New Polypodal Polycarboxylic Ligands − Complexation of Rare-Earth Ions in Aqueous Solution

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The complexation ability of new polypodal carboxylic ligands prepared from 2,2-bis(hydroxymethyl)-1-alkanols was investigated in aqueous solution with Y^{3+} and various lanthanide ions (La^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}). The values of the stability constants for ML complexes are of the order of 10^4 for the hexadentate trioxypropionates, 10^7 for the hexadentate trioxyacetates and 10^{14} for the nonadentate triaminohexaacetates. In these systems, the nitrogens of the amino groups seem to be better chelating atoms than the oxygens of the ether groups. The hydration numbers of dysprosium(III) complexes have been determined from the Dy^{III}-induced ^{17}O NMR water shifts.

Introduction

The design of polypodal ligands capable of forming stable complexes for in vivo use has received increasing interest in the past decade.[1] With a view towards the preparation of new complexing systems for yttrium and some lanthanide ions which could be used for radiotherapy or as biomedical radiolabels (^{90}Y, ^{153}Sm, ^{165}Dy), we undertook the synthesis of a series of new polypodal ligands: trioxypropionic acids (TOTP),[2] trioxytriacetic acids (TOTA)[3] and triaminohexaacetic acids (TAHA) (see Figure 1). Derivatives of these compounds for conjugation with a biomolecule (such as a monoclonal antibody or peptide) should be easier to prepare than macrocyclic ligands bearing carboxylate groups, which are well-known for their ability to complex yttrium and lanthanides ions.[1,4]

In this paper we report the stability constants of the 1:1 complexes (ML) of these ligands with yttrium(III) and a variety of lanthanide(III) cations in aqueous solution as determined potentiometrically. The ^{17}O NMR spectra in variable pH solutions of the Dy^{III} complexes are also described in order to gain information on their coordination sphere in aqueous solution. A shift of the water ^{17}O NMR signal (Dysprosium Induced Shift, DIS) is induced upon addition of dysprosium(III) complexes to water that is related to the number of coordinated water molecules in the complex.

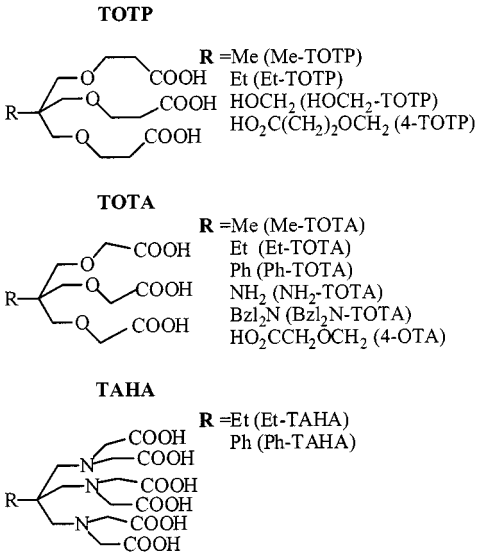

Figure 1. Structure of the ligands

Results

Ligands Synthesis

The TOTP and TOTA ligands are easily accessible from inexpensive commercial primary polyols. These preparations involve two steps: the formation of the ether linkages followed by the hydrolysis of the *tert*-butyl ester functions with formic acid. The polyetherifications were accomplished by phase-transfer catalysis[2,3] with *tert*-butyl acrylate or *tert*-butyl bromoacetate (Figure 2).

The TAHA ligands were also synthesised from primary polyols using the procedure described by Hellmann and coworkers[5] to prepare 1,1,1-tris(aminomethyl)phenylmethane (Figure 3).

Ligand Deprotonation Constants

The deprotonation constants K_a of the ligands investigated in this research were studied by potentiometric titration. Analysis of the potentiometric titration curves (see Figure 4 for examples and details in Exp. Section) by the SUPERQUAD[6] program yielded the pK_{an} values defined by Equation (1) and (2) (charges omitted for clarity):

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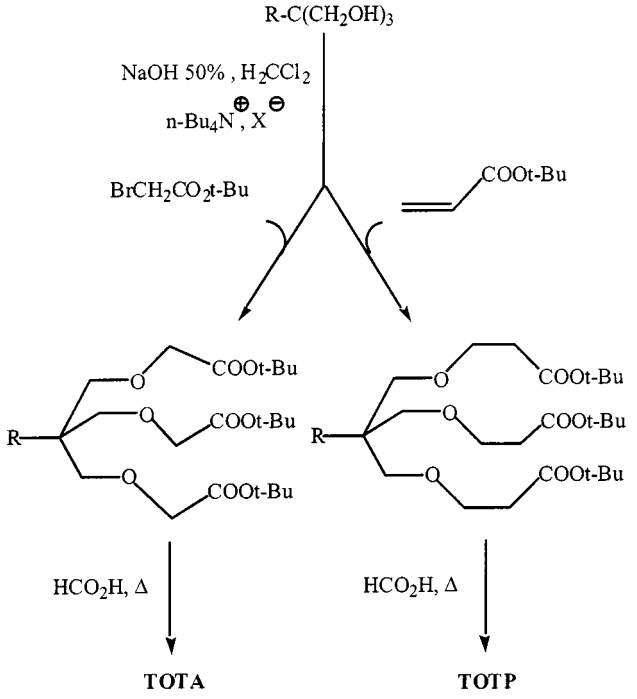

Figure 2. Synthesis of the ligands TOTP and TOTA

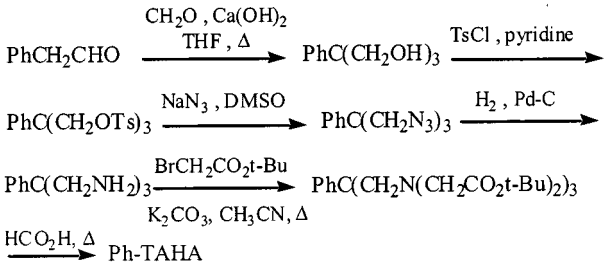

Figure 3. Synthesis of the ligand Ph-TAHA

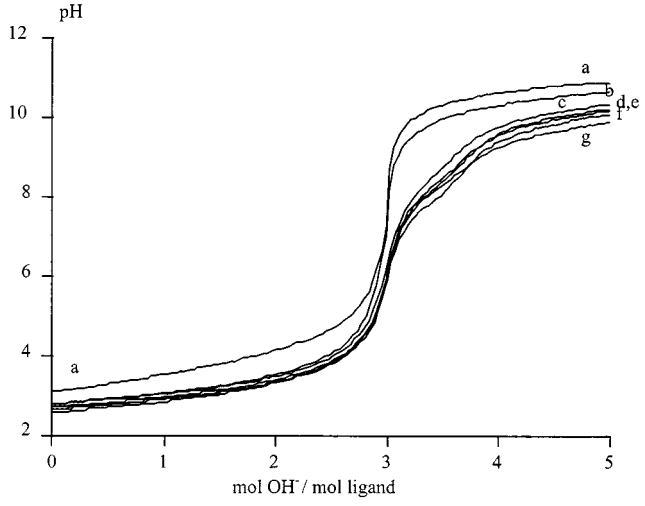

Figure 4. Potentiometric titration curves for (a) 1 mM ligand Me-TOTA and (1:1 1 mM Me-TOTA + (b) La^{3+}, (c) Sm^{3+}, (d) Eu^{3+}, (e) Tb^{3+}, (f) Gd^{3+}, (g) Y^{3+}; all solutions were at 25 °C and $I = 0.1$ M (NMe_4Cl); the data were refined by the SUPERQUAD program

$$LH_n \rightleftarrows LH_{n-1} + H^+ \quad (1)$$

$$K_{an} = [LH_{n-1}]\,[H^+]/[LH_n] \quad (2)$$

The ligands of the series TOTA and TOTP possess three carboxylic groups and the tetrapodal ligands 4-OTA and 4-TOTP four carboxylic groups. The two derivatives NH_2-TOTA and $N(Bz)_2$-TOTA have a fourth ionizable proton in an ammonium group. The pK_{an} values are listed in Table 1. For the ligand of the tri(amino)hexaacetic acid series, denoted Ph-TAHA, the pK_{an} of three carboxylic groups (three pK_{an} values are too low to be determined from potentiometric titration) and of the three ammonium groups have been determined. The values are reported in Table 2 together with literature data for tripodal ligands incorporating aminocarboxylate groups.

Table 1. Deprotonation constants of the *R*-TOTA ligands[a]

Ligand	pK_{a4}	pK_{a3}	pK_{a2}	pK_{a1}
Me-TOTA		2.89(4)	3.56(1)	4.36(1)
Et-TOTA		2.35(9)	3.40(1)	4.29(1)
Ph-TOTA		3.05(1)	3.53(1)	4.26(1)
NH_2-TOTA	2.64(5)	3.03(2)	3.96(2)	9.49(1)
$N(Bz)_2$-TOTA	2.90(5)	3.19(3)	4.08(2)	7.74(1)
4-OTA	2.71(2)	3.75(2)	3.92(1)	4.98(1)
Me-TOTP		4.02(1)	4.63(1)	5.46(1)
Et-TOTP		4.14(1)	4.64(1)	5.50(1)
$HO(CH_2)$-TOTP		4.25(1)	4.83(1)	5.85(1)
4-TOTP	3.87(2)	4.41(1)	5.00(1)	5.68(1)

[a] All values are determined at 25 °C and $I = 0.1$ M (NMe_4Cl); standard deviation of the last significant digit (95% confidence level) in parentheses.

Table 2. Deprotonation constants of the ligand Ph-TAHA and comparison with literature data

Ligand	pK_{a6}	pK_{a5}	pK_{a4}	pK_{a3}	pK_{a2}	pK_{a1}
Ph-TAHA	2.2(1)	3.36(7)	3.71(5)	4.81(5)	7.20(4)	11.17(4)
TAPHA[b]	2.0	2.42	3.52	4.3	8.26	9.88
TAMETA[c]				4.69	7.73	10.73

[a] All values are determined at 25 °C and $I = 0.1$ M (NMe_4Cl); standard deviation of the last significant digit (95% confidence level) in parentheses. – [b] 1,2,3-Triaminopropanehexaacetic acid, ref.[11] – [c] 1,1,1-Tris(aminomethyl)ethane-*N*,*N*′,*N*′′-triacetic acid, ref.[10]

Stability Constants of the Metal Complexes

The stability constants were determined from potentiometric titration of ligand/metal solutions at a molar ratio of 1:1. (for example, as shown in Figure 4). The titration curves for ligands Me, Et, Ph-TOTA and *R*-TOTP exhibit buffer regions over the pH range 7−10 owing to the formation of hydroxo complexes; however, a precipitation occurred and precluded the use of the data in refinement program. No precipitation was observed below pH 10 for ligands the NH_2, $N(Bz)_2$ and 4-OTA. Analysis of the titration curves was performed over the pH range 2.5−6 by the

SUPERQUAD program[6] yielding the K_{ML} and K_{MLH_n} constants. The best fit of the data was obtained by considering only the 1:1 species. The values are reported in Table 3 (*R*-TOTA) and Table 4 (*R*-TOTP).

served DIS represents a concentration-weighted average of the shifts of the individual species in solution. The experimental DIS was measured for different Dy^{III} concentrations. The plot of the DIS versus [Dy^{III}] gives a straight line

Table 3. Stability constants and protonation constants of lanthanide complexes[a] with TOTA ligands and pSm values[b]

Ligand	Y^{3+}	La^{3+}	Sm^{3+}	Eu^{3+}	Gd^{3+}	Tb^{3+}	pSm
Me-TOTA log *K*(ML)[c]	6.6(1)	5.7(1)	6.8(1)	6.8(1)	6.8(1)	7.0(1)	4.90
Et-TOTA log *K*(ML)	6.65(5)	6.05(6)	7.1(1)	7.0(1)	7.2(1)	7.2(1)	5.05
Ph-TOTA log *K*(ML)	6.6(1)	5.0(1)	6.7(1)		7.0(1)	7.0(1)	4.85
NH_2-TOTA log *K*(ML)	8.40(5)	8.01(7)	9.21(2)	9.15(2)	9.10(3)	9.22(1)	4.90
log K(MLH)[d]	4.12(3)	4.74(5)	3.56(2)	3.76(2)	3.85(3)	3.74(2)	
$N(Bz)_2$-TOTA log *K*(ML)	8.81(3)	7.57(3)	9.06(4)	8.69(1)	8.72(2)	8.59(1)	5.45
log *K*(MLH)	3.64(2)	4.02(3)	3.43(3)	3.31(1)	3.28(1)	3.38(1)	
4-OTA log *K*(ML)	8.13(2)	6.55(2)	8.10(2)	7.87(5)	7.42(3)	7.33(2)	5.55
log *K*(MLH)	3.77(2)	4.4 (1)	3.82(4)	3.8(1)	3.87(6)	3.88(4)	

[a] All values are determined at 25 °C and I = 0.1 M (NMe_4Cl); standard deviation of the last significant digit (95% confidence level) in parentheses. – [b] calculated for $[L]_{tot} = 10^{-3}$ M, $[Sm^{3+}]_{tot} = 10^{-3}$ M and pH = 7. – [c] $K(ML) = [ML]/[M][L]$. – [d] $K(MLH) = [MLH]/[ML][H]$.

Table 4. Stability constants and protonation constants of lanthanide complexes[a]

Ligand	Y^{3+}	La^{3+}	Sm^{3+}	Eu^{3+}	Gd^{3+}	Tb^{3+}
Me-TOTP log *K*(ML)[b]	3.65(3)	3.22(3)	3.82(8)	3.95(2)	3.72(2)	3.7(1)
log *K*(MLH)[c]	4.30(5)		4.08(6)	4.35(6)	4.20(2)	4.25(6)
Et-TOTP log *K*(ML)	3.63(5)	3.21(2)	3.88(1)	3.97(1)		3.79(1)
log *K*(MLH)	4.60(2)	4.75(2)	4.68(2)	4.61(2)		4.57(3)
$HO(CH_2)$-TOTP log *K*(ML)	4.52(2)	4.23(2)	5.04(3)	4.23(2)	4.50(4)	4.57(2)
log *K*(MLH)		4.1(1)				
4-OTP log *K*(ML)	4.7(1)	3.90(3)	5.0(1)	5.20(1)	4.9(1)	4.8(1)
log *K*(MLH)	4.95(5)	5.1(1)	5.00(5)	4.95(2)	5.01(8)	5.02(9)

[a] All values are determined at 25 °C and I = 0.1 M (NMe_4Cl); standard deviation of the last significant digit (95% confidence level) in parentheses. – [b] with TOTP ligands$K(ML) = [ML]/[M][L]$. – [c] $K(MLH) = [MLH]/[ML][H]$.

With the ligand Ph-TAHA we investigated the complexation of the cations Sm^{3+}, for comparison with the other ligands, Dy^{3+}, for comparison with the NMR study, and Ce^{3+}. No precipitation occurred above pH 7 and the titration data were refined over the pH range 2.5–8. A comparison of the titration curves of the ligand solution and of the metal-ligand solution (Figure 5) indicates that no hydroxo complexes are formed at basic pH's, which is contrary to the ligands of the TOTA and TOTP series. The stability constants are shown in Table 5.

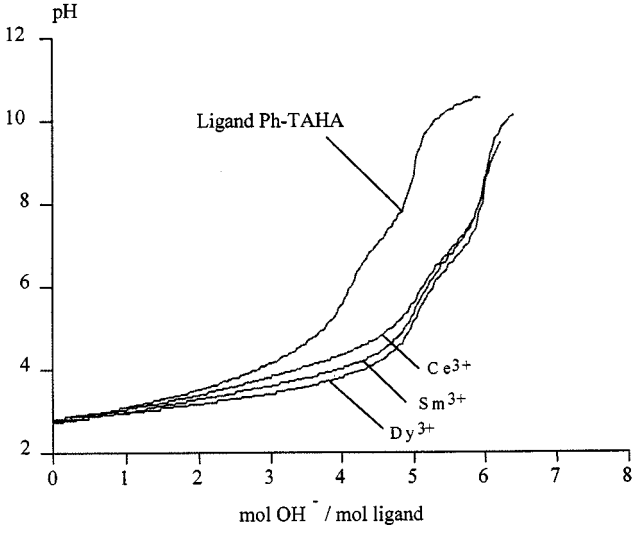

Figure 5. Potentiometric titration curves for 1 mM Ph-TAHA and Ph-TAHA + Ln^{III} (1:1; 1 mM); all solutions were at 25 °C and I = 0.1 M (NMe_4Cl); the data were refined by the SUPERQUAD program

^{17}O NMR Spectroscopy

The number of water molecules present in the lanthanide complexes was investigated by dysprosium-induced ^{17}O shift measurements. From these a qualitative picture of the lanthanide coordination sphere can be deduced. Addition of Dy^{III} complexes to water results in a shift of the water signal in the ^{17}O NMR spectrum to lower frequencies. Peters and co-workers[7] have shown that the dysprosium-induced shift of water (DIS) can be utilised to estimate the number of bound water molecules associated with the lanthanide complexes. The exchange between bound and bulk water is fast on the NMR time scale. Accordingly, the ob-

Table 5. Stability constants and protonation constants of lanthanide complexes[a] and pSm values[b] for the ligand Ph-TAHA

Ligand		Ce^{3+}	Sm^{3+}	Dy^{3+}	pSm
Ph-TAHA	log K (ML)[c]	11.14(3)	12.71(1)	14.85(7)	5.64
	log K(MLH)[d]	6.90(2)	6.62(1)	6.51(7)	
	log K(MLH_2)[d]	4.53(2)	4.17(1)	3.95(7)	
	log K(MLH_4)[e]	7.30(6)	6.88(2)	6.59(9)	

[a] All values are determined at 25 °C and I = 0.1 M (NMe_4Cl); standard deviation of the last significant digit (95% confidence level) in parentheses. – [b] calculated for $[L]_{tot} = 10^{-3}$ M, $[Sm^{3+}]_{tot} = 10^{-3}$ M and pH = 7. – [c] K(ML) = [ML]/[M][L]. – [d] $K(MLH_n) = [MLH_n]/[MLH_{n-1}][H]$. – [e] $K(MLH_4) = [MLH_4]/[MLH_2][H]^2$.

with a slope proportional to the number of bound water molecules in the complex. DIS measurements were carried out for the ligands Ph-TOTA, 4-OTP and Ph-TAHA. Following the procedure described by Peters,[7] the DIS for solutions of the hydrated cation $[Dy(H_2O)_8]^{3+}$ at pH 6.5 were measured, these results produced a slope of 358 ppm M^{-1} (Figure 6a or 7a) which is in excellent agreement with that obtained in Peters's paper (−357 ppm M^{-1}). Therefore, a value of 45 ppm M^{-1} can be calculated for one coordinated water molecule if the cation is hydrated by eight water molecules.[8] DIS measurements were also performed for solutions of the complex $[Dy(ligand)(H_2O)_q]^{3+}$ over the pH range 1−5 for Ph-TOTA, 2−5 for 4-OTP and 1−9 for Ph-TAHA. The experimental slopes p of the plots of the DIS versus $[Dy(ligand)(H_2O)_q]$ (Figure 6 and 7) were determined. The p values and the calculated water coordination numbers, q, are shown in Table 6. The q values indicate that the number of coordination positions for the ligand increase as the pH increases (Table 6) which agrees with the existence of different protonated species.

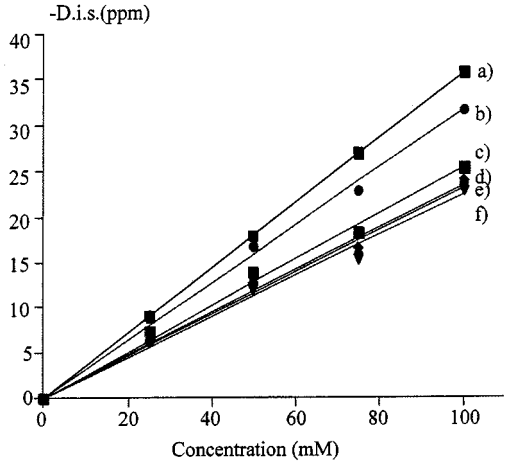

Figure 6. ^{17}O NMR shifts of water versus dysprosium complex concentration for solutions of: (a) $Dy(H_2O)_8$, (b) Dy(Ph-TOTA) at pH 1, (c) pH 2, (d) pH 3, (e) pH 4, (f) pH 5

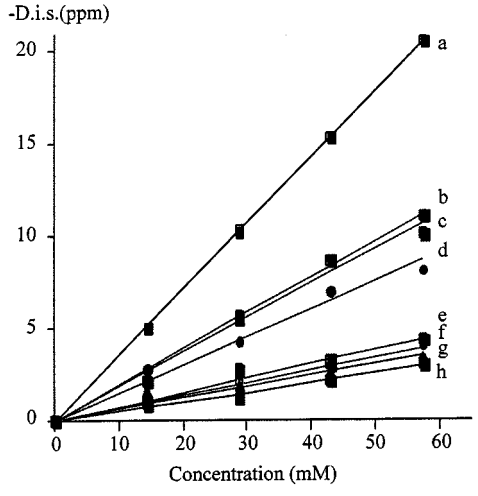

Figure 7. ^{17}O NMR shifts of water versus dysprosium complex concentration for solutions of: (a) $Dy(H_2O)_8$, (b) Dy(Ph-TAHA) at pH 1, (c) pH 2, (d) pH 3, (e) pH 4, (f) pH 5, (g) pH 6, (h) pH 9

Table 6. Values of the water coordination number q in Dy^{III} complexes derived from ^{17}O chemical shifts

Ligand	pH	p[a]	q
H_2O	6.5	−358	8
Ph-TOTA	1	−317	7.1
	2	−255	5.7
	3	−235	5.3
	4	−231	5.2
	5	−224	5.0
4-OTP	2−4	−288	6.4
Ph-TAHA	1	−195	4.4
	2	−186	4.2
	3	−152	3.4
	4	−77	1.7
	5	−68	1.5
	6	−62	1.4
	9	−51	1.1

[a] Slope of the plot of DIS versus the concentration of the complex (in ppm M^{-1}).

Discussion

Ligand Deprotonation Constants

For the ligands of the series *R*-TOTA it should be noted that the average values of the carboxylic pK_a over a range 3.21−3.61 in the tripodal ligands are close to the value of 3.31 for the analogous monodentate methoxyacetic acid.[9] For the ligands of the series TOTP the pK_{an} values are higher than those for the ligands *R*-TOTA, owing to the presence of a second methylene group which acts as an electron donor. A similar effect is observed in 3-hydroxypropionic acid (pK_a = 4.32[9]) in comparison to hydroxyacetic acid (pK_a = 3.63).[9] Furthermore, the separation of the pK_{an} values for a given ligand is in the range 0.39−1.05 [except for a value of 0.29 for $N(Bz)_2$-TOTA]. This reflects a rough statistical separation (log 3 or 0.48), which is consistent with noninteracting pendant arms. For the ligand Ph-TAHA, the three highest and the three lowest pK_a values are attributed to the ammonium and carboxylic

groups, respectively. The acidities of the ammonium groups are close to those of the ligands TAMETA [1,1,1-tris-(aminomethyl)ethane-*N*,*N*′,*N*′′-triacetic acid][10] which has a similar tripodal structure. The pK_a's of the carboxylic groups are comparable to those of the ligand TAPHA (1,2,3-triaminopropanehexaacetic acid)[11] which contains three aminodiacetic arms.

Stability Constants of the Metal Complexes

A small change in the stability constant *K*(ML) is observed on going from Sm^{3+} to Tb^{3+} and for Y^{3+}. The values for La^{3+} are smaller than those for the other cations owing to its larger size and its lower Lewis acidity. The same trend has been observed for EDTA complexes.[9] The ligands TOTP appear to be poor chelators of Ln^{III} ions, since their stability constants are two or three orders of magnitude lower than those of the TOTA series. This result shows that the presence of longer chains in TOTP than in TOTA ligands makes the complexation of Ln^{III} ions unfavorable, probably due to their higher flexibility. Furthermore, the coordination of carboxylic groups could be hindered by their weak acidity. Accordingly, the water coordination number of 6 (Table 6) (corresponding to a ligand coordination number of 2 or 3) for 4-OTP, as determined by ^{17}O NMR spectroscopy, is not unexpected even for a tetrapodal ligand. For the complex with Ph-TOTA, the value $q = 5$ suggests a coordination of three carboxylates in the ML species. These results clearly show that the ether oxygens are not involved in the coordination to the cation. The complexation constants for the lanthanides with ligands having the same backbone as the TOTA series, each arm containing one or two ethylenoxy moieties ($CH_2CH_2OCH_2CH_2CO_2H$ or $CH_2CH_2OCH_2CH_2OCH_2CH_2CO_2$ H), have also been measured (not described here).[12] These ligands were found to have stability constants *K*(ML) approximately equal to those for the *R*-TOTP ligands. This result also supports the fact that the ether oxygens are not involved in the complexation to the cations and that the carboxylate anions alone are responsible for the complexation.

Since the ligands are weak acids, proton competition occurs depending on their pK_a and the pH. The pM (= −log[M]) is thus a more reliable parameter for a comparison of ligand effectiveness under given conditions of pH, M and L concentrations: the larger the pM values, the more effective the ligand. The pM values for the cation Sm^{3+} ($[L]_{tot} = 10^{-3}$ M, $[Sm^{3+}]_{tot} = 10^{-3}$ M) over the pH range 3−7 for the ligands *R*-TOTA and 3−9 for the ligand Ph-TAHA have been calculated and are presented on the plot of pSm vs. pH in Figure 8. The values of pSm at pH 7 are reported in Table 3 and 5. For the ligands *R*-TOTA, the pSm values for R = Me, Et or Ph are very close and much higher than those for the ligands NH_2-TOTA and $N(Bz)_2$-TOTA at acidic pH. These ligands are protonated at the nitrogen and the positive charge is assumed to reduce the affinity of the ligand for the lanthanide cation. However, at pH > 6 for $N(Bz)_2$-TOTA and pH > 7 for NH_2-TOTA, this affinity is greatly enhanced. This could be ascribed to the coordination of the cation with the nitrogen of the amine. The pSm value for the ligand 4-OTA is about 0.5 units higher than those for the *R*-TOTA ligands, showing that a fourth arm increases the affinity for the cation, and is probably involved in the coordination. Furthermore, it can be seen that these three ligands prevent the precipitation of hydroxo complexes in neutral and basic mediums.

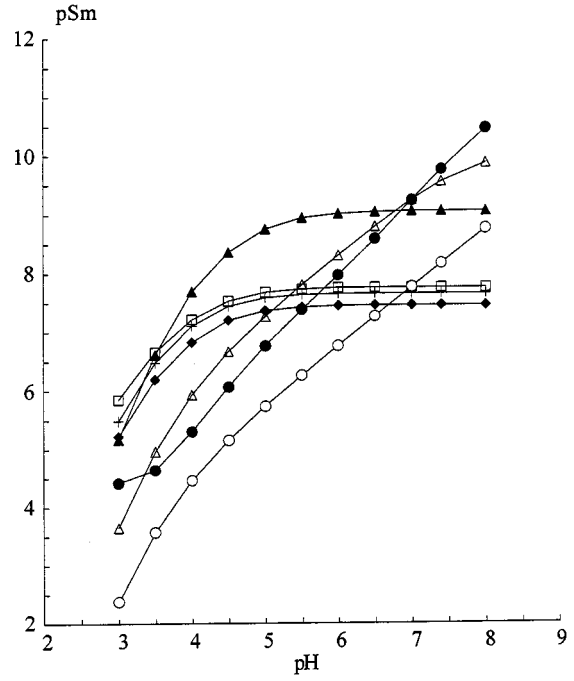

Figure 8. Plot of pSm vs. pH for the ligands; pSm was calculated for $[L]_{tot} = 10^{-3}$ M, $[Sm^{3+}]_{tot} = 10^{-3}$ M using the known constants of Me-TOTA (◆), Et-TOTA (□), Ph-TOTA (+), NH_2-TOTA (○), NBz_2-TOTA (Δ), 4-OTA (▲) and Ph-TAHA (●)

The pSm vs. pH curve indicates that the ligand Ph-TAHA is a poorer complexing agent in an acidic medium, but is more efficient at pH above 7, than the other ligands described in this paper. The obvious advantage of this ligand is much greater efficiency of complexation in basic media and an increase in the coordination number as the pH increases, as determined by the ^{17}O NMR study. The water coordination number *q* varies from a mean value of 4 over the pH range 1−3 to a mean value of 1.5 over the pH range 4−6. The latest value reflects the contribution of several species, among which [DyLH] is predominant with a hexadentate ligand. At pH 9, the *q* value of 1.1 attributed to the [DyL] species indicates that the coordination to the ligand is heptadentate with one water molecule. This suggests that the six carboxylate oxygens and one nitrogen are involved in the coordination to the cation. As a consequence, no hydroxo complexes are formed at basic pH. This reflects the low Lewis acidic character of the cation in the complex ML.

Conclusion

Our results have shown that the coordination to the lanthanides ions involves the carboxylate groups alone and not

the oxygens from the ether groups (TOTP or TOTA), while the nitrogen in similar polypodal ligands does participate in the chelation (TAHA). The presence of amino groups enhances the stability constants of the complexes, thus preventing the formation of hydroxo complexes or precipitation of metal hydroxides at pH close to or above 7. The formation of 1:1 complexes leads to a relatively high hydration number as determined by Dy^{III}-induced ^{17}O NMR water shifts. A value of 5 has been obtained for the tris(carboxylate) ligands and a value of 2 for the tris(aminocarboxylate) ligands. This hydration state is expected to provide interesting relaxivity properties for these complexes. However, the results in terms of the potentialities of these ligands in biomedical applications are still not satisfactory. With some ligands of the TOTP series, the bioconjugation with a fragment of monoclonal antibody [*anti*-ACE(F6)] has been realized.[2] A study of ^{111}In radiolabelling indicated that one ligand is linked to the $F(ab')_2$ but the complexes of these bioconjugated polypodal ligands with ^{153}Sm are not stable enough to be purified.

Experimental Section

Materials: Hydrated lanthanide chlorides were obtained from Aldrich. All other compounds were of reagent grade and were used without further purification.

Instrumentation: ^1H NMR spectra (200, 250 and 300 MHz) are referenced to TMS and recorded on Bruker AC 200, Bruker AWM 250 or Bruker AM 300 spectrometers. ^{17}O NMR spectra (natural abundance) were recorded at 54.24 MHz on a Bruker AM 300 spectrometer, using 5 mm sample tubes. Mass spectra were obtained with a NERMAGT1OC quadrupolar instrument. Infrared spectra were obtained as KBr disks for solid compounds and as pure film products between two NaCl discs for oils on a Nicolet Impact 400 spectrometer driven by the OMNIC program on a PC. Elemental analyses were performed by Le Service Central d'Analyses du CNRS in Vernaison (France). Melting points were measured on a Buchi-Tottoli apparatus and are uncorrected.

Synthesis of *R*-TOTP and *R*-TOTA: These ligands were prepared by acid hydrolysis from the corresponding polyesters, which were themselves prepared by a previously described method.[2,3]

Synthesis of Ph-TAHA

1,1,1-Tris(hydroxymethyl)phenylmethane: A solution of phenylacetaldehyde (10.75 g, 0.09 mol), paraformaldehyde (16.8 g, 0.56 mol) and $Ca(OH)_2$ (5.2 g, 0.70 mol) in THF (60 mL) was stirred at 60−65 °C for four days. After cooling to room temperature, the mixture was filtered through celite gel and the solvent was evaporated under vacuum (0.05 Torr). The residual oil was dissolved in hot ethyl acetate. Under standing at room temperature the product crystallized as a white powder (9.55 g, 58%), m.p. 80−81 °C. − $C_{10}H_{14}O_3$ (182.22): calcd. C 65.32, H 7.50; found C 65.93, H 7.69. − ^1H NMR ($CDCl_3$): δ = 3.92 (s, 6 H, CH_2OH), 7.18−7.46 (m, 5 H, aromatic CH). − ^{13}C NMR ($CDCl_3$): δ = 48.7 (C_6H_5-*C*), 65.8 (CH_2OH), 126.7, 127.2, 129.0 and 138.4 (C_6H_5).

1,1,1-Tris[(4-tolylsulfonyl)methyl]phenylmethane: *p*-Toluenesulfonyl chloride (36.5 g, 0.191 mol) was slowly added to a solution of 1,1,1-tris(hydroxymethyl)phenylmethane (8.717 g, 0.048 mol) in cold pyridine (90 mL) at a rate such that the temperature remained between 5 and 10 °C. After stirring at room temperature for half an hour, the mixture was poured into 15 mL of cold water. The product obtained was filtered, washed with diethyl ether (3 × 20 mL) and dried in vacuo for a few days to yield 27.76g (90%) of a white powder, m.p. 136 °C. − $C_{31}H_{32}O_9S_3$ (644.77): calcd. C 57.66, H 5.06; found C 57.75, H 5.00. − ^1H NMR ($CDCl_3$): δ = 2.45 (s, 9 H, CH_3), 4.18 (s, 6 H, CH_2), 6.94−7.19 (m, 5 H, C_6H_5), 7.29 and 7.32 (d, *J* = 7.13 Hz, 6 H, Tos-H^2), 7.60 and 7.62 (d, *J* = 6.34 Hz, 6 H, Tos-H^3). − ^{13}C NMR ($CDCl_3$): δ = 21.7 (CH_3), 46.0 (C_6H_5-*C*), 68.9 (CH_2), 126.1 (C_6H_5-C^4), 127.6 (Tos-C^2), 127.9 (Tos-C^3), 128.7 (C_6H_5-C^2), 130.0 (Tos-C^3), 131.6 (Tos-C^4), 134.7 (C_6H_5-C^1), 135.2 (Tos-C^1).

1,1,1-Tris(azidomethyl)phenylmethane: 1,1,1-Tris[(4-tolylsulfonyl)-methyl]phenylmethane (3.22 g, 5 mmol) and NaN_3 (2.94 g, 45 mmol) in DMSO (50 mL) were stirred at 90 °C for three hours. After cooling to room temperature the solution was poured into water (250 mL). The suspension was then extracted with ethyl acetate (250 mL). The organic fraction was washed with a saturated ammonium chloride solution, and dried with anhydrous sodium sulfate. The solution was filtered and the solvent was carefully removed under vacuum yielding 1.471 g of a crude transparent oil which was used directly in the following step. − ^1H NMR ($CDCl_3$): δ = 3.65 (s, 6 H, C−CH_2N_3), 7.22−7.40 (m, 5 H, aromatics CH). − ^{13}C NMR ($CDCl_3$): δ = 46.9 (C_6H_5-*C*), 54.3 (CH_2), 126.2, 127.8 and 128.8 (aromatics CH), 138.3 (aromatic C). − IR (NaCl disk): ν_{N_3} = 2103 cm^{-1}.

1,1,1-Tris(aminomethyl)phenylmethane: 10% Pd/C (0.500 g) was added to a solution of 1,1,1-tris(azidomethyl)phenylmethane (1.28 g, 5 mmol) in anhydrous ethanol (25 mL) under argon. The solution was stirred under H_2 for 6 hours and then filtered through Celite, which was then washed with more ethanol. The solvent was removed under vacuum to yield 0.075g (99%) of a transparent oil. − $C_{10}H_{17}N_3$ (179.26): calcd. C 66.66, H 9.86, N 23.25; C 67.00, H 9.56, N 23.44. − ^1H NMR ($CDCl_3$): δ = 3.05 (s, 6 H, CH_2), 7.19−7.42 (m, 5 H, aromatics CH). − ^{13}C NMR ($CDCl_3$): δ = 45.4 (CH_2), 48.1 (C_6H_5-C), 125.2, 127.1 and 128.4 (aromatics CH), 141.7 (aromatic C).

1,1,1-Tris[di(*tert*-butylcarboxymethyl)aminomethyl]phenylmethane: 1,1,1-Tris(aminomethyl)phenylmethane (0.803 g, 4.72 mmol) was dissolved in acetonitrile (25 mL) by warming slightly. Potassium carbonate (7.834 g, 12 equiv.) and *tert*-butyl bromoacetate (11.056g, 12 equiv.) were then added and the solution stirred for one week at 60 °C. After cooling to room temperature the organic products were extracted with ethyl acetate. The organic layer was washed with saturated NaCl solution and dried with anhydrous sodium sulfate. After filtration the solvent was removed under vacuum to yield a crude oil, which was purified by column chromatography with silica gel and a cyclohexane/ethyl acetate gradient from 10:0 to 8:2. The product was obtained as an oil (0.590 g, 14.5%). MS (D/IC, NH_3 + isobutane): *m*/*z* = 864 $[M + 1]^+$. − ^1H NMR ($CDCl_3$): δ = 1.42 (s, 54 H, CH_3), 3.23 (s, 12 H, CH_2), 3.27 (s, 6 H, CH_2), 7.14−7.40 (m, 5 H, aromatics CH). − ^{13}C NMR ($CDCl_3$): δ = 28.18 (CH_3), 48.49 (C_6H_5-*C*), 56.44 (N*C*H_2CO_2tBu), 59.29 (C*C*H_2N), 80.40 [*C*(CH_3)$_3$], 125.82, 126.61 and 128.55 (aromatics CH), 144.69 (aromatic C), 171.19 (*C*O_2tBu).

1,1,1-Tris[di(carboxymethyl)aminomethyl]phenylmethane: 1,1,1-Tris[di(*tert*-butyl)aminomethyl]phenylmethane (0.200g, 0.232 mmol) in formic acid (5 mL, 97%) was stirred for three hours at 60 °C. The solvent was removed under vacuum and the residual product was dried overnight under vacuum to yield 0.120 g (98%) of product. − $C_{22}H_{29}N_3O_{12}\cdot 3H_2O$ (581.55): calcd. C 45.14, H 5.97,

N 8.70, O 36.63; C 45.43, H 6.02, N 7.23, O 41.30. Potentiometric analysis was consistent with this molecular weight. – ^{1}H NMR (D_2O, pD = 1.0): δ = 3.45 (s, 12 H, NCH_2CO$_2$H), 3.54 (s, 6 H, CCH_2N), 7.17–7.42 (m, 5 H, aromatics CH). – ^{13}C NMR (D_2O, pD = 1.0): δ = 48.00 (C_6H_5-*C*), 58.59 (N*C*H$_2$CO$_2$H), 63.30 (C*C*H$_2$N), 128.90, 130.79 and 132.06 (aromatics CH), 140.44 (aromatic C), 175.04 (CO$_2$ H). MS (FAB): *m*/*z* = 528 [M + 1]$^+$.

Potentiometric Titrations: All the measurements were made at 25 °C and the solutions were prepared with deionized and doubly distilled water. The ionic strength was fixed at I = 0.1 M with tetramethylammonium chloride (PROLABO puriss). The potentiometric titrations were performed using an automatic titrator system, DMS 716 Titrino (Metrohm), equipped with a combined glass electrode (Metrohm, filled with NaCl saturated solution) and connected to an IBM Aptiva microcomputer. The electrodes were calibrated to read pH according to the classical method.[13] The ligand and its metal(III) complex of ca. 0.001 M were titrated with standardized 0.025 M tetramethylammonium hydroxide. Argon was bubbled through the solutions to exclude CO_2 and O_2. Tetramethylammonium hydroxide solution was standardized against potassium hydrogenphthalate. Carbonate content was checked by Gran's method. The titration data were refined by the non-linear least-squares refinement program SUPERQUAD[6] to determine the deprotonation and/or stability constants (σ_{fit} in the range 1–5). Typically, about 150 data points were collected over the pH range 2.5–10.5 for the ligand solution and 100 points over the pH range 2.5–7 for the metal-ligand solution since precipitation occurred at a pH of about 7.5. The pK_{an} values were calculated from the cumulative constants determined by the program. The values of the stability constants are the mean values calculated from two or three titrations.

NMR Measurements: ^{17}O spectra (natural abundance) were recorded at 54.24 MHz on a Bruker AM 300 using 5 mm samples tubes. The ^{17}O shifts were referenced to the external $D_2{}^{17}O$ signal. The dysprosium-induced shifts (DIS) were obtained from the observed shift by making a correction for the bulk magnetic susceptibility of the solution. The solutions of the dysprosium complexes at a Dy^{III}/ligand molar ratio of 1 were prepared in doubly distilled water containing 20% D_2O. Concentrations ranged from 0.025 to 0.1 M. The pH was adjusted with tetramethylammonium hydroxide or HCl solution and measured by a pH meter employing a combined microelectrode and calibrated with standard buffers at pH 4.01, 6.86 and 10.01 from Polylabo.

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