Synthesis and solution thermodynamic study of rigidified and functionalised EGTA derivatives†

Lorenzo Tei,[a] Zsolt Baranyai,[b] Mauro Botta,[a] Laura Piscopo,[a] Silvio Aime[b] and Giovanni B. Giovenzana*[c]

Received 11th March 2008, Accepted 28th March 2008
First published as an Advance Article on the web 30th April 2008
DOI: 10.1039/b804195d

The synthesis of a new series of EGTA (ethyleneglycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid) derivatives incorporating aromatic and functionalized aromatic moieties into the oxoethylenic bridge is described. A solution thermodynamic study was carried out to determine the influence of structural modifications on the coordinating ability towards lanthanide and alkaline earth metal ions. The presence of remote functional groups on the aromatic moiety would allow the conjugation of the complexes to macromolecules or other biological targets.

Introduction

The chelation of metal ions by highly polydentate ligands has been widely investigated in the last thirty years. The interest in such ligands is mainly driven by their ability (depending on the nature of the donor atoms) to form metal complexes with tunable physicochemical and functional properties engaged in a wide range of applications as inorganic medicinal compounds,[1] anion or molecular receptors,[2] catalysts for organic transformations,[3] molecular sensors,[4] and mimics for enzymes catalysing redox and hydrolytic processes.[5] Among them, polyaminocarboxylate ligands have been extensively employed as chelators for lanthanide(III) ions. The ligands may be tailored to fine-tune the kinetic and thermodynamic stabilities of the resulting complexes according to the application in which they are to be used, *e.g.* as sequestering agents or as diagnostic or therapeutic tools in medicine.

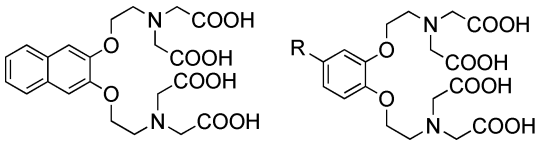
Among the latter applications, the use as contrast-enhancing agents (CAs) for magnetic resonance imaging (MRI) has attracted much attention in recent years.[6] In fact, CAs have been shown to cause a dramatic variation of the water proton relaxation rates, thus providing physiological information well beyond the impressive anatomical resolution commonly obtained in the uncontrasted images. In the class of paramagnetic CAs, the attention has been focused on Gd(III) complexes, which must have extremely high kinetic and thermodynamic stabilities to preserve their integrity for the time they stay in the patient body. A large database has already been acquired concerning the stability constants of Ln(III) polyaminocarboxylate complexes,[7] and interest has been recently renewed by the discovery of a new disease (named as Nephrogenic Systemic Fibrosis/Nephrogenic Fibrosing Dermopathy, NSF/NFD) referred to the release of free Gd^{3+} after administration of the Gd-based contrast agent Omniscan.[8]

The specific application as CA for MRI, but also other possible uses in biomedicine, prompted the search for the relationships between the details of the solution structure and the physico-chemical properties of the metal chelates. More importantly, a rational modification of the ligand backbone with different donor groups or different structural moieties can affect not only its coordinating ability but also the size, the charge, and the lipophilicity of the corresponding complexes, thus affecting their affinity for different biological targets.[6] The poly(aminocarboxylate) ligands such as the linear DTPA (diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid) or the macrocyclic DOTA (1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) and their derivatives represent the most common and studied class of chelators for biomedical applications, as they are octadentate and form highly stable Ln(III) complexes with one additional water molecule coordinated by the metal ion.[6] In the search for new and efficient contrast agents for MRI, a systematic investigation of the solution structure and dynamics and relaxation properties of the Ln(III) complexes of the simple acyclic ligand EGTA has been previously reported by our group.[9] Its Gd(III) complex is potentially a very efficient CA, as it presents an optimal value of one of the key factors influencing the efficacy, *viz.* the residence lifetime of the coordinated water molecule. EGTA has also been reported to exhibit interesting patterns of metal-binding selectivity, especially related with the alkaline earth metal ions Ca^{2+} and Mg^{2+}.[10] In fact, it is highly selective towards Ca^{2+} compared to the other alkaline earth metal ions, with a stability constant six orders of magnitude higher for the binding of Ca^{2+} over Mg^{2+}, larger than that typically exhibited by calcium-binding proteins. Despite the really interesting properties of EGTA both as an MRI CA and as Ca^{2+} sequestering agent, only a few papers have been published on EGTA derivatives in the last twenty years.[11] The reasons may be found in the fact that EGTA lacks functional groups suitable for covalent linking to macromolecules or biological targets, and that the corresponding Gd(III) complex suffers from low thermodynamic stability and selectivity, precluding practical and safe *in vivo* MRI applications.

[a]*Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale "Amedeo Avogadro", Via Bellini 25/G, I-15100, Alessandria, Italy*
[b]*Dipartimento di Chimica IFM, Università degli Studi di Torino, Via P. Giuria 7, I-10125, Torino, Italy*
[c]*DiSCAFF & DFB Center, Università degli Studi del Piemonte Orientale "Amedeo Avogadro", Via Bovio 6, I-28100, Novara, Italy. E-mail: giovenzana@pharm.unipmn.it*

† Electronic supplementary information (ESI) available: Experimental details and procedures related to the synthetic protocol reported in Scheme 2. See DOI: 10.1039/b804195d

Herein, we report synthesis and characterization of five derivatives of EGTA where structural variations were introduced in order to: i) investigate the effects on the stability of the corresponding Ln(III) complexes by rigidification of key sites of the flexible EGTA molecule or by functional group modification; and ii) allow conjugation of the corresponding complexes to macromolecules or biological targets, introducing additional remote functional groups. In the new **L1–L5** ligands (Scheme 1) the basic structure of EGTA was modified in the central ethylenic moiety, rigidified by fusion with an aromatic ring.



L1

L2 R = H
L3 R = NHC(=S)NHCH(CH$_2$Ph)COOH
L4 R = NHC(=S)NHC$_{12}$H$_{25}$
L5 R = NHCOC$_{17}$H$_{35}$

Scheme 1 Structure of **L1–L5**.

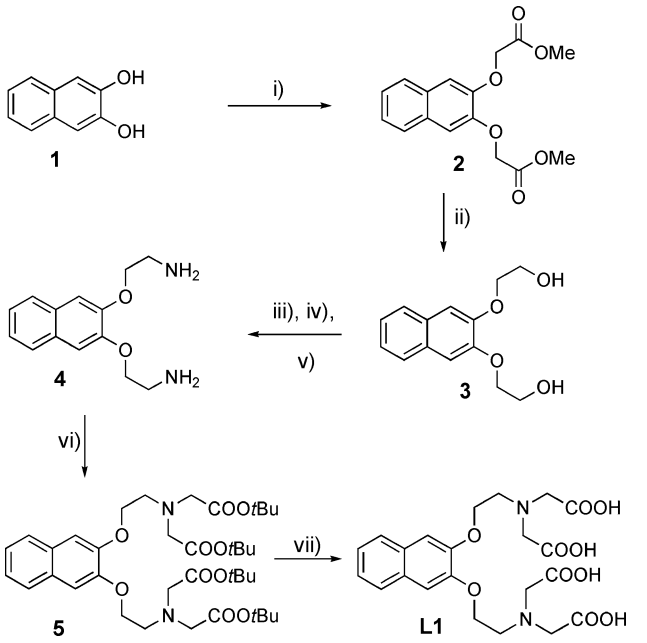
The modified ligands were prepared from 1,2-arenediols. **L1** was obtained from 2,3-naphthalenediol, **L2** from 1,2-benzenediol, whereas **L3**, **L4** and **L5** were synthesised starting from 4-nitrocatechol, in which the nitro group, once reduced, represents a useful site for further functionalization. Two synthetic protocols to access these molecules are described and compared. Moreover, solution studies on the protonation of **L2** and **L3** and their stability constants with lanthanide ions across the series and with Ca^{2+} and Mg^{2+} are reported in order to check the ability of these ligands to form stable complexes with Ln^{3+} ions and/or to complex selectively other metal ions.

Results and discussion

Synthesis of L1–L5

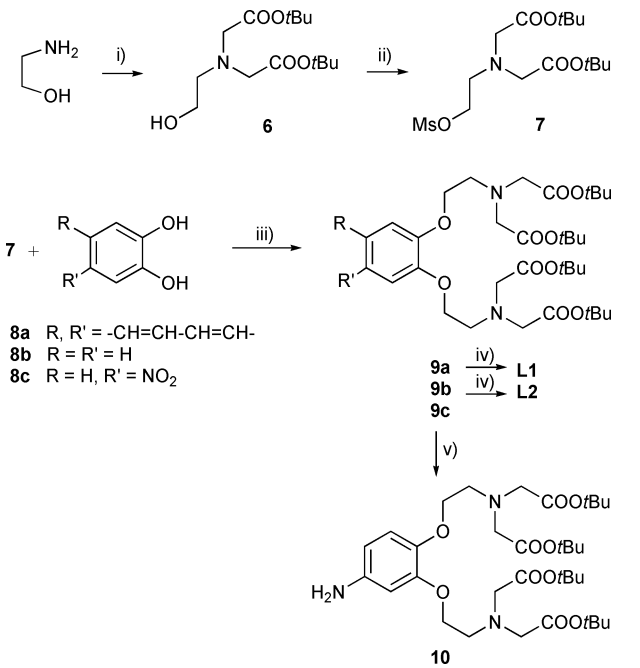
Two synthetic approaches were employed to synthesize the EGTA-derivatives **L1–L5**. Commercially available 1,2-arenediols (catechols) are the preferred starting materials for these syntheses. The first strategy, outlined in Scheme 2, relies on a multistep introduction of the aminoethyl group onto the phenolic oxygen atoms.

2,3-Naphthalenediol **1** was used as a model compound to explore this synthetic protocol. Alkylation of both phenolic oxygen atoms was performed with a slight excess of methyl bromoacetate in refluxing acetone and in the presence of potassium carbonate. Reduction of ester **2** with sodium borohydride in refluxing ethanol afforded diol **3**. The latter was converted to the dimesyl derivative trying to follow the strategy employed by Brunet, Rodríguez-Ubis *et al.*[12] to synthesize a fluorescent derivative of EGTA; unfortunately, attempted alkylation of *tert*-butyl iminodiacetate with this compound led to sluggish reactions and isolation of only minute amounts of the monoalkylation product. The dimesyl derivative was then redirected toward a more classical Gabriel protocol, obtaining the overall conversion of diol **3** to the diamine **4**. Straightforward exhaustive alkylation with *tert*-butyl bromoacetate/potassium carbonate and *tert*-butyl group



Scheme 2 *Reagents and conditions*: i) BrCH$_2$COOMe, K$_2$CO$_3$, Me$_2$CO, reflux, 2 h; ii) NaBH$_4$, EtOH, reflux, 3 h; iii) CH$_3$SO$_2$Cl, *i*Pr$_2$NEt, AcOEt, 0 °C, 3 h; iv) PhtN$^-$K$^+$, K$_2$CO$_3$, DMF, 100 °C, 6 h; v) N$_2$H$_4$·H$_2$O, EtOH, reflux, 2 h; vi) BrCH$_2$COO*t*Bu, K$_2$CO$_3$, CH$_3$CN, r.t., 48 h; vii) TFA/PhOCH$_3$ (4 : 1), r.t., 24 h.

removal with TFA/anisole gave ligand **1** in overall 10–11% yield. As multigram amounts of these ligands are needed to carry out a full characterization of their complexes, a shorter synthesis with a higher throughput was then sought. In order to reduce the number of synthetic steps, we envisaged a convergent synthesis, outlined in detail in Scheme 3.

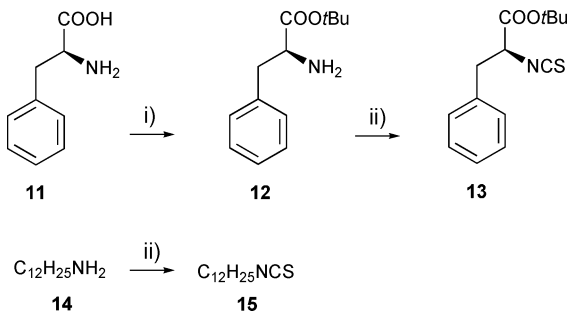


8a R, R' = -CH=CH-CH=CH-
8b R = R' = H
8c R = H, R' = NO$_2$

Scheme 3 *Reagents and conditions*: i) Ref. 13; ii) CH$_3$SO$_2$Cl, *i*Pr$_2$NEt, AcOEt, 0 °C, 3 h; iii) K$_2$CO$_3$, 18-crown-6, PhCH$_3$, r.t., 72 h; iv) TFA/PhOCH$_3$ (4 : 1), r.t., 24 h; v) H$_2$, 10% Pd/C, MeOH, r.t., 2 h.

In this alternative strategy, the lateral bis(carboxymethyl)aminoethyl arms were preformed in two steps and implanted onto the phenolic oxygen atoms through a solid–liquid phase transfer catalyzed (PTC) alkylation. Removal of *tert*-butyl esters completed the four-step synthesis. The protocol was applied to 2,3-naphthalenediol, 1,2-benzenediol and 4-nitrocatechol, obtaining **L1**, **L2** and the interesting *tert*-butyl ester **9c**, respectively; the latter was catalytically hydrogenated to compound **10**. This second synthetic pathway is shorter and allows direct access from catechols to ligand esters in one single step, using the alkylating agent **7**. Overall yield is significantly higher, being about 25% for **L1** (from catechol **1**), and hence more than doubled with respect to the linear seven-step protocol. Further optimization, especially of the PTC alkylation step, may lead to an additional improvement of the overall yield of the convergent strategy. Aminoester **10** is a key intermediate as it allows the possibility to conjugate this class of EGTA derivatives to specific targets through the free primary amine. This nucleophilic reactive group is placed strategically on the aromatic ring because: i) this position is remote with respect to the coordinating group and should not disturb the coordination of metal ions or the dynamic processes associated to the complex; ii) it is directly and firmly bound to the ligand backbone, warranting a rigid linkage with the target.

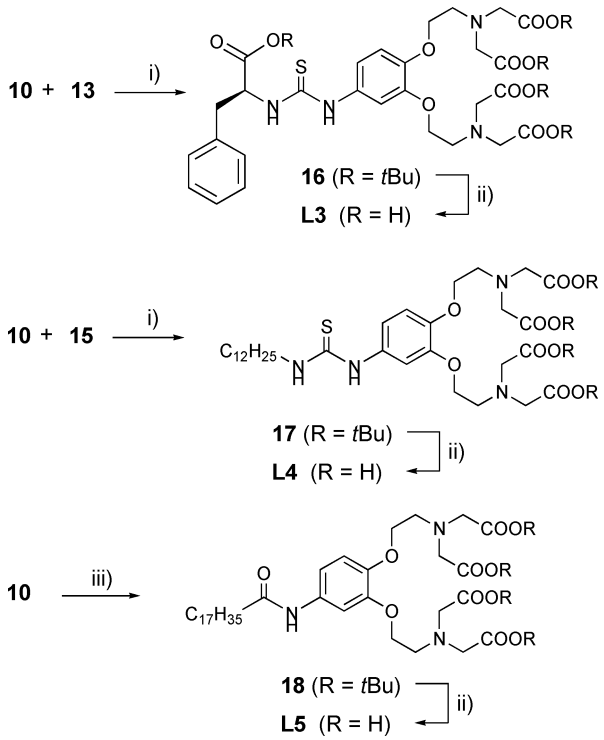
To demonstrate the conjugability and the potential improvements in CA performance, compound **10** was converted into ligands **L3–L5**. To this purpose, we selected three different electrophiles to be reacted with compound **10** to impart specific properties to the resulting ligand. We synthesized two isothiocyanates, **13** and **15**, based on phenylalanine and dodecylamine, respectively, according to Scheme 4.



Scheme 4 *Reagents and conditions*: i) *t*BuOAc, 70% aq $HClO_4$, r.t., 24 h; ii) $CSCl_2$, CH_2Cl_2–sat. aq. $NaHCO_3$, r.t., 1 h.

We selected these compounds in order to provide the EGTA-like ligand with substructures with a known affinity for the binding sites of Human Serum Albumin (HSA). Binding a paramagnetic contrast agent to a slowly tumbling macromolecule increases its relaxivity, extends its lifetime in the plasma and its residence in blood vessels. HSA is known to bind reversibly aromatic carboxylic acids and hydrophobic aliphatic chains and this explains the choice of phenylalanine, dodecylamine and stearic acid as recognition moieties conjugated to **10** in order to obtain **L3–L5**. We have recently exploited the strong interaction with HSA and the restricted local rotation upon HSA binding of the Gd complex of **L1** thanks to its naphthyl moiety. For this complex, an unprecedented high relaxivity close to that predicted by theory was observed.[14]

Scheme 5 shows the synthesis of **L3–L5** starting from **10**. Reaction of **10** with isothiocyanates **13** and **15** was carried out in dichloromethane, while acylation with stearoyl chloride was performed under Schotten–Baumann conditions in a biphasic mixture of dichloromethane and aqueous sodium carbonate. Purification of the esters and deblocking of *tert*-butyl esters led to the desired ligands **L3–L5**, subsequently used for metal ion complexation.



Scheme 5 *Reagents and conditions*: i) CH_2Cl_2, r.t., 24–48 h; ii) TFA/$PhOCH_3$ (4 : 1), r.t., 24 h; iii) $C_{17}H_{35}COCl$, CH_2Cl_2–10% aq. Na_2CO_3, r.t., 2 h.

Solution thermodynamic study

In the chemistry of lanthanides, it is generally accepted that non-ionic donor atoms are more weakly bound than ionic donor atoms. In EGTA derivatives, nitrogen atoms alone cannot coordinate to the lanthanide ion to form an energetically stable five-membered chelate ring, as typically found in other polyaminocarboxylate complexes (*e.g.* $[Ln(EDTA)]^-$). According to the literature, the stability constants of the lanthanide(III) complexes formed with diamino-tetracarboxylate ligands decrease with the increase of the distance between the two nitrogen atoms. The value of the stability constants of the $[Ln(EGTA)]^-$ complexes ($\log K_{LnL} = 15$–18) are comparable with those of the corresponding $[Ln(EDTA)]^-$ complexes ($\log K_{LnL} = 15$–20), but significantly higher than those reported for the $[Ln(HMDTA)]^-$ chelates ($\log K_{LnL} = 8$–11) (HMDTA= hexamethylenediamine-*N*,*N*,*N*′,*N*″-tetraacetic acid; the length of the ligand backbone between the two terminal nitrogen atoms is similar to that of EGTA). These data suggest that the coordination of the ethereal oxygen atoms contributes to the thermodynamic stability of $[Ln(EGTA)]^-$ complexes.[7a] In addition, the stability of the lanthanide complexes is influenced

Table 1 Protonation constants of the ligands **L2**, **L3**, EGTA and EDTA as determined by potentiometric titration at 25 °C[a]

	L2	**L3**	EGTA[b]	EDTA[b]
$\log K_1^H$	8.96 (0.02)	8.75 (0.01)	9.47	10.19
$\log K_2^H$	8.41 (0.01)	8.26 (0.01)	8.85	6.13
$\log K_3^H$	2.99 (0.02)	3.34 (0.02)	2.26	2.69
$\log K_4^H$	2.05 (0.01)	2.23 (0.02)	2.00	2.0
$\log K_5^H$	1.72 (0.02)	2.05 (0.02)	—	—

[a] Ionic strength 0.1 M KCl in all cases. [b] Ref. 7*a*.

by their coordination geometry, which is dictated by the ligand structure and flexibility. The presence of the aromatic ring increases the stereochemical rigidity of the ligands and this could affect their coordinating ability towards the Ln(III) cations.

Protonation constants. The protonation constants ($\log K_i^H$) of **L2** and **L3** were determined by pH-potentiometry and are reported in Table 1 with those of EGTA and EDTA for comparison (standard deviations are shown in parentheses). The protonation constants are defined as follows:

$$K_i^H = \frac{[H_iL]}{[H_{i-1}L][H^+]} \quad i = 1,2,3\ldots \tag{1}$$

A comparison of the protonation constants of **L2**, **L3** and EGTA, obtained in similar media, reveals that the $\log K_3^H$ and $\log K_4^H$ values are quite similar, whereas the values of $\log K_1^H$ and $\log K_2^H$ slightly differ. To explain these findings, we may assume that, in analogy with the protonation scheme of EGTA,[15,16] the first and second equivalents of acid protonate the two nitrogen atoms, while the third and fourth protonation processes involve the carboxylate oxygens. The third protonation process in **L3** is likely to correspond to the protonation of the carboxylate oxygen of the pendant phenylalanine moiety. The small differences found between the corresponding protonation constants of **L2** and **L3** indicate a slightly different basicity of the nitrogen atoms imposed by the substituent on the aromatic ring. On the other hand, the protonation constants of the nitrogen atoms in **L2** and **L3** are somewhat lower than those of EGTA, likely as a result of their lower basicity arising from the electron- withdrawing character of the aromatic group. Moreover, the similar values of $\log K_1^H$ and $\log K_2^H$ are simply accounted for in terms of two distinct protonation processes originating from the large distance between the two nitrogen atoms.

Stability and protonation constants of complexes. The stability and protonation constants of the metal complexes formed with **L2** and **L3** are defined by eqns (2) and (3):

$$K_{ML} = \frac{[ML]}{[M][L]} \tag{2}$$

$$K_{MH_iL} = \frac{[MH_iL]}{[MH_{i-1}L][H^+]} \quad i = 1,2,3 \tag{3}$$

The protonation and stability constants obtained by direct pH-potentiometric titration are reported in Table 2. The protonation and stability constants of [Ln(**L2**)]$^-$ and [Ln(**L3**)]$^-$ were calculated from the titration curves, obtained with a 1 : 1 metal-to-ligand concentration ratio. The best fitting was performed by using the model that includes the formation of the ML, MHL and MH_2L species in the equilibrium.

The **L2** and **L3** ligands possess two amino nitrogens, four carboxylate oxygens (or five in the case of **L3**) and two ethereal oxygen donor atoms. The presence of the aromatic ring in the ligands not only results in a decrease of the basicity of the oxygen and nitrogen donor atoms, but it also increases the stereochemical rigidity of the backbone, which in turn could affect the geometry of the coordination polyhedron.

The values of the stability and protonation constants of the complexes of Mg(II) with **L2** and EGTA are rather similar (Table 2), whereas the values of $\log K_{ML}$ and $\log K_{MHL}$ for [Ca**L2**]$^{2-}$, [Ln**L2**]$^-$ and [Ln**L3**]$^-$ are about one or two orders of magnitude lower than those reported for [Ca(EGTA)]$^{2-}$ and [Ln(EGTA)]$^-$. The lower stability of [Mg(EGTA)]$^{2-}$ and [Mg**L2**]$^{2-}$ compared to [Mg(EDTA)]$^{2-}$ is explained in terms of the larger size of EGTA and **L2**, which prevents the coordination of all the donor atoms to the small Mg^{2+} ion due to the electrostatic repulsion between the carboxylate groups. In the case of [Ln**L2**]$^-$ and [Ln**L3**]$^-$, coordination of all donor atoms of the ligands (except the carboxylate group of the phenylalanine moiety in **L3**) is clearly suggested by the results of solution NMR or X-ray diffraction studies on the corresponding [Ln(EGTA)]$^-$ complexes.[9,10] The lower basicity of the amine N and ethereal O donor atoms results in a lower stability of [Ca**L2**]$^{2-}$, [Ln**L2**]$^-$ and [Ln**L3**]$^-$ compared to the complexes with EGTA (Table 2). The stability constants of [Ln**L2**]$^-$ complexes increase from La^{3+} to Er^{3+}, where a plateau is reached. This behaviour differs from that found for the [Ln(EGTA)]$^-$ complexes whose $\log K_{ML}$ values increase

Table 2 The thermodynamic stability constants of complexes formed between **L2**, **L3**, EGTA, EDTA and selected lanthanide and alkaline earth metal ions at 25 °C[a]

	L2		**L3**			EGTA[b]		EDTA[c]
	$\log K_{ML}$	$\log K_{MHL}$	$\log K_{ML}$	$\log K_{MHL}$	$\log K_{MH_2L}$	$\log K_{ML}$	$\log K_{MHL}$	$\log K_{ML}$
Mg^{2+}	5.12 (0.01)	7.35 (0.02)	—	—	—	5.28	7.62	8.69
Ca^{2+}	9.99 (0.01)	4.64 (0.03)	—	—	—	10.86	3.79	10.61
La^{3+}	—	—	14.07 (0.03)	3.20 (0.04)	3.04 (0.05)	15.55	—	15.46
Ce^{3+}	14.62 (0.01)	3.09 (0.02)	—	—	—	15.70	—	15.95
Nd^{3+}	15.26 (0.01)	3.05 (0.02)	—	—	—	16.28	—	16.56
Gd^{3+}	15.76 (0.02)	2.33 (0.05)	15.25 (0.04)	3.03 (0.04)	2.09 (0.07)	16.97	—	17.35
Er^{3+}	16.21 (0.01)	2.34 (0.02)	—	—	—	17.40	—	18.83
Lu^{3+}	16.23 (0.01)	2.31 (0.04)	15.43 (0.04)	3.12 (0.05)	—	17.81	—	19.80

[a] Ionic strength 0.1 M KCl for **L2**, **L3** and EDTA; 0.1 M KNO_3 for EGTA. [b] Ref. 17. [c] Ref. 7*a*.

monotonically from La^{3+} to Lu^{3+}. These data clearly indicate an influence of the ligand "rigidity" on the trend of the $\log K_{ML}$ values across the lanthanide series and the presence of an optimal size match for the Er^{3+}–Lu^{3+} cations. On the other hand, only a small change in the stability constants for the complexes with **L3** is observed on passing from Gd to Lu. It could be hypothesized that the lower stability of the [Ln**L3**]$^-$ complexes is caused by the presence of an intramolecular "stacking" interaction between the aromatic moieties of **L3**. This interaction could further decrease the flexibility of the chelator, with negative effects particularly in the case of the coordination to the smaller Ln^{3+} ions.

Conclusions

In this paper we have reported the synthesis of a new series of EGTA derivatives incorporating an aromatic group into the ligand backbone. Ligands **L3–L5** feature a further functionalization on the aromatic group, leading to bifunctional chelating agents of potential utility for biomedical applications. Metal complexes of **L4** and **L5** are expected to aggregate and form micelles as well as to interact non-covalently with HSA: both these types of macromolecular conjugates are of potential interest for contrast-enhanced MRI applications.[6] In fact, preliminary ^1H and ^{17}O relaxometric data on the corresponding Gd(III) complexes of **L1–L5** are consistent with the presence of one coordinated water molecule in fast exchange with the bulk.[14] The relaxivity increases as a function of the molecular weight (Gd**L1–L4**) and it is strongly enhanced in the presence of human serum albumin (Gd**L2–L5**). A full account of these results will be presented in a subsequent paper. The structural modifications of the parent ligand do not compromise the coordinating ability towards lanthanide and alkaline earth metal ions, as demonstrated by solution thermodynamic studies. Minor variations are explained in terms of increased stereochemical rigidity imposed by the aromatic moiety. Future work will be directed to further increasing the stability of the complexes, which are still inadequate for medical applications.

Experimental

All chemicals were purchased from Sigma-Aldrich Co. and were used without purification unless otherwise stated. NMR spectra were recorded on a JEOL ECP 300 spectrometer (operating at 7.05 Tesla). ESI mass spectra were recorded on ThermoFinnigan LCQ-Deca XP-Plus and melting points (uncorrected) with Stuart Scientific SMP3 apparatus.

The synthesis and characterisation of compounds **1–4** (Scheme 2) are reported in the ESI†.

Compound 7

Compound **6**[13] (5.0 g, 17.3 mmol) was dissolved in dry ethyl acetate (30 mL); ethyldiisopropylamine (3.23 mL, 19.0 mmol) was added to the solution and the mixture was cooled to 0 °C. Methanesulfonyl chloride (1.18 mL, 17.3 mmol) was slowly added dropwise and the mixture stirred at 0 °C for 3 h. The solvent was removed *in vacuo* and the oily product was purified by flash chromatography (eluant petroleum ether–ethyl acetate 7 : 3) giving compound **7** (4.88 g, 77% yield). Due to its instability, compound **7** was not characterized and was used as such for the following steps.

Compound 5

2,3-Naphthalenediol (500 mg, 3.1 mmol), powdered potassium carbonate (700 mg, 12.5 mmol) and 18-crown-6 (165 mg, 0.6 mmol) were mixed in toluene (40 mL), under a nitrogen atmosphere. Compound **7** (2.82 g, 7.8 mmol) was slowly added to the stirring suspension, previously cooled to 0–5 °C. After the addition the stirring mixture was left to reach room temperature and followed by HPLC. After 72 h the mixture was filtered and evaporated *in vacuo*. The residue was dissolved in dichloromethane (20 mL) and washed with 5% aq. K_2CO_3 (3 × 25 mL), aq. $NaHSO_3$ (3 × 25 mL) and water (3 × 25 mL). The organic phase was then dried over Na_2SO_4, filtered and evaporated. The oily crude product was purified by flash chromatography (silica gel, eluant petroleum ether–ethyl acetate–2-propanol 85 : 10 : 5), providing pure **5** as a light yellow oil (899 mg, 41% yield). MS (ESI) 703.1 (MH^+). Calc. for $C_{38}H_{52}N_2O_{10}$: 702.1. ^1H-NMR ($CDCl_3$): 7.60 (m, 2H), 7.28 (m, 2H), 7.13 (s, 2H), 4.24 (t, 4H, $J = 6.3$ Hz), 3.58 (s, 8H), 3.24 (t, 4H, $J = 6.3$ Hz), 1.44 (s, 36H). ^{13}C-NMR ($CDCl_3$): 170.8 (C), 148.9 (C), 129.2 (C), 126.2 (CH), 123.9 (CH), 107.7 (CH), 80.9 (C), 67.9 (CH_2), 57.0 (CH_2), 53.2 (CH_2), 28.1 (CH_3).

Ligand L1

Compound **5** (350 mg, 0.49 mmol) was dissolved in a mixture of anisole and trifluoroacetic acid (1 : 4, 5 mL) and stirred at room temperature for 24 h. Volatiles were evaporated *in vacuo* and the crude product was redissolved in methanol (1 mL); slow addition of excess diethyl ether led to precipitation of **L1** as a white amorphous solid, isolated by centrifugation. Dissolution in methanol and precipitation with diethyl ether was repeated thrice, obtaining analytically pure **L1**. Yield 0.147 g. M.p. 151–152 °C (dec.). MS (ESI) 479.0 (MH^+). Calc. for $C_{22}H_{26}N_2O_{10}$: 478.1. ^1H-NMR (D_2O): 7.65 (m, 2H), 7.25 (m, 2H), 7.23 (s, 2H), 4.25 (t, 4H, $J \sim 5$ Hz), 3.78 (s, 8H), 3.35 (t, 4H, $J \sim 5$ Hz). ^{13}C-NMR (D_2O): 169.9 (C), 147.0 (C), 129.1 (C), 126.6 (CH), 125.0 (CH), 108.4 (CH), 63.1 (CH_2), 57.5 (CH_2), 54.6 (CH_2).

Compound 9b

Prepared in 44% yield following the procedure reported for compound **5**, starting from catechol **8b**. Light yellow oil. MS (ESI) 653.2 (MH^+). Calc. for $C_{34}H_{56}N_2O_{10}$: 652.1. ^1H-NMR ($CDCl_3$): 6.84–6.75 (m, 4H), 4.05 (t, 4H, $J \sim 6.0$ Hz), 3.48 (s, 8H), 3.09 (t, 4H, $J \sim 6.1$ Hz), 1.45 (s, 36H). ^{13}C-NMR ($CDCl_3$): 170.7 (C), 148.7 (C), 121.7 (CH), 113.8 (CH), 80.9 (C), 68.2 (CH_2), 56.8 (CH_2), 53.4 (CH_2), 28.1 (CH_3).

Compound 9c

Prepared in 27% yield following the procedure reported for compound **5**, starting from 4-nitrocatechol **8c**. Light yellow oil. MS (ESI) 698.4 (MH^+). Calc. for $C_{34}H_{55}N_3O_{12}$: 697.1. ^1H-NMR ($CDCl_3$): 7.86 (dd, 1H, $J_1 = 8.8$ Hz, $J_2 = 2.6$ Hz), 7.76 (d, 1H, $J = 2.6$ Hz), 6.93 (d, 1H, $J = 8.9$ Hz), 4.24 (t, 2H, $J = 6.1$ Hz), 4.21 (t, 2H, $J = 5.8$ Hz), 3.57 (s, 4H), 3.56 (s, 4H), 3.22 (t, 2H, $J = 5.9$ Hz), 3.21 (t, 2H, $J = 6.1$ Hz), 1.46 (s, 18H), 1.44 (s, 18H).

^{13}C-NMR ($CDCl_3$): 170.6 (2 × C), 154.2 (C), 148.3 (C), 141.3 (C), 117.8 (CH), 111.1 (CH), 107.9 (CH), 81.1 (C), 81.0 (C), 68.7 (2 × CH_2), 57.0 (CH_2), 56.9 (CH_2), 53.1 (2 × CH_2), 28.1 (2 × CH_3).

Ligand L2

Compound **9b** (950 mg, 1.46 mmol) was dissolved in a mixture of anisole and trifluoroacetic acid (1:4, 10 mL) and stirred at room temperature for 24h. Volatiles were evaporated *in vacuo* and the crude product was redissolved in methanol (2 mL); slow addition of excess diethyl ether led to precipitation of **L2** as a white amorphous solid, isolated by centrifugation. Dissolution in methanol and precipitation with diethyl ether was repeated thrice, obtaining analytically pure **L2**. Yield 0.485 g. M.p. 132 °C (sint.), 180 °C (dec.). MS (ESI) 451.1 (MNa^+), 429.2 (MH^+). Calc. for $C_{18}H_{24}N_2O_{10}$: 428.1. ^{1}H-NMR (D_2O): 7.04 (m, 4H), 4.43 (bt, 4H), 4.18 (s, 8H), 3.85 (bt, 4H). ^{13}C-NMR (D_2O): 169.2 (C), 146.8 (C), 122.6 (CH), 113.7 (CH), 63.1 (CH_2), 57.5 (CH_2), 56.7 (CH_2), 55.3 (CH_2).

Compound 10

Compound **9c** (697 mg, 1.00 mmol) was dissolved in methanol (20 mL) and Pd/C (10%, 100 mg) was added. The mixture was introduced into a hydrogenation bottle, purged with nitrogen and then stirred under hydrogen (1 atm) until HPLC analysis showed complete reduction of the substrate (~2 h). The catalyst was removed by filtration on Celite® and the solvent by evaporation *in vacuo* leaving the aminoester **5** as a light orange oil. Yield 510 mg (76% yield). MS (ESI) 668.4 (MH^+). Calc. for $C_{34}H_{57}N_3O_{10}$: 667.1. ^{1}H-NMR (CD_3OD): 6.74 (d, 1H, J = 8.5 Hz), 6.44 (d, 1H, J = 2.4 Hz), 6.27 (dd, 1H, J_1 = 8.9 Hz, J_2 = 2.4 Hz), 4.07 (t, 2H, J = 5.4 Hz), 4.01 (t, 2H, J = 5.4 Hz), 3.55 (s, 4H), 3.54 (s, 4H), 3.12 (t, 2H, J = 5.4 Hz), 3.07 (t, 2H, J = 5.4 Hz), 1.46 (s, 36H). ^{13}C-NMR (CD_3OD): 171.0 2x(C), 149.7 (C), 142.7 (C), 141.1 (C), 116.4 (CH), 107.5 (CH), 102.5 (CH), 80.94 (C), 80.90 (C), 69.1 (CH_2), 67.5 (CH_2), 56.4 (CH_2), 56.3 (CH_2), 53.7 (CH_2), 53.6 (CH_2), 27.1 (2 × CH_3).

L-Phenylalanine *tert*-butyl ester (12)

L-Phenylalanine (3.00 g, 18.2 mmol) was suspended in *tert*-butyl acetate (100 mL) and 70% aq. $HClO_4$ (2.0 g, 20 mmol) and the mixture stirred at room temperature. After 24 h the reaction mixture was washed with 5% aq. Na_2CO_3 (50 mL), brine (50 mL) and water (50 mL), then dried over Na_2SO_4, filtered and evaporated *in vacuo* to provide **12** as a colorless oil (2.18 g, 54%), used without further purification in the following step.

Compound 13

A solution of thiophosgene (1.37 g, 11.8 mmol) in dichloromethane (5.0 mL) was slowly added dropwise to a cooled (0–5 °C) flask containing a stirring mixture of phenylalanine *tert*-butyl ester (**12**, 2.37 g, 10.7 mmol), dichloromethane (5 mL) and sat. aq. $NaHCO_3$ (10 mL). Stirring was maintained for 1 h and then the organic layer was separated, washed thrice with water (10 mL), dried over Na_2SO_4, filtered and evaporated *in vacuo*. Compound **13** was obtained as an amorphous off-white solid. Yield 1.93 g (68%). MS (ESI) 264.2 (MH^+). Calc. for $C_{14}H_{17}NO_2S$: 263.1. ^{1}H-NMR ($CDCl_3$): 7.37–7.23 (m, 5H), 4.33 (dd, 1H, J_1 = 7.9 Hz, J_2 = 5.2 Hz), 3.20 (dd, 1H, J_1 = 13.8 Hz, J_2 = 5.2 Hz), 3.11 (dd, 1H, J_1 = 13.8 Hz, J_2 = 7.9 Hz), 1.47 (s, 9H). ^{13}C-NMR ($CDCl_3$): 166.7 (C), 137.8 (C), 135.3 (C), 129.5 (CH), 128.6 (CH), 127.5 (CH), 83.7 (C), 61.3 (CH), 39.5 (CH_2), 27.9 (CH_3).

Compound 15

Obtained as off-white amorphous solid following the same procedure adopted for compound **13**, starting from dodecylamine **14** (1.98 g, 10.8 mmol). Yield 1.86 g (76%). MS (ESI) 228.0 (MH^+). Calc. for $C_{13}H_{25}NS$: 227.1. ^{1}H-NMR ($CDCl_3$): 3.50 (t, 2H, J = 6.6 Hz), 1.70 (quint, 2H, J = 6.8 Hz), 1.41 (m, 2H), 1.27 (m, 16H), 0.88 (bt, 3H, J = 6.7 Hz). ^{13}C-NMR ($CDCl_3$): 129.7 (C), 45.1 (CH_2), 31.8 (CH_2), 30.0 (CH_2), 29.6 (2 × CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 28.8 (CH_2), 26.5 (CH_2), 22.7 (CH_2), 14.1 (CH_3).

Compound 16

Compound **10** (435 mg, 0.65 mmol) was dissolved in dichloromethane (5.0 mL) and stirred at 0–5 °C under nitrogen. A solution of **13** (175 mg, 0.66 mmol) in dichloromethane (5.0 mL) was added dropwise and the resulting solution was stirred for 48 h. The solvent was removed *in vacuo* and the oily residue was purified by flash chromatography (petroleum ether–ethyl acetate–2-propanol 85 : 10 : 5) to provide the conjugate **16** as a yellow oil. Yield 314 mg (52%). MS (ESI) 931.5 (MH^+). Calc. for $C_{48}H_{74}N_4O_{12}S$: 930.5. ^{1}H-NMR ($CDCl_3$): 7.63 (bs, 1H), 7.30–6.40 (m, 9H), 5.19 (m, 1H), 4.10 (t, 2H, J = 5.6 Hz), 4.00 (bt, 2H), 3.55 (s, 4H), 3.54 (s, 4H), 3.29 (dd, 1H, J_1 = 13.9 Hz, J_2 = 6.6 Hz), 3.20–3.14 (m, 5H), 1.44 (s, 36H), 1.37 (s, 9H). ^{13}C-NMR ($CDCl_3$): 170.6 (2 × C), 170.2 (C), 149.7 (C), 148.0 (C), 136.0 (C), 129.5 (CH), 128.6 (C), 128.3 (CH), 126.9 (CH), 117.9 (CH), 113.9 (CH), 111.0 (CH), 82.3 (C), 81.0 (2 × C), 68.5 (2 × CH_2), 58.9 (CH), 56.9 (CH_2), 56.7 (CH_2), 53.4 (CH_2), 53.2 (CH_2), 37.5 (CH_2), 28.1 (2 × CH_3), 27.9 (CH_3).

Compound 17

Obtained as a yellow oil following the same procedure adopted for compound **16**, starting from aminoester **10** (450 mg, 0.67 mmol) and isothiocyanate **15** (161 mg, 0.71 mmol). Yield 341 mg (57%). MS (ESI) 895.5 (MH^+). Calc. for $C_{47}H_{82}N_4O_{10}S$: 894.5. ^{1}H-NMR ($CDCl_3$): 6.97–6.68 (m, 3H), 7.73 (bs, 1H), 6.38 (bs, 1H), 4.09 (bt, 4H), 3.58 (s, 4H), 3.56 (s, 4H), 3.48 (bt, 2H), 3.18 (bt, 4H), 1.58 (m, 2H), 1.41 (s, 36H), 1.33–1.21 (m, 18H), 0.88 (bt, 3H, J = 6.3 Hz). ^{13}C-NMR ($CDCl_3$): 170.5 (C), 170.3 (C), 148.6 (C), 146.6 (C), 134.7 (C), 117.8 (CH), 113.8 (CH), 111.8 (CH), 81.9 (C), 81.1 (C), 67.8 (2 × CH_2), 56.6 (2 × CH), 53.7 (CH_2), 53.6 (CH_2), 44.9 (CH_2), 31.8 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.3 (CH_2), 29.0 (CH_2), 28.0 (4 × CH_2), 27.9 (2 × CH_3), 22.6 (CH_2), 14.0 (CH_3).

Compound 18

Aminoester **10** (520 mg, 0.78 mmol) was dissolved in a stirring mixture of dichloromethane (10.0 mL) and 10% aq. Na_2CO_3, cooled in an ice bath under an N_2 atmosphere. A solution of stearoyl chloride (245 mg, 0.80 mmol) in dichloromethane

(5.0 mL) was slowly added dropwise and the resulting suspension was stirred for 2 h. The organic layer was separated and washed with 10% aq. Na_2CO_3 (3 × 20 mL), dried over Na_2SO_4, filtered and evaporated *in vacuo*. The conjugate **18** was obtained as a light yellow oil. Yield 662 mg (91%). MS (ESI) 934.5 (MH^+). Calc. for $C_{52}H_{91}N_3O_{11}$: 933.8. ^{1}H-NMR ($CDCl_3$): 7.52 (bs, 1H), 7.28 (s, 1H), 7.10 (d, 1H, $J = 8.3$ Hz), 6.81 (d, 1H, $J = 8.7$ Hz), 4.12 (t, 2H, $J = 6.0$ Hz), 4.09 (t, 2H, $J = 6.0$ Hz), 3.54 (s, 4H), 3.53 (s, 4H), 3.14 (t, 4H, $J = 5.9$ Hz), 2.30 (bt, 2H, $J = 7.2$ Hz), 1.69 (quint, 2H, $J = 6.9$ Hz), 1.44 (s, 18H), 1.42 (s, 18H), 1.40–1.25 (m, 28H), 0.86 (bt, 3H, $J = 6.4$ Hz). ^{13}C-NMR ($CDCl_3$): 171.3 (C), 170.7 (C), 170.6 (C), 148.8 (C), 145.1 (C), 132.3 (C), 114.3 (CH), 112.2 (CH), 107.0 (CH), 81.0 (C), 80.9 (C), 68.5 (CH_2), 68.4 (CH_2), 56.7 (CH_2), 56.6 (CH_2), 56.5 (CH_2), 53.4 (CH_2), 37.7 (CH_2), 31.9 (CH_2), 29.6–29.3 (12 × CH_2), 28.11 (CH_3), 28.07 (CH_3), 25.3 (CH_2), 22.6 (CH_2), 14.1 (CH_3).

Ligand L3

Obtained as an amorphous light yellow powder following the same procedure adopted for the *tert*-butyl group removal giving **L1**, starting from ester **16** (126 mg, 0.13 mmol). Yield 94 mg. M.p. 158–159 °C (dec.). MS (ESI, negative ion mode) 649.2 ($M - H^+$). Calc. for $C_{28}H_{34}N_4O_{12}S$: 650.2. ^{1}H-NMR (DMSO-d_6): 10.52 (bs, 4H), 9.69 (bs, 1H), 7.31–6.88 (m, 8H), 6.33 (bd, 1H), 6.13 (bs, 1H), 5.22 (m, 1H), 4.03–3.84 (m, 4H), 3.55 (s, 8H), 3.46 (m, 1H), 3.17–2.93 (m, 5H). ^{13}C-NMR (D_2O): 173.9 (C), 173.2 (C), 148.6 (C), 148.2 (CH), 134.8 (C), 130.3 (CH), 129.5 (CH), 128.6 (CH), 121.4 (CH), 113.8 (CH), 113.0 (CH), 68.4 (CH_2), 68.1 (CH_2), 60.4 (CH), 55.9 (CH_2), 53.2 (CH_2), 36.7 (CH_2).

Ligand L4

Obtained as an amorphous yellow powder following the same procedure adopted for the *tert*-butyl group removal giving **L1**, starting from ester **17** (243 mg, 0.27 mmol). Yield 147 mg. MS (ESI) 671.5 (MH^+). Calc. for $C_{31}H_{50}N_4O_{10}S$: 670.5. ^{1}H-NMR (D_2O): 6.89 (m, 2H), 6.78 (d, 1H, $J = 8.8$ Hz), 4.03 (bt, 4H), 3.47 (bt, 2H), 3.17 (s, 8H), 2.92 (bt, 4H), 1.54 (m, 2H), 1.24 (m, 18H), 0.85 (bt, 3H), $J = 6.1$ Hz).

Ligand L5

Obtained as an amorphous white powder following the same procedure adopted for *tert*-butyl group removal giving **L1**, starting from ester **18** (402 mg, 0.43 mmol). Yield 249 mg. M.p. 160 °C (dec.). MS (ESI) 711.0 (MH^+). Calc. for $C_{36}H_{59}N_3O_{11}$: 709.8. ^{1}H-NMR (DMSO-d_6): 11.30 (bs, 4H), 9.67 (bs, 1H), 7.28 (d, 1H, $J = 1.7$ Hz), 7.07 (dd, 1H, $J_1 = 8.6$ Hz, $J_2 = 1.9$ Hz), 6.86 (d, 1H, $J = 8.7$ Hz), 3.98 (t, 2H, $J = 5.7$ Hz), 3.97 (t, 2H, $J = 6.0$ Hz), 3.53 (bs, 8H), 3.05 (t, 2H, $J = 5.7$ Hz), 3.02 (t, 2H, $J = 5.7$ Hz), 2.24 (t, 2H, $J = 7.4$ Hz), 1.56 (m, 2H), 1.31–1.15 (m, 28H), 0.86 (bt, 3H, $J = 6.4$ Hz). ^{13}C-NMR (DMSO-d_6): 173.2 (2 × C), 171.2 (C), 148.5 (C), 144.3 (C), 133.8 (C), 114.5 (CH), 111.6 (CH), 106.0 (CH), 68.4 (CH_2), 68.0 (CH_2), 56.0 (2 × CH_2), 53.3 (2 × CH_2), 36.8 (CH_2), 31.7 (CH_2), 29.5–29.1 (12 × CH_2), 25.2 (CH_2), 22.5 (CH_2), 14.4 (CH_3).

Potentiometric studies

The chemicals used for the experiments were of the highest analytical grade. The $LnCl_3$ solutions were prepared from $LnCl_3 \cdot xH_2O$ ($x = 5$–7). The concentration of the $MgCl_2$, $CaCl_2$, and $LnCl_3$ solutions were determined by complexometric titration with standardized Na_2H_2EDTA and xylenol orange ($LnCl_3$), Patton & Reeder ($CaCl_2$) and eriochrome black T ($MgCl_2$) as indicator. The concentration of **L2** ligand was determined by pH-potentiometric titration in the presence and absence of a large excess (40-fold) of $CaCl_2$. The protonation and the stability constants of the metal complexes formed with **L2** were determined by pH-potentiometric titration. The metal-to-ligand concentration ratios were 1 : 1 with a concentration of ligand generally of 0.002 M.

pH measurements and titrations were performed on a CRISON micro pH 2002 pH-meter, a CRISON micro BU2030 autoburette and a Metrohm-6.0233.100 combined electrode. Equilibrium measurements were carried out at a constant ionic strength (0.1 M KCl) in 10 mL sample at 25 °C. The solutions were stirred with N_2 bubbling. The titrations were carried out in the pH range 1.7–11.7. For the calibration of the pH meter, buffer standard solution, color-coded "pink" (pH = 4.010) and buffer standard solution, color coded "yellow" (pH = 7.000) buffers were used. For the calculation of $[H^+]$ from the measured pH values, the method proposed by Irving *et al.* was used.[18] A 0.01 M HCl solution was titrated with the standardized KOH solution. The differences between the measured and calculated pH values were used to obtain the H^+ concentration from the pH values, measured in the titration experiments. The protonation and stability constants were calculated with the program PSEQUAD.[19]

Acknowledgements

Financial support from PRIN 2005 is gratefully acknowledged. This work was carried out under the auspices of CIRCMSB and COST Action D38.

References

1 Z. J. Guo and P. J. Sadler, *Adv. Inorg. Chem.*, 2000, **49**, 183; T. Storr, K. H. Thompson and C. Orvig, *Chem. Soc. Rev.*, 2006, **35**, 534; E. Meggers, *Curr. Opin. Chem. Biol.*, 2007, **11**, 287; S. M. Cohen, *Curr. Opin. Chem. Biol.*, 2007, **11**, 115.

2 *Supramolecular Chemistry of Anions*, ed. A. Bianchi, K. Bowman-James and E. Garcia-Espana, Wiley-VCH, New York, 1997; J. L. Sessler, P. A. Gale and W.-S. Cho, *Anion Receptor Chemistry*, RSC Publishing, Cambridge, 2006; K. Bowman-James, *Acc. Chem. Res.*, 2005, **38**, 671; K. Wichmann, B. Antonioli, T. Sohnel, M. Wenzel, K. Gloe, K. Gloe, J. R. Price, L. F. Lindoy, A. J. Blake and M. Schroder, *Coord. Chem. Rev.*, 2006, **250**, 2987; P. Gamez, T. J. Mooibroek, S. J. Teat and J. Reedijk, *Acc. Chem. Res.*, 2007, **40**, 435.

3 Recent reviewed examples: S. F. Liu and J. L. Xiao, *J. Mol. Catal. A: Chem.*, 2007, **270**, 1; A. Fihri, P. Meunier and J. C. Hierso, *Coord. Chem. Rev.*, 2007, **251**, 2017; S. Gladiali and E. Alberico, *Chem. Soc. Rev.*, 2006, **35**, 226; V. Dragutan, I. Dragutan, L. Delaude and A. Demonceau, *Coord. Chem. Rev.*, 2007, **251**, 765.

4 Selected books and reviews: B. Valeur and I. Leray, *Coord. Chem. Rev.*, 2000, **205**, 3; *Chemosensors of Ion and Molecule Recognition*, ed. J. P. Desvergne and A. W. Czarnik, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1997; A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515; L. Fabbrizzi, F. Foti, S. Patroni, P. Pallavicini and A. Taglietti, *Angew. Chem., Int. Ed.*, 2004, **43**, 5073.

5 P. Chaudhuri, K. Wieghardt, T. Weyhermuller, T. K. Paine, S. Mukherjee and C. Mukherjee, *Biol. Chem.*, 2005, **386**, 1023; P. Chaudhuri and K. Wieghardt, *Prog. Inorg. Chem.*, 2001, **50**, 151; D. Fiedler, D. H. Leung, R. G. Bergman and K. N. Raymond, *Acc. Chem. Res.*, 2005, **38**, 349.
6 P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev.*, 1999, **99**, 2293; S. Aime, S. Geninatti Crich, E. Gianolio, G. B. Giovenzana, L. Tei and E. Terreno, *Coord. Chem. Rev.*, 2006, **250**, 1562; S. Aime, M. Botta and E. Terreno, *Adv. Inorg. Chem.*, 2005, **57**, 173; *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, ed. E. Toth and A. E. Merbach, Wiley, New York, 2001; S. Aime, M. Botta, M. Fasano and E. Terreno, *Chem. Soc. Rev.*, 1998, **27**, 19; R. Artali, M. Botta, C. Cavallotti, G. B. Giovenzana, G. Palmisano and M. Sisti, *Org. Biomol. Chem.*, 2007, **5**, 2441.
7 (*a*) A. E. Martell and R. M. Smith, *Critical Stability Constants*, vol. 4, Plenum Press, New York, 1974; (*b*) A. Bianchi, L. Calabi, F. Corana, S. Fontana, P. Losi, A. Maiocchi, L. Paleari and B. Valtancoli, *Coord. Chem. Rev.*, 2000, **204**, 309.
8 See for example: J.-M. Idee, M. Port, I. Raynal, M. Schaefer, S. Le Greneur and C. Corot, *Fundam. Clin. Pharmacol.*, 2006, **20**, 563; S. K. Morcos, *Br. J. Pharmacol.*, 2007, **80**, 73.
9 S. Aime, A. Barge, A. Borel, M. Botta, S. Chemerisov, A. E. Merbach, U. Müller and D. Pubanz, *Inorg. Chem.*, 1997, **36**, 5104.
10 C. K. Schauer and O. P. Anderson, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1986, **42**, 760; C. K. Schauer and O. P. Anderson, *J. Am. Chem. Soc.*, 1987, **109**, 3646.
11 E. Brunet, M. T. Alonso, O. Juanes, O. Velasco and J. C. Rodriguez-Ubis, *Tetrahedron*, 2005, **57**, 3105; S. R. Adams, J. P. Y. Kao, G. Grynkiewicz, A. Minta and R. Y. Tsien, *J. Am. Chem. Soc.*, 1988, **110**, 3212; G. C. R. Ellis-Davies and J. H. Kaplan, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 187; G. C. R. Ellis-Davies, *Tetrahedron Lett.*, 1998, **39**, 953; W. Li, S. Fraser and T. Meade, *J. Am. Chem. Soc.*, 1999, **121**, 1413; R. Y. Tsien, *Biochemistry*, 1980, **19**, 2396.
12 E. Brunet, M. T. Alonso, O. Juanes, O. Velasco and J. C. Rodríguez-Ubis, *Tetrahedron*, 2000, **57**, 3105.
13 M. A. Williams and H. Rapoport, *J. Org. Chem.*, 1993, **58**, 1151.
14 S. Avedano, L. Tei, A. Lombardi, G. B. Giovenzana, S. Aime, D. Longo and M. Botta, *Chem. Commun.*, 2007, 4726.
15 S. Aime, A. Barge, M. Botta, L. Frullano, U. Merlo and K. I. Hardcastle, *J. Chem. Soc., Dalton Trans.*, 2000, 3435.
16 J. Felcman and J. da Silva, *Talanta*, 1983, **301**, 565.
17 J. L. Mackey, M. A. Hiller and J. E. Powell, *J. Phys. Chem.*, 1962, **66**, 311.
18 H. M. Irving, M. G. Miles and L. Pettit, *Anal. Chim. Acta*, 1967, **28**, 475.
19 L. Zékány and I. Nagypál, in *Computational Methods for Determination of Formation Constants*, ed. D. J. Legett, Plenum, New York, 1985, p. 291.

