6.90–7.10 (m, 4H, C$_{ar}$*H*); δ_C ($CDCl_3$) 28.2 (15*C*H_3), 48.9 (*C*H_2), 51.9 (*C*H_2), 53.5 (2*C*H_2), 53.6 (*C*H_2), 56.1 (2*C*H_2), 80.6 (2*C*), 81.0 (3*C*), 120 + 121.5 + 122.3 + 122.5 (4*C*$_{ar}$H), 141.3 + 141.8 (2*C*$_{ar}$N), 170.3 (2*C*O), 170.7 (2*C*O), 171.0

(*C*O); MS *m/z*: 722 (100, M^+), 608, 450; HRMS (DCI) *m/z* calcd. for $C_{38}H_{63}N_3O_{10}$ $[M]^+$ 722.4592, found 722.4593.

o-Phenylene-*N*,*N*-bis(ethylenediamine-*N*,*N*,*N*′-tri(*tert*-butyl)triacetate) (4b)

Compound **3b** (143 mg, 0.73 mmol), potassium carbonate (1 g, 7.33 mmol), potassium iodide (24.9 g, 0.15 mmol) and *tert*-butylbromoacetate (1.43 g, 7.33 mmol) were dissolved in anhydrous acetonitrile (15 cm^3). The mixture was stirred for 96 h at 80 °C under argon. After evaporation under reduced pressure, the residue was taken up in dichloromethane (40 cm^3) and the organic phase was washed with water (20 cm^3) and dried over magnesium sulfate. The solution was filtered and evaporated under reduced pressure. The residue was purified by chromatography with petroleum ether–dichloromethane–triethylamine (93 : 5 : 2) to afford compound **4b** as a colorless oil. (264 mg, 41%): IR (KBr): 2978, 2933, 1742, 1148 cm^{-1}; δ_H ($CDCl_3$) 1.36 (s, 18H, 6CH_3), 1.44 (s, 36H, 12CH_3), 2.81 (t, 4H, 2CH_2, $J = 7.0$ Hz), 3.40 (m, 12H, 6CH_2), 4.08 (s, 4H, 2CH_2), 6.88 (m, 2H, 2C*H*), 7.06 (m, 2H, 2C*H*); δ_C ($CDCl_3$) 28.3 (18*C*H_3), 49.1 (2*C*H_2), 52.1 (2*C*H_2), 53.6 (2*C*H_2), 56.1 (4*C*H_2), 80.6 (2*C*), 81.0 (4*C*), 121.4 + 122.2 (4*C*H), 141.7 (2C_{ar}N), 170.7 (4*C*O), 170.9 (2*C*O); MS *m/z*: 879 $[M + H]^+$, 607, 362, 336 (100); HRMS $[M + Na^+]$ *m/z* calcd. for $C_{46}H_{78}N_4O_{12}$ $[M]^+$ 901.5514, found 901.5512.

N-(2-*N*,*N*-Dicarboxymethylaminophenyl)ethylenediamine-*N*,*N*′,*N*′-triacetic acid trichloride (5a)

A mixture of **4a** (190 mg, 0.26 mmol), ethanol (12 cm^3) and 2 M aqueous solution of NaOH (12 cm^3) was stirred 12 h at 60 °C. The solution was evaporated to dryness, and the residue was dissolved in cool water (12 cm^3). Three molar hydrochloric acid was then added until pH < 7 and the solution had evaporated to dryness. Acetone was added (40 cm^3) and the solution was stirred for 10 min at rt. NaCl was filtered on a frit and the filtrate, evaporated to dryness to give acid **5a** as a white powdery solid, which was vacuum dried and kept under nitrogen. Acid **5a** is an hygroscopic compound. (127 mg, 88%): mp: 125–127 °C; IR (KBr): 3328, 3005, 1734, 1419, 1218 cm^{-1}; δ_H (D_2O) 3.47 (br s, 2H, CH_2), 3.52 (br s, 2H, CH_2), 4.07 (s, 2H, CH_2), 4.20 + 4.26 (2s, 8H, 4CH_2), 7.09–7.17 (m, 4H, 4C$_{ar}H$); δ_C (D_2O) 51.3 (*C*H_2), 56.6 (*C*H_2), 56.7 (2*C*H_2), 58.2 (4*C*H_2), 125.1 + 126.3 + 128.2 + 129.1 (4C_{ar}H), 144.3 + 146.1 (2C_{ar}N), 172.3 (2*C*O), 178.0 (2*C*O), 178.3 (*C*O); L-SIMS$^+$ *m/z*: M + H^+ − 3HCl = 442.0, M + Na^+ − 3HCl = 464.1. L-SIMS$^-$: M − H^+ − 3HCl = 440.2, M − H + Na^+ − 3HCl = 464.1

o-Phenylene-*N*,*N*-bis(ethylenediamine-*N*,*N*,*N*′-triacetic) acid tetrachloride (5b)

Compound **4b** (120 mg, 0.137 mmol) was dissolved in ethanol (5 cm^3). 2 M NaOH (5 cm^3) was added while stirring, and the mixture was heated at 60 °C for 12 h. After evaporation under reduced pressure, the residue was dissolved in water (5 cm^3) at 0 °C. 6 M HCl was added until the solution became acidic. The solution was then evaporated under reduced pressure and the residue, stirred at rt in a ethanol–dichloromethane (9 : 1) solution for 40 min. After filtration on a frit, the filtrate was evaporated under reduced pressure. The residue was taken in three times in 6 M HCl to afford compound **5b** as an off-white hygroscopic solid. (92 mg, 90%): mp: >300 °C; IR (KBr): 3419, 2969, 1733, 1256 cm^{-1}; δ_H (D_2O) 3.49 + 3.68 (2m, 8H, 4CH_2), 4.09 (s, 4H, 2CH_2), 4.19 (s, 8H, 4CH_2), 7.13 (m, 4H, 4C$_{ar}H$); δ_C (D_2O) 50.4 (2CH_2), 56.5 (2CH_2), 57.3 (2CH_2), 58.5 (4CH_2), 125.7 (2C*H*), 128.8 (2C*H*), 145.0 (2*C*N), 171.9 (4*C*O), 178.2 (2*C*O); L-SIMS$^+$ *m/z*: M + Na^+ − 4HCl = 542.0.

UV spectroscopy

A slight excess (1.1 eq.) of chelating agent [Ph-DTPA (**5a**) or Ph-TTHA (**5b**)] was added to a solution of the appropriate metal trichloride (2 mg, *ca.* 5.10^{-5} M) in deionized water (10 cm^3). The final metal concentration of each solution ranged from 5.10^{-4} to 10^{-3} M in a final volume of 15 cm^3. Solutions were stirred at rt for 1 h. Absorbance intensity was monitored between 200 and 800 nm with a SHIMADZU-UV 2501 PC spectrophotometer.

Radiolabelling

Stock solutions of each chelate (2, 0.2, 0.02 and 0.002 $mg.cm^{-3}$) were prepared in 0.1 M sodium acetate, pH 5.5. Radionuclide–chelating agent complexes were formed by adding a defined volume of the appropriate radionuclide stock solution to increasing amounts of each chelating agent [DTPA, Cy-DTPA, DOTA, CHX-DTPA, Ph-DTPA (**5a**), and Ph-TTHA (**5b**)]. The final volume was adjusted to 500 μL with a 0.1 M solution of sodium acetate. The final pH of each solution ranged from 5.5 to 5.8 with a radionuclide concentration ranging from 10^{-6} to 10^{-10} M. Solutions were incubated at 37 °C for 1 h, 1 μL of each sample was taken, and complexation was measured with a PhosphorImager 445SI after thin-layer chromatography on silica plates (MERCK Art. 5714) by elution with 0.1 M sodium acetate (pH 5.5).

Radionuclides were produced by Cis-Bio International for ^{153}Sm and ^{111}In, by MDS Nordion for ^{90}Y, by Ansto for ^{177}Lu and by Transuraniens Institute for ^{213}Bi and ^{225}Ac.

Serum stability

40 to 120 μL of previously prepared buffered solutions of radionuclide-Ph-DTPA (**5a**) and radionuclide-Ph-TTHA (**5b**) complexes (pH 5.5) were added to fresh human serum (4 cm^3). Serum samples were incubated at 37 °C. Portions of the serum solution (500 μL) were periodically removed and analyzed on a PD10 column (size exclusion chromatography). The column was eluted with phosphate buffer solution at a flow rate of 1 cm^3 min^{-1}. The amount of radioactivity in each sample was determined using an automatic PerkinElmer WALLAC WIZARD 3 counter.

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69 Compound **3a** is now commercially available (Interchim Intermediates).