Quantitative Two-Dimensional EXSY Spectroscopy and Dynamic Behavior of a Paramagnetic Lanthanide Macrocyclic Chelate: YbDOTA (DOTA = 1,4,7,10-Tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic Acid)

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YbDOTA (DOTA: 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid) is a rigid lanthanide chelate that adopts two conformations in slow exchange. The intra- and intermolecular exchanges within and between the two isomeric forms of YbDOTA have been investigated by a quantitative analysis of the pure absorption mode two-dimensional exchange ^{1}H spectra. The dynamic matrix was completely solved and the activation parameters for all exchanges were deduced from variable temperature measurements. Useful information on the dynamic processes were obtained despite the paramagnetism of Yb(III) and despite the short relaxation times and the very large NMR shifts induced by this ion. As suggested previously (Aime et al. *Inorg. Chem.* 1992, *31*, 4291), the inter- and intramolecular exchange peaks were assigned respecively to a reorientation of the acetate substituents and to a complete inversion of the conformation of the chelate. The activation energy for the major → minor species exchange is higher than for the minor → major species exchange ($\Delta G^{\ddagger}$ = 65.6 vs 61.4 kJ mol^{-1}) presumably because of the greater steric crowding of the minor isomer. The accuracy of the measurements is discussed and the data are compared with previously published analyses.

Introduction

Trivalent lanthanides are known to form stable but highly fluxional chelates with a variety of ligands. Their lability results in the appearance of one set of NMR peaks only for a large number of geometries in fast exchange. Consequently, NMR spectroscopy is often not suited for unraveling the solution structure of lanthanide chelates, as it is difficult to take into account the geometry of each species, its relative concentration, and its dynamic behavior. This difficulty is encountered with the lanthanide shift reagents and has led to their relative unpopularity in quantitative analyses.[1] However, the selection of sterically demanding ligands affords entry to rigid lanthanide complexes whose solution structure can be reliably established by a quantitative analysis of the NMR shifts induced by paramagnetic metal ions. For instance, the macrocyclic ligand 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid, DOTA,
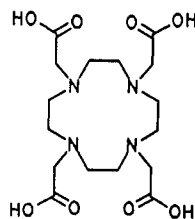
DOTA

forms highly symmetric and exceedingly rigid lanthanide complexes. This rigidity is due to the strong preference of the 12-membered tetraaza cycle of DOTA for a square [3.3.3.3] geometry,[2] and the ethylenic and the acetate groups are prevented from inverting their configuration rapidly at room temperature. The relative magnitudes of the paramagnetic shifts are particularly useful for elucidating the solution structure of these complexes.[2] In some cases, the steric constrains that a ligand imparts to its

complexes are so important that the exchange between conformational isomers becomes slow compared to the observation time. For instance, separate peaks are observed for different solution species in the case of the paramagnetic lanthanide complexes with 2,2',2''-terpyridine[3] and with DOTA[2] and its derivatives.[4] This unsual behavior has been investigated by NMR[4] and by luminescence spectroscopy.[4,5] Very recently, two-dimensional exchange spectroscopy (EXSY) has also been used to investigate the dynamic properties of the lanthanide DOTA chelates. Aime *et al.*[6] and Hoeft *et al.*[7] suggested that the two sets of peaks exhibited by the paramagnetic complexes YbDOTA and EuDOTA should be assigned to two isomers that differ in the position of the acetate groups but not in the conformation of the tetraaza cycles. This hypothesis is reasonable in view of crystallographic structures that show that the tetraza ring and the acetate arms of ligands derived from 1,4,7,10-tetraazacyclododecane can be oriented independently. For instance, the gadolinium chelate of (1*R*,4*R*,7*R*)-α,α',α''-trimethyl-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, DO3MA, adopts two conformations in the solid state:[8] the three acetate substituents of DO3MA are in the same orientation in both conformers, but the macrocyclic rings are mirror images of each other.

According to Aime *et al.*[6] and Hoeft *et al.*,[7] the inversion of the configuration of the acetate pendant arms of the DOTA chelates takes place at the same rate or faster than the conformational exchange in the tetraaza ring. These results were published while the present work was in progress.[9] We adopted here a quantitative approach in the analysis of the EXSY spectra. Indeed, qualitative studies of EXSY spectra that are based solely on the presence or the absence of peaks are open to question

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because cross-peaks can be small and difficult to distinguish from noise specially in the case of paramagnetic compounds. We report here on a detailed study of the EXSY spectra of YbDOTA recorded at different temperatures. Because of the short longitudinal relaxation times T_1 and the large induced shifts exhibited by the paramagnetic lanthanide complexes, there are very few quantitative studies of their two-dimensional NMR spectra.[10,11] There is however a growing number of two-dimensional studies of paramagnetic complexes of other metal ions such as iron(III).[12] A study of the dynamic behavior of the YbDOTA chelate allows to test the feasibility of quantitative EXSY for compounds exhibiting very large paramagnetic shifts and to gain a better insight into the conformational processes taking place in compounds that are effective contrast agents for magnetic resonance imaging.

Experimental Section

The ligand DOTA was synthesized as described previously.[2] Solutions of the Yb(III) and Eu(III) complexes were prepared by mixing equimolecular amounts of $YbCl_3$ or $EuCl_3$ (Aldrich) and of DOTA and adjusting the pH to 7 with NaOH. Each mixture was taken to dryness on a rotatory evaporator and redissolved in enough D_2O to yield a 0.1 M solution of complex. The solutions were approximately 0.4 M in NaCl. It is noteworthy that the presence of a salt modifies the relative concentration of the two isomers.[7,9]

Bruker AM400 and AM300 spectrometers were used for collection of both 1D and 2D ^1H NMR spectra. The variable-temperature accessories were calibrated with an ethylene glycol sample. Each calibration was repeated three times, and the quoted temperatures are accurate to ±0.7 °C. An inversion–recovery pulse sequence (180°–τ–90°–acq) was used to obtain the ^1H and ^{13}C relaxation times T_1 and to estimate the relaxation delay to be used between each pulse sequence ($\tau_{delay} \simeq 0.5$ s). Pure absorption mode EXSY spectra were recorded using the conventional NOESY 90°–t_1–90°–τ_{mix}–90°–acq phase sensitive pulse sequence with 2048 t_2 and 1024 t_1 data points and with time-proportional phase incrementation (TPPI). Both dimensions were apodized by a 60°-phase shifted sine-bell-window function and zero-filled to 2048 t_2 × 2048 t_1. The final 2D spectra were obtained after Fourier transformation. The mixing times at individual temperatures were optimized by trial and error to give maximum intensity of cross-peaks (τ_{mix}= 5–8 ms). The NMR tubes were left in the probe during 15 min to allow for temperature equilibration and were not rotated to avoid ridges of noise along the ν_1 dimension. Zero-quantum coherences if any were suppressed by varying the mixing time τ_{mix} randomly over a small range. The integrated volume under each peak was computed with the software supplied by Bruker. As expected, the strength of the radiofrequency field B_1 was insufficient to produce a uniform 90° pulse rotation angle[13] across entire NMR spectra exhibiting resonances spread out over 250 ppm. Distortions of peak intensities were observed at both ends of the spectra and had to be corrected. The three 90° pulses of the NOESY sequence bring about a $\sin^3 \beta$ correction for each diagonal peak where β is the real pulse rotation angle. A calibration of the pulse rotation angle β vs $|\omega - \omega_{RF}|$, the difference between the frequency of a diagonal peak and the carrier frequency, was perfomed by recording a 2D spectrum with a very short mixing time τ_{mix} $\rightleftharpoons$ 10 μs so that the exchange processes had not yet taken place. A $\sin^2 \beta_A \sin \beta_B$ correction was then applied to each cross-peak resulting from an exchange from nucleus A to nucleus B. The corrections amounted to a maximum of 50% for the most shifted peaks at high and low fields and are unavoidable because of the insufficient strength of the B_1 field over the complete spectral window. The kinetic constants were computed as described by Perrin and Gipe.[14] The integrated amplitude $I_{k,l}(\tau_m)$ of a cross-peak is given by[13]

$$I_{kl}(\tau_m) = e^{L_{kl}\tau_m} M_l^0 \qquad (1)$$

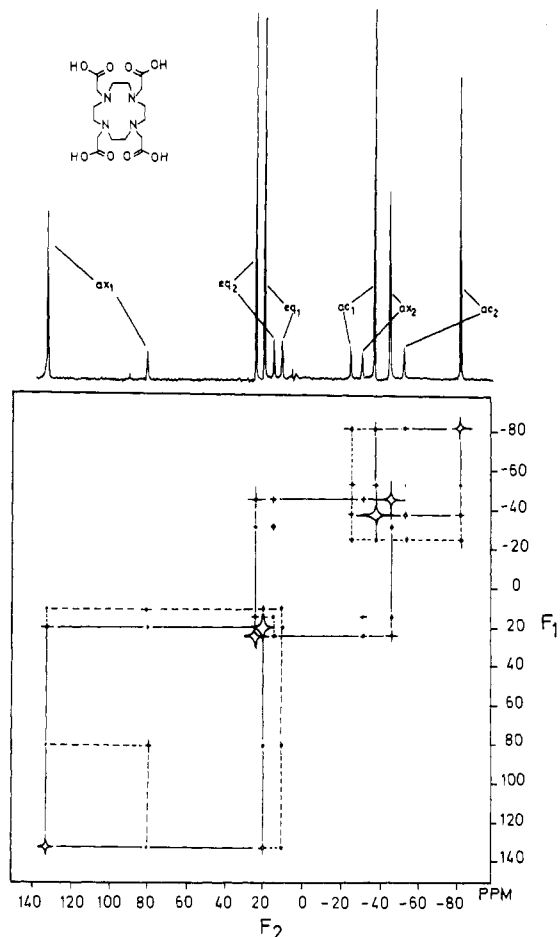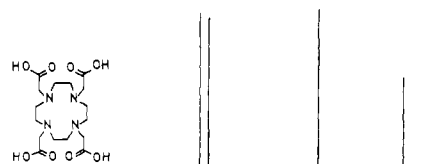where L_{kl} is the dynamic matrix. Provided there is no cross-relaxation,

Figure 1. Magnitude EXSY spectrum of YbDOTA at 303 K. The assignments of the peaks are taken from ref 4.

the off-diagonal elements of L_{kl} are $L_{kl} = -k_{lk}$ where k_{lk} is the first-order rate constant for the exchange from site l to site k. The equilibrium magnetization in site l, M_l^0, was measured experimentally in a spectrum with a very short mixing time τ_{mix} and thus with no exchange peaks. Equation 1 was solved by computing matrix A whose elements are the normalized amplitudes of the peaks $a_{ij} = I_{ij}/M_j^0$. Matrix A was diagonalized so that $D = X^{-1}AX$ where D is the diagonalized matrix of the eigenvalues and where X is the eigenvector matrix. The dynamic matrix L was then solved with the equation

$$L = \frac{1}{\tau_m} \ln A = \frac{1}{\tau_m} X(\ln D) X^{-1} \qquad (2)$$

All calculations were performed with the Mathcad 4.0 program (MathSoft).

Results

As reported previously,[4,5] the spectrum of YbDOTA exhibits two series of peaks that are due to a major isomer and a minor isomer (see Figure 1). Each species give rise to six peaks that are assigned to the two equatorial and the two axial protons of the tetraaza ring and to the two protons of the acetate groups in complexes of C_4 symmetry. The complete assignment of the NMR peaks was made after analysis of the spectra[4,15] and by comparison with the solid-state structure of EuDOTA.[15] Aime et al[6] recently reported an analysis of the spectrum of YbDOTA and proposed

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(16) Using the polar coordinates θ_i and r_i and the susceptibility factor D given by these authors, the calculated shifts are –57.5 and –59.1 ppm rather than –62.6 and –33.7 ppm as listed in Table 5 in ref 6. On the contrary, using the crystallographic coordinates of EuDOTA, the calculated shifts become –27.7 and –59.1 ppm and are thus much closer to the experimental values, –33.7 and –61.9 ppm respectively.
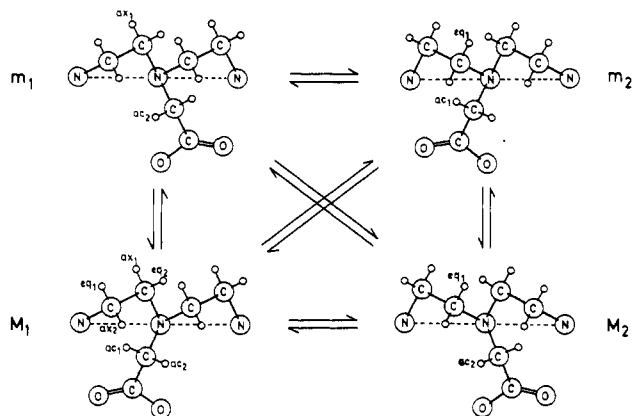
Figure 2. Longitudinal relaxation times T_1 of the ^{13}C nuclei of LuDOTA: top, CH_2 peaks; bottom, $C=O$ peaks ($\bullet$, major isomer; $\bigcirc$, minor isomer).

the same assignments for the paramagnetic shifts except for the two acetate peaks of the minor isomer for which the assignments were inverted. The analyses of the NMR spectrum of YbDOTA were based on the dipolar equation that links the induced paramagnetic shift $\Delta \nu_i$ to the magnetic susceptibility term D and to the polar coordinates θ_i and r_i of proton i relative to the axes of the susceptibility tensor

$$\Delta \nu_i = \left(\frac{3 \cos^2 \theta_i - 1}{r^3} \right) D \tag{3}$$

The values of θ_i and r_i can be computed directly from crystallographic structures[15] or the internal coordinates of a geometrical model of the complex under investigation can be adjusted until the best agreement is reached between the experimental and the calculated NMR shifts.[6] In both approaches, the contact contribution is neglected on the basis that it is small compared to the dipolar contribution in the Yb(III) chelates. Even if it is small, the contact contribution can lead to spurious results when a fine adjustment of the interatomic distances and angles is performed. Difficulties also arise when detailed molecular structures are deduced from relaxation measurements.[17] In addition, the application of eq 3 does not lead[16] to the assignment of the acetate protons of the minor form of YbDOTA as proposed by Aime *et al.*[6] The exact geometry of the minor isomer is thus difficult to unravel completely. We will assume here that the difference between the major and the minor isomers lies only in the position of their acetate groups as suggested by Aime *et al.*[6], but we will not make any assumption on the exact position of the acetate groups except that they adopt a four-bladed propeller-like conformation that is either clockwise or counterclockwise. It should be noted that the acetate groups are probably not exact mirror images of each other in the two geometries. Indeed, the magnetic susceptibility tensor depends essentially on the position and nature of the coordinating groups. Protons of two isomers with acetate arms in mirror image positions would then have the same susceptibility factor D and would thus exhibit the same paramagnetic shifts. This assumption is in agreement with crystallographic data.[8] The similarity of the structures of the two isomers is supported by the relaxation studies reported in Figure 2: the T_1 values of the ^{13}C nuclei of both isomers of LuDOTA are identical within the limits of the errors. It should be noted here that these measurements were carried out after the addition of NaCl. As noted previously,[7,9] adding inorganic salts to solutions of YbDOTA modifies the relative concentrations of the isomers. The errors on the relaxation data shown in Figure 2 are very silmilar because the T_1 values were

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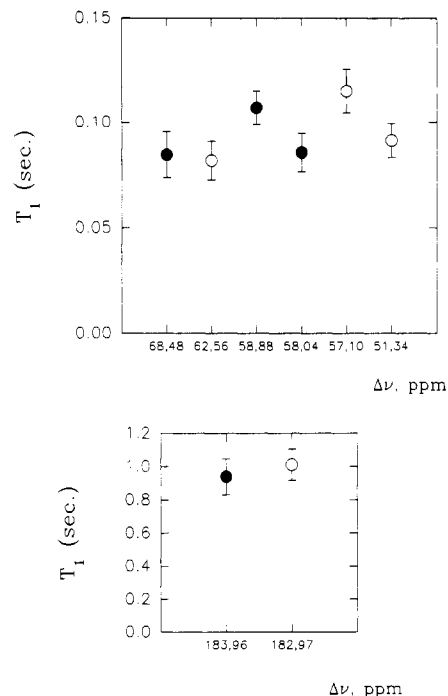

Figure 3. Schematic presentation of the conformational processes in the YbDOTA chelate. The symbols M and m designate the major and the minor isomers respectively. The metal ions are left off for clarity and the atom labeling is the same as in ref 4 (ax = axial, eq = equatorial, ac = acetate). In the $M_1 \rightleftarrows m_1$ and $M_2 \rightleftarrows m_2$ exchanges, the dipolar geometric factors (see eq 3) of the acetate protons are modified while they remain unchanged in the case of the ring protons. This process leads to exchanges such as $ac_1(M) \rightleftarrows ac_2(m)$ and $ax_1(M) \rightleftarrows ax_1(m)$. The $M_1 \rightleftarrows m_2$ and $M_2 \rightleftarrows m_1$ processes give rise to exchanges such as $ac_1(M) \rightleftarrows ac_1(m)$ and $ax_1(M) \rightleftarrows eq_1(m)$. The dipolar geometric factors remain unchanged in the intramolecular exchanges $M_1 \rightleftarrows M_2$ and $m_1 \rightleftarrows m_2$. These exchanges give rise to EXSY cross-peaks for mechanisms such as $ac_1(M) \rightleftarrows ac_2(M)$ and $ax_1(M) \rightleftarrows eq_1(M)$.

collected in the presence of enough NaCl to ensure that all the ^{13}C peaks of LuDOTA had about the same intensity.

The exchange processes that are assumed to take place between the major (M) and the minor (m) forms of YbDOTA are illustrated in Figure 3 along with the labeling of the atoms. The assignment of the relative orientations of the acetate and the ethynenic groups to one of the two forms of YbDOTA is supported by the measurements as will be shown later. This scheme is in keeping with the two-dimensional EXSY spectrum of YbDOTA reproduced in Figure 1. Each proton of the major and the minor isomers features three cross-peaks because it is exchanging with another proton in the same isomer and with two protons of the other isomer, one of them being located in a similar environment (for instance, M_{ax}–m_{ax}) and the other one being oriented differently (for instance, M_{ax} –m_{eq}). The EXSY spectrum we recorded for EuDOTA is similar to the the spectrum reported by Hoeft *et al.*[7] except that all exchanges were observed: EuDOTA features again three cross-peaks per diagonal peak. Hoeft *et al.*[7] proposed an exchanging scheme analogous to the one illustrated in Figure 3. However, these authors assumed that no intramolecular exchanges ($M_1 \rightleftarrows M_2$) can take place but rather that the exchange between the two mirror geometries of, e.g., the major isomer proceeds by an inversion of the configuration of the acetate groups that leads to the formation of a minor isomer ($M_1 \rightarrow m_2$) followed by an inversion of the configuration of the ring ($m_2 \rightarrow M_2$). Should this assumption be correct, the curve representing the peak intensity versus the mixing time τ_{mix} should have an S shape for the protons of the major isomer since an indirect pathway implies that there should be an induction period during which the first exchange occurs. This induction period should be observed as an initial zero slope curve for short mixing times ($\tau_{mix} \lesssim 1/k_{ex}$).
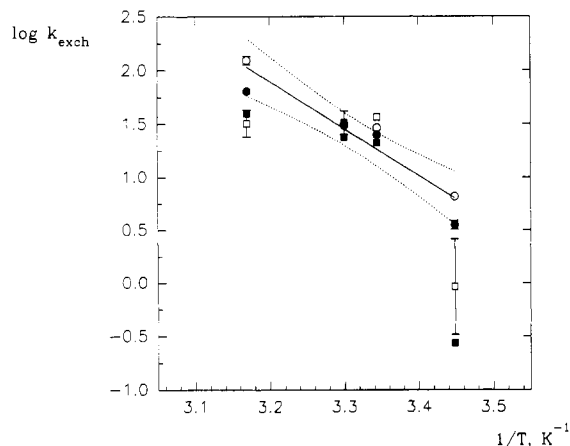
Figure 4. Arrhenius plot for the exchange between the two mirror-image conformations of the major isomer $M_1 \rightleftharpoons M_2$ (O, ring protons; ●, acetate protons) and of the minor isomer (□, ring protons; ●, acetate protons). These processes involve an inversion of the configuration of the tetraaza ring and of the acetate groups. All measurements have been performed at 400 MHz except for the kinetic constants measured at 299 K that were collected at 300 MHz. The 95% confidence interval is displayed by dashed lines.

Such a situation has already been described in the literature[18,19] and was not reported by Hoeft *et al.*[7]

We analyzed quantitatively the pure aborption-mode EXSY spectrum of YbDOTA by solving completely the dynamic matrix L_{kl} as described in the Experimental Section (eqs 1 and 2). At each temperature, one 2D spectrum was recorded with a very short mixing time τ_{mix} to obtain the equilibrium magnetization of each proton, M_i^0, and one or two EXSY spectra were recorded with τ_{mix} = 5–10 ms. The matrix A containing the normalized intensities $a_{ij} = I_{ij}/M_j^0$ (eq 2) was then calculated. It is a 12 × 12 square matrix that is easily rearranged into a block-diagonal matrix containing three 4 × 4 matrices in keeping with the EXSY spectra that show that the protons are exchanging in groups of four. The temperature dependance of the kinetic constants for the exchange between the mirror-image conformations of the major ($M_1 \rightleftharpoons M_2$) and of the minor ($m_1 \rightleftharpoons m_2$) isomers is reproduced in Figure 4. The errors on the volumes of the peaks assigned to the $m_1 \rightleftharpoons m_2$ exchange are large because these peaks are due to a species in small concentration and because there is no transfer of intensity from the major isomer to these peaks. No attempt was made at fitting the data for the $m_1 \rightleftharpoons m_2$ exchange to an Arrhenius equation

$$\ln k_{exch} = -\frac{E_a}{RT} + \ln A \tag{4}$$

where E_a is the activation energy and A is the frequency factor. On the contrary, a reasonably good linear relationship was obtained for the intramolecular exchange of the major isomer $M_1 \rightleftharpoons M_2$. Within the limits of the errors, no distinction can be made between the dynamic behavior of the tetraaza ring and of the acetate arms. The same conclusion was reached when analyzing the intermolecular exchanges between the major and the minor isomers, whether the conformational process involves an inversion of the acetate groups only ($M_1 \rightleftharpoons m_1$ and $M_2 \rightleftharpoons m_2$) or of the ethylenic groups only ($M_1 \rightleftharpoons m_2$ and $M_2 \rightleftharpoons m_1$) and whether the exchange proceeds from the major to the minor isomer or vice versa (Figures 5 and 6). The Arrhenius parameters and the activation parameters deduced from Eyring's equation are listed in Table 1 along with the standard errors on these values.
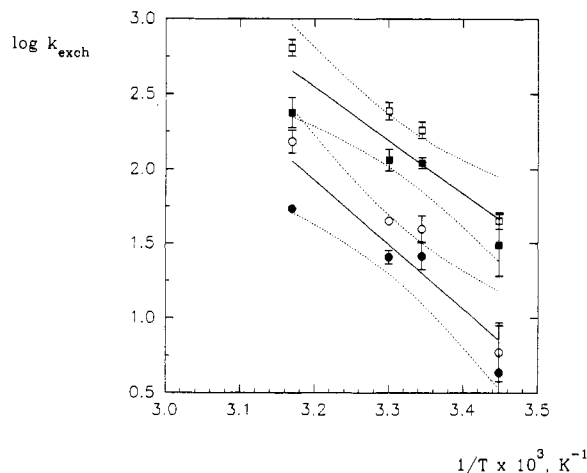
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Figure 5. Arrhenius plot for the exchange $M_1 \rightarrow m_1$ and $M_2 \rightarrow m_2$ between the major and the minor isomer (O, ring protons; ●, acetate protons) and $m_1 \rightarrow M_1$ and $m_2 \rightarrow M_2$ between the minor and the major isomers (□, ring protons; ■, acetate protons). These processes involve an inversion of the configuration of the acetate groups. The experimental conditions are the same than in Figure 4.
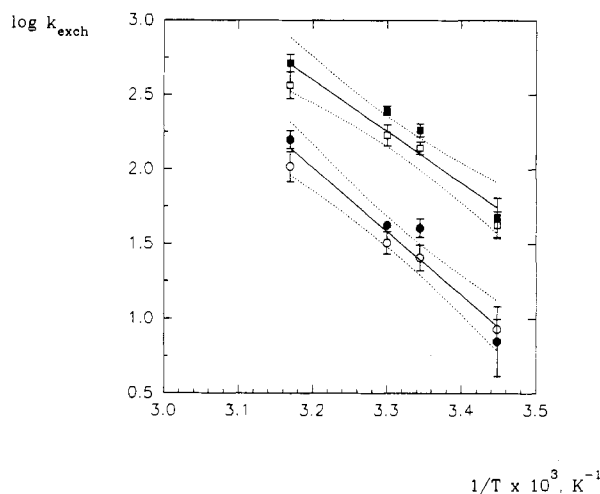

Figure 6. Arrhenius plot for the exchange $M_1 \rightarrow m_2$ and $M_2 \rightarrow m_1$ between the major and the minor isomer (O, ring protons; ●, acetate protons) and $m_1 \rightarrow M_2$ and $m_2 \rightarrow M_1$ between the minor and the major isomers (□, ring protons; ■, acetate protons). These processes involve an inversion of the configuration of the tetraaza ring. The experimental conditions are the same as those in Figure 4.

A rough assessment of the errors on the kinetic constants k_{exch} can be gained by comparing the data collected at two different mixing times τ_{mix} = 5 and 8 ms. These errors range from 5 to 40% and are within or somewhat above the usual limits for EXSY spectroscopy.[14] Moreover, an additional source of errors on the activation parameters stems from the narrow range of temperatures in which reliable measurements can be carried out (in the present case, 30 °C). This is a well-known limitation of the EXSY measurements.[20] Despite these difficulties, the values of the kinetic constants found in Figures 4–6 seem to be reliable since reasonably good Arrhenius relationships were obtained and since the ratio $k_{M \rightarrow m}/k_{m \rightarrow M}$ = 4.2 is close to the ratio of the relative concentrations of the two isomers as determined from the areas of the NMR peaks (major/minor = 4.0 under our experimental conditions).

Two-dimensional nuclear Overhauser effect spectra (NOESY) and exchange spectra (EXSY) are obtained with the same pulse sequence, and there is always a danger that 2D spectra will contain both NOE and exchange cross-peaks. The rotational correlation

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Table 1. Thermodynamic Parameters of the Exchanges between the Major and Minor Isomers of YbDOTA[a]

	ln A	E_a (kJ mol^{-1})	ΔH^* (kJ mol^{-1})	ΔS^* (J mol^{-1} K^{-1})	ΔG^* (kJ mol^{-1})[b]
M$_1$ ⇌ M$_2$	37 ± 2	84 ± 5	82 ± 12	52 ± 39	65.9 ± 1.0
M$_1$ → m$_1$ M$_2$ → m$_2$	39 ± 7	90 ± 17	79 ± 8	46 ± 26	65.1 ± 0.8
m$_1$ → M$_1$ m$_2$ → M$_2$	34 ± 6	72 ± 15	64 ± 8	9 ± 27	61.2 ± 0.6
M$_1$ → m$_2$ M$_2$ → m$_1$	35 ± 5	80 ± 12	80 ± 15	49 ± 51	65.7 ± 1.3
m$_1$ → M$_2$ m$_2$ → M$_1$	31 ± 2	65 ± 6	66 ± 13	14 ± 45	61.6 ± 1.1

[a] Mean values of the thermodynamic parameters calculated for all the protons in the EXSY spectra along with the standard errors. [b] Mean value of ΔG^* calculated directly from the Eyring equation $\Delta G^* = RT \ln(k_B T/hk_{exch})$; the errors are the standard deviations on the mean values.

times of the isostructural chelates GdDOTA and YDOTA[21] are of the order of 90 ps in keeping with the Debye–Stokes equation. Thus, the DOTA chelates give rise to spectra for which the extreme narrowing limit applies, and cross-peaks due to NOE effects if any should be very weak and negative. The absence of significant NOE effects in the EXSY spectra of YbDOTA is substantiated by several experimental observations. First, the ratio $k_{M\rightarrow m}/k_{m\rightarrow M}$ is close to the ratio of the peak areas of the two isomers of YbDOTA. Second, no cross-peaks were found for two protons attached to the same carbon atom in each isomer.[7] This last observation also indicates that no cross-peak arises from scalar couplings.

Discussion

The activation parameters for the inversion of the conformation in the tetraaza ring of LaDOTA have been determined by a band shape analysis of the ethylenic ^{13}C NMR peaks.[2] LaDOTA is an exceedingly rigid chelate with $\Delta H^* = 59.4 \pm 0.8$ kJ mol^{-1}, $\Delta S^* = -4.6 \pm 3.3$ J K^{-1} mol^{-1}, and $\Delta G^* = 60.7 \pm 1.2$ kJ mol^{-1}. This lack of lability is also found in the present EXSY investigation: the activation free energy ΔG^* of YbDOTA and LaDOTA are similar (see Table 1). The EXSY study has the advantage of being based on the analysis of the cross-peak intensities of both the ethylenic and the acetate protons while the band shape study was limited to the ethylenic ^{13}C peaks that are the only ones that undergo spectral changes during the exchange processes. However, the standard errors on the activation parameters are higher in the EXSY analysis than in the band shape investigation. Despite these errors, the data collected in Table 1 indicate that the kinetics is faster in the minor → major exchange than in the major → minor exchange and accordingly, the free energy of activation ΔG^* is higher for the M → m process. As seen in Figure 2, the acetate groups in the minor species are assumed to be located close to a CH$_2$ group that is near to the mean plane of the nitrogen atoms. The minor isomer could thus be regarded as being more crowded, and the M → m conversion would then be sterically more hindered and would require a higher activation energy. On the other hand, the m → M exchange proceeds faster because it leads to a sterically less crowded structure.

As expected, the errors on ΔS^* are large, and comparisons between different DOTA complexes could be of little value. However, one fact stands out: the entropy of activation is very small in the case of LaDOTA[2] while a positive value indicative of a decrease in order was systematically obtained for YbDOTA (mean value in Table 1: $\Delta S^* = 34$ J mol^{-1} K^{-1}). A large positive entropy of activation ($\Delta S^* = 30.6 \pm 2.8$ J mol^{-1} K^{-1}) and an enthalpy of activation similar to the ΔH^* values listed in Table 1 ($\Delta H^* = 60.4 \pm 0.7$ kJ mol^{-1}) were also found by Pittet *et al.*[22] in a band-shape analysis of the NMR spectrum of the Pb(II)

complex of N,N',N'',N'''-tetrakis(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane. Should the difference between the ΔS^* values of LaDOTA and YbDOTA be real, it could be assumed that the formation of the activation complex is attended by the loss of a water molecule. Presumably, the inversion of the configuration of the acetate groups proceeds through the formation of a prismatic polyhedron in which the carboxylate oxygen atoms remain coordinated at the normal metal–oxygen distance. This geometrical arrangement leaves less space for the water molecule that belongs to the first coordination sphere of the metal.[15] The same phenomenon might not have be observed in the case of LaDOTA because the larger ionic size of La(III) leaves more space for a water molecule in the activation complex. The entropy variation corresponding to the loss of a water molecule from the first coordination sphere of a lanthanide ion was estimated[23] to be in the range 40–60 J mol^{-1} K^{-1}. Structural inferences must of course be accepted with reservation because large errors on the ΔS^* values are unavoidable. Moreover, other phenomena such as a rupture of some metal–oxygen bonds could also account for the calculated ΔS^* values. We reported previously[2] a band shape analysis of the NMR spectra of LaDOTA, but such an analysis would be difficult in the case of LuDOTA. Indeed, the ^{13}C NMR of LaDOTA can be analyzed in terms of a simple two-sites exchange because the concentration of the minor isomer is exceedingly small.[6] On the contrary, the full scheme reproduced in Figure 3 would have to be used to interpret the variable temperature ^{1}H and ^{13}C spectra of LuDOTA with strongly coupled protons.and with peaks that overlap as soon as they start to broaden. This would require an extensive study with computer simulations and would not necessarily lead to the sought results.[20] A 2D approach avoids these difficulties and seems more appropriate despite the large errors on the activation parameters inherent in studies on paramagnetic compounds.

Within the limits of the errors, the inversions of the configuration of the acetate groups and of the ethylenic moieties in the tetraaza ring proceed at the same rate and are characterized by the same thermodynamic parameters (see for instance M$_1$ → m$_1$ and M$_1$ → m$_2$ in Table 1). The two processes are probably concerted mechanisms, and it is thus not totally unexpected that they have similar activation barriers. According to Aime *et al.*,[6] the acetate arms are exchanging their geometrical arrangements faster than the ethylenic moities. This hypothesis rests on a qualitative analysis of the ^{13}C spectrum of NdDOTA and of the EXSY spectrum of YbDOTA. For the latter complex at 0 °C, these authors found cross-peaks between the protons assigned to the acetate groups of the major and minor isomers but no cross-peaks for the ethylenic groups. We could not reproduce these measurements and in addition, it seems dangerous to base a spectral interpretation on the presence or the absence of cross-peaks in a 2D spectrum at low temperature because cross-peaks can be so small that they are easily lost in the noise. Moreover, the inversion of the acetate groups automatically causes a

(21) Pittet, P. A.; Laurence, G. S.; Lincoln, S. F.; Turonek, M. L.; Wainwright, K. P. *J. Chem. Soc., Chem. Commun.* **1991**, 1205–1206.
(22) Bertha, S. L.; Choppin, G. R. *Inorg. Chem.* **1969**, *8*, 613–617.
(23) Goldman, S.; Morss, L. R. *Can. J. Chem.* **1975**, *53*, 2695–2700.

modification of the environment of the ethylenic protons that should thus resonate at a different frequency and should also give rise to cross-peaks such as M(ax$_2$)–m(ax$_2$) as shown in Figure 3. This remains true wether one adopts the peak assignments we published earlier[2] or the assignments proposed by Aime *et al.*[6]

Conclusion

The steric requirements of the macrocyclic ligand DOTA are at the origin of the unusual rigidity of its lanthanide chelates. Not only intramolecular exchange processes but also intermolecular exchanges between isomers are readily observed by NMR. Quantitative two-dimensional EXSY spectroscopy is particularly useful for investigating the dynamic behavior of the paramagnetic YbDOTA chelate although the errors on the thermodynamic activation parameters are larger than for the band shape studies of the diamagnetic LaDOTA complex. All conformational exchanges can be analyzed simultaneously and the full exchange scheme is readily established. We have observed slow intra- and/ or intermolecular exchanges for a range of macrocyclic lanthanide complexes in which the DOTA ligand is substituted in the ring or in the acetate arms.[4] The same observations were made recently in the case of bi- and tricyclic macrocyclic complexes of paramagnetic lanthanide ions, and EXSY spectroscopy should be quite useful for a greater understanding of the dynamic behavior of these compounds despite the difficulties encountered in studying paramagnetic species by two-dimensional techniques.