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Multinuclear Magnetic Resonance Study on the Structure and Dynamics of Lanthanide(III) Complexes of Cyclic DTPA Derivatives in Aqueous Solution[1]

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The lanthanide coordination of the macrocyclic ligands cy(DTPA-EN) and cy(DTPA-EN-DTPA-EN) was studied in aqueous solution. ^{17}O NMR measurements on the Dy^{III} complexes showed that, in both complexes, the first coordination sphere of the Ln^{III} ion contains one water molecule, leaving eight coordination sites for the ligand molecule. ^{89}Y and ^{139}La NMR analysis confirmed that the coordination mode of cy(DTPA-EN-DTPA-EN) is similar to that of the acyclic DTPA-bis(amide) derivatives. However, as a result of the cyclic nature of the ligands considered, the number of isomers in solution is lower than for the acyclic compounds. From variable-temperature ^{1}H and ^{13}C NMR data, we conclude that, in the respective Ln^{III} complexes in solution, the cy(DTPA-EN) ligand is present in two rapidly interconverting isomers, whereas the cy(DTPA-EN-DTPA-EN) ligand exists in four isomeric forms. Two types of exchange processes are observed for the cy(DTPA-EN-DTPA-EN) complexes; one is fast on the NMR time scale and does not require decoordination of the ligand, and the second is relatively slow and decoordination is necessary to realize the interconversion. The complexes of cy(DTPA-EN) and the lighter Ln^{III} ions ($Ln = La \rightarrow Eu$) precipitated, probably due to the formation of binuclear complexes. Comparisons are made with the previously studied acyclic DTPA-bis(amides).

Introduction

The development of contrast agents for magnetic resonance imaging (MRI) has become of great interest since this technique is a very powerful tool in medical diagnosis.[2−4] Several chelates of Gd^{III}, such as Gd^{III}(DTPA)[5] and Gd^{III}(DOTA),[6] have been approved for use as MRI contrast agents in humans and are applied routinely nowadays. These paramagnetic complexes contain one inner-sphere water molecule[7−10] that exchanges rapidly with the bulk water in the body,[11] thereby providing an efficient mechanism for the enhancement of the water proton relaxation rates ($1/T_1$ and $1/T_2$). The latter parameters contribute to the intensity of the MRI signal and thus to the contrast of the image.

The negative charge of both Gd^{III} complexes mentioned above leads to a high osmolality under physiological conditions, which may give rise to adverse effects. Consequently, efforts have been made on derivatizing the ligands in such a way that neutral Ln^{III} complexes can be formed. There are several possibilities to modify the ligand molecules,[12,13] but the formation of amide derivatives appeared to be a simple method. The DTPA-bis(amides) have been studied by several research groups,[14−17] and suitable contrast agents of this type have been developed, for example Gd^{III}(DTPA-BMA).[18]

The rapid development of the MRI technique has led to an increasing demand for more effective contrast agents. The efficiency with which the complex enhances the proton relaxation rates of the water protons, or the relaxivity of the complex, is governed by several parameters; the rotational correlation time and the exchange rate of water molecules between the Gd^{III} complex and the bulk are the most important ones.[2] Therefore, efforts to develop contrast agents with a higher relaxivity are directed toward compounds with a higher molecular volume, such as cy(DTPA-EN) and cy(DTPA-EN-DTPA-EN)[1,19,20] (Figure 1).

This paper reports a multinuclear NMR study on the structure and dynamics of Ln^{III} complexes of the cy(DTPA-EN) and cy-

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(1) Abbreviations: DTPA = diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid; DTPA-BEA = DTPA-bis(ethylamide); DTPA-BMA = DTPA-bis(methylamide); DTPA-BPA = DTPA-bis(propylamide); DTPA-BGLUCA = DTPA-bis(glucamide); DTPA-dien = 1,4,7-tris(carboxymethyl)-9,17-dioxo-1,4,7,10,13,16- hexaazacyclooctadecane; DOTA = 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*- tetraacetic acid; cy(DTPA-EN) = 1,4,7-tris(carboxymethyl)-9,14-dioxo-1,4,7,10, 13-pentaazacyclopentadecane; cy(DTPA-PN) = 1,4,7-tris(carboxymethyl)-9,15- dioxo-1,4,7,10,14-pentaazacyclohexadecane; cy(DTPA-EN-DTPA-EN) = 1,4,7, 16,19,22-hexakis(carboxymethyl)-9,14,24,29-tetraoxo-1,4,7,10,13,16,19,22,25,28- decaazacyclotriacontane.

(2) Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901.

(3) Tweedle, M. F. In *Lanthanide Probes in Life, Chemical and Earth Sciences;* Bünzli, J.-C. G., Choppin, G. R., Eds.; Elsevier: Amsterdam, 1989; Chapter 5.

(4) Watson, A. D. *J. Alloys Comp.* **1994**, *207/208*, 14.

(5) Gries, H.; Rosenberg, D.; Weinmann, H.-J. U.S. Patent 4,647,447, 1987.

(6) Bousquet, J. C.; Saini, S.; Stark, D. D.; Hahn, P. F.; Nigam, M.; Wittenberg, J.; Ferrucci, J. T. *Radiology* **1988**, *166*, 693.

(7) Bryden, C. C.; Reilley, C. N. *Anal. Chem.* **1982**, *54*, 610.

(8) Peters, J. A. *Inorg. Chem.* **1988**, *27*, 4686.

(9) Geraldes, C. F. G. C.; Sherry, A. D.; Cacheris, W. P.; Kuan, K. T.; Brown, R. D., III; Koenig, S. H.; Spiller, M. *Magn. Reson. Med.* **1988**, *8*, 191.

(10) Chang, C. A.; Brittain, H. G.; Telser, J.; Tweedle, M. F. *Inorg. Chem.* **1990**, *29*, 4468.

(11) Micskei, K.; Helm, L.; Brücher, E.; Merbach, A. E. *Inorg.Chem.* **1993**, *32*, 3844.

(12) Peters, J. A.; Huskens, J.; Raber, D. J. *Prog. NMR Spectrosc.* **1996**, *28*, 283.

(13) Sherry, A. D.; Cacheris, W. P.; Kuan, K. T. *Magn. Reson. Med.* **1988**, *8*, 180.

(14) Konings, M. S.; Dow, W. C.; Love, D. B.; Raymond, K. N.; Quay, S. C.; Rocklage, S. M. *Inorg. Chem.* **1990**, *29*, 1488.

(15) Geraldes, C. F. G. C.; Urbano, A. M.; Alpoim, M. C.; Hoefnagel, M. A.; Peters, J. A. *J. Chem. Soc.,Chem. Commun.* **1991**, 656.

(16) Geraldes, C. F. G. C.; Urbano, A. M.; Hoefnagel, M. A.; Peters, J. A. *Inorg. Chem.* **1993**, *32*, 2426.

(17) Geraldes, C. F. G. C.; Urbano, A. M.; Alpoim, M. C.; Sherry, A. D.; Kuan, K.-T.; Rajagopalan, R.; Maton, F.; Muller, R. N. *Magn. Reson. Imaging* **1995**, *13*, 401.

(18) Quay, S. C. U.S. Patent 4,687,659, 1987.

(19) Carvalho, J. F.; Kim, S.-H.; Chang, C. A. *Inorg. Chem.* **1992**, *31*, 4065.

(20) Carvalho, J. F.; Crofts, S. P.; Vadarajan, J. WO 08707, 1992.

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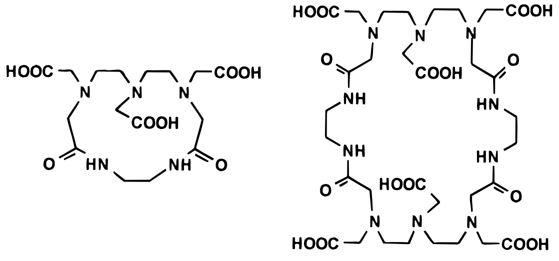

cy(DTPA-EN) cy(DTPA-EN-DTPA-EN)

Figure 1. Chemical structures of the ligands.

(DTPA-EN-DTPA-EN) ligands in aqueous solution. Since the Ln^{III} complexes of a particular ligand are usually isostructural, one can profit from the different NMR properties of various Ln^{III} ions while studying these complexes.[12,21,22] The stoichiometry of the complexes and the water coordination number was determined from Dy^{III}-induced ^{17}O water shifts. The coordination geometry was deduced using ^{89}Y and ^{139}La NMR, and variable-temperature measurements were performed in order to obtain information on the dynamics of the complexes in solution. Comparisons are made with the results from previous studies on Ln[DTPA][8] and Ln[DTPA-bis(amide)][15,16] complexes.

Experimental Section

Chemicals. Hydrated lanthanide chlorides were purchased from Aldrich Chemical Co. (Milwaukee, WI); other starting materials were purchased from Acros Chimica (Geel, Belgium) and used without further purification.

Synthesis of the Ligands. The ligand cy(DTPA-EN) was synthesized by condensation of ethylenediamine with DTPA-bis(anhydride), according to the procedure reported by Carvalho *et al.*[19,20] An aqueous solution of the reaction product at neutral pH was filtered through a UTC-60 membrane filter (Toray Industries Inc., Tokyo, Japan) under 20 bar of N_2 pressure and freeze dried. The white solid obtained was recrystallized from a 10:7 EtOH−acetone mixture. We obtained a yield of 43%, which is notably higher than that mentioned in literature (30%).[19] ^1H-NMR (300 MHz, D_2O, pH = 1.07): δ 3.26 (s, 4H, N_1CH_2CONH), 3.20 (s, 4H, N_1CH_2COOH), 2.80 (s, 2H, N_2CH_2COOH), 2.57 (m, 8H, $N_1CH_2CH_2N_2$), 2.28 (m, 4H, $N_3CH_2CH_2N_4$). ^{13}C-NMR (100 MHz, D_2O, pH = 0.93): δ 175.2 (1C, N_2COOH), 170.8 (2C, N_1COOH), 168.4 (2C, NHCO), 57.4 (2C, $N_1CH_2CH_2N_2$), 56.3 (2C, $N_1CH_2CH_2N_2$), 55.7 (1C, N_2CH_2COOH), 54.2 (2C, N_1CH_2COOH), 51.2 (2C, N_1CH_2CONH), 40.8 (2C, $NHCH_2CH_2NH$). FAB-MS: *m*/*e* = 418 (M + 1).

The cy(DTPA-EN-DTPA-EN) ligand was made by a condensation of EN-DTPA-EN (14-imino-3-[2-[(2-aminoethyl)amino]-2-oxoethyl]-6,9-bis(carboxymethyl)-11-oxo-3,6,9,12-tetraazatetradecanoic acid) and DTPA-bis(anhydride), also following a procedure from Carvalho *et al.*[19] EN-DTPA-EN was synthesized via a condensation of DTPA-bis(anhydride) and ethylenediamine; the latter was first treated with di-*tert*-butyl dicarbonate to protect one of the amine functions from reaction with the carboxylate functions of the DTPA molecule. The final reaction product cy(DTPA-EN-DTPA-EN) was obtained as a yellow solid after membrane filtration and lyophilization. ^1H-NMR (300 MHz, D_2O, pH = 12.83): δ 2.64 (s, 8H, $N_3CH_2CH_2N_4$), 2.54 (s, 8H, CH_2CONH), 2.44 (m, 12H, NCH_2COOH), 1.94 (m, 16H, $N_1CH_2CH_2N_2$). ^{13}C-NMR (100 MHz, D_2O, pH = 12.3): δ 180.7 (6C, N_1CH_2COOH), 175.9 (4C, CONH), 60.1 (10C, NCH_2CO), 53.4 (8C, $N_1CH_2CH_2N_2$), 39.8 (4C, $NHCH_2CH_2NH$). FAB-MS: *m*/*e* = 901 (M + 1 + $3Na^+$)

Preparation of the Lanthanide(III) Complexes. The Ln^{III} complexes of both ligands studied were prepared by adding an equimolar amount of $LnCl_3 \cdot xH_2O$ to a solution of the ligand (pH about 6) in D_2O, followed by adjustment of the pH to about 6.5 with a solution of NaOD in D_2O. The pH of the samples was measured at room temperature using a Corning 125 pH meter and a calibrated microcombination probe purchased from Aldrich Chemical Co. There were no corrections made for the deuterium isotope effect.

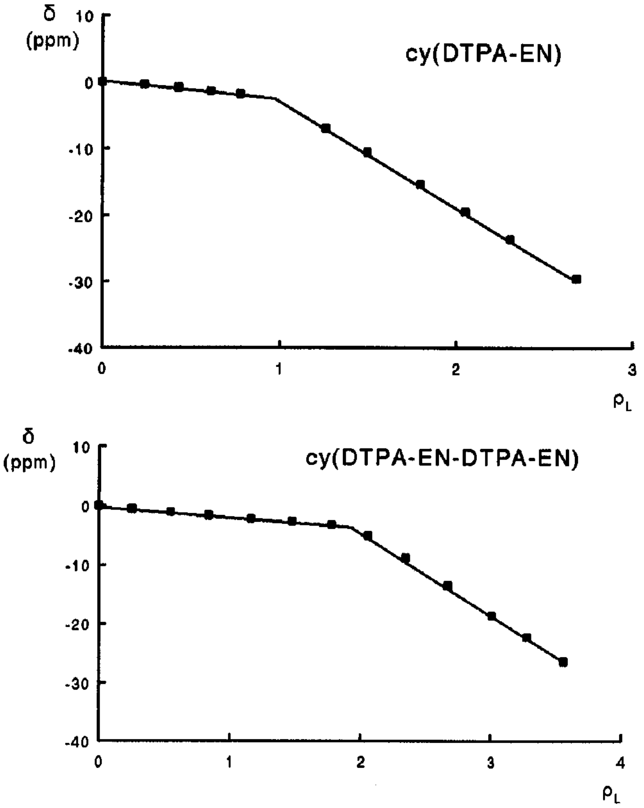

Figure 2. Plot of the Dy^{III}-induced water ^{17}O NMR shift *vs* the molar ratio Dy^{III}/ligand (ρ_L) for 0.05 M ligand solutions in D_2O at 76 °C.

NMR Measurements. The NMR spectra were recorded by a Nicolet NT-200 WB, a Varian INOVA 300, or a Varian VXR-400 S spectrometer. For the ^1H and ^{13}C NMR spectra, *tert*-butyl alcohol was used as internal standard (methyl signal at 1.2 ppm (^1H) or 31.2 ppm (^{13}C)). The ^{17}O chemical shifts were determined with respect to D_2O as external standard, by fitting the observed signals with a Lorentzian line function. The ^{89}Y NMR spectra were recorded at 19.6 MHz using 71 K data points, a spectral width of 12 kHz, and an acquisition time of 3 s. The ^{89}Y chemical shifts were referred to the resonance of 1 M YCl_3 in D_2O as external reference. The ^{139}La NMR spectra were recorded at 56.5 MHz using 6 K data points, a spectral width of 80 kHz, and an acquisition time of 0.04 s. The ^{139}La chemical shifts were determined with respect to the resonance signal of a 0.1 M solution of $LaCl_3$ in D_2O as external standard. ^1H variable-temperature measurements were performed for Yb^{III} complexes of both ligands. The spectra of these compounds were recorded at 400 MHz, 60 K data points, a spectral width of 100 kHz, and an acquisition time of 0.3 s. The water signal was suppressed by a spin-echo pulse sequence.

Results and Discussion

Dy^{III}-Induced ^{17}O Water NMR Shifts. Upon addition of Dy^{III} to a solution of cy(DTPA-EN) in D_2O, the water ^{17}O shift decreased linearly as the molar ratio Dy^{III}/ligand (ρ_L) increased (see Figure 2), indicating that the exchange between a Dy^{III}-bound water molecule and the bulk is fast on the NMR time scale. At $\rho_L = 1.0$ a steep increase in the magnitude of the (negative) shift was observed. This sudden change can be ascribed to the formation of 1:1 Dy[cy(DTPA-EN)] complexes. If ρ_L exceeds the value 1.0, additional Dy^{III} ions no longer bind to a ligand molecule and, therefore, appear as $Dy^{III}(H_2O)_8$ aquo complexes.

For the ligand cy(DTPA-EN-DTPA-EN), the ^{17}O-induced shift as a function of ρ_L showed also a sharp change of the

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[21] Peters, J. A.; Kieboom, A. P. G. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 381.

[22] Sherry, A. D.; Geraldes, C. F. G. C. In *Lanthanide Probes in Life, Chemical and Earth Sciences*; Bünzli, J.-C. G., Choppin, G. R., Eds.; Elsevier: Amsterdam, 1989; Chapter 4.

Table 1. Hydration Numbers q of Ln^{III} Complexes of DTPA and DTPA Derivatives

	NMR (Dy^{III} complex)		luminescence (Eu^{III} complex)	
ligand (L)	q	[L], M	q	[L]
DTPA	1.3(±0.2)[a]	0.05	1.2(±0.2)[d]	0.1 M
DTPA-BPA	1.0(±0.2)[b]	0.05	1.0(±0.2)[d]	0.1 M
cy(DTPA-EN)	1.2(±0.2)[c]	0.05	2.3(±0.5)[e]	10 μM
cy(DTPA-EN-DTPA-EN)	0.8(±0.2)[c]	0.05	1.2(±0.5)[e]	5 μM

[a] Reference 25. [b] Reference 16. [c] This study. [d] Reference 9. [e] Reference 24.

shift magnitude but now at $\rho_L = 2.0$ (see Figure 2). This can be interpreted as the cy(DTPA-EN-DTPA-EN) ligand forming Dy^{III} complexes with a 2:1 metal−ligand stoichiometry. Notably, there is no change in the slope at $\rho_L = 1.0$, suggesting that the two DTPA moieties of the ligand act completely independent of each other.

In both cases, the slope of the curve after the break point is comparable with the slope obtained for a similar experiment in the absence of an organic ligand. This supports the statement that at this point in the titration free Dy^{III} ions appear in the solution.

In order to calculate the hydration number from these plots, it is important to note that the Dy^{III}-induced ^{17}O shifts of Dy^{III}-bound oxygens are predominantly of contact origin (>85%).[23,25] These contact shifts of Ln^{III}-bound oxygens are almost independent of the nature of the ligand in question and the presence of other ligands in the complex. Therefore, it can be deduced that the initial slopes of plots of Dy^{III}-induced ^{17}O water shifts *vs* the molar ratio Dy^{III}/H_2O (ρ_w) are proportional to the number of water molecules bound in the first coordination sphere of the complex.[23] For the cy(DTPA-EN) ligand the slope is −2467 ppm ($\rho_L < 1$), and the plot for the cy(DTPA-EN-DTPA-EN) ligand has a slope of −1793 ppm ($\rho_L < 2$). Under the conditions applied in the present study, the slope per Dy^{III}-bound water molecule is −2140 ppm. It can be concluded that the Dy[cy(DTPA-EN)] complex contains on the average 1.2 (=−2467/−2140) inner-sphere water molecules, and the Dy_2[cy(DTPA-EN-DTPA-EN)] complex contains 0.8 (=−1793/−2140) water molecules per Dy^{III} ion. The latter complex contains about two water molecules, each of them bound to a Dy^{III} ion. It should be noted that the accuracy of the hydration numbers determined following this procedure is ±0.2.

The number of Ln^{III}-bound water molecules in the cyclic complexes provides information on the coordination mode of the ligand, since the coordination number of the Ln^{III} ion in polyamine polycarboxylate complexes is in general 9. In the complexes of both cyclic ligands one coordination site of each Ln^{III} ion is occupied by a water molecule and eight sites are available for the ligand molecule. By binding of three amine nitrogen atoms of the DTPA backbone and three carboxylate and two amide oxygen atoms, a similar coordination mode as found for the previously studied acyclic DTPA-bis(amide) ligands is attained.[16]

Surprisingly, the hydration number q obtained for cy(DTPA-EN) ($q = 0.8$) is lower than that obtained in a luminescence study on the corresponding Eu^{III} complexes ($q = 2.3$)[24] (see Table 1). Generally, the results of the ^{17}O NMR technique are in good agreement with those obtained by other methods such as luminescence and relaxivity measurements.[12,25] Therefore, we assume that the water coordination number changes along the lanthanide series, and ^{17}O NMR measurements for each Ln^{III} ion would be needed to check this. Unfortunately, the cy(DTPA-EN) complexes of the lighter lanthanides ($La^{III} \rightarrow Eu^{III}$) precipitate in aqueous solution at concentration ranges necessary for ^{17}O NMR measurements (≥0.05 M).

Inoue *et al.* have studied the solid Gd^{III} complex of cy(DTPA-EN) with single-crystal X-ray analysis.[26] This complex appears as a binuclear chelate, in which each Gd^{III} ion is coordinated to one amide oxygen, two carboxylate oxygens, and two amine nitrogen atoms of one cy(DTPA-EN) molecule and an amide oxygen, a carboxylate oxygen, and an amine nitrogen atom of a second molecule. On the other hand, the Gd^{III} complex of cy(DTPA-PN) is monomeric in the solid state, with a similar binding mode of the organic ligand as the acyclic bis(amides).[26] The coordination occurs in an octadentate fashion *via* three carboxylate groups and two amide oxygen and the three amine nitrogen atoms. Each Gd^{III} ion is also coordinated by one water molecule. It was noted that the Gd[cy(DTPA-PN)] complex has a higher solubility in water than the Gd[cy(DTPA-EN)] complex.

The difference in binding mode between these two ligands can be ascribed to steric factors. The smaller cy(DTPA-EN) ligand cannot be wrapped around a Gd^{III} ion in an octadentate fashion, and therefore, the metal face is relatively exposed, which results in a tendency to oligomerize. Similar phenomena have been observed in the X-ray crystal structures of lanthanide complexes of the 18-membered DTPA-dien ligand.[27] Most likely, the presently studied Eu[cy(DTPA-EN)] complex occurs predominantly in a monomeric form under the highly diluted conditions applied in the luminescence study (10 μM).[24] The organic ligand is probably bound in a heptadentate fashion under these conditions, which leaves space for the coordination of two water molecules.

With the smaller Ln^{III} ions ($Dy^{III} \rightarrow Lu^{III}$), binding of cy(DTPA-EN) in an octadentate mode is possible and monomeric species are predominant, which is reflected in the dramatic increase of solubility and in a hydration number $q = 1$ (for the first coordination sphere of the Ln^{III} ion).

^{89}Y NMR and ^{139}La NMR. In order to obtain support for the proposed lanthanide(III) coordination of cy(DTPA-EN-DTPA-EN), we studied the complexes of this ligand by ^{89}Y and ^{139}La NMR.

The chemical properties and the ionic radius of Y^{III} (0.89 Å) are comparable to that of the Ln^{III} ions (1.06−0.85 Å), and thus, Y^{III} complexes have a coordination geometry that is closely related to the geometry of the Ln^{III} complexes. ^{89}Y is a spin $I = {}^{1}/_{2}$ nucleus, and consequently, the NMR signals are usually very narrow (<5 Hz). The NMR receptivity is very low (0.007 with respect to ^{13}C), but this is compensated by a natural abundance of 100%.

The ^{89}Y NMR spectra of the Y^{III} complex of the cy(DTPA-EN-DTPA-EN) ligand at 25 °C for a 1:1 and 2:1 M:L ratio both show a broad peak at 81 ppm. The peak consists of at least three overlapping signals, from which two are narrow. This results from the presence of different isomers in solution (see below). Similar chemical shifts were measured for some Y^{III} complexes of acyclic DTPA-bis(amides), such as DTPA-BEA ($\delta = 82.5$ ppm) and Y^{III}(DTPA) ($\delta = 82.2$ ppm),[28] and for DTPA-BGLUCA ($\delta = 82$ ppm).[29] This confirms that the coordination of Y^{III} in these complexes is similar.

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(23) Vijverberg, C. A. M.; Peters, J. A.; Kieboom, A. P. G.; van Bekkum, H. *Recl. Trav. Chim. Pays-Bas* **1980**, *99*, 403.

(24) Frey, S. T.; Chang, C. A.; Carvalho, J. F.; Varadarajan, A.; Schultze, L. M.; Pounds, K. L.; Horrocks, W. DeW., Jr. *Inorg. Chem.* **1994**, *33*, 2882.

(25) Alpoim, M. C.; Urbano, A. M.; Geraldes, C. F. G. C.; Peters, J. A. *J. Chem. Soc., Dalton Trans.* **1992**, 463.

(26) Inoue, M. B.; Inoue, M.; Muñoz, I. C.; Bruck, M. A.; Fernando, Q. *Inorg. Chim. Acta* **1993**, *209*, 29.

(27) Franklin, S. J.; Raymond, K. N. *Inorg. Chem.* **1994**, *33*, 5794.

(28) Holz, R. C.; Horrocks, W. DeW., Jr. *J. Magn. Reson.* **1990**, *89*, 627.

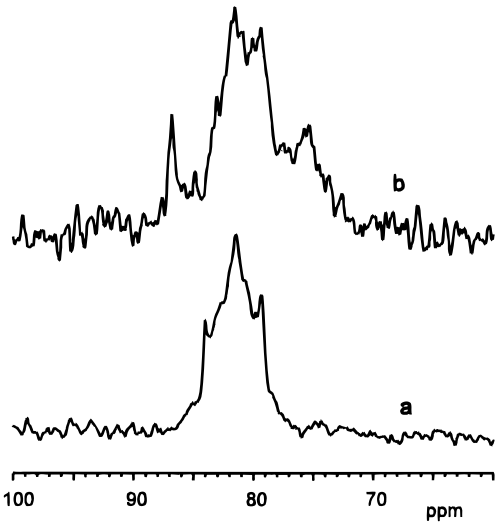

Figure 3. ^{89}Y NMR spectra of Y_2[cy(DTPA-EN-DTPA-EN)] and (Y,Tm)[cy(DTPA- EN-DTPA-EN)] (0.1 M) at 25 °C and pH = 8.

For mixed complexes, prepared by adding $YCl_3 \cdot 6H_2O$ and $TmCl_3 \cdot 6H_2O$ to a solution of cy(DTPA-EN-DTPA-EN) in different ratios Y:Tm:L, the position of the ^{89}Y signals was hardly affected by the presence of the paramagnetic Tm^{III} in the complex ($\delta = 81$ ppm), but the signals broaden (see Figure 3). Apparently, the Tm^{III}-induced pseudocontact shift at the Y^{III} nucleus is negligible due to the large distance between these metal ions. Horrocks and co-workers[24] determined this distance by Eu^{III} luminescence to be about 7.1 Å.

We can conclude from these data that the binding of a metal ion at one coordination site has almost no influence on the second coordination site. As mentioned in the discussion of the ^{17}O NMR experiments, the two coordination sites in the ligand molecule behave independently from each other.

Among the trivalent lanthanide ions, only La^{III} has favorable NMR properties, a high natural abundance (99.91%) and a high NMR receptivity (336 relative to ^{13}C). ^{139}La is a quadrupole nucleus with spin $I = {}^{7}/_{2}$ and a relatively high magnetic moment ($Q = 0.21 \times 10^{-28}$ m^2), leading to broad resonances as a consequence of quadrupolar relaxation. The ^{139}La chemical shift is very sensitive to its coordination.[12,30,31] Empirically, it was found that the chemical shift in polyamino polycarboxylate chelates is determined by the number of bound carboxylate groups and ethylenediamine units. The line width of the resonance signal is very sensitive to the environment of the ^{139}La nucleus;[12] the electric field gradients produced by the surrounding ligands and the molecular tumbling govern the relaxation. Consequently, ^{139}La NMR is very suitable for probing the coordination in Ln^{III} complexes.

The ^{139}La NMR spectrum of La$_2$[cy(DTPA-EN-DTPA-EN)] at 80°C exhibits a broad resonance signal ($\Delta\nu_{1/2} = 17\,000$ Hz) at 220 ppm. For the 1:1 M−L complex a similar signal was found, although it was slightly more shielded ($\delta = 240$ ppm). The spectral data are close to those of La^{III} complexes of acyclic DTPA-bis(amides), such as La[DTPA-BGLUCA], which has a ^{139}La chemical shift of 210 ppm and $\Delta\nu_{1/2} = 9850$ Hz.[29] This once again suggests that the binding mode of cy(DTPA-EN-DTPA-EN) is similar to that in Ln^{III} complexes of acyclic DTPA-bis(amides), which supports the conclusions of the ^{17}O and ^{89}Y NMR studies. Unfortunately, we were not able to investigate the Y^{III} and La^{III} complexes of cy(DTPA-EN) by ^{89}Y and ^{139}La NMR, respectively, since the solubility of these complexes was too low.

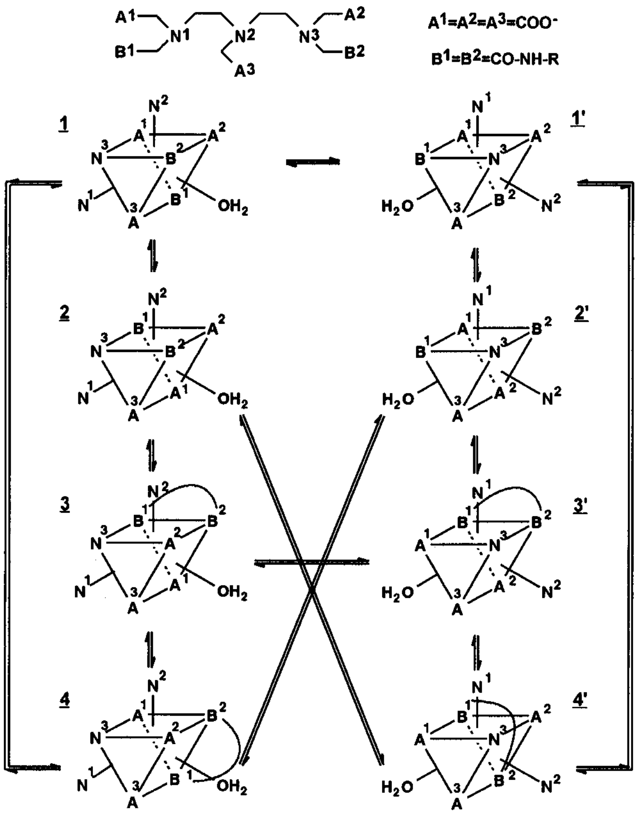

Figure 4. Coordination polyhedrons of the Ln[DTPA-bis(amide)] complexes[16] assuming a tricapped trigonal prism coordination geometry. The complexes of the macrocyclic ligands can only occur as isomers **3**, **3′**, **4**, and **4′**, and in these structures the cyclic nature of the ligand is indicated.

Variable Temperature ^{13}C and ^{1}H NMR. The ^{17}O, ^{89}Y, and ^{139}La NMR data indicate that, in solution, the DTPA-bis(amide) units in cy(DTPA-EN) and cy(DTPA-EN-DTPA-EN) bind Ln^{III} ions in an octadentate fashion by the three amine nitrogen atoms and three carboxylate and two amide oxygen atoms. By binding in this way, inversion of the three nitrogen atoms of the diethylenetriamine backbone is precluded. Therefore, these atoms are chiral and the DTPA-bis(amide) unit can occur in eight enantiomeric forms. If it is assumed that the coordination polyhedron of the nine-coordinated Ln^{III} in these complexes can be described by a distorted tricapped trigonal prism, this gives rise to the eight complex geometries schematically depicted in Figure 4.[16] Similar isomers are possible for a monocapped square antiprism. Previously, it has been shown that all eight isomers occur for Ln^{III} complexes of the acyclic DPTA-BPA in aqueous solution.[16] Two dynamic processes play an important role: (i) the interconversions **1** ⇄ **1′**, **2** ⇄ **4′**, **3** ⇄ **3′**, and **4** ⇄ **2′**, which are associated with the racemization of the two gauche conformations of the ethylenediamine bridges (“wagging motion”); (ii) racemization at the terminal nitrogen atoms via the interconversions **1** ⇄ **2** ⇄ **3** ⇄ **4** ⇄ **1** and **1′** ⇄ **2′** ⇄ **3′** ⇄ **4′** ⇄ **1′**, which is relatively slow because it requires decoordination of a nitrogen and its two neighboring oxygen atoms (Figure 4).

Some differences are expected in the presently studied systems, due to the steric constraints caused by the macrocyclic nature of the ligand. Inspection of Dreiding models shows that isomers **1**, **1′**, **2**, and **2′** can be definitely excluded on steric grounds.

The ^{13}C NMR spectrum of the Lu[cy(DTPA-EN)] complex at high temperature (90 °C) is very similar to that of the previously studied Lu(DTPA)$^-$ complex: only two carboxylate

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(29) Lammers, H.; van der Heijden, A. M.; Geraldes, C. F. G. C.; van Bekkum, H.; Peters, J. A. To be published.

(30) Evans, D. F.; Missen, P. H. *J. Chem. Soc., Dalton Trans.* **1982,** 1929.

(31) Geraldes, C. F. G. C.; Sherry, A. D. *J. Magn. Reson.* **1986**, *66,* 274.

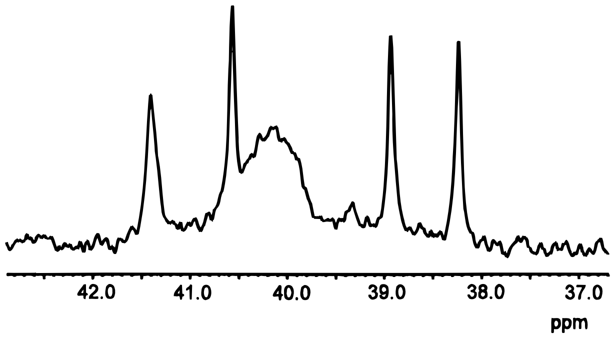

Figure 5. ^{13}C NMR spectrum of La_2[cy(DTPA-EN-DTPA-EN)] (0.1 M) at 85 °C and pH = 7.

(δ = 180.75, 180.45 ppm), one amide carbonyl (δ = 171.41 ppm), and six methylene signals were observed. Apparently, an exchange process is operative which results in an effective mirror plane through the middle glycine unit. By cooling of the sample to 15 °C, the signals broadened and coalesced. A further lowering of the temperature to −10 °C, after diluting the sample with CD_3OD (1:1 v/v), resulted in a splitting of the amide resonance into two peaks of equal intensity. From the coalescence temperature, the $\Delta G^{\ddagger}$ associated with the concerning exchange process is estimated to be 57 kJ mol^{-1}. The magnitude of this free enthalpy barrier is the same as that previously observed for the "wagging" process in the linear bis(amide) complexes.[16] This process causes a "shuffling" of the coordinated acetate groups without ligand decoordination. The only exchange process possible for the present system that does not involve decoordination is the exchange between **3** and **3′**. We conclude that these are the major species present in solutions of Lu[DTPA-EN]. Molecular models confirmed this explanation. The steric constraints imposed by the rather small ethylene bridge precludes the formation of the other stereoisomers.

The situation for cy(DTPA-EN-DTPA-EN), however, is more complicated due to the presence of two DTPA-bis(amide) units, which each can occur in four *diastereomeric* forms upon coordination to a Ln^{III} ion. Furthermore, the ethylene bridges that are linking the two DTPA units may also occur in various conformations.

The ^{13}C NMR spectrum of La_2[cy(DTPA-EN-DTPA-EN)] at 85 °C indicates that there are various species present in solution. Figure 5 shows the ^{13}C resonance signals of the ethylenediamine bridges between two DTPA moieties. The other signals in the spectrum have a similar pattern, e.g. for the amide NH ^{1}H-NMR signals. Each cluster consists of four sharp signals superimposed on a broad peak. By the lowering of the temperature, the sharp signals remained sharp, whereas the broad peak seems to decoalesce into two broad peaks. This phenomenon can be explained by the existence of two exchange processes in solution: one is relatively fast on the NMR time scale and gives rise to the broad signal, and the second one is slower; even at 85 °C narrow signals arise in the spectrum.

We assume that the fluxionality of the system leads to a pseudo mirror plane between the two DTPA units (through the centers of the ethylene bridges). The broad methylene signal in Figure 5 is assigned to the relatively fast equilibrium between the diastereomers **3** and **3′**, which in a static situation could give four signals. This interconversion, described earlier as the "wagging motion" of the diethylenetriamine backbone of the DTPA unit, is relatively fast on the NMR time scale at 85 °C, since no decoordination of the ligand is required. The four narrow signals are assigned to diastereomers **4** and **4′**, which cannot interconvert into each other in the way **3** and **3′** do. For these isomers, in a first step, one of the terminal nitrogen atoms rearranges *via* decoordination (**4** ⇄ **3**); second, the coordination polyhedron is rearranged *via* the so called "wagging motion" (**3** ⇄ **3′**), and in the final step, there occurs a second decoordination at a terminal nitrogen atom (**3′** ⇄ **4′**). Since this exchange process requires twice a partial decoordination of the ligand, it is much slower on the NMR time scale than the exchange process between **3** and **3′**.

Similar phenomena have been observed by Franklin and Raymond[27] for the Ln^{III} complexes of an 18-membered macrocyclic DTPA-bis(amide) formed by a 1:1 condensation of DTPA-bis(anhydride) and diethylenetriamine. These complexes occur in solution as two enantiomeric pairs, a so-called "dynamic" and a "static" pair.

It should be noted that the Ln^{III} coordination polyhedron of similar macrocyclic DTPA-bis(amide) complexes in solid-state structures reported up to now[26,27] always correspond with **3/3′** (see Figure 3), whereas the acyclic bis(amide) complexes always have geometry **1/1′**.

Frey *et al.*[24] have studied the coordination of cy(DTPA-EN) and cy(DTPA-EN-DTPA-EN) using Eu^{III} luminescence spectroscopy and molecular mechanics. It was concluded that both compounds occur in a single conformation. The present NMR study clearly shows that the Ln^{III} complexes of both ligands exist in more than one form in solution. Apparently, luminescence is not able to discriminate between enantiomeric/diastereomeric complexes.

^{13}C NMR spectra for the heavier paramagnetic lanthanides (Gd → Yb) were not observable due to excessive broadening. This phenomenon is common for DTPA derivatives.[8,16] The spectra for the lighter Ln^{III} ions were very complicated, showing many overlapping signals. The pseudo-mirror plane between the two DTPA units is no longer present, due to the larger chemical shift differences between the various isomers as a result of the influence of the paramagnetic metal ion.

The ^{1}H NMR spectrum of Yb_2[cy(DTPA-EN-DTPA-EN)] also contained a huge amount of signals. At room temperature, a set of 11 signals of relatively high intensity could be observed next to numerous other smaller signals (between +90 and −50 ppm). The set of 11 signals broadened somewhat by lowering the temperature to 0 °C. Most likely, these signals can be assigned to the complex in which both DTPA units are coordinated in isomeric forms **3** ⇄ **3′**, thus a "dynamic−dynamic" pair in the nomenclature of Franklin and Raymond.[27] The large amount of remaining signals can be ascribed to the "dynamic−static" and "static−static" forms.

Conclusions

The present study clearly demonstrates the power of NMR in the determination of the solution structures of Ln^{III} chelates. It appears that the structures of the complexes is dependent on the ring size of the macrocycle. Both the 30-membered macrocycle cy(DTPA-EN-DTPA-EN) and the 18-membered macrocycle DTPA-dien, previously studied by Franklin and Raymond,[27] form complexes that are similar to those of linear DTPA-bis(amides). However, it should be noted that the number of isomers in these complexes is limited, as a result of the constraints imposed by the macrocycle. In the 15-membered ring cy(DTPA-EN) the strain is such that it is not possible to bind the larger lanthanide ions in a similar way as the acyclic counterparts. In this case precipitation occurs, probably as a result of oligomerization.

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